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CARACTERISATION ET VARIABILITE DES PLANTES A PARFUM AROMATIQUES ET MEDICINALES DE CORSE ET DE L'OUEST ALGERIEN

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A mes parents

A mes frères, et à ma sœur

AVANT PROPOS

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SOMMAIRE

A. INTRODUCTION

I. Cadre général

L'histoire des plantes à parfums, aromatiques et médicinales « PPAM » est associée à l'évolution des civilisations. Dans toutes les régions du monde, l'histoire des peuples montre que ces plantes ont toujours occupées une place importante dans la vie quotidienne : préparations culinaires, médecine empirique puis médecine chimique, médecine curative et médecine de bien-être, parfums…

Les années 90 ont été marquées par une prise de conscience générale en faveur de la santé de l'homme et de la qualité de l'environnement. L'agriculture biologique, la phytothérapie et l'aromathérapie ont suscité un regain d'intérêt pour la culture des PPAM pour leur utilisation (plante entière) ou encore sous forme d'huile essentielle ou d'extrait.

Les deux rives du bassin méditerranéen possèdent une flore riche et variée dans laquelle il existe de nombreuses PPAM qui suscitent de grands intérêts, dans des domaines aussi divers que la pharmacie, la cosmétique, la parfumerie ou l'agroalimentaire, pour leurs propriétés thérapeutiques, organoleptiques et odorantes, ou encore pouvant être utilisées comme source d'isolats pour les hémisynthèses. Ces plantes aromatiques sont, donc, à l'origine de produits à forte valeur ajoutée (H.E., extraits, résines…) qui se présentent presque toujours comme des mélanges complexes dont il convient d'analyser la composition avant leur éventuelle valorisation.

A l'instar de nombreux pays et contrées, l'Algérie et la Corse possèdent une flore abondante, riche et variée dans laquelle il a été dénombré de nombreuses espèces aromatiques susceptibles de fournir des huiles essentielles. De même, les hydrolats, sous-produits de l'hydrodistillation des végétaux sont devenus une valeur montante du marché des PPAM au vue de leur importance notamment en aromathérapie (1). Depuis quelques années, la valorisation des huiles essentielles est devenue un facteur de développement économique. De nombreux pays émergents, tentent de pénétrer ce secteur. Dans ce contexte, la tendance actuelle du marché international des huiles essentielles va dans le sens d'une production dont la qualité est constante et contrôlée. Il est donc indispensable que le démarrage d'une activité industrielle de ce type se fasse sur des bases solides s'appuyant sur des données objectives.

II. Contexte national et international

Le secteur des PPAM et de la production des huiles essentielles en Algérie est mal connue (manque d'informations et de statistiques), il reste l'œuvre d'initiatives personnelles (herboristes, distillateurs ambulants…). Mais depuis 2003, la politique agricole de l'Algérie encourage la culture et la valorisation des PPAM par la mise en œuvre de projets de développement rural (2). Le secteur PPAM en Algérie est aujourd'hui en pleine structuration et son expansion permettra d'atténuer l'exode rural en augmentant le revenu des cultivateurs et en générant des emplois pour les habitants de la région. Il doit permettre de résorber une forte demande d'emplois qualifiés.

Contrairement à l'Algérie, la France et plus particulièrement la Corse possède des organismes bien structurés et expérimentés dans le secteur des PPAM et de la production des huiles essentielles originaires de cette région. Dans le cadre d'une dynamique d'échange et de collaboration entre les pays des deux rives de la Méditerranée, ce travail de thèse qui rentre aussi dans le cadre d'échange interuniversitaire permet un transfert d'information et surtout de compétence autour de la production et de la valorisation des PPAM. Ainsi, outre l'intérêt de ma formation et de mon apprentissage dans le domaine des PPAM, mon travail de thèse s'inscrit dans une dynamique socio-économique et aussi de protection et de gestion de l'environnement. En effet :

- Il permet la disponibilité et la diffusion d'informations fiables et objectives relatives à l'identification des plantes à cultiver et aux produits que les « petits » professionnels seront amenés à commercialiser.
- Il favorise l'émergence d'une plateforme technique de transfert de technologie et d'une équipe de recherche spécialisée dans ce domaine et, donc, la production d'un travail intellectuel à forte valeur ajoutée.
- Il comporte un intérêt d'un point de vue environnemental en contribuant à l'accroissement de l'utilisation de la biomasse et à l'étude de la biodiversité végétale. Il présente un impact sur l'aménagement et la valorisation du territoire en favorisant la création et le développement, au niveau artisanal voire semi-industriel, d'unités de production délocalisées.

III. Rappel du contexte de la coopération et des relations existantes

Créé il y a 25 ans, le laboratoire de Chimie des Produits Naturels de l'Université de Corse Pascal Paoli (CPN-UCPP) a développé la thématique des PPAM. Dans ce secteur, il se prévaut, aujourd'hui, d'un savoir-faire important en termes de méthodes de caractérisation des plantes à travers l'identification des constituants des huiles essentielles et des extraits. Il dispose d'un plateau technique analytique performant basé, en premier lieu, sur l'analyse par couplage chromatographie en phase gazeuse / spectrométrie de masse (CPG/SM : méthodologie labellisée au niveau international) mais aussi sur l'analyse spectrométrique par résonance magnétique nucléaire (RMN ^{1}H et ^{13}C, 1D et 2D). Afin d'approcher la réalité de la composition chimique des fractions volatiles en minimisant les altérations hydrothermales, le laboratoire dispose des équipements permettant la concentration des volatils par micro extraction en phase solide (MEPS) et d'un désorbeur thermique automatique (ATD).

Le Laboratoire des Substances Naturelles & Bioactives (LASNABIO) de l'Université Abou Bekr Belkaïd de Tlemcen se donne comme objectifs la recherche et le développement de la filière PPAM en Algérie. Ses unités se chargent de l'étude des huiles essentielles et des extraits de PPAM ainsi que la mise en place de protocole pour la réalisation de différents types d'activité biologique sur ces dernières, tout en apportant une dimension supplémentaire par la synthèse et la modélisation des substrats actifs trouvés dans ces PPAM.

Dans cette optique, mon travail de thèse à débuté dans le cadre d'une convention de recherche entre les Universités de Corse et de Tlemcen donnant lieu à une thèse en cotutelle réalisée au sein des laboratoires CPN et LASNABIO. Ce travail a été financé par l'Agence Universitaire de la Francophonie (AUF) et il s'est étendu au travers d'un projet PHC TASSILI 2011-2013. L'intérêt de cette coopération réside dans le fait qu'elle met en œuvre la complémentarité des compétences des deux équipes de recherche des deux pays et des potentialités de ceux-ci pour la prise en compte de la filière dans sa globalité : inventaire des plantes endémiques, extraction par divers procédés, analyses par plusieurs techniques, étude de la variabilité, mesure des activités biologiques, sélection de plants économiquement intéressants en vue d'une mise en culture et optimisation des procédés de production.

IV. Problématique de recherche

Les huiles essentielles sont des produits à forte valeur ajoutée utilisées dans des domaines aussi divers que la parfumerie, les cosmétiques, l'agro-alimentaire ou encore l'aromathérapie et la pharmacie. Elles se présentent sous forme de mélanges complexes de plusieurs dizaines voire de plusieurs centaines de composés en général terpéniques présents dans des proportions variables. La production et la caractérisation des huiles essentielles, le contrôle de leur qualité tout autant que la mise en évidence d'une éventuelle spécificité nécessite la mise en œuvre des méthodes de préparation et d'analyses les plus modernes.

Dans ce travail de thèse, trois grands volets ont été développés : le principal volet est chimique, il concerne la caractérisation des compositions chimiques des huiles essentielles, les deux autres volets sont totalement complémentaires : (i) un volet génétique dont le but est d'établir les séquences génétiques des plantes étudiées et examiner l'impact du patrimoine génétique sur la production de métabolites secondaires (ii) un volet microbiologique basé sur la mise en évidence d'activités antimicrobiennes des huiles essentielles étudiées. Pour cela :

i) nous avons développé une séquence analytique permettant la caractérisation chimique des huiles essentielles, en optimisant tout autant l'étape de préparation de l'échantillon que l'étape d'identification proprement dite. L'hydrodistillation est la technique de référence pour l'obtention des huiles essentielles, cette étape est délicate et elle nécessite un meilleur contrôle. Dans certain cas, elle peut être substituée par des techniques dites alternatives telle que la Micro-Extraction en Phase Solide. Cette méthode permet d'accéder aux composés volatils émis par le végétal, composés qui jouent un rôle écologique important. De manière conventionnelle, la caractérisation chimique des huiles essentielles est réalisée à l'aide de techniques chromatographiques telles que la Chromatographie en Phase Gaz (CPG) et la CPG couplée à la Spectrométrie de Masse (CPG/SM), qui sont les techniques de référence. Nous avons développé une méthodologie du laboratoire basées sur l'utilisation combinée de techniques complémentaires pour l'identification des composés absents des bibliothèques de spectres.

ii) différents marqueurs sont utilisés pour étudier la diversité génétique des espèces végétales et par la même estimer le polymorphisme et prédire l'évolution des populations végétales. L'avancée dans la connaissance du génome des plantes a favorisé le développement de marqueurs moléculaires ciblant différentes régions des génomes nucléaire, chloroplastique et mitochondrial. Le développement de la génomique donne accès aux gènes contrôlant des

caractères adaptatifs, très importants pour la gestion des ressources génétiques. Les objectifs sont d'évaluer les facteurs génétiques contribuant à la colonisation rapide de nouveaux domaines. Dans cette optique, des réponses aux questions suivantes méritent d'être apportées :

- le polymorphisme peut-il jouer un rôle ? Dans l'affirmative, dans quelle mesure ?
- quel est le degré de flux génétique entre les différentes populations ?
- quelle a été l'évolution des adaptations végétales ?

Il existe à l'heure actuelle un large éventail de stress, destiné à s'intensifier dans un avenir proche, qui menace les populations végétales (3). Ceci va donc influencer les stratégies de conservation. Dans les régions tempérées, l'instabilité climatique attendue et la sédimentation des polluants sont des facteurs indirects importants qui influencent à la fois la composition des espèces et les fréquences des gènes au sein des espèces, même dans des communautés totalement intactes (3). La tâche capitale de la conservation des ressources génétiques consiste à préserver le potentiel évolutif/adaptatif des espèces, des communautés et des écosystèmes. Ceci est surtout vrai pour les PPAM qui se caractérisent par une distribution limitée et fragmentée.

La conservation des ressources génétiques des communautés autochtones grâce aux méthodes *in situ*, c'est-à-dire à la préservation du *status quo*, se base sur le concept suivant : les fréquences alléliques les plus adaptées à un environnement donné évoluent dans les conditions naturelles. Les populations locales résistent au stress environnemental et aux maladies mieux que les autres. Ce concept conduit implicitement à la conclusion que la diversité génétique originelle dans son ensemble est la véritable ressource à préserver. Rien donc, ne justifie d'attacher des valeurs à certains caractères dans la mesure où la valeur d'adaptation des caractères est très difficile à évaluer, même des caractères apparemment « neutres » ou des allèles rares peuvent prendre de l'importance face à d'autres situations sélectives.

En conséquence, la constitution génétique des populations autochtones qui se sont développées naturellement devrait être conservée inchangée, car les végétaux ont le grand avantage de posséder une extraordinaire plasticité phénotypique. Dans ce cas, les objectifs de la conservation semblent relativement simples : sur la base d'inventaires génétiques, les stratégies et les méthodes de conservation doivent être développées de façon à assurer la survie des populations localement adaptées et à éviter la disparition des allèles rares.

iii) nous avons cherché à mettre en évidence les activités biologiques des huiles essentielles étudiées. En effet, le regain d'intérêt pour l'utilisation et la consommation de produits « bio », a conduit les scientifiques à s'intéresser aux huiles essentielles et en particulier aux activités biologiques de leurs constituants. Ces composés présentent l'intérêt d'avoir une faible toxicité, d'être facilement acceptés par les consommateurs et d'avoir un potentiel multi-usage important. En Europe, les huiles essentielles sont surtout utilisées dans l'industrie alimentaire en tant qu'additifs aromatisants, dans l'industrie des parfums et dans l'industrie pharmaceutique pour leurs propriétés fonctionnelles. Si les propriétés antimicrobiennes sont reconnues depuis la fin du 19° siècle, c'est plus récemment que des études ont montré les activités antivirales, antimitotiques, antioxydantes, antiparasitaires, ou encore insectifuges des huiles essentielles (2). Avec la résistance accrue des bactéries aux antibiotiques, les constituants des huiles essentielles apparaissent comme des produits de substitutions très intéressants. Il est donc indispensable de chercher des agents actifs contre les bactéries résistantes. De plus, la sécurité alimentaire est l'une des plus importantes préoccupations en santé publique. Des études montres que 30% de la population des pays industrialisés souffrent d'intoxications alimentaires dues à la présence de micro-organismes au sein des aliments (4). Par ailleurs, l'oxydation des lipides observée pendant le traitement et le stockage des produits alimentaires est responsable de la détérioration de la qualité des aliments mais surtout est néfaste pour la santé humaine. L'utilisation d'antioxydants synthétiques, tels que les dérivés du toluène ou de l'anisole, utilisés pour permettre une meilleure conservation de l'aliment est limitée du fait de leurs propriétés cancérigènes (5). Les huiles essentielles peuvent donc jouer un rôle important et nouveau dans la préservation de la qualité des produits de l'industrie alimentaire (6-9).

Afin de mettre en application ces différentes approches, nous nous sommes intéressés à l'étude de quelques espèces et sous-espèces d'un genre appartenant à la famille des Lamiacées : le genre *Teucrium*. Ce genre est connu pour sa richesse en huiles essentielles, de plus il s'agit d'un genre très répandu dans le bassin méditerranéen, en particulier en Corse et en Algérie.

Il est à noter qu'un travail préalable a été mené par l'équipe du laboratoire CPN de Corse sur l'étude de la composition chimique des huiles essentielles de deux espèces de *Teucrium* de Corse, ainsi mon travail s'inscrit dans une continuité de cette étude tout en approfondissant et élargissant notre recherche aux autres espèces de *Teucrium* présentes en Corse et leur

équivalentes poussant dans l'ouest algérien. Ces études visent aussi bien les huiles essentielles, les hydrolats et les fractions volatiles et ainsi :

✓ nous avons étudié la composition chimique de la fraction volatile de plusieurs espèces appartenant au genre *Teucrium* de Corse et de l'ouest algérien ;

✓ nous avons caractérisé et étudié la variabilité chimique et génétique par une approche interdisciplinaire chimie/génétique des espèces et sous-espèces du genre *Teucrium* ;

✓ nous avons recherché une valorisation des huiles essentielles étudiées en développant une approche pluridisciplinaire chimie/activité biologique au travers de la mise en évidence des activités biologiques des huiles essentielles.

Dans ce manuscrit, après un paragraphe consacrée à l'étude bibliographique (B), je me propose de vous présenter une synthèse des principaux résultats obtenus sur l'analyse des mélanges complexes volatils (huiles essentielles, hydrolats, fraction volatile) (C), l'apport de la génétique dans une approche interdisciplinaire chimie/génétique afin mieux appréhender la diversité végétale (D) et la valorisation des huiles essentielles au travers de l'étude de leurs activités biologiques (E).

B. ETUDE BIBLIOGRAPHIQUE

Le genre *Teucrium* comprend plus de 300 espèces généralement aromatiques poussant à l'état spontané dans diverses régions du globe. Il est largement présent dans le bassin méditerranéen et plus particulièrement en Algérie et en Corse où sont recensées respectivement 21 et sept espèces (10, 11). Le genre *Teucrium* appartient à la famille des *Lamiaceae* ou labiées qui comptent également des végétaux plus communs tels que le thym (*Thymus herba baronna*) et la lavande sauvage (*Lavandula stoechas*). Il est à noter que les *Teucrium* se différentient généralement par leurs caractéristiques morphologiques communes, notamment l'indumentum et les glandes qui sécrètent des huiles essentielles (ce qui fait des *Teucrium*, un genre riche en huile essentielle), ainsi que par leurs richesses en certaines grandes familles de composés chimiques, notamment les diterpènes de squelette néoclérodanes caractéristiques de ce genre. Dans ce paragraphe, nous présentons les caractéristiques botaniques et les utilisations traditionnelles des espèces étudiées au cours du travail de thèse, ainsi qu'une synthèse bibliographique des travaux relatifs à la caractérisation chimique de leur partie volatile.

I. *Teucrium massiliense* L.

Germandrée de Marseille est une espèce endémique à la Corse, à la Sardaigne et aux Baléares (11, 12). Elle se présente sous la forme d'un sous arbrisseau d'une quarantaine de centimètre au maximum. Plante vivace très aromatique avec des feuilles persistantes d'un vert un peu grisâtre et des fleurs roses en grappe qui s'épanouissent depuis le mois de mai jusqu'au mois d'aout. Elle croit dans les endroits pierreux et boisés. Très rare et très peu utilisée, cette plante est considérée comme une plante protégée en Corse (13). Pour cette raison cette plante est très peu utilisée en médecine traditionnelle, toutefois la partie aérienne est utilisée en décoction pour son effet fébrifuge et anti malarique (11, 14, 15).

Un seul travail rapporte la composition chimique de l'huile essentielle de *T. massiliense* originaire de Sardaigne (15) dans laquelle 34 composés ont été identifiés et les composés majoritaires sont la 3,7-diméthyloctan-2-one (15,2%), le butyrate de 2-butyle (12,1%), le linalol (10,6%) et l'acétate de linalyle (7,1%). La littérature rapporte aussi l'identification de quelques diterpènes possédant le squellete néoclérodane extraits aux solvants et connus, entre autre, pour leur activité antifongique et antimicrobienne (16,17).

Figure 1 : Représentation schématique et photo de *Teucrium massiliense* L.

II. *Teucrium marum* L.

Germandrée marine ou Pivarella en Corse, est une espèce endémique à la Corse, à la Sardaigne, aux Baléares et aux îles d'Hyères (11, 12). Elle se présente sous la forme d'un sous arbrisseau d'une trentaine de centimètre au maximum, qui ressemble fortement au thym d'ou son surnom de thym aux chats. Elle doit également cette appellation au fait qu'elle entraîne une certaine forme d'ivresse pouvant aller jusqu'à l'endormissement chez les félidés (18, 19). On la trouve aussi bien au bord de mer qu'en montagne et elle affectionne les terrains en éboulis à roches apparentes aussi bien secs et chauds qu'humides et ombragés. Elle fleurie du début de mois de juin au mois d'août en donnant de petites fleurs roses disposées à l'extrémité des rameaux. Ses feuilles sont d'un diamètre d'un centimètre au maximum, leur face supérieure est parsemée de glandes sécrétrices et recouverte sur toute sa surface d'une fine couche de poils qui forme une cuticule blanchâtre à l'origine de son aromaticité (12, 18,19).

T. marum est connue pour ses utilisations populaires et notamment en médecine traditionnelle. Ainsi, la plante servait autrefois aux bergers à protéger les fromages des mouches (20) et elle était utilisée en infusion pour prévenir des rhumes, soulager les maux de tête, faire baisser la fièvre et traiter les symptômes de la malaria. Aujourd'hui, elle est encore utilisée en cuisine pour parfumer les plats mais aussi en aromathérapie.

Les composés volatils issus de *Teucrium marum*, ont fait l'objet de plusieurs travaux dans lesquels des phenylpropanoïdes, des diterpènes de la famille des néoclérodanes et des iridoides monoterpéniques comme le dolichodial, la teucréine et la dolicholactone ont été identifiés. A noter que les iridoïdes sont connus pour posséder de puissantes propriétés biologiques à savoir, lacrymogènes, insecticides et bactéricides ou encore psychotropes pour les félidés (18, 19,21). Un seul travail décrit la composition chimique de l'huile essentielle de *Teucrium marum* originaire de Sardaigne obtenue par hydrodistillation dans laquelle les composés majoritaires sont l'isocaryophyllène (20,2 %), le (E)-β-bisabolène (14,7 %), le β-sésquiphellandrène (11,2 %), l'α-santalène (10,9 %) et le dolichodial (9,3 %) (22).

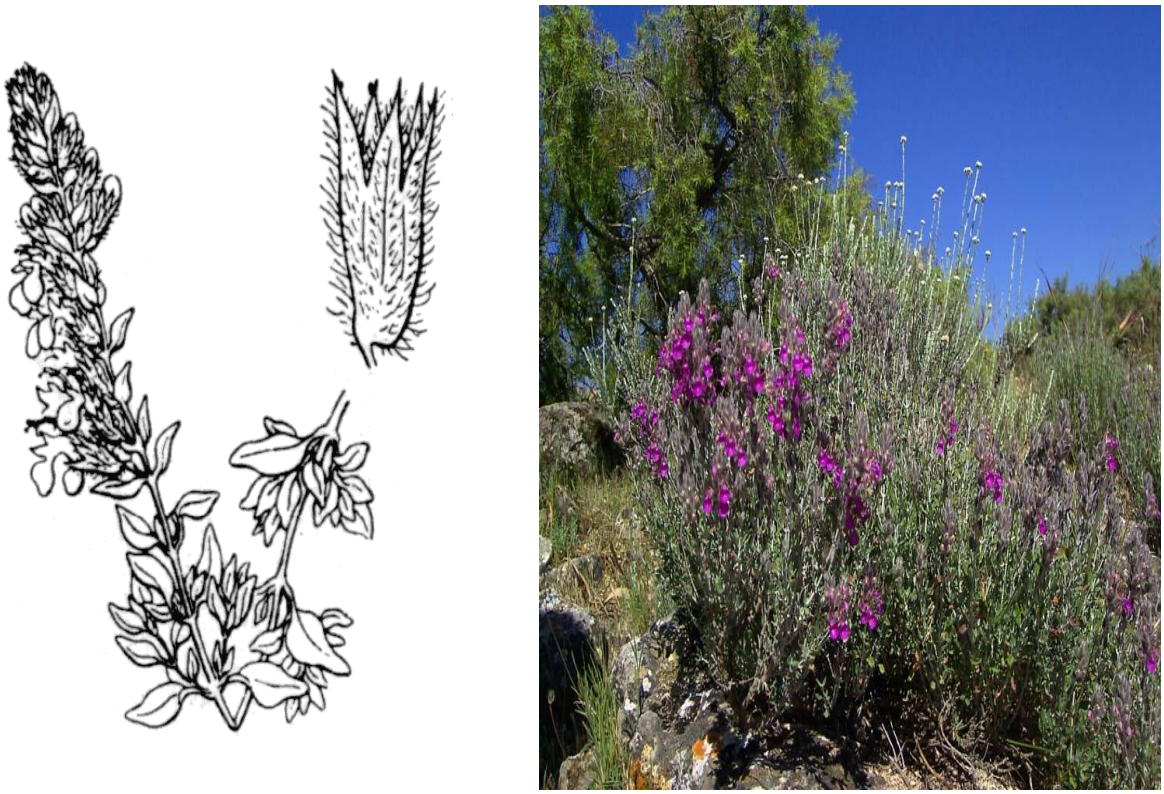


Figure 2 : Représentation schématique et photo de *Teucrium marum* L.

III. *Teucrium scorodonia* L.

Cette espèce est connue pour posséder plusieurs appellations : Germandrée des Bois, Sauge d'Ail, Large-Leaved Germander… *T. scorodonia* pousse de façon spontanée en Corse et en Algérie, dans les lieux rocailleux. Cette plante est un bon indicateur des sols acides et plutôt secs (23,24). Il s'agit d'une plante vivace de 20 à 40 cm de hauteur, qui fleurit de juin à septembre et que l'on trouve jusqu'à une altitude de 1500 m. *T. scorodonia* est présente, dans le basin Méditerranéen, seulement sous forme de deux sous-espèces : *T. scorodonia* ssp.

scorodonia et *T. scorodonia* ss. *baeticum* (10, 11, 12, 25, 26). De façon générale, les fleurs font moins de 1 cm de longueur, sont de couleur oscillante entre le jaune et le vert, elles sont dotées de poils et possèdent des étamines très longues dépassant largement la corolle pour ce qui est de la ssp. *baeticum*, alors que pour la ssp. *scorodonia* les poils possèdent des étamines moins longues et moins abondantes. Les feuilles quant à elles sont opposées, en forme de cœur, dentées, très ridées, vertes sur les deux faces et à longs pétioles. La tige est de section carrée. C'est une plante qui, lorsqu'elle est froissée, exhale une odeur d'ail (24).

Teucrium scorodonia possède plusieurs actions thérapeutiques : il s'agit d'une plante fortifiante, vermifuge, diurétique, elle est utilisée comme expectorant et en tant que spasmolytique. Elle stimule l'appétit et était autrefois utilisée pour nettoyer et désinfecter de vieilles blessures, on la prescrivait également dans le traitement des ulcères. Couramment employée en homéopathie, elle est notamment utilisée pour traiter les polypes de la muqueuse nasale (24).

La ssp. *scorodonia* est présente, entre autre, en Corse, en Espagne et en Italie (11, 12, 26), par contre la ssp. *baeticum* est présente seulement en Algérie et en Espagne (10, 25). Concernant la bibliographie, un premier travail décrit la composition chimique des deux sous-espèces originaire d'Espagne (25) et un second rapporte celle de la ssp. *scorodonia* d'Italie (26). Toutes sont caractérisées par une forte présence de sesquiterpènes hydrocarbonés. Les compositions chimiques des deux sous-espèces d'Espagne sont qualitativement semblables et différent seulement quantitativement sur les pourcentages des composés majoritaires : on distingue pour (i) la ssp. *scorodonia* : un mélange d'aristolène et de (*E*)-β-caryophyllène (12,3–21,0% et 35,1–39,7%, respectivement), l'aromadendrène (0,9–6,5% et 10,6–14,0%, respectivement) et (ii) pour la ssp *baeticum :* un mélange d'oxyde de caryophyllène et de spathulénol (4,9–13,0% et 5,0–9,1%, respectivement). Pour la ssp. *scorodonia* d'Italie, les composés majoritaires sont : le germacrène B (26,2%), le (*E*)-β-caryophyllène (25,2%), l'α-humulène (8,0%), l'α-cubebène (8,0%) et le germacèene D (6,3%). Par ailleurs, deux autres travaux (27,28) décrivent l'identification de 3 dérivés furanoditerpéniques possédant un squelette néoclérodane.

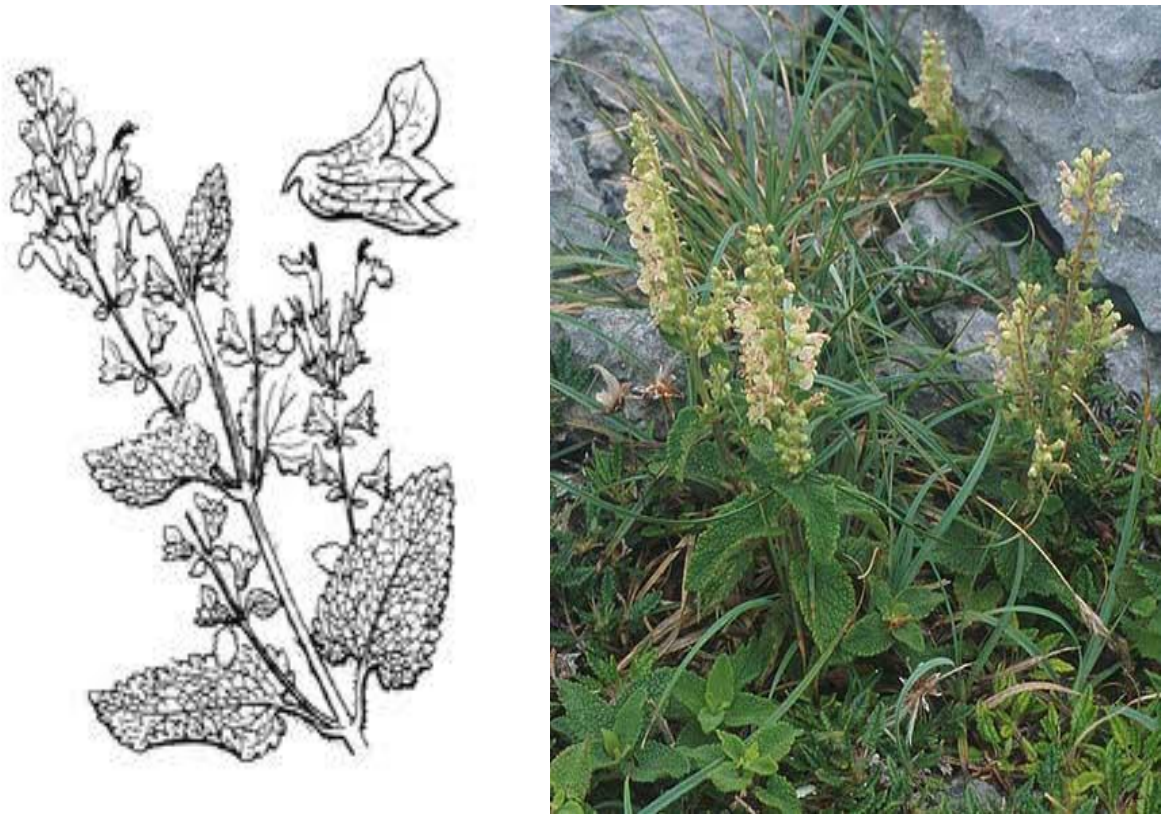


Figure 3 : Représentation schématique et photo de *Teucrium scorodinia* L.

IV. *Teucrium flavum* L.,

Il s'agit de la Germandrée jaunâtre, une espèce très répandue dans le basin méditerranéen et plus particulièrement sur les coteaux pierreux de cette région (11). La Corse présente la particularité d'être le seul territoire où l'on trouve les deux sous-espèces *glaucum* et *flavum*. En effet, en Italie la ssp. *flavum* est seulement présente sur le continent alors que la ssp. *glaucum* est présente seulement sur le territoire insulaire (Sardaigne). Ces deux sous-espèces se distinguent par des caractères morphologiques différents et une répartition au sein de l'île différente. La ssp. *glaucum* est la plus répandue, elle présente des feuilles glabres en dessus et glauques en dessous, alors que la ssp. *flavum* est très rare et très localisé et elle présente des tiges ligueuses à la base avec des petites feuilles crénelées, velues-cendrées sur les deux faces. Les fleurs sont d'un jaune pâle ou verdâtre, parfois tachées de rouge (11, 12). Cette plante est utilisée en générale dans les bordures, ou comme plante semencière pour revégétalisation de talus et espaces dégradés.

Plusieurs travaux ont été réalisés sur les compositions chimiques des huiles essentielles de *T. flavum* originaires de différentes régions du monde. Synthétiquement, on peut dire que ces huiles essentielles sont caractérisés par la présence de monoterpènes tels le linalol mais surtout des monoterpènes hydrocarbonés tels l'α et le β-pinène et le limonène. Il existe aussi

quelques travaux qui rapportent comme composés majoritaires des sesquiterpènes hydrocarbonés tels le (E)-β-caryophyllène, le (*E*)-β-farnesène, le (*E*)-β-bergamotène ou le germacrène D, ou d'autres qui rapportent exclusivement la présence de sesquiterpènes oxygénés tels l'oxyde de caryophyllène et le spathulénol.

Figure 4 : Représentation schématique et photo de *Teucrium flavum* L.

V. *Teucrim polium* L.

Elle est connue sous le nom de Germandrée tomenteuse en français ou Djaïda en arabe, c'est une petite plante blanchâtre, cotonneuse, assez commune dans l'espace méditerrano-saharienne, très polymorphe, elle croit dans les lieux rocailleux et secs. Cette espèce est représentée par de nombreuses sous-espèces dont la détermination reste assez délicate (10). En Algérie, pas moins de douze sous-espèces parmi lesquelles la ssp. *polium* qui est la plus répandue, sont recensées. En Corse, seule la ssp. *capitatum* est décrite. La différence entre les deux sous-espèces réside dans le fait que la ssp. *polium* possède un calice à dents courtes et obtuses contrairement à celui de la ssp. *capitatum* ou les dents sont plus longues. Signalons que dans l'ouest algérien, les fleurs de la ssp. *polium* présentent la particularité d'avoir une variabilité chromatique corrélée à une fréquence de présence, on distingue des spécimens à fleurs mauves très répandus et des spécimens à fleurs blanches beaucoup plus localisés (10).

La ssp. *capitatum* de Corse, présente une plus grande stabilité morphologique, elle est caractérisé par des capitules de 1 cm au plus qui sont nombreuse au somment des tiges, avec des fleurs blanches et des feuilles en général vertes en-dessus et blanchâtre en dessous. (10, 11). Cette plante est connue pour ses vertus thérapeutiques, entre autre, anti-inflammatoire, astringente, détersif, fébrifuge (paludisme…), hypoglycémiante…

Concernant la composition chimique des huiles essentielles de *T. polium*, il est à noter que cette espèce est la plus étudiée dans le genre *Teucrium* nous avons répertorié 22 travaux traitant de l'identification des constituants de son huile essentielle. Deux types d'huile essentielle sont ainsi distinguées : (i) une est riche en composés monoterpéniques tels l'α et β-pinène, le limonène, le myrcène, et le linalol, le terpinèn-4-ol et la carvone et (ii) l'autre est dominée par des composés sesquiterpéniques comme le (*E*)-*β*-caryophyllène, le germacrène D et l'α-humulène et l'oxyde de caryophyllène, l'α et le τ-cadinol et le spathulènol.

Figure 5 : Représentation schématique et photo de *Teucrium polium* L.

C. L'ANALYSES DES MELANGES COMPLEXES DES VOLATILS DES PLANTES

Les huiles essentielles et la fraction volatile émise par une plante aromatique constituent des mélanges complexes de volatils qui suscitent un intérêt permanent. La valorisation de ces mélanges passe nécessairement par une étape de caractérisation chimique. Pour cela, il apparaît que l'étape de préparation de l'échantillon est tout aussi fondamentale que celle de l'analyse proprement dite des constituants (29-32) pour plusieurs raisons :

- ces mélanges volatils sont généralement des mélanges complexes constitués majoritairement de molécules terpéniques (mono- et/ou sesquiterpènes et plus rarement des diterpènes), mais aussi de composés non terpéniques (chaînes linéaires, dérivés phénylpropanoïques, etc.) (33). L'identification de ces molécules nécessite donc des techniques analytiques fiables et efficaces (32),
- la complexité des ces mélanges vient également du fait qu'ils sont constitués de plusieurs dizaines, voire plusieurs centaines de composés présents à des concentrations parfois extrêmement faibles. Classiquement la concentration perceptible pour les molécules odorantes peut être inférieure au nanogramme par litre, en conséquence les procédures analytiques doivent présenter des sensibilités extrêmement élevées (34, 35),
- certaines molécules odorantes présentent une instabilité chimique sous l'action de la lumière, de la température, en condition oxydante ou au cours d'une étape de transformation avant commercialisation. Ainsi, à pression atmosphérique le temps de vie d'un monoterpène soumis à une exposition lumineuse est estimé entre moins de 5 min pour l'α-terpinène à 3 h pour l'α- et le β-pinène (31). Les procédures de préparation des échantillons doivent être adaptées à la nature des constituants de la matrice à étudier,
- pour les composés volatils générés à partir de sources biologiques, telles que les plantes ou les animaux, des difficultés analytiques surgissent du fait de la nature dynamique de ces systèmes (36). Le fait que la production et l'émission des composés volatils d'une plante soient affectées par des facteurs comme la lumière, la température, un stress hydrique, une activité enzymatique ou la présence de polluants, introduit des difficultés dans l'analyse. Ainsi, des procédés analytiques ont été développés permettant l'échantillonnage *in vivo* de la fraction volatile des plantes (37).

Conformément à l'esprit d'une thèse soutenue sur article, je me propose de décrire dans cette partie une synthèse des résultats obtenus au cours de mes travaux. Ces résultats sont extraits de six publications [**PT1-6**] présentées en fin de document. J'ai choisi d'insérer les résultats à la suite de l'inventaire des techniques analytiques généralement mises en œuvre pour l'analyse des mélanges volatils. Je me propose de suivre les différentes étapes de la séquence analytique, à savoir, (i) l'étape de préparation de l'échantillon avec notamment la mise en œuvre de la micro-extraction en phase solide comme méthode complémentaire à l'hydrodistillation, (ii) l'étape de séparation puis d'identification proprement dite en insistant sur l'intérêt de combiner plusieurs techniques analyse pour identifier les composés absents de nos bibliothèques de spectres, (iii) l'étape d'interprétation des résultats en montrant l'intérêt d'utiliser l'outil statistique pour montrer la variabilité chimique des échantillons au travers d'une approche interdisciplinaire chimie/génétique et enfin (iv) l'étape de valorisation au travers d'une approche pluridisciplinaire chimie/microbiologie qui met en évidence le potentiel antimicrobien des huiles essentielles analysées.

I. LES METHODES DE PREPARATION DE L'ECHANTILLON

Les méthodes de préparation des échantillons ont fait l'objet de nombreuses revues récentes qui décrivent les potentialités et les limites des procédures généralement mises en œuvre pour l'étude des composés volatils issus de plantes (31, 37-47). Nous distinguerons les méthodes d'extraction produisant des matrices liquides telles que les huiles essentielles et les extraits, des méthodes permettant d'échantillonner les analytes dans la phase gazeuse de matrices solides ou liquides.

I.1. Hydrodistillation : Une technique de référence

A l'échelle industrielle, l'entraînement à la vapeur d'eau est l'un des procédés les plus anciens, d'extraction ou de séparation de certaines substances organiques, apporté par les Arabes au IXe siècle. Cette opération s'accomplit dans un alambic. La méthode est basée sur l'existence d'un azéotrope de température d'ébullition inférieure aux points d'ébullition des deux composés purs pris séparément. Le but est d'entraîner avec la vapeur d'eau les constituants volatils des produits bruts. La vapeur détruit la structure des cellules végétales, libère les molécules contenues et entraîne les plus volatiles en les séparant du substrat cellulosique. La vapeur, chargée de l'essence de la matière première distillée, se condense

dans le serpentin de l'alambic avant d'être récupérée dans un essencier (vase de décantation pour les huiles essentielles). Les parties insolubles dans l'eau de condensation sont décantées pour donner l'huile essentielle surnageant. La partie contenant les composés hydrosolubles est appelée eau de distillation (ou hydrolat). (48)

A l'échelle du laboratoire, les huiles essentielles sont obtenues par hydrodistillation dans un appareil de type Clevenger (49), toutefois de nombreuses techniques ont été développées dans le but de limiter le temps d'extraction, la consommation en eau et en énergie, d'augmenter le rendement d'extraction et d'améliorer la qualité de l'huile essentielle en évitant les dégradations thermiques et hydrolytiques (50-52). Des méthodes telles que la distillation-extraction utilisant un appareil de type Lickens-Nickerson (31), de l'extraction par fluide supercritique (42) et de l'extraction assistée par micro-ondes (53-55) ont été développées, elles ne produisent pas d'huiles essentielles mais elles permettent d'obtenir des extraits contenant les composés volatils d'une plante.

I.2. Micro-extraction en phase solide « MEPS » : Un échantillonnage en phase gazeuse

Avec l'apparition de la CPG dans les années 50 et l'expansion du pouvoir séparatif des colonnes capillaires dans les années 80, les techniques d'extraction de composés volatils ont connu de nombreux développements (56, 57). Nous décrirons plus particulièrement les techniques de pré-concentration en « espace de tête » plus connues sous le vocable anglo-saxon « headspace». L'abréviation EdT sera utilisée dans la suite du document pour désigner l'« espace de tête ». La première utilisation du terme « headspace » date de 1960 et la première application de ce concept combinée à la CPG date de 1958 (43). Au cours des 20 dernières années, ce procédé d'extraction a connu un regain d'intérêt qui coïncide avec le succès toujours croissant que connaissent les techniques d'extractions « vertes » (43). L'extraction par exposition de l'EdT consiste à prélever les composés volatils contenus dans la phase gazeuse en équilibre (ou non) avec une matrice solide ou liquide, avant leur caractérisation. Traditionnellement, le prélèvement s'opère en mode statique ou en mode dynamique.

Le développement de techniques de prélèvement qui augmentent la capacité de concentration des analytes dans l'EdT a permis d'associer la simplicité, la reproductibilité et la facilité d'automatisation de l'EdT-statique, à la sensibilité et la sélectivité de l'EdT-dynamique (58). Elles permettent l'accumulation statique ou dynamique de composés volatils sur des polymères qui opèrent par absorption ou adsorption, ou plus rarement sur des solvants

(43). Ainsi il a été développé pour l'étude des composés volatils des matrices végétales, plusieurs procédés de prélèvement à Haute Capacité de Concentration (HCC) dans l'EdT, (58-70), parmi lesquels, la MEPS qui a été la première technique à HCC développée.

L'EdT-MEPS est une technique de préparation d'échantillon sans solvant, simple, rapide, sensible, reproductible, peu couteuse et nécessitant une faible quantité d'échantillon. Elle a été développée par Arthur et Pawliszyn en 1990 (71) et appliquée au prélèvement dans l'EdT en 1993 (72). Sa combinaison aisée avec des techniques chromatographiques comme la CPG, la CPG/SM, la chromatographie liquide haute performance (CLHP), la CLHP/SM ou encore la CPG/olfactométrie, explique son succès pour l'étude des composés polaires et apolaires volatils contenus dans des matrices complexes, solides ou liquides d'origine végétale (36, 39, 43, 73-77).

L'extraction par EdT-MEPS consiste en une adsorption et/ou un partage des molécules volatiles sur une fibre de silice fondue revêtue d'un polymère après leur distribution entre la matrice (solide ou liquide) et la phase gazeuse qui constitue l'EdT. Au pouvoir de séparation du polymère est associée la désorption thermique avant l'analyse. Les phénomènes d'adsorption et/ou de partage mis en jeu ne correspondent pas à une extraction totale des composés volatils car l'extraction par exposition dans l'EdT est basée sur un phénomène thermodynamique qui implique deux équilibres : le premier concerne l'équilibre entre la matrice et la phase gazeuse (EdT) et le second correspond à l'équilibre entre la phase gazeuse et le revêtement de la fibre.

Du fait que la MEPS est une méthode simple de préparation des échantillons, il serait réducteur de penser que l'extraction est un processus simple à réaliser. La nature et la concentration des analytes ainsi que la complexité des matrices à partir desquels ils sont extraits, détermine le niveau de difficulté du processus d'extraction (77). Ainsi, quelles que soient les matrices végétales étudiées, la mise en œuvre de la EdT-MEPS nécessite une étape d'optimisation des paramètres d'extraction afin d'obtenir une bonne reproductibilité et la meilleure sensibilité. Les paramètres à examiner sont la nature de la fibre (polymères liquides et/ou poreux), le volume d'échantillon (variable selon la nature solide ou liquide de la matrice), les conditions d'extraction (pH, agitation, ajout de sels), le temps d'équilibre et d'extraction (variables selon la concentration de l'analyte et son coefficient de distribution), la température, paramètre primordial comme nous montrerons par la suite et enfin, les conditions de désorption.

L'influence de ces paramètres a été démontrée sur le rendement qualitatif et quantitatif de l'extraction (73, 78-93). Selon la littérature, il apparaît que le meilleur rendement d'extraction par exposition dans l'EdT, est obtenu avec les fibres triples constituées d'une phase liquide, le Polydiméthylsiloxane (PDMS) pour les composés les moins polaires et de deux phases solides poreuses, le Carboxen (CAR) et le Divinylbenzène (DVB) pour les composés les plus polaires. Ce type de fibre permet l'extraction de composés volatils sur une large gamme, s'étendant de C_3 à C_{20}. Pour l'étude des composés volatils extraits d'une plante, la préparation de l'échantillon est généralement réalisée à température ambiante (TA) sur du végétal frais, coupé en morceau dont le rapport entre la masse de la matrice et le volume de l'espace de tête sont compris entre 0.01 et 0.1 (75, 84-90). Il est à signaler qu'il existe une grande variation dans les conditions de température et de temps d'équilibre et d'extraction selon les auteurs et la nature de la matrice.

Nous avons appliqué l'EdT-MEPS à l'étude des composés volatils extraits des parties aérienne du matériel végétal qui fait l'objet d'études. Nous avons choisi de présenter deux travaux qui concernent (i) l'influence des paramètres de la MEPS sur la composition finale de la fraction volatile au travers de l'étude des composés volatils de *T. massiliense* de Corse [**PT1**] et (ii) la différence entre les compositions chimiques de l'huile essentielle et de la fraction volatile au travers des résultats obtenus lors de l'étude des constituants volatils de *T. marum* de Corse [**PT2**].

La température guide l'extraction des composés volatils en MEPS : L'optimisation des paramètres d'extraction a été réalisée sur la base de la réponse maximale mesurée en CPG-FID c'est à dire la somme des aires de tous les pics chromatographiques intégrés sur le chromatogramme. La mise au point du protocole expérimental d'extraction est une étape essentielle qu'il faut appréhender avec méthode. A titre d'exemple, après avoir fixé la nature de la fibre et le volume d'échantillon, l'optimisation du temps et de la température d'extraction, a nécessité pas moins de 30 injections en CPG pour l'étude des composés volatils contenus dans les parties aériennes de *T. massiliense* de Corse. Le tableau 1 met en relation les proportions des classes de composés volatils contenus dans le végétal en fonction des paramètres MEPS utilisés pour l'optimisation.

Tableau 1 : Paramètres MEPS optimisés pour l'étude de la fraction volatile des parties aériennes de *T. massiliense* de Corse

	Paramètres MEPS									
	Temps d'équilibre t_{eq} min (T_{ext}= 70 °C - t_{ext} = 15min)				Temps d'extraction t_{ext} min (T_{ext}= 70 °C t_{eq} = 40min)		Température T_{ext} °C (t_{ext}= 30 min - t_{eq} = 40 min)			
Classes de composés	20	40	60	80	30	45	30	50	70	90
Composés hydrocarbonés	33,5	46,9	48,5	36,3	38,7	27,6	19,9	23,7	38,7	33,0
Composés oxygénés	57,7	49,3	49,0	61,3	57,8	64,1	76,0	72,7	57,8	59,3
Composés non-terpéniques	53,0	43,6	42,6	53,0	53,2	55,5	70,5	66,7	53,2	53,2
Monoterpènes hydrocarbonés	6,5	5,7	5,8	6,6	6,1	5,5	8,9	7,2	6,1	4,4
Monoterpènes oxygénés	7,3	5,4	5,1	7,1	4,3	7,2	5,5	5,8	4,3	5,2
Sesquiterpènes hydrocarbonés	27,0	41,2	42,7	29,7	32,6	22,1	11,0	16,5	32,6	28,6
Sesquiterpènes oxygénés	0,9	0,9	1,3	1,1	0,2	1,4	-	0,2	0,2	0,9
Total identification (%)	91,2	96,2	97,5	97,6	96,5	91,7	95,9	96,4	96,5	92,3
Aire totale 10^5	586	885	802	745	1267	1062	516	706	1267	1192

Afin d'assurer de la reproductibilité de la méthode, les analyses des différentes fractions volatiles ont été systématiquement réalisées trois fois dans les mêmes conditions. La reproductibilité de la méthode, exprimée par le coefficient de variation (CV), indique la fiabilité des résultats. Le calcul du CV de l'aire totale du signal CPG-Ir et les CV des aires de plusieurs composés représentatifs du mélange ont été systématiquement réalisés et des CV toujours inférieurs à 15%, ont permis de valider la reproductibilité de la MEPS dans les deux matrices citées.

Le travail mené sur la fraction volatile émise à partir des parties aériennes et les organes séparés de *T. massiliense* [**PT1**] confirme que la température d'extraction est le paramètre le plus important à maîtriser et démontre qu'elle peut être utilisée pour favoriser l'extraction spécifique de classes de composés, ainsi nous obtenons des modifications quantitatives sur les classes de composés suivant les conditions choisis :

- à une température comprise entre 30 et 50°C, les proportions de monoterpènes hydrocarbonés et de composés acycliques non terpéniques sont optimales,
- à une température moyenne (50-70°C), la proportion de sesquiterpènes hydrocarbonés augmente,
- à température élevée (70-90°C), l'extraction des sesquiterpènes oxygénés est exaltée.

La MEPS apporte donc des informations qualitatives sur la composition de la fraction volatile d'une plante et ce à partir d'une quantité de végétal très faible (1 gramme). C'est une

méthode bien adaptée pour obtenir la fraction volatile des plantes qui produisent peu ou pas d'huile essentielle ou des plantes très rares dont la matière végétale est peu disponible comme c'est le cas de *T. massiliense*.

La question qui se pose légitimement est celle de la corrélation entre les compositions chimiques des mélanges complexes des volatils obtenus par hydrodistillation et ceux extraits par MEPS. Il est difficile d'établir une corrélation directe puisque la première technique est une technique d'épuisement de la matrice alors que la seconde est régie par un double équilibre et une compétition entre les molécules interférentes au niveau des sites de fixation. La MEPS permet de donner une composition de la fraction volatile pour une température donnée. En fait la meilleure réponse est donnée par l'étude de l'influence de la température d'extraction sur l'abondance relative des différentes familles de composés. La température optimale d'extraction est seulement un compromis basé sur la quantité maximale d'analyte absorbée. Dans le cas de *T. massiliense*, les compositions chimiques de l'huile essentielle obtenue par hydrodistillation et de la fraction volatile obtenue par MEPS, sont très peu différentes, seules des différences quantitatives sont à remarquer sur certain constituant. C'est un constat totalement différent que nous avons fait au cours de l'étude des composés volatils de *T. marum* de Corse.

La MEPS comme méthode de préparation de l'échantillon complémentaire : L' analyse de l'huile essentielle de *T. marum* de Corse a permis l'identification de 93 composés représentant 92.2 % de la composition chimique. Cette dernière est dominée par une forte proportion de composés oxygénés (plus de 60 %), parmi lesquels 26,8 % de composés non terpéniques, 18,3 % de sesquiterpènes et 14,0 % de monoterpènes. Les constituants majoritaires sont : l'oxyde de caryophyllène (9,8 %), le (E)-α-bergamotène (8,2 %), le β-bisabolène (7,5 %), l' (E)-β-caryophyllène (5,3 %), le β-sesquiphéllandrène (3,7%) et le (3Z,6E,10E)-α-springène (3,2 %). La composition chimique de l'huile essentielle de *T. marum* de Corse diffère de celle de la Sardaigne (22) dans laquelle il a été identifié deux iridoïdes caractéristiques de la plante : le dolichodial et son épimère, l'épi-dolichodial (respectivement 9,1 et 2,0 %) alors que dans les échantillons originaires de Corse, ces composés n'ont jamais été repérés.

Par contre, il est intéressant de signaler que l'étude de la fraction volatile extraite par MEPS à partir du même matériel végétal a révélé la présence du dolichodial et de son épimère dans les 25 stations étudiées. Le dolichodial est toujours le composé majoritaire, il dépasse le

plus souvent 50% de la composition totale de la fraction volatile. Nous concluons donc que cet iridoïde, dialdéhyde pentacyclique, est bien présent comme composé volatile dans *T. marum* mais qu'il est absent dans son huile essentielle.

L'hypothèse avancée pour expliquer l'absence du dolichodial dans l'huile essentielle et sa présence dans la fraction volatile, est basée sur la probable perte du dolichodial par transformation chimique sous l'effet de la chaleur et du pH acide qu'impose l'hydrodistillation. Ainsi, au cours de l'extraction, les deux fonctions aldéhydes du dolichodial sont hydratées et la forme di-hydrate produite est alors transférée dans l'hydrolat. C'est pour cette fraction aqueuse que l'hydrate a vraisemblablement une plus grande affinité. Cette hypothèse est confortée par (i) le calcul du Log P à l'aide d'un logiciel de simulation ACD/Labs V11.02 (© 1994-2012 ACD/Labs) (+1.45±0.37) pour le dolichodial et (-1.27±0.56) pour sa forme hydrate confirmant le caractère lipophile du premier et la forte affinité pour l'eau du second (ii) la présence du dolichodial, de la dolicholactone et de l'épi-dolichodial, identifiés en tant que composés majoritaires dans l'extrait d'hydrolat obtenu par extraction liquide/liquide à l'éther à partir de l'hydrolat (iii) la présence du dolichodial et de la dolicholactone, identifiés en tant que composés majoritaires dans l'extrait héxanique de la plante et (iv) l'absence de protons aldéhydiques dans le spectre de RMN du proton enregistré dans un mélange hydrolat/l'eau deuterrée confirme l'absence de la forme aldéhydique du dolichodial [**PT2**].

Il apparait donc important de bien maitriser l'étape de préparation de l'échantillon et ce travail démontre la nécessité d'adapter une stratégie de préparation de l'échantillon pour la caractérisation complète des composés volatiles d'une plante. De plus, dans l'optique de déterminer de nouveau types chimiques ou de caractériser les volatils d'une plante non encore étudiée, il peut être intéressant de mettre en œuvre au moins deux méthodes de préparation de l'échantillon.

II. LES METHODES D'IDENTIFICATION DES CONSTITUANTS DES MELANGES COMPLEXES DE VOLATILS

L'analyse d'un mélange complexe de volatils s'effectue classiquement par le couplage « en ligne » d'une technique chromatographique, généralement la CPG, avec une technique d'identification spectrale, généralement la SM ou, quelques fois, la Spectrométrie Infrarouge par Transformée de Fourier (IRTF). Cette procédure est privilégiée lors de la réalisation d'analyse « de routine » d'un échantillon dont les constituants ne présentent pas de difficultés

d'identification (30, 32). Dès lors que l'étape d'identification se complexifie, la procédure nécessite un fractionnement de l'échantillon par chromatographie liquide sur colonne (CLC) ou mieux encore en utilisant la chromatographie flash automatisée (CFA), qui peut se poursuivre jusqu'à la purification d'un constituant. L'objectif étant d'aboutir à son élucidation structurale par les techniques spectroscopiques usuelles : RMN-^{1}H et ^{13}C, SM, IRTF, etc.

II.1. Chromatographie en phase gazeuse (CPG)

La CPG est une méthode d'analyse par séparation qui s'applique aux composés gazeux ou susceptibles d'être vaporisés par chauffage sans décomposition (91). C'est la technique de séparation la plus utilisée car elle permet d'effectuer l'individualisation des constituants à partir d'échantillons de l'ordre du milligramme voire du microgramme. La séparation a lieu dans des colonnes capillaires qui possèdent un fort pouvoir résolutif parfaitement adaptée aux mélanges complexes volatils.

Chaque constituant est caractérisé par des indices calculés à partir d'une gamme d'alcanes ou plus rarement d'esters méthyliques linéaires, à température constante (indice de Kováts IK) ou en programmation de température (indices de rétention, Ir). Cependant, la comparaison des indices de rétention avec ceux de la littérature ne suffit pas à identifier formellement un composé. Le développement des phases stationnaires chirales (colonne de cyclodextrines) et de la CPG multidimensionnelle a permis de surmonter certaines difficultés de séparation et d'identification des composés volatils (92). La CPG, est aujourd'hui, un outil incontournable pour l'analyse des composés volatils en mélange.

II.2. Chromatographie en phase gazeuse/Spectrométrie de masse (CPG/SM)

Depuis 1975, les performances du couplage en ligne n'ont cessé d'évoluer ; nombreux sont les domaines d'application : agroalimentaire (aliments, eau), produits pétroliers (carburants, matières synthétiques), produits naturels (parfumerie, cosmétique, médecine).

L'analyseur de masse le plus fréquent pour l'analyse des composés volatils est le « quadripôle » qui utilise la stabilité des trajectoires pour séparer les ions selon le rapport masse sur charge m/z (93). Les détecteurs les plus courants sont les channeltrons (multiplicateurs d'électrons) et les photomultiplicateurs ; ils convertissent les impacts ioniques en signaux. Le multiplicateur de photons permet la détection des ions positifs et,

dans certains cas, des ions négatifs. L'ordinateur enregistre les signaux visualisés sous forme de pics d'intensités variables, rangés sur une échelle de masses.

Le couplage de la chromatographie en phase gazeuse avec la spectrométrie de masse (CPG/SM) permet d'effectuer simultanément la séparation et l'analyse des différents constituants d'un mélange complexe. Pour l'analyse des huiles essentielle, le spectromètre de masse fonctionne selon deux méthodes d'ionisation : l'ionisation par impact électronique (IE) et l'ionisation chimique (IC). Dans ce dernier cas, deux modes sont distingués : L'ionisation chimique positive (ICP) et l'ionisation chimique négative (ICN).

II.3. Résonance magnétique nucléaire (RMN)

C'est la technique de choix pour la caractérisation des molécules organiques ; elle permet l'accès à des informations concernant le squelette et la fonctionnalisation des molécules. Dans cette optique les données de la littérature constituent une base intéressante permettant la comparaison avec les valeurs des déplacements chimiques du carbone 13 des composés absents de nos bibliothèques de données, mais aussi elles proposent les valeurs de déplacements chimiques de molécules « modèles » à partir desquelles des reconstitutions de spectres sont possibles.

III. METHODOLOGIE D'ANALYSE

Au laboratoire CPN, l'identification des constituants d'une huile essentielle ou de la fraction volatile extraite d'une matrice d'origine végétale est réalisée par des techniques d'analyses conventionnelles. Celle-ci est basée sur l'utilisation conjointe de la CPG/Ir et de la CPG/SM-IE (figure 1).

Le mélange complexe des volatils (fractionné ou non dans le cas des huiles essentielles) est analysé simultanément par CPG/Ir et CPG/SM-IE. Le calcul des Ir, polaires et apolaires, et la quantification des composés s'effectuent par CPG/Ir. L'analyse par CPG/SM permet d'obtenir les spectres de masse des divers constituants qui, à l'aide d'un logiciel, sont ensuite comparés à ceux répertoriés dans des bibliothèques, dont une élaborée au laboratoire et les autres, commerciales, en éditions traditionnelles ou informatisées [Jennings et Shibamoto (94) Joulain (95, 96), Wiley (97, 98), Adams (99), Nist (100)]. Afin de rendre performante l'identification, il est préconisé de posséder une bibliothèque riche mais surtout adaptée au

domaine d'investigation (30). La bibliothèque « Arômes » construite au laboratoire, est élaborée à partir de spectres de masse enregistrés dans les mêmes conditions opératoires que celles utilisées pour l'analyse des mélanges complexes, assurant ainsi une fiabilité accrue dans l'identification. Elle contient actuellement les indices de rétention sur deux colonnes de polarité différentes et les spectres de masse de plus de 1000 composés volatils dont plus de 700 molécules terpéniques. Cette bibliothèque a été constituée à partir de molécules disponibles dans le commerce et elle est enrichie continuellement par des molécules isolées par fractionnement à partir des huiles essentielles ou encore obtenues par hémi-synthèse et dans tous les cas, identifiées par RMN.

Chaque proposition du logiciel de comparaison des spectres de masse est assortie d'une note de concordance qui reflète la validité de la structure proposée. Si la note de concordance est correcte, les indices de rétention du constituant proposé sont comparés à ceux présents dans la bibliothèque élaborée au laboratoire, ou dans les bibliothèques commerciales [Jennings (94), Joulain (95, 96), Adams (99)], ou encore répertoriés dans la littérature. Toutefois, se limiter à la note de concordance n'est pas suffisant; il faut systématiquement procéder à l'examen du spectre de masse du composé recherché afin d'en tirer les principales informations : masse de l'ion moléculaire, fragmentations caractéristiques ou encore mise en évidence de co-élutions. A ce stade, trois approches différentes (**a**, **b**, et **c**) sont envisagées (figures 6 et 7) :

- (**a**), le spectre de masse du constituant et ses indices de rétention correspondent à ceux d'un composé présent dans la bibliothèque élaborée au laboratoire. L'identification du constituant est réalisée sans ambiguïté. Cette démarche est systématiquement mise en œuvre quelque soit la nature du mélange complexe (huile essentielle, extrait au solvant ou fraction volatile extraite par MEPS).
- (**b**), les données spectrales et les indices de rétention du constituant ne correspondent à ceux d'aucun composé de la bibliothèque du laboratoire mais correspondent à ceux d'un composé présent dans les bibliothèques commerciales (ou dans la littérature). Dans ce cas nous vérifions, par l'étude des fragmentations principales, si le spectre de masse du produit proposé est bien en accord avec la structure de ce dernier. Cette approche mécanistique peut être complétée, lorsque cela s'avère possible, soit par une étape d'hémi-synthèse suivie de l'exploitation de l'analyse du composé synthétisé, soit par le recours à la RMN ^{13}C dans le cas de l'analyse d'une huile essentielle

- (**c**), les données spectrales et les indices de rétention du constituant ne correspondent à ceux d'aucun composé d'aucune bibliothèque. Dans ce cas deux stratégies, uniquement envisageables pour l'analyse des constituants d'une huile essentielle, sont imaginables :
  - soit le composé est présent dans les bibliothèques RMN ^{13}C (auquel cas il est identifié sans ambiguïté),
  - soit le composé est absent des bibliothèques RMN ^{13}C, auquel cas nous n'avons d'autre ressource que le schéma classique de purification du constituant dans l'optique d'une étude structurale.

Afin de palier les limites analytiques du couplage CPG/SM-IE, l'ionisation chimique a été utilisée pour l'identification des constituants des huiles essentielles (101-121). Il s'agit d'un mode d'ionisation plus doux que l'impact électronique dans lequel il est recherché des réactions ions-molécules entre les molécules de l'échantillon en phase gazeuse et les ions d'un plasma obtenus à partir d'un gaz réactant. La réaction plasma/molécule produit des ions positifs ou négatifs qui sont repérés sur des spectres de masse plus simples et surtout plus informatifs que ceux obtenus en IE (114-117). L'ionisation peut se faire par transfert de proton, réactions d'association ou formation d'adduits, perte ou abstraction d'un hydrure ou échange de charge (115-121). La contribution à chacune de ces réactions d'ionisation dépend de la nature de la substance à analyser et du gaz réactant.

Le grand avantage de cette technique est sa flexibilité. En effet, en faisant varier les conditions expérimentales, à savoir la nature du gaz réactant, la pression et la température de la source, il est possible d'observer l'ion quasi-moléculaire des molécules (114). La faible quantité d'énergie transférée lors de l'ionisation limite les fragmentations et permet ainsi une meilleure différenciation des isomères (195, 116). De plus, la sensibilité de l'IC peut être particulièrement affectée par le choix du gaz réactant, phénomène qui peut permettre de résoudre des problèmes de co-élutions observés en CPG/SM-IE lors d'analyse de mélanges complexes (104).

Figure 6 : Identification des constituants de mélanges complexes de volatils par combinaison les techniques CPG et CPG/SM

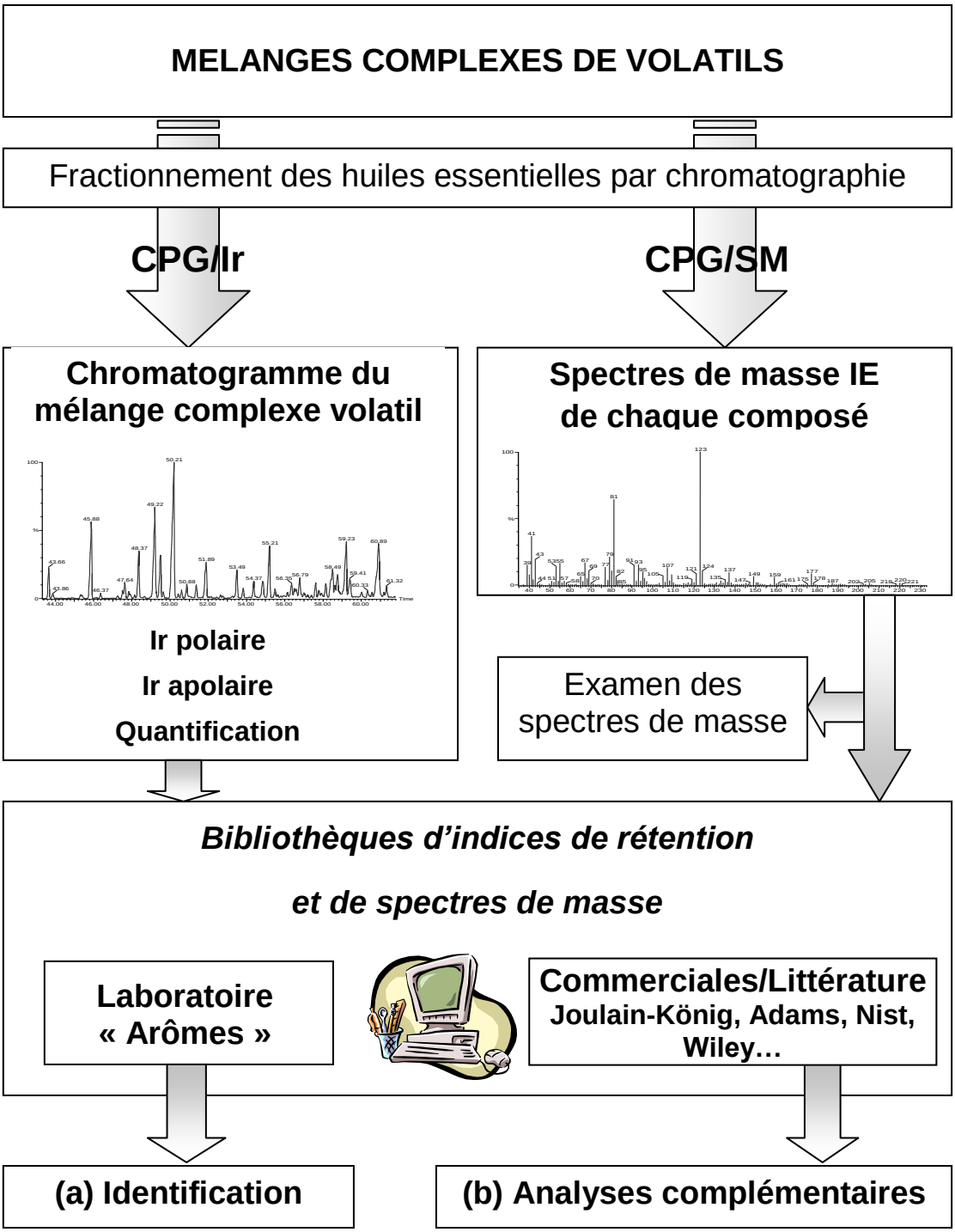
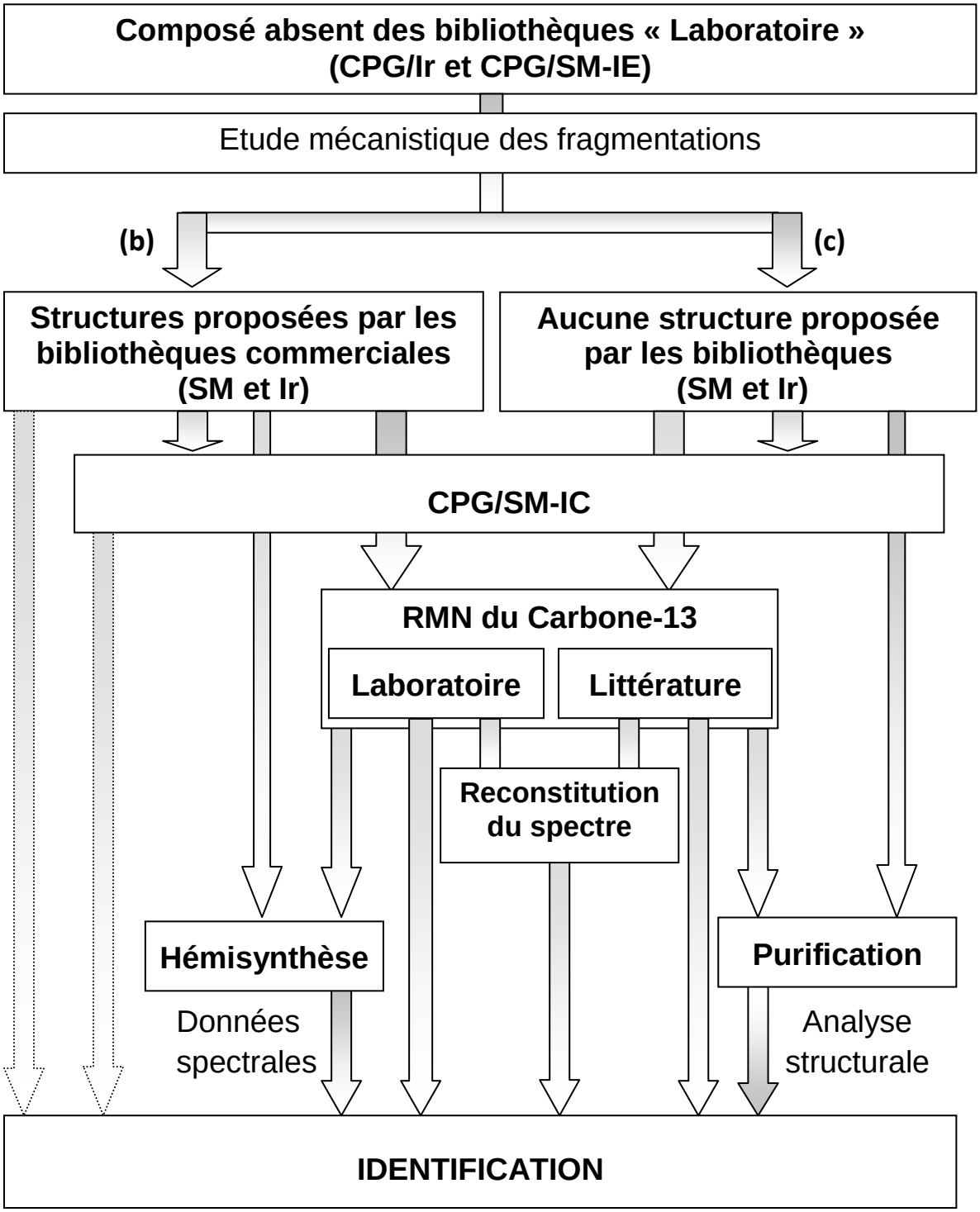


Figure 7 : Analyses complémentaires mises en œuvre pour l'identification des constituants d'une huile essentielle.



C'est cette méthodologie que nous avons mis en œuvre pour l'analyse des huiles essentielles et des fractions volatiles des différentes espèces de *Teucrium*. Du fait de la complexité des mélanges volatiles issus des espèces du genre *Teucrium*, nous n'avons pratiquement pas eu recours à l'analyse « de routine » qui est généralement réalisée pour l'étude des mélanges de volatils dont l'identification des constituants ne présente pas de difficultés majeures. Elle est réalisée conjointement par CPG et CPG/SM sans fractionnement sur colonne pour les huiles essentielles. L'identification des constituants du mélange est réalisée, pour la plupart d'entre eux, à partir des données spectrales présentes dans la bibliothèque d'indices de rétention et de spectres de masses « Arômes » construite au laboratoire. Le plus souvent cette méthode est plus dédiée au suivi de la qualité des huiles essentielles commerciales. Dans le cadre du présent travail, seules les fractions volatiles obtenues par MEPS ont été analysées directement par contre toutes les analyses des huiles essentielles produites ont été réalisées par combinaison des techniques chromatographiques et spectroscopiques.

L'analyse par combinaison des techniques associe le pouvoir de séparation des techniques chromatographiques (CLC, CPG) à la puissance d'identification des techniques spectroscopiques (SM et RMN) dans le but d'optimiser la performance de notre méthodologie d'analyse. Cette combinaison est généralement mise en œuvre pour l'analyse des huiles essentielles dont l'identification des constituants est difficile, notamment lorsque les limites d'identification de la bibliothèque du laboratoire sont atteintes. Au travers de plusieurs exemples, nous illustrerons les démarches analytiques complémentaires basées sur l'utilisation conjointe de la chromatographie sur colonne (CLC), l'ionisation chimique (IC), la RMN du carbone-13 et la synthèse organique afin d'identifier les composés absents de nos bibliothèques.

- ***La chromatographie liquide sur colonne (CLC), une dimension analytique supplémentaire.***

Le fractionnement par CLC des huiles essentielles est une opération réalisée de manière fréquente au laboratoire. Cette séparation est mise en œuvre en fonction de la quantité essentielle dont nous disposons et surtout en fonction de la complexité du mélange. Dans tous les cas, le fractionnement permet d'améliorer le rendement quantitatif mais surtout qualitatif de l'identification. Le mélange binaire *n*-pentane/éther diéthylique est le mélange le plus utilisé

pour le fractionnement des huiles essentielles. Dans le tableau 2, nous avons regroupé le nombre de composés identifiés et le pourcentage d'identification avant et après fractionnement.

Tableau 2 : Apport de la CLC à l'identification des constituants d'huiles essentielles.

	Fractions obtenues par CLC	Nombre de composés identifiés et taux d'identification		
		avant CLC	après CLC	%
T. scorodonia ssp. baeticum	2	43	51	98,8
T. scorodonia ssp. scorodonia	6	64	80	98,2
T. flavum ssp. glaucum	2	76	81	94,9
T. polium ssp. capitatum	12	49	86	92,7
T. massiliense	16	78	137	93,3
T. marum	26	46	93	95,5

Il est opportun de séparer les grandes familles de composés (analyse des huiles essentielles de *T. scorodonia ssp. baeticum, T. scorodonia ssp. scorodonia* et *T. flavum ssp. glaucum*) ou de manière plus fine de concentrer un composé dans une fraction (analyse des huiles essentielles de *T. massiliense, T. marum* et *T. polium* ssp. *capitatum*). La chromatographie sur colonne permet de simplifier le mélange complexe initial en concentrant les composés minoritaires dans les fractions. Ainsi, après une simple partition des composés hydrocarbonés et oxygénés de l'huile essentielle *T. massiliense* de Corse [**PT1**], l'analyse par CPG/Ir et CPG/SM de la fraction apolaire a permis l'identification de 11 alcanes acycliques présents à l'état de trace dans le mélange complexe.

La CLC permet également de s'affranchir de certains cas de co-élution. En effet, la partition sur colonne, à l'aide d'un éluant de polarité croissante : C_5H_{12}/Et_2O (98/2) et (95/5), de l'huile essentielle de *T. massiliense* de Corse permet de séparer un oxyde (théaspirane I) d'un aldéhyde ((E,E)-2,4-décadienal), deux composés qui co-éluent sur la colonne apolaire utilisée en CPG [**PT1**].

- ***Bibliothèques de données commerciales : Un savoir faire à acquérir***

Si l'identification d'un constituant d'un mélange complexe volatil est réalisée de manière non ambiguë par comparaison de ses indices de rétention et de ses spectres de masse avec ceux de molécules de référence contenues dans la bibliothèque « Arômes », l'identification à partir des bibliothèques commerciales nécessite une attention particulière. Les bibliothèques commerciales que nous utilisons présentent un niveau de performance inégal car elles sont

construites dans des conditions expérimentales non standardisées (nature du quadripôle, conditions d'ionisation, balayage de masses variables…) et différentes de celles utilisées au laboratoire. Les bibliothèques commerciales informatisées telles que NIST (100) et Wiley (97, 98) sont construites par compilation de différentes collections de données d'origines variées, qui renferment les spectres de masse de plusieurs milliers de molécules organiques dont le taux de réplicas relativement important limite inévitablement la pertinence de l'identification (126, 127). Par contre, les bibliothèques commerciales informatisées Joulain (95, 96) et Adams (99) sont plus performantes et sont directement exploitables. Elles contiennent certes un nombre moins élevé de spectres de masse (respectivement plus de 1200 et plus de 1600) mais qui correspondent tous à des molécules volatiles identifiées dans des huiles essentielles. De plus, les spectres de masse répertoriés ont tous été enregistrés dans des conditions expérimentales voisines de celles utilisées au laboratoire. Enfin, ces deux bibliothèques présentent l'énorme avantage de lister les indices de rétention des composés mesurés, en programmation de température, sur des colonnes apolaire respectivement de type DB-5 et CpSil 5 : conditions chromatographiques voisines de celles utilisées au laboratoire. Toutefois, c'est l'analyste qui, en dernier ressort, valide la proposition à partir de la note de concordance fournie par le logiciel de comparaison, de la prise en compte des indices de rétention de la littérature et de l'examen du spectre de masse. A titre d'exemple, le germacrène B, qui est un des composés majoritaire dans l'huile essentielle de *T. scorodonia* ssp. *scorodonia* de Corse est identifié à l'aide de la bibliothèque de données Joulain [**PT3**]. De même, l'isovalérate de benzyle est identifié dans l'huile essentielle de *T. marum* de Corse à l'aide de la bibliothèque de données Adams [**PT2**].

Certes, les bibliothèques Joulain (95, 96) et Adams (99) apparaissent comme les plus pertinentes en termes d'identification. Cependant, les autres bibliothèques commerciales peuvent apporter des informations essentielles à l'identification d'un constituant d'une huile essentielle.

- ***L'apport de la résonance magnétique nucléaire du carbone-13***

La RMN du carbone-13 est la technique d'identification par excellence car elle permet d'accéder aux valeurs de déplacements chimiques de chaque carbone de la molécule. Par ailleurs, il est fort aisé de comparer les valeurs de déplacements chimiques avec celles décrites dans la littérature et ainsi identifier des structures partielles « modèles » utilisables pour effectuer une reconstitution de spectre.

L'identification du (3Z, 6E, 10E)-*α*-springène dans l'huile essentielle de *T. marum* de Corse est un bon exemple pour illustrer l'apport de la RMN du carbone-13 pour l'identification des composés absents de nos bibliothèques. L'identification du (3Z, 6E, 10E)-*α*-springène a été réalisée par comparaison de ses valeurs de déplacements chimiques avec celles de deux molécules choisies comme modèles : le (Z, E)-*α*-farnesène et le (E)-*β*-farnesène. La procédure de reconstitution de ce diterpène hydrocarboné peu commun dans le règne végétal, est présentée dans la figure 8 [**PT2**].

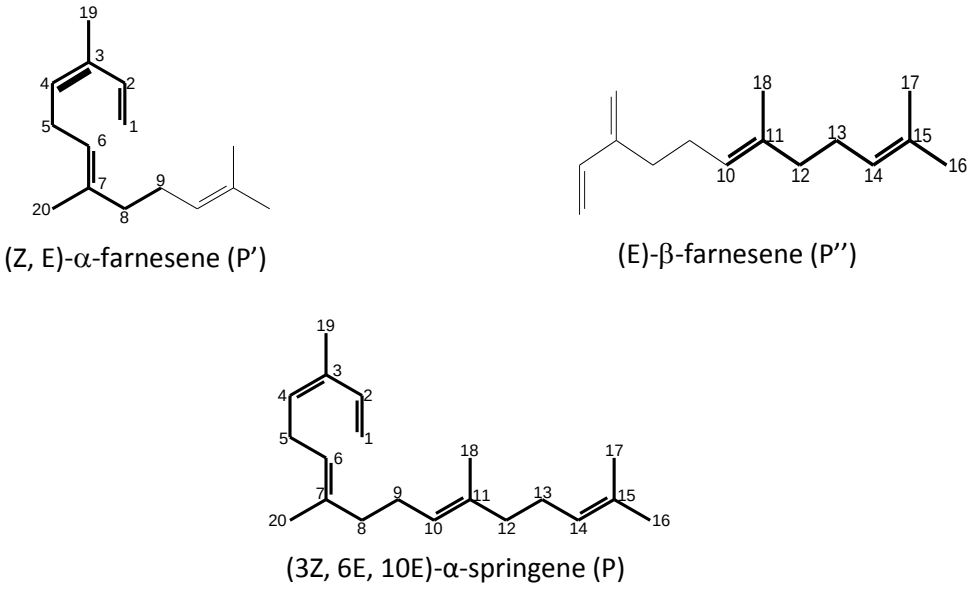


Figure 8 : Reconstitution de spectre du (3Z, 6E, 10E)-*α*-springène.

- ***Apport de l'ionisation chimique et de la synthèse.***

Les informations fournies par les bibliothèques commerciales peuvent contribuer à la décision d'hémisynthèses afin d'accéder aux données spectrales de composés absents des bibliothèques. Dans certain cas on peut même arriver à faire de la synthèse quand l'hémisynthèse n'est pas possible. C'est précisément la procédure analytique qui a été mise en œuvre pour identifier le composé majoritaire de *T. massiliense* [**PT1**].

A la suite de l'analyse préliminaire le composé majoritaire reste non identifié car il est vraisemblablement absent de nos bibliothèques. Seule la bibliothèque Wiley suggère la 3,7-diméthyloctan-2-one. Rappelons qu'en ce qui concerne la composition chimique de l'huile essentielle de *T. massiliense*, un seul travail a été réalisé sur un échantillon originaire de Sardaigne dans lequel la 3,7-diméthyloctan-2-one a été identifiée en tant que composé majoritaire (15). Sur le spectre de masse enregistré en impact électronique, l'ion moléculaire

n'est vraisemblablement pas visible et nous observons le pic de base à m/z 43 qui suggère un composé contenant une fonction acétate.

Un fractionnement sur CLC, avec un gradient de solvants *n*-pentane/éther diéthylique (98:2) a permis de concentrer le composé majoritaire de l'huile essentielle dans une fraction (52,7%) riche en ester, ce qui confirme que cette molécule est un ester. L'examen des spectres de masse en ionisation chimique positive avec comme gaz ionisant le méthane et l'ammoniac permet d'observer respectivement les ions pseudomoléculaires à m/z 113 $[M + H\text{-}RCOOH]^+$ en ICP-CH_4 et à m/z 190 $[M + NH_4]^+$ en ICP-NH_3 et de déduire la masse moléculaire du composé [M= 172 u].

Les spectres RMN du carbone-13 et DEPT de la fraction permettent d'obtenir les valeurs de déplacements chimiques ainsi que la multiplicité des 10 signaux constitutifs de la molécule. Ces informations nous laissent supposer la présence d'un acétate ramifié de formule $C_{10}H_{20}O_2$. Cette hypothèse est confirmée par réduction en présence de $LiAlH_4$ de la fraction contenant le composé majoritaire de l'huile essentielle, réduction qui conduit à une molécule identifiée dans l'huile essentielle, le 6-méthylheptan-3-ol. Ce résultat nous oriente donc vers l'acétate correspondant. L'identification de l'acétate de 6-méthyl-3-héptyle a été réalisée par comparaison de ses données spectrales avec celles de la molécule synthétisée. En collaboration avec le Dr Nicolas Baldovini de l'Université de Nice, le composé majoritaire de l'huile essentielle de *T. massiliense* a été obtenu à partir de la séquence suivante :

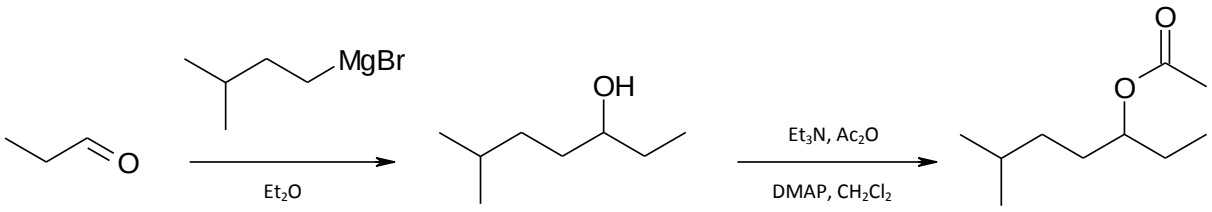


Figure 9 : Séquence de synthèse de l'acétate de 6-méthyl-3-heptyle

IV. QUANTIFICATION DES CONSTITUANTS DES HUILES ESSENTIELLES

Le développement qui touche le secteur des huiles essentielles fait que les professionnels tels que les firmes pharmaceutiques, agro-alimentaires, cosmétiques ou encore les parfumeurs souhaitent connaître la teneur vraie en principes actifs dans les huiles essentielles. Ainsi l'exigence de connaitre les concentrations exactes des différents constituants est demandée,

c'est ce que nous appelons la quantification vraie (122). Si dans son principe, la quantification des constituants d'un mélange est simple, il en va autrement de son application aux huiles essentielles. En effet, il est irréaliste de réaliser la quantification vraie de tous les constituants d'une huile essentielle par manque des standards correspondant. Nous verrons que des approches permettent de palier à ce défaut.

Les aspects quantitatifs de l'analyse des huiles essentielles ne sont pas faciles à traiter, non seulement parce que la quantification a longtemps été considéré comme moins importante que l'élucidation de la structure de nouveaux odorants, mais aussi parce que plusieurs aspects de quantification ont été et le sont encore, ambiguës. L'approche quantitative de la plus part des huiles essentielles est rapportée dans la littérature en termes de rapport d'abondance relative de pourcentage, bien que malheureusement cette approche est d'une valeur limitée.

En effet, la composition des huiles essentielles est généralement donnée par des pourcentages relatifs à l'aide d'un Détecteur à Ionisation de Flamme (DIF). Ce dernier restitue une réponse sous forme d'un signal électrique qui est ensuite convertie par un électromètre en un signal digital analysable par l'ordinateur. A partir de ce signal, chaque composé est désigné par un pic dont l'aire est proportionnelle à la quantité de matière. L'équation de base de toute méthode chromatographique étant masse injectée = Aire du pic x facteur de réponse (RF), il apparait que le détecteur idéal serait celui qui a un facteur de réponse égal à 1 pour toutes les classes de composés. Dans le cas du DIF, les facteurs de réponse peuvent varier jusqu'à 60% selon la nature et la structure du produit chimique détecté. Pour l'utilisation du DIF de la CPG à des fins de quantifications il devient plus judicieux d'établir des RF pour chaque classe de composés.

Pour cela, nous avons constitué trois solutions mères à partir des 30 composés listés dans le tableau 2. Chaque solution mère comprend 10 composés, regroupés en fonction de leur masse molaire, à des concentrations identiques ($2.10.\ 10^{-4}$ $mol.L^{-1}$). Les solutions mères sont ensuite diluées de manière à obtenir un rapport de concentration entre les standards et l'étalon interne qui, lui, est introduit de façon similaire quelle que soit la dilution de la solution. Dans notre cas, le tridécane a été choisi comme étalon interne car il possède un temps de rétention situé dans une zone où les co-élutions sont peu probables. Préalablement, il faut s'assurer de son absence dans l'huile essentielle. Enfin, il doit être introduit à une concentration proche des autres constituants, concentration qu'il faut optimiser. Les composés choisis pour l'étude

sont sélectionnés en fonction des standards disponibles mais aussi de manière à être représentatifs des familles chimiques (voir tableau 3) présentes usuellement dans les huiles essentielles.

Le rapport des concentrations [tridécane/composé] varie de 0,5 à 80 ce qui est représentatif des variations possibles de concentrations d'un composé dans une huile essentielle. Les solutions ainsi obtenues sont analysées par CPG. Les courbes de calibration issues du rapport des aires [tridécane/composé] en fonction du rapport des concentrations ont été établies (figure 10).

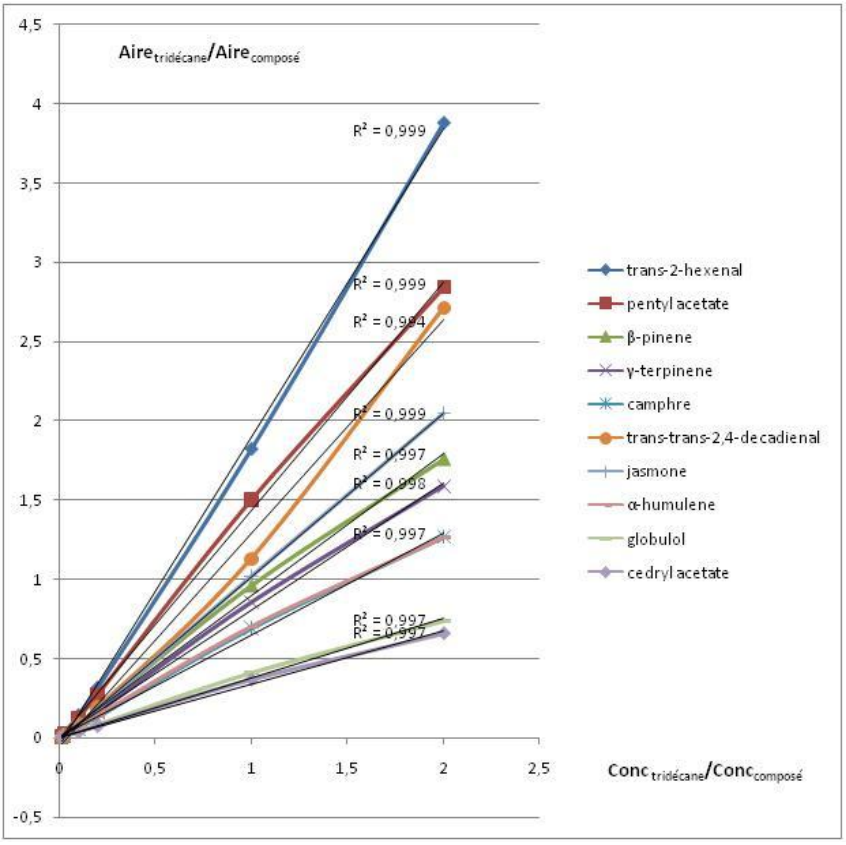


Figure 10 : Courbes de calibration et droites de corrélation correspondantes à 10 composés.

Les coefficients de corrélation (R^2) ont été définis pour chacune des courbes, de manière à vérifier la linéarité sur l'ensemble des concentrations. La figure 3 montre les droites obtenues pour dix composés d'une solution mère. L'ensemble des courbes établies pour 30 composés sélectionnés sont linéaires sur toute la gamme de concentration. Le tableau 2 donne les coefficients de corrélation.

Les facteurs de réponses pour l'ensemble des 30 composés sont calculés à partir de l'équation ci-dessous établie par Costa et Coll. (124) :

$$\mathbf{RF} = \mathbf{C_{analyte}}/[(\mathbf{A_{abs\ analyte}}/\mathbf{A_{abs\ i.s.}})] \times \mathbf{C_{i.s.}} \quad (124)$$

Avec : RF : facteur de réponse ; $C_{analyte}$: concentration du composé ; $A_{abs\ analyte}$: aire du pic du composé ; $C_{i.s}$: concentration du standard interne ; $A_{absi.s}$: aire pic du standard interne.

Les valeurs des RF obtenues sont mentionnées dans le tableau 2. Les valeurs moyennes des facteurs de réponse sont très proches de celles trouvées dans la littérature (122, 124). Ainsi à partir des RF moyens des différentes familles de composés, il est possible de quantifier un composé dont on ne dispose pas le standard en lui appliquant le RF moyen de la famille auquel il appartient. Au préalable, il faut s'assurer que le composé quantifié et celui auquel on fait référence possède la même formule brute.

Signalons enfin, que l'utilisation d'un étalon interne contribue à standardiser les résultats d'une série d'échantillon. En effet, lorsqu'on souhaite comparer des compositions chimiques et mettre en œuvre l'outil statistique en vue d'examiner la variabilité chimique des huiles essentielles, il est indispensable que la matrice de données soit standardisée par l'ajout d'un étalon interne à une concentration donnée dans tous les échantillons. Il est alors possible de calculer des pourcentages relatifs normalisés et standardisés d'après la méthodologie décrite par Bicchi et col. (122). C'est précisément ce que nous avons réalisé dans les études des compositions chimiques des huiles essentielles de *Teucrium* qui ont fait l'objet d'une étude statistique [**PT2-PT6]**.

Tableau 3 : Coefficients de corrélation R^2 des courbes de calibration et les facteurs de réponses RF des 30 composés sélectionnés.

		Courbe de calibration	Facteur de réponse	
	Composés	**R^2**	**RF**	**Moyenne**
Monoterpènes hydrocarbonés	néo-allo-ocimène	0.999	1.00±0.01	1.01±0.05
	α-pinène	0.999	1.04±0.01	
	β-pinène	0.997	1.08±0.01	
	γ-terpinène	0.998	1.01±0.01	
	limonène	0.999	0.94±0.01	
Aromatiques hydrocarbonés	p-cymène	0.999	0.93±0.04	0.93±0.04
Sesquiterpènes hydrocarbonés	β-caryophyllène	0.999	0.98±0.01	1.00±0.03
	α-humulène	0.997	1.01±0.01	
	aromadendrène	0.999	1.03±0.01	
Monoterpènes et sesquiterpènes Alcools	nérol	0.991	1.31±0.02	1.34±0.04
	lavandulol	0.994	1.32±0.02	
	trans-3-hexèn-1-ol	0.999	1.40±0.02	
	cédrol	0.999	1.31±0.02	
	globulol	0.997	1.34±0.02	
Monoterpènes et sesquiterpènes Esters	acétate de pentyle	0.999	1.53±0.01	1.55±0.03
	acétate de néryle	0.999	1.54±0.01	
	acétate de *trans*-myrtènyle	0.991	1.53±0.01	
	acétate de cédryle	0.997	1.59±0.01	
Cétones monoterpéniques et non terpéniques	artemisia cétone	0.999	1.30±0.01	1.30±0.02
	décan-2-one	0.999	1.28±0.03	
	camphre	0.998	1.31±0.01	
	jasmone	0.999	1.32±0.01	
Oxydes monoterpéniques	oxyde d'isobornyle et de méthyle	0.999	1.25±0.01	1.24±0.01
	1,8-cinéole	0.999	1.25±0.03	
	oxyde de carvacryle et de méthyle	0.999	1.23±0.01	
Oxydes sesquiterpéniques	oxyde de caryophyllène	0.999	1.59±0.01	1.59±0.01
Aldéhydes non terpéniques	*trans*-hex-2-ènal	0.999	1.39±0.01	1.40±0.02
	trans-trans-décadièn-2,4-al	0.994	1.42±0.01	
	trans-décén-2-al	0.999	1.40±0.01	

Parallèlement, une méthode basée sur l'utilisation des RF a été décrite par Costa et al. (124), dans laquelle les auteurs réalisent une semi-quantification des constituants des huiles essentielles dont les concentrations sont exprimées en gramme pour 100 grammes d'huile essentielle. Cette procédure est à rapprocher de la quantification vraie dans le sens où elle fournit une concentration, toutefois son inconvénient majeure, c'est qu'elle est peu utilisée et par conséquent elle rend difficile la comparaison avec les données de la littérature exprimées en pourcentages. Les concentrations des constituants des huiles essentielles collectives de *T. massiliense* [**PT1**], *T. scorodonia* [**PT3**], *T. flavum* [**PT4**] *et T. polium* [**PT5**] ont été calculées à partir de l'équation suivante :

$$\mathbf{C_{VOC}} = \{[(\mathbf{A_{abs\ VOC}}/\mathbf{A_{abs\ i.s.}}) \times \mathbf{C_{i.s.}} \times \mathbf{RF}]/\ \mathbf{W_{oil}}\} \times \mathbf{100} \quad (124)$$

Avec : C_{VOC} est la concentration en g/100g du composé quantifié ; $A_{abs\ VOC}$ représente l'aire du pic de ce même composé et W_{oil} est la masse de l'huile analysée en g ; $C_{i.s}$: concentration du standard interne ; RF : facteur de réponse.

V. OUTILS STATISTIQUES

Les travaux de recherche actuels sur les compositions chimiques des huiles essentielles et des fractions volatiles pré-concentrées par MEPS, intègrent de plus en plus des variations de composition chimique induites par les critères environnementaux (altitude, nature du sol,…), physiologiques (morphologie du végétal) ou encore génétiques. Pour étudier cette variabilité chimique de l'huile essentielle ou de la fraction volatile d'une plante, il est indispensable d'effectuer un traitement statistique de la matrice constituée par les pourcentages normalisés et standardisés des constituants des mélanges volatils. Pour cela, les deux outils les plus fréquemment utilisés sont l'Analyse en Composante Principale (ACP) et la Classification Ascendante Hiérarchique (CAH) (125). Ces deux outils permettent d'avoir une vision synthétique, des similitudes ou des différences au sein d'un ensemble d'échantillons. Sans entrer dans les théories régissant les statistiques, nous allons montrer le parti que le chimiste peut en tirer.

L'ACP est un outil permettant de convertir un nuage de points d'un espace multivarié en un nuage de points dans un espace à 2 dimensions. Les points et les différentes variables de

cet espace sont projetés selon les 2 axes perpendiculaires, les plus représentatifs de l'ensemble du nuage (123). Concrètement, dans notre domaine d'étude, les variables sont généralement les constituants du mélange tandis que les points sont les diverses stations de récolte.

La CAH est une méthode d'agglomération de différents points dans un espace varié. Tout d'abord la dissimilarité entre chaque point *via* des calculs de distance est établie, ensuite, le système choisi agglomère les points entre eux de façon itérative et aboutit à la formation de plusieurs groupes. Les agglomérations successives sont représentées sous forme d'arborescence sur le dendrogramme issu de la CAH. Ceci permet ainsi de classer les échantillons selon plusieurs groupes, en fonction de l'importance, de la dissimilarité entre chaque rameau de l'arbre (125).

L'utilisateur doit faire preuve de prudence dans les conclusions tirées des représentations issues de son échantillonnage géographique. Dans une ACP, il est important que les deux axes choisis aient un pourcentage de représentation suffisamment élevé pour que l'ACP soit jugée pertinente. Si le pourcentage est trop faible, les conclusions qui découlent de la représentation sont erronées. De même, la CAH, bien que permettant d'établir différents groupes, ne fournit strictement aucune information concernant les affinités de ces groupes avec les diverses variables utilisées.

Dans notre cas, nous avons systématiquement établi les groupes les plus représentatifs en CAH et confirmé ces groupes en ACP. Une fois les groupes et leur composition fixés, les affinités de chaque groupe avec les composés discriminants ont été déterminées par l'ACP. *In fine*, et selon la classification ainsi faite, chaque échantillon a été confronté aux données de la matrice afin d'éviter les aberrations toujours possibles en ACP. A titre d'exemple, nous avons choisi de présenter les résultats de l'étude de la variabilité chimique des huiles essentielles de *T. scorodonia* de Corse et d'Algérie. La composition chimique de la ssp. *scorodonia* de Corse est très semblable de celle d'Italie présente dans la littérature (26), les composés majoritaires sont : le (*E*)-β-caryophyllène (17,3–25,2%), l'α-cubebène (6,2–11,3%), le germacrène D (4,6–10,1%), le germacrène B (4,2–8,8%), l'α-humulène (4,6–8,3%) et le γ- élémène (2,6–5,7%). De même, la composition chimique de l'huile essentielle de la ssp. *baeticum* d'Algérie est très semblable à celle originaire d'Espagne décrite dans la littérature (25), les composés majoritaires sont : le (*E*)-β-caryophyllène (31,5–37,1%), le germacrène D (18,1–26,8%), l'α-

humulène (7,2–10,2%), l'oxyde de caryophyllène (2,15–6,8%) et le linalol (2,1–6,2%). Cette analyse a permis de déceler une forte présence de sesquiterpènes hydrocarbonés toujours supérieure à 70% de la composition chimique des huiles essentielles des deux sous-espèces. L'outil statistique a permis de distinguer deux groupes de compositions chimiques. Le groupe I (10 échantillons) regroupe les échantillons originaires de Corse et le groupe II (17 échantillons) inclut les échantillons d'Algérie (voir figure 11). L'élément discriminant est la présence du germacrène B et *γ*-élémène comme composés majoritaires dans la ssp. *scorodonia* mais jamais détectés dans la ssp. *baeticum*. Cette étude montre l'impact du facteur environnemental sur la variation des compositions chimiques des huiles essentielles. En effet, il est possible de relier la production des sesquiterpènes hydrocarbonés au pH et à la composition élémentaire du sol sur lequel le végétal se développe **[PT3]**.

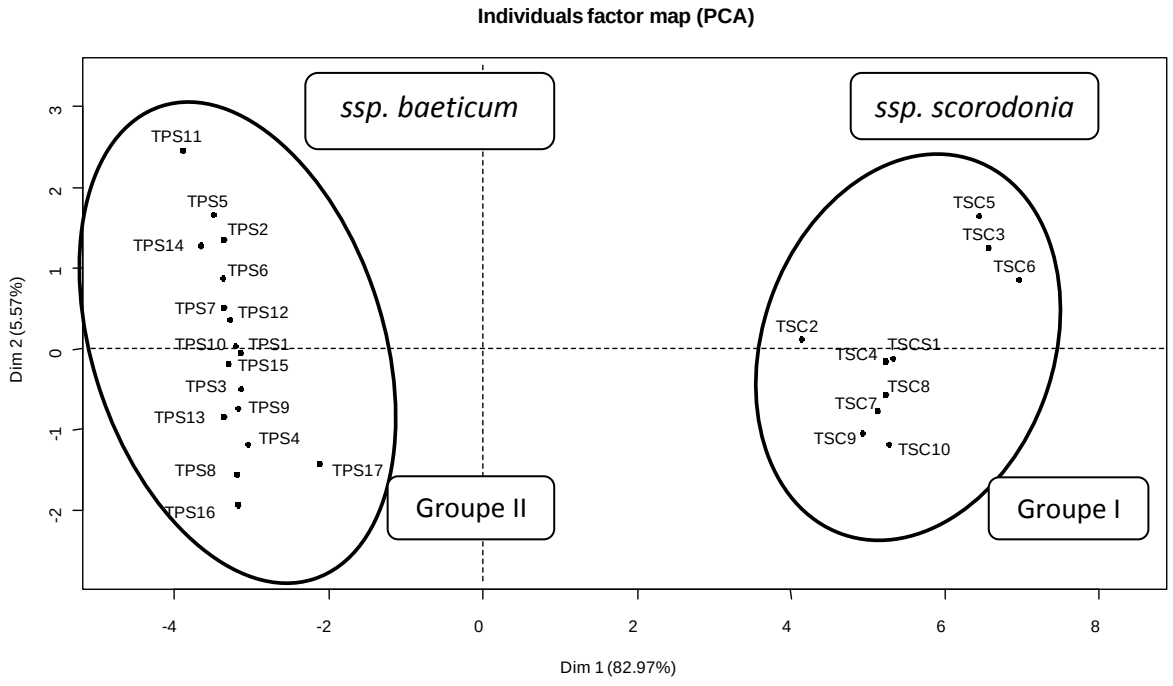


Figure 11 : ACP de la représentation des échantillons d'huiles essentielles des deux sous-espèces *scorodonia* et *beaticum* de *T. scorodonia* de Corse (TSC-10) et d'Algérie TPS (1-17).

D. L'APPROCHE INTERDISCIPLINAIRE CHIMIE/GENETIQUE

I. APPORT DE LA GENETIQUE

La conservation *in situ* permet aux plantes de se développer dans leur milieu naturel par la genèse de processus évolutif, source de diversité génétique, essentielle à leur capacité d'adaptation. Pour cette raison, la conservation *in situ* peut être considérée comme un système dynamique qui permet de préserver la capacité d'adaptation. Sans être figée, cette diversité évolue en fonction des mutations et des flux de gènes (à l'intérieur et entre les populations).

Le rôle de la recherche dans la conservation, la caractérisation et l'utilisation de la diversité génétique végétale est primordiale. La caractérisation détaillée de la diversité, dépendante ou indépendante de l'environnement, peut être obtenue grâce aux différentes méthodes permettant d'analyser l'ADN.

L'étude de la variabilité génétique a été menée, dans une première approche, en utilisant le polymorphisme des zones non codantes de l'ADN chloroplastique (ADNcp). Ce choix présente l'avantage de ne considérer que la lignée maternelle, écartant, d'une part, les phénomènes de recombinaison et d'autre part, limitant les flux géniques par la faible dissémination des graines. Cette approche a été développée à l'aide de deux types de marqueurs chloroplastiques, TRNL-TRNF et RPL32-TRNL.

De la même sorte, et pour l'étude de l'ADN nucléique, un type de marqueur nucléique à été sélectionné, il s'agit du marqueur ITS (ITS1 et ITS4). A noter que le choix de ces trois marqueurs est dicté par leur neutralité vis-à-vis des facteurs environnementaux.

Comment aborder une analyse génétique des PPAM ?

Différents types de marqueurs moléculaires ont été utilisés pour répondre à cette question par l'analyse des polymorphismes, tout d'abord en utilisant des isoenzymes (technique de faible coût), puis par l'analyse de l'ADN (avec les techniques RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Lenght Polymorphism), ISSR (Inter-Simple Sequence Repeats) et séquençage…). Celles-ci permettront une large analyse des génomes et l'identification d'une empreinte caractéristique de chaque espèce.

L'analyse des isoenzymes est une méthode robuste et reproductible, marqueurs codominants, ils sont utilisables pour estimer tous les paramètres de génétique des populations. La limitation majeure de l'analyse des isoenzymes est le faible nombre de marqueurs utilisables, du fait de l'absence de tests biochimiques permettant leur détection. Un autre inconvénient des isoenzymes est leur relation avec le phénotype et donc influençables par les facteurs environnementaux. Les différences de développement de la plante peuvent ainsi affecter l'interprétation des résultats. En revanche, les marqueurs de l'ADN permettent de surmonter ces inconvénients et de distinguer différents génotypes (128).

L'AFLP est basée sur la révélation du polymorphisme de position des sites de restriction. Suivant le génome étudié, la taille des fragments obtenus peut varier en jouant sur la combinaison des enzymes de restriction.

L'ADN génomique est dans un premier temps hydrolysé par un couple d'enzymes de restriction, à sites de reconnaissance de 6 et 4 paires de bases. Deux adaptateurs d'une vingtaine de paires de bases sont ensuite flanqués aux fragments obtenus. Dans un deuxième temps, les fragments subissent une amplification par PCR (Polymerase Chain Reaction) en utilisant des amorces correspondant à la séquence des adaptateurs, plus 1 ou 2 bases sélectives arbitraires en 3' (vers l'intérieur du fragment à amplifier).

Le polymorphisme est révélé à la fois par les enzymes de restriction et les bases sélectives, la plupart des fragments correspondent à des positions uniques dans le génome.

Les avantages de cette technique sont les suivants :

- seule une faible quantité d'ADN est nécessaire,
- à l'inverse de la RAPD, qui utilise des amorces arbitraires avec une faible température d'hybridation pendant la PCR et conduit à des résultats peu reproductibles, les conditions de haute stringence en AFLP et l'utilisation de deux amorces, correspondant à des adaptateurs, permettent d'obtenir une bonne reproductibilité,
- de nombreux fragments amplifiés peuvent être révélés en changeant le couple d'enzymes de restriction ou les bases sélectives,
- aucune connaissance particulière de l'ADN génomique n'est requise.

L'ISSR est une technique s'appuyant sur la présence de nombreux microsatellites dans les génomes végétaux. Les microsatellites sont des séquences de 2 à 5 paires de bases répétées en tandem.

Le polymorphisme est déterminé par le nombre d'unités de répétitions. La technique ISSR consiste à révéler en masse des microsatellites. Les amorces sont constituées d'une partie d'une séquence de microsatellite (4 à 6 répétitions selon sa taille) et d'1 à 3 bases arbitraires sélectives en 3' ou en 5'. L'amplification par PCR va révéler de nombreux fragments flanqués de part et d'autre du même microsatellite en orientation inversée. Le polymorphisme dépend ici du nombre d'unité de répétitions ; il est très élevé pour une technicité et un coût faible. L'avantage de cette technique est qu'elle ne nécessite pas, comme l'AFLP, de connaissance particulière des séquences d'ADN et que les cibles des amorces sont très abondantes dans le génome des plantes (129).

Les séquences nucléaires ITS (ITS1 et ITS4) des ARN ribosomial (ARNr) 18S, 5.8S et 28S présentent chez les plantes un polymorphisme permettant des études phylogénétiques au niveau intraspécifique et interspécifique. Les gènes ribosomiaux existent en plusieurs centaines de copies répétées en tandem sur plusieurs chromosomes. Ces copies d'ADNr évoluent le plus souvent de la même façon et accumulent donc les mêmes mutations montrant ainsi une séquence identique (130).

L'analyse comparée des variations des séquences de l'ADN chloroplastique (ADNcp) est une méthode de plus en plus utilisée pour estimer les relations phylogénétiques chez les végétaux. L'analyse de certaines régions non codantes, caractérisées par des taux de mutations plus élevés que les régions codantes, permet d'étendre le domaine d'utilisation de l'ADNcp à des niveaux taxonomiques hiérarchiquement bas. L'avantage de l'ADNcp réside dans son mode de transmission uniparental permettant l'étude phylogénétique par filiation unique.

Le plus souvent la transmission de l'ADNcp se fait par voie maternelle. Le polymorphisme de l'ADNcp peut être analysé par le séquençage de zones non codantes par PCR-RFLP (Restriction Fragment Lenght Polymorphism) et par l'analyse de microsatellites (131).

L'étude précise de microsatellites nucléaires nécessite une bonne connaissance du génome et donc un immense travail en amont avec l'établissement de cartes génétiques. Ceci a été réalisé pour beaucoup de végétaux présentant un intérêt économique mais pas encore pour les PPAM.

Très utilisés jusqu'à présent, les marqueurs décrits succinctement ci-dessus ne reflètent qu'une partie infime du génome des plantes. A juste titre, leur interprétation pourrait être critiquée. L'utilisation de gènes codants uniques, d'origine biparentale, constituerait un avantage précieux dans la connaissance de la diversité génétique. L'identification de ces

gènes végétaux est actuellement en plein essor comme le montre le nombre croissant de séquences contenues dans différentes bases de données génétiques. L'utilisation de cette information permettra dans les années à venir de mieux préciser la structure, l'évolution et les relations des génomes végétaux. Cette information sur l'expression génétique constitue une première base pouvant, dans un proche avenir, être complétée et utilisée dans la mise en place de différentes actions de sélection variétale.

Les études actuelles se sont focalisées sur les corrélations entre diversité génétique, structure du paysage, perturbation de l'habitat, ainsi que des rapports génétiques entre espèces et sous-espèces. En dehors de cet aspect scientifique, le but est également de contribuer à appliquer des mesures de conservation ainsi qu'à l'établissement de programmes d'amélioration génétique à des fins commerciales.

II. APPLICATION AU GENRE *TEUCRIUM*

En collaboration avec l'équipe de génétique de l'Université de Corse, l'étude des séquences génétiques, de tous les échantillons des différentes espèces de *Teucrium* a été réalisée à l'aide de trois marqueurs : deux marqueurs chloroplastiques (TrnL-F et RPL32-TRNL) et un marqueur nucléique (ITS). L'analyse des séquences génétiques a donné lieu à 12 publications dans la banque de données GenBank. Les études comparatives, ont été établies entre les séquences des différentes sous-espèces d'une même espèce, notamment pour *T. scorodonia* [**PT3**], *T. flavum* [**PT4**] et *T. polium* [**PT5**]. Nos objectifs étaient de corréler les empreintes génétiques avec les compositions chimiques des H.E. de chaque *Teucrium*. Cette approche est plutôt originale et n'est pas souvent réalisée. De nos jours, encore très peu d'étude font ce type de corrélation (3, 132-135) et les résultats trouvés sont très intéressants.

Dans le paragraphe précédant, nous avons présenté la variabilité chimique de l'huile essentielle des deux sous-espèces de T. *scorodonia* originaires de Corse et d'Algérie. L'étude de la diversité génétique de ces 2 sous-espèces par l'intermédiaire de trois marqueurs choisis, conduit à un résultat qui est en accord avec celui de la chimie. Elle renseigne sur le fait que la variabilité chimique des huiles essentielles étudiées dépend autant du facteur génétique que des facteurs environnementaux.

En complément, il nous a semblé intéressant de présenter l'étude de la variabilité chimique des huiles essentielles et de la diversité génétique de deux sous-espèces de *T. flavum* présentent un même environnement, limitant de fait l'impact du facteur environnemental dans l'éventuelle variabilité chimique de l'huile essentielle. La Corse est le seul territoire du bassin

méditerranéen qui présente la particularité de posséder les deux sous-espèces *flavum* et *glaucum* de *T. flavum*. Les compositions chimiques des deux sous-espèces sont qualitativement semblables, elles diffèrent quantitativement par les proportions de leurs composés majoritaires. Pour la ssp. *glaucum* les majoritaires sont le limonène (21,1 - 31,8 %), l'*α*-pinène (8,5 - 17,6 %), le *β*-pinène (9,7 - 12,4 %), le (Z)-*β*-ocimène (2,4 - 8,1 %) et le (E)-phytol (2,4 - 5,5 %) alors que pour la ssp. *flavum* les majoritaires sont l'*α*-pinène (19,8 - 21,5 %), le limonène (21,9 - 22,3 %), le *β*-pinène (16,4 - 18,1 %) et le (Z)-*β*-ocimène (14,5 - 17,0 %). L'analyse statistique des données issues de l'analyse chimique conduisent à considérer deux groupes faiblement discriminés (notés respectivement I et II) dans lesquels on retrouve respectivement les échantillons des sous-espèces *glaucum* et *flavum*. Par contre l'établissement des séquences génétiques permet de distinguer, de manière beaucoup plus évidente, l'existence de ces deux groupes avec des distances génétiques importantes entre les deux populations constituées des échantillons des deux sous-espèces. Par ailleurs, l'approche chimie-génétique montre une corrélation directe des résultats avec la répartition géographique des deux sous-espèces sur le territoire insulaire. La large répartition des échantillons du groupe I est en accord avec la répartition de la sous-espèce *glaucum* et à l'inverse la localisation restreinte des échantillons du groupe II est en accord la localisation restreinte des échantillons de la sous-espèce *flavum* uniquement répertoriée par les botanistes dans la région de Porto-Vecchio. Les résultats chimie/génétique montrent que les différences observées dans la composition chimiques des huiles essentielle des *T. flavum* ssp. *glaucum* et *T. flavum* ssp. *flavum* dépendent plus du facteur génétique que du facteur environnemental.

Le troisième exemple concerne l'espèce *T. polium*, connue pour être très fortement polymorphe **[PT5]**. Nous avons comparé les diversités chimiques et génétiques des espèces *capitatum* poussant en Corse et *polium* poussant dans l'ouest algérien. Le polymorphisme de cette espèce se traduit notamment par des caractères morphologiques qui attribuent aux spécimens de la sous-espèce *polium* de l'ouest algérien deux couleurs de fleurs que l'on peut relier à une répartition géographique : on retrouve des spécimens à fleurs mauves sur le littoral et en montagne alors que ceux à fleurs blanches sont localisés uniquement dans la région montagneuse de Beni Senous.

L'examen des compositions chimiques des huiles essentielles deux sous-espèces au travers du filtre statistique (ACP, CHA) révèle l'existence de deux groupes (respectivement notés I et II) constitués respectivement par les échantillons de Corse et d'Algérie. Signalons, qu'au sein des échantillons algériens, deux sous-groupes d'échantillons (IIa et IIb) avec des

compositions chimiques différentes se distinguent. Les composés majoritaires de la sous-espèce *capitatum* de Corse (groupe I) sont l'α-pinène (20,2-28,5 %), le β-pinène (8,1-10,1 %), l'α-thujène (6,1-10,3 %), le terpinèn-4-ol (5,1-6,3%), le limonène (4,2-5,9 %), le sabinène (3,7-5,2 %) et le p-cymène (2,6-4,7 %). Concernant la sous-espèce *polium* d'Algérie, le groupe IIa est caractérisé par un fort taux de sesquiterpène tels le germacrène D (21,8-35,8 %), le bicyclogermacrène (1,4-17,9 %) et le spathulénol (4,4-16,7 %), alors que le groupe IIb est caractérisé par une forte proportion de monoterpènes hydrocarbonés tels le β-pinène (20,8-40,5 %), le limonène (6,4-14,2 %) et le myrcène (2,4-9,1 %). Signalons que les données chimiques ne permettent pas d'établir une corrélation directe entre les compositions des huiles essentielles, les caractères morphologiques et les paramètres environnementaux. On distingue dans le même sous-groupe IIb, des spécimens à fleurs mauves et blanches et des échantillons récoltés sur le littoral et en montagne.

L'établissent des séquences génétiques a permis de différencier les échantillons des deux sous-espèces de Corse et d'Algérie et surtout elle confirme le regroupement d'échantillon obtenu à partir des données de la chimie. On remarque une faible distance génétique entre les spécimens du sous-groupe IIb alors que morphologiquement ils diffèrent par la couleur de leurs fleurs. Cette différence peut être attribuée à une différence de la nature du sol. Dans le cas de *T. polium* deux observations sont établies :

(i) les différences observées dans la composition chimique des huiles essentielle de *T. polium* ssp. *polium* de l'ouest algérien et *T. polium* ssp. *polium* de Corse dépendent plus du facteur génétique,

(ii) par contre les différences observées dans les compositions chimiques des échantillons de la sous-espèce *polium* récoltés dans trois grandes zones d'étude (littorale, montagne et montagne de Beni Senous) sont plus a relier à des facteurs environnementaux qu'au facteur génétique [**PT5**].

E. VALORISATION DES HUILES ESSENTIELLES : ACTIVITE BIOLOGIQUE

Ce travail a été réalisé dans le cadre d'une collaboration avec nos collègues microbiologistes de l'UCPP. Il est évident que ce travail est générateur de retombées importantes sur le plan de la santé publique en général et sur le plan de la sécurité alimentaire, en particulier.

De plus, les plantes ont de tous temps, été utilisées en médecine traditionnelle pour le traitement de nombreux troubles (136, 137). Avant « l'aire synthétique » des années 1990, 80% des médicaments étaient extraits à partir des racines, écorces et feuilles de plantes. Malgré le développement de la chimie de synthèse, les produits naturels restent une source importante de molécules biologiques actives puisque 60% des anticancéreux et 70% des anti-infectieux usités de nos jours proviennent de produits naturels (138). Un grand nombre de travaux ont été menés pour identifier les principes actifs responsables des activités pharmacologiques des plantes communément utilisées en médecine traditionnelle.

L'étude des activités biologiques des huiles essentielles est l'un des principaux moyens de valoriser commercialement ces dernières ou d'expliquer son utilisation en médecine traditionnelle. Les activités biologiques les plus couramment observées sont les activités antibactériennes. Les deux méthodes les plus usitées pour les évaluer sont la diffusion sur gel et la dilution en milieu liquide (139).

I. METHODE DE DIFFUSION SUR GEL

Des disques de papier buvard imprégnés des matrices à tester, sont déposés à la surface d'un milieu gélosé, préalablement ensemencé avec une culture pure de la souche à étudier. Dès l'application des disques, la matrice diffuse de manière uniforme si bien que sa concentration est inversement proportionnelle à la distance du disque. Après incubation, les disques s'entourent de zones d'inhibition circulaires correspondant à une absence de culture.

Pour les tests standards, nous considérons qu'une huile essentielle, fraction ou molécule est active pour une souche donnée si le diamètre d'inhibition (D.I.) est supérieur ou égal à 15 mm (140).

Cette technique est la plus couramment utilisée dans le domaine de recherche des huiles essentielles, elle présente l'avantage d'être rapide, d'une grande simplicité à mettre en œuvre

et de nécessiter qu'une faible quantité de matrice. S'agissant des inconvénients, une huile très visqueuse, voire une huile qui cristallise, aura un diamètre d'inhibition faible ou nul même si elle est fortement bactéricide. Cela est dû, dans ces cas à un problème de diffusion sur le gel.

II. METHODE DE DILUTION EN MILIEU LIQUIDE

Cette méthode consiste à mettre un inoculum bactérien standardisé au contact de concentrations croissantes de matrice à tester, selon une progression géométrique de raison 2. L'inoculum bactérien est distribué de façon égale dans une série de tubes (méthode de macrodilution) contenant la matrice testée. Après incubation, la Concentration Minimale Inhibitrice (CMI) est indiquée par le tube qui contient la plus faible concentration de matrice où aucune croissance n'est visible.

Pour les tests standards, nous considérons qu'une huile essentielle, fraction ou molécule est active pour une souche donnée pour une CMI inférieure à 1000 µg/mL (140).

Cette technique, complémentaire de la méthode de diffusion, donne directement la CMI mais, par rapport à la méthode précédente, présente l'inconvénient d'être grande consommatrice en temps d'expérience et en quantité de matrice nécessaire.

III. HUILES ESSENTIELLES DES DIFFERENTS *TEUCRIUM*

Nous avons évalué le potentiel antibactérien des huiles essentielles des espèces du genre *Teucrium* poussant Corse : *T. marum* (TM), *T. massiliense* (TMC), *T. polium* subsp. *capitatum* (TP), *T. flavum* subsp. *glaucum* (TFG), *T. scorodonia* subsp. *scorodonia* (TSC) et *T. chamaedrys* (TCH). L'objectif complémentaire est de mettre en évidence une corrélation entre leurs compositions chimiques et l'effet d'inhibiteur observé sur les souches étudiées. Pour cela six souches ont été sélectionnées : *Staphylococcus aureus* (CIP 53.156), *Staphylococcus epidermidis* (CIP 53124), *Listeria innocua* (CIP 80.11), *Campylobacter jejuni* (CIP 107370), *Enterobacter aerogenes* (CIP 60.86) et une souche multi-résistante *Enterobacter aerogenes* (EAEP289) [**PT6**].

Les D.I. mesurés pour des différentes huiles essentielles affichent de grandes variations selon l'espèce de *Teucrium* étudiée mais ils restent généralement beaucoup plus faible que celles des antibiotiques, excepté pour *C. jejuni* qui est sensible aux huiles essentielles de TM, TMC, TP et TFG avec des D.I. supérieurs à 39 mm (vs. 42 mm pour l'antibiotique).

Les mesures de CMI montrent que :

- *S. aureus* est sensible aux huiles essentielles de TM, TMC, TP et TFG et modérément sensible aux huiles essentielles de TSC et TCH,
- *S. epidermidis* est sensible aux huiles essentielles de TMC et TFG. Cette souche n'a été que modérément sensible à l'huile essentielle de TP, et n'est pas sensible aux huiles essentielles de TM, TSC et TCH,
- *L. innocua* est sensible aux huiles essentielles de TMC et TFG et est modérément sensible aux huiles essentielles de TM, TP et TCH, par contre elle n'est pas sensible à TSC,
- La souche EAEP289 est particulièrement sensible à toutes les huiles essentielles des *Teucrium* sauf celle de TCH. Le même profil a été observé avec *E. aerogenes*. En effet toutes les huiles essentielles sont actives sur cette souche à l'exception de TMC qui est modérément active,
- Enfin l'agent pathogène d'origine alimentaire *C. jejuni* est très sensible pour les H.E de TP et TFG, et est aussi sensible à toutes les autres huiles essentielles.

L'activité d'une huile essentielle est directement liée à sa composition chimique, on considère que les huiles essentielles riches en composés oxygénés sont celles qui donnent les meilleures activités et à l'inverse celles riches en hydrocarbonés ont une activité modérée (4). Une analyse statistique menée sur les données des compositions chimiques de huiles essentielles a permis d'établir l'existence de deux groupes d'échantillons : le premier est caractérisé par un fort taux de composés hydrocarbonés, on trouve les huiles essentielles de TFG, TP, TCH et TSC et le second est caractérisé par un fort taux de composés oxygénés caractéristique des huiles essentielles de TMC et TM.

Dans le but de corréler la composition chimique des huiles essentielles et l'activité enregistrée sur les différentes souches, l'analyse statistique réalisée en intégrant les données de la microbiologie a permis de distinguer deux groupes : le premier inclus les échantillons de TSC, TCH et TM qui sont modérément actives voir pas actives sur la plupart des souches étudiées et le deuxième inclus les échantillons de TMC, TP et TFG qui semblent être actives sur toutes les souches. On remarque que ce classement ne confirme pas la relation composés oxygénés/forte activité puisque les huiles essentielles de TP et TFG sont très riches en composés hydrocarbonés et malgré ça elles donnent de très bonnes activités et à l'inverse l'huile essentielle de TM est riche en composés oxygénés mais elle ne donne pas une bonne

activité. Cela peut s'expliquer par la nature des composés oxygénés minoritaires qui contribuent à l'activité du mélange [**PT6**].

L'enjeu majeur de la recherche des principes actifs présents dans les huiles essentielles, a pour objectif la compréhension des mécanismes d'action des ces principes actifs afin de lutter contre les multi-résistances que développent les micro-organismes. Un des phénomènes mis en jeu par les micro-organismes pour développer cette résistance aux antibiotiques est le mécanisme d'efflux à travers de la membrane cellulaire afin de rejeter l'antibiotique à l'extérieur et se protéger de ce dernier (145). Un travail en cours de réalisation, engagé avec nos collègues microbiologistes de l'UCPP met en évidence la présence de molécules susceptibles d'inhiber l'action des pompes à efflux d'*Enterobacter aerogenes*, important pathogène résistant aux antibiotiques et aux antiseptiques, dans l'hydrolat de *Teucrium marum* de Corse. La séparation et l'identification des molécules responsables de cette activité est en cours de réalisation.

F. CONCLUSIONS

La richesse de la flore de Corse et de l'ouest algérien n'est plus à démontrer. Le bassin méditerranéen est l'un des « points chauds » ou hotspot dans lesquels se trouve concentrée la biodiversité. A l'instar des autres zones rouges régionales, ces deux régions sont caractérisées par une richesse spécifique, un taux d'endémisme élevé mais aussi par des menaces anthropiques fortes et en augmentation rapide.

Ce travail de thèse repose sur l'étude des huiles essentielles et des fractions volatiles de 9 espèces et sous-espèces du genre *Teucrium*: *Teucrium marum*, *Teucrium massiliense*, *Teucrium chamaedrys*, *Teucrium scorodonia* subsp. *scorodonia*, *Teucrium scorodonia* ssp. *baeticum*, *Teucrium polium* subsp. *capitatum*, *Teucrium polium* ssp. *polium*, *Teucrium flavum* subsp. *glaucum*, *Teucrium flavum* subsp. *flavum.* Notre objectif est de contribuer à l'amélioration des connaissances des ressources naturelles issues de la biomasse végétale en fournissant des informations scientifiques objectives. Ce travail de thèse a été l'occasion de développer un travail méthodologique et appliqué totalement complémentaire :

- Aspect méthodologique :(i) optimiser la méthodologie d'analyse du laboratoire tant au niveau de la préparation des échantillons que de l'analyse de leurs constituants et enrichir les bases de données et les bibliothèques scientifiques en molécules, (ii) de développer une interdisciplinarité d'étude Chimique/Génétique, en établissant une corrélation entre les diversités chimiques des huiles essentielles et génétiques des espèces végétales étudiées,
- Au niveau appliqué : (i) de permettre la diversification des sources de produits naturels susceptibles d'être valorisés afin d'élargir les gammes commerciales à travers l'étude de leur spécificité et de la biodiversité, (ii) apporter une dimension supplémentaire à la valorisation des huiles essentielles par l'étude de leur activité biologique.

L'étude menée sur *T. massiliense* a confirmé l'intérêt de mettre en œuvre la MEPS en tant que méthode d'extraction simple et rapide pour accéder aux composés volatils d'une plante produisant peu ou pas d'huile essentielle. Cette méthode permet d'obtenir à partir d'une très faible quantité de matière végétale, l'empreinte volatile d'une plante dont la composition est proche de celle de l'huile essentielle. Le travail mené sur les composés de *T. marum* met en défaut cette observation puisque les deux mélanges de volatils (l'huile essentielle et la fraction volatile) diffèrent dans ce cas fortement. Ce travail a permis de

signaler la vraisemblable transformation du dolichodial au cours de l'hydrodistillation et donc l'importance de la maitrise de l'étape de préparation des échantillons.

Parallèlement, signalons la présence de l'acétate de 6-méthyl-3-heptyle, composé majoritaire de l'huile essentielle de *T. massiliense*, décrit pour la première fois comme composé naturel et l'identification du (3E, 6E, 10E)-*α*-springène, diterpène hydrocarboné de l'huile essentielle de *T. marum* est décrit pour la première fois dans le genre *Teucrium*. L'identification de ces deux composés absents de nos bibliothèques de spectres a été réalisée par combinaison des techniques, CPG, CPG/SM, RMN du carbone-13 et synthèse.

De manière plus originale, nous avons développé une approche interdisciplinaire chimie/génétique qui a permis d'évaluer la diversité chimique des huiles essentielles et la génétique d'une partie du génome de *T. scorodonia*, *T. flavum* et *T. polium.* Nous avons mis en évidence que selon le cas, la production des métabolites secondaires peut être influencée par des facteurs environnementaux mais plus souvent liée à la diversité génétique du végétal.

Enfin, les huiles essentielles des différentes espèces du genre *Teucrium* ont fait l'objet d'un criblage biologique. Les tests ont été menés sur des microorganismes impliqués dans des infections nosocomiales et alimentaires. Une corrélation composition chimique des huiles essentielles/activité biologique a été discutée, les résultats ont démontré le potentiel intéressant des huiles essentielles de *Teucrium* sur plusieurs pathogènes alimentaires.

G. REFERENCES

1. Associated products, Indian essential oil industry 2005, *Focus on Surfactants*, 2005, 4.

2. Algerian Minister of Agriculture and Rural Development, 2003. Phase B du plan de gestion I 2000-2005.

3. Zaouali Y., Boussaid M., Isozyme markers and volatiles in Tunisian *Rosmarinus officinalis* L. (Lamiaceae): a comparative analysis of population structure, 2008, **36**, 11-21.

4. Burt S. Essential oils: their antibacterial properties and potential applications in foods - a review, ***Int. J. Food Microbiol.***, 2004, **94**, 223-253.

5. Observatoire national de l'épidémiologie de la résistance bactérienne aux antibiotiques (ONERBA). Résistance bactérienne aux antibiotiques. Données de l'observatoire national de l'épidémiologie de la résistance bactérienne aux antibiotiques (ONERBA), *Médecines et maladies infectieuses*, 2005, **35**, 155-169.

6. Tepe B., Daferera D., Sokmen A., Sokmen M., Polissiou M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae), *Food Chem*., 2005, **90**, 333-340.

7. Souza E.L., Stamford T.L.M., Lima E.O., Trajano V.N. Effectiveness of *Origanumvulgare* L. essential oil to inhibit the growth of food spoiling yeasts, *Food control*, 2007, **18**, 409-413.

8. Ipek E., Zeytinoglu H., Okay S., Tuylu B. A., Kurkcuoglu M., Baser K. H. C. Genotoxicity and antigenotoxicity of *Origanum* oil and carvacrol evaluated by *Ames Salmonella*/microsomal test, *Food Chem.*, 2005, **93**, 551-556.

9. Smith-Palmer A., Stewart J., Fyfe L. The potential application of plant essential oil as natural food preservatives in soft cheese, *Food Microbiol.*, 2001, **18**, 463-470.

10. Quezel P., Santa S., *Nouvelle flore de l'Algérie*, Tome II, 1963, Edition CNRS, Paris.

11. Jeanmonod D., Gamisans J., *Flora Corsica*, 2007, Edisud, Aix en Provence.

12. Coste H., Flore *descriptive et illustrée de la France, de Corse et des contrées limitrophes*, 1909, Edition Blanchard, Paris.

13. Arrêté du 20 janvier 1982 modifié relatif à la liste des espèces végétales protégées sur l'ensemble du territoire. Liste de portée nationale. Articles L411-1 et 2 du code de l'environnement. Journal officiel du 13 mai 1982.

14. Camarda I., Ricerche etnobotaniche nel comune di Dorgali (Sardegna centro-orientale). *Boll. Soc. Sarda Sci. Nat.* 1990, **27**, 147-204.

15. Giamperi L., Bucchini A., Fraternale D., Cara P., Ricci D., Epifano F., Genovese S., Curini M., Chemical Composition and Antioxydant Activity of the Essential Oil of *Teucrium massiliense* L., *J. Essent. Oil Res.*, 2008, **20**, 446-469.

16. Bruno M., Piozzi F., Rodriguez B., Savona G., De la Torre M.C., Servettaz O., Neo-clerodane diterpenes from *Teucrium* species. *Phytochemistry,* 1992, **31**, 4366-4367.

17. Fontana G., Paternostro M.P., Savona G., Rodriguez B., De la Torre M.C., Neoclerodene Diterpenes from *Teucrium massiliense*. *J. Nat. Prod.* 1998, **61**, 1242-1247.

18. Beaupin C., Granger R., Rossi J.C., Passet J., Peyron L., L'huile essentielle de *Teucrium marum* L. de Corse, *Rivista Italiana*, EPPOS, 1977, **59**, 369-373.

19. Beaupin C., Rossi J.C. Passet J., Granger R., Peyron L., Monoterpènes cyclopentanique : synthèse des dolichodials et de leurs analogues structuraux, *Rivista Italiana*, EPPOS, 1978, 93-96.

20. Conrad M., L'ethnobotanique en Corse, 1983, Etudes corses Xème année.

21. Grandi R., Pagnoni U.M., Pinetti A., Trave R., Biosynthesis of dolicholactone in *Teucrium marum. Phytochemistry,* 1983, **22**, 2723-2726.

22. Ricci D., Fratermale D., Giamperi L., Bucchini A., Epifano F., Burini G., Curini M., Chemical composition antimicrobial and antioxidant activity of the essential oil of *Teucrium marum* (lamiaceae). *Journal of ethnopharmacology*, 2008, **98**, 195-200.

23. Bayer E., Buttler K.P., Furkenzellzer X., Gran J., Guide de la Flore Méditerranéenne, 1990, Delachaux et Niestlé Ed., Paris.

24. Fresson-Laskar R., Essai Biosystématique sur *T. scorodonia* (L.) en Belgique, Mémoire de licence présenté au Laboratoire de botanique systématique et de phytogéographie de l'Université libre de Bruxelles, 1963.

25. Velasco-Negueruela A., Perez-Alonso M., The Volatiles of Six *Teucrium* Species from the Iberian Peninsula and the Balearic Islands, *Phytochemistry*, 1990, **29**, 1165-1169.

26. Maccioni S., Baldini R., Tebano M., Cioni P.L., Essential oil of *Teucrium scorodonia* L. ssp. *scorodonia* from Italy. Food Chemistry, **2007**, 104, 1393-1395.

27. Marco J.L., B., Savona G. and Piozzi F., Diterpenoïds from *Teucrium scorodonia,* Three Neo-clerodane derivatives, *Phytochemistry*, 1982, **21**, 1116-1119.

28. Marco J.L., Rodriguez B., Savona G., Piozzi F., Pascual C., Teuscorodin, Teuscorodonin and 2-hydroxy Teuscorolide, Neo-clerodane diterpenoïds from *Teucrium scorodonia, Phytochemistry,* 1983, **22**, 1421-1426.

29. Muñoz-Olivas R., Screening analysis: an overview of methods applied to environmental, clinical and food analyses, *Trends Anal. Chem*., 2004, **23**, 203-216.

30. Mariott P.J., Shellie R., Cornwell C., Gas chromatographic technologies for the analysis of essential oils, *J. Chromatogr. A*., 2001, **936**, 1-22.

31. Augusto F., Leite e Lopes A., Zini C.A., Sampling and sample preparation for analysis of aromas and fragrances. *Trends Anal. Chem*., 2003, **22**, 160-169.

32. Joulain D., Method for Analysing Essential Oil. Modern Analysis Methodologies: Use and Abuse, *Perfum. Flavor*, 1994, **19**, 5-17.

33. Bauer K., Garbe D., Surburg H., Common Fragrance and Flavor Materials, Preparation, Properties and Uses, *Verlag Chemie Int.*, 1990, Second Edition, New-York.

34. Takeoka G.R., Buttery R.G., Ling L.C., Wong L.Y., Dao L.T., Edwards R.H., Berrios J.J., Odor thresholds of various unsaturated branched esters, *Lebensm.-Wiss. u-Technol.*, 1998, **31**, 443-448.

35. Nagata Y., Measurement of Odor Threshold by Triangle Odor Bag Method, *Bulletin of Japan Environmental Sanitation Center*, 1990, **17**, 77-89.

36. Tholl D., Boland W., Hansel A., Loreto F., Röse U.S.R., Schnitzler J-P., Practical approaches to plant volatile analysis, *Plant J.*, 2006, **45**, 540-560.

37. Augusto F., Valente A.L.P. Applications of solid-phase microextraction to chemical analysis of live biological samples, *Trends Anal. Chem*., 2002, **21**, 428-438.

38. Wilkes J.G., Conte E.D., Kim Y., Holcomb M., Sutherland J.B., Miller D.W., Sample preparation for the analysis of flavors and off-flavors in foods, *J. Chromatogr. A*, 2000, **880**, 3-33.

39. Kataoka H., Lord H.L., Pawlisyn J., Applications of solid-phase micro-extraction and gas chromatography in food analysis., *J. Chromatogr. A*, 2000, **800**, 35-62.

40. Sides A., Robards K., Helliwell S., Developments in extraction techniques and their application to analysis of volatiles in foods, *Lebensm.-Wiss. u-Technol*., 2000, **19**, 322-329.

41. Pillonel L., Bosset J.O., Tabacchi R., Rapid preconcentration and enrichment techniques for the analysis of food volatile. A review, *Lebensm.-Wiss. u-Technol*., 2002, **35**, 1-14.

42. Pourmortazavi S.M., Hajimirsadeghi S.S. Supercritical fluid extraction in plant essential and volatile oil analysis, *J. Chromatogr. A*, 2007, **1163**, 2-24.

43. Bicchi C., Cordero C., Sgorbini B., Rubiolo P., Headspace sampling of the volatile fraction of vegetable matrices, *J. Chromatogr. A*, 2007, in press.

44. Buldini P.L., Ricci L., Sharma J.L., Recent applications of sample preparation techniques in food analysis, *J. Chromatogr. A*, 2002, **975**, 47-70.

45. Lang Q., Wai C.M., Supercritical fluid extraction in herbal and natural product studies – a pratical review, *Talanta*, 2001, **53**, 771-782.

46. Ridgway K., Lalljie S.P.D., Smith R.M. Sample preparation for the determination of trace residues and contaminants in foods, *J. Chromatogr. A*, 2007, **1153**, 36-53.

47. Mendiola J.A., Herrero M., Cifuentes A., Ibañez E. Use of compressed fluids for sample preparation: Food applications, *J. Chromatogr. A*, 2007, **1152**, 234-246.

48. Roux D., Conseil en Aromathérapie, 2ème édition, 2008, Editions Pro-Officina, Ruel-Malmaison, France

49. Pharmacopée Européenne, Conseil de l'Europe, Strasbourg, 5ième Edition, 2004, Supplément 5.8.

50. Diaz-Maroto M.C., Perez-Coello M.S., Cabezudo M.D. Supercritical carbon dioxide extraction of volatiles from spices. Comparison with simultaneous distillation-extraction, *J. Chromatogr. A*, 2002, **947**, 23-29.

51. Jimenez-Carmona M.M., Ubera J.L., Luque de Castro M.D. Comparison of continuous subcritical water extraction and hydrodistillation of marjoram essential oil, *J. Chromatogr. A*, 1999, **855**, 625-632.

52. LucchesiM.E., Chemat F., Smadja, J. Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation, *J. Chromatogr. A*, 2004, **1043**, 323-327.

53. Collin G.J., Lord D., Allaire J., Gagnon D. Huiles essentielles et extraits « micro-ondes ». *Parfums, Cosmét. Arômes*, 1991, **97**, 105-112.

54. Chen S.S., Spiro M. Study of microwave extraction of essential oil constituents from plants materials, *J. Microw. Electromagn. Energy*, 1994, **29**, 231-241.

55. Paré J.R., Belanger M.R., Stafford S. S. Microwave-Assisted Process (MAP™): a new tool for the analytical laboratory, *Trends Anal. Chem.*, 1994, **13**, 176-184.

56. Kolb B., Headspace sampling with capillary columns, *J. Chromatogr. A*, 1999, **842**, 163-205.

57. SnowN.H., Slack G.C., Head-space analysis in modern gas chromatography, *Trends Anal. Chem.*, 2002, **21**, 608-617.

58. Bicchi C., Cordero C., Liberto E., Rubiolo P., Sgorbini B. Automated headspace solid-phase dynamic extraction to analyse the volatile fraction of food matrice, *J. Chromatogr. A*, 2004, **1024**, 217-226.

59. Musshoff F., Lachenmeier D.W., Kroener L., Madea B. Automated headspace solid-phase dynamic extraction for the determination of amphetamines and synthetic designer drugs in hair samples, *J. Chromatogr. A*, 2002, **958**, 231-238.

60. Segal A., Gorecki T., Mussche P., Lips J., Pawliszyn J., Development of membrane extraction with a sorbent interface–micro gas chromatography system for field analysis,*J. Chromatogr. A*, 2000, **873**, 13-27.

61. Wang L., Lord H., Morehead R., Dorman F., Pawliszyn J. Sampling and Monitoring of Biogenic Emissions by *Eucalyptus* Leaves Using Membrane Extraction with Sorbent Interface (MESI),*J. Agric. Food Chem*., 2002, **50**, 6281-6286.

62. Liu X., Pawliszyn R., Wang L., Pawliszyn J. On-site monitoring of biogenic emissions from *Eucalyptus dunnii* leaves using membrane extraction with sorbent interface combined with a portable gas chromatograph system**.** *Analyst.* 2004, **129**, 55-62.

63. Bicchi C., Cordero C., Iori C., Rubiolo P., Sandra P. Headspace sorptive extraction (HSSE) in the headspace analysis of aromatic and medicinal plants, *J. High Resol. Chromatogr*., 2000, **23**, 539-546.

64. Bicchi C., Cordero C., Liberto E., Rubiolo P., Sgorbini B., David F., Sandra P. Dual-phase twisters: A new approach to headspace sorptive extraction and stir bar sorptive extraction, *J. Chromatogr. A*, 2005, **1094**, 9-16.

65. Cavalli J.F., Fernandez X., Lizzani-Cuvelier L., Loiseau A.M., Comparison of static headspace, headspace solid phase microextraction, headspace sorptive extraction, and direct thermal desorption techniques on chemical composition of French olive oils, *J. Agric. Food Chem.*, 2003, **51**, 7709-7716.

66. Ishikawa M., Ito O., Ishizaki S., Kurobayashi Y., Fujita A., Solid-phase aroma concentrate extraction (SPACE^{TM}): a new headspace technique for more sensitive analysis of volatiles, *Flav. Fragr. J.*, 2004, **19**, 183-187.

67. Ishizaki S., Ito O., Fujita A., Kurobayashi Y., Ishikawa M. Proceedings of the 10th Weurman Flavor Research Symposium, Le QuereJ.-L., Etievant P. (Eds.), Beaune, France, 2002, Tec & Doc, Paris, 2003, p. 634.

68. Bicchi C., Cordero C., Liberto E., Rubiolo P., Sgorbini B., Sandra P. Sorptive tape extraction in the analysis of the volatile fraction emitted from biological solid matrices, *J. Chromatogr. A*, 2007, **1148**, 137-144.

69. Theis A.L., Waldack A.J., Hansen S.M., Jeannot M.A., Headspace Solvent Microextraction, *Anal. Chem*. 2001, **73**, 5651-5654.

70. Besharati-Seidani A., Jabbari A., Yamini Y., Saharkhiz M.J., Rapid extraction and analysis of volatile organic compounds of Iranian feverfew (*Tanacetum parthenium*) using headspace solvent microextraction (HSME), and gas chromatography/mass spectrometry,*Flav. Fragr. J*., 2006, **21**, 502-509.

71. Arthur C.L., Pawliszyn J. Solid-phase microextraction with thermal desorption using fused silica optical fibers, *Anal. Chem*., 1990, **62**, 2145-2148.

72. Zhang Z., Pawliszyn J. Headspace solid-phase microextraction, *Anal. Chem*., 1993, **65**, 1843-1852.

73. Stashenko E.E., Martinez J.R., Sampling volatile compounds from natural products with headspace/solid-phase micro-extraction, *J. Biochem. Biophys. Methods*, 2007, **70**, 235-242.

74. Pawliszyn J., *Applications of Solid phase microextraction*, Royal Society of Chemistry, Cambridge, 1999.

75. Jackobsen H.B., *Plant volatile analysis. Modern methods of plant analysis*. Vol. 19, Springer, Dordrecth, 1997.

76. Wardencki W., Micheluc M., Curylo J., A review of theoretical and practical aspects of solid-phase microextraction in food analysis,*Int. J. Food Sci. Technol*., 2004, **39**, 703-717.

77. Musteata J., Pawliszyn J. *In vivo* sampling with solid phase microextraction, *J. Biochem. Biophys. Methods*, 2007, **70**, 181-193.

78. Penalver R., Pocurull E., Borrul P., Marce R.M., Evaluation of parameters in solid phase microextraction process, *Chromatogr.*, 1999, **50**, 685-688.

79. Bicchi C., Cordero C., Liberto E., Rubiolo P., Rubiolo P. Reliability of fibres in solid-phase microextraction for routine analysis of the headspace of aromatic and medicinal plants, *J. Chromatogr. A*, 2007, **1152**, 138-149.

80. Dietz C., Sanz J., Camara C. Recent developments in solid-phase microextraction coatings and related techniques, *J. Chromatogr. A*, 2006, **1103**, 183-192.

81. BruheimI., Liu X., Pawliszyn J., Thin-Film Microextraction, *Anal. Chem*. 2003, **75**, 1002-1010.

82. Cuevas-Glory L.F., Pino J.A., Santiago L.S., Sauri-Duch E., A review of volatile analytical methods for determining the botanical origin of honey, *Food Chem*., 2007, **103**, 1032-1043.

83. Ai J., Solid Phase Microextraction for Quantitative Analysis in Nonequilibrium Situations, *Anal. Chem*., 1997, **69**, 1230-1236.

84. Bicchi C., Drigo S., Rubiolo P., Influence of fibre coating in headspace solid-phase microextraction-gas chromatographic analysis of aromatic and medicinal plants, *J. Chromatogr*. A, 2000, **892**, 469-485.

85. Bertoli A., Pistelli L., Morelli I., Fraternale D., Giamperi L., Ricci D., Volatile constituents of different parts (roots, stems and leaves) of *Smyrnium olusatrum* L., *Flav. Fragr. J*., 2004, **19**, 522-525.

86. Demirci B., Demirci F., Baser H.C., Headspace-SPME and hydrodistillation of two fragrant *Artemisia* sp., *Flav. Fragr. J*., 2005, **20**, 395-398.

87. Bertili A., Pistelli L., Morelli I., Fraternale D., Giamperi L., Ricci D., Volatile constituents of micropropagated plants of *Bupleurum fructicosum* L., *Plant Sci*., 2004, **167**, 807-810.

88. Flamini G., Cioni P.L., Morelli I., Use of solid-phase micro-extraction as a sampling technique in the determination of volatiles emitted by flowers, isolated flower parts and pollen, *J. Chromatogr. A*, 2003, **998**, 229-233.

89. Flamini G., Cioni P.L., Morelli I., Maccioni S., Baldini R., Phytochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory, *Food Chem*., 2004, **85**, 599-604.

90. Flamini G., Cioni P.L., Morelli I., Composition of the essential oils and in vivo emission of volatiles of four *Lamium* species from Italy: *L. purpureum, L. hybridum, L. bifidum* and *L. amplexicaule, Food Chem*., 2005, **91**, 63-68.

91. Arpino P., Perevot A., Serpinet J., Tranchant J., Vergnol A., Witier P., Manuel pratique de chromatographie en phase gazeuse, Ed. Masson, Paris, 1995, p. 700.

92. Tranchant J., Manuel pratique de chromatographie en phase gazeuse, 4iéme édition. Ed. Masson, Paris-Milan-Barcelone, 1995, p. 699.

93. De Hoffman E., Charrette J., Strootbant V., Spectrométrie de masse. Librairie Dunod, Paris, 1999, p. 339.

94. Jennings W., Shibamoto T., Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography, Ed. Jovanovitch H.B., Academic Press, New-York, 1980.

95. Joulain D., König W.A., *The atlas of spectral data of sesquiterpene hydrocarbons, Hambourg*, Ed. E.B. Verlag, 1998.

96. Köning W.A., Hochmuth D.H., Joulain D., *Terpenoids and related constituants of essential oils*, Library of MassFinder 2.1, Institute of organic chemistry, Hambourg, Germany, 2001.

97. McLafferty F.W., Stauffer D.B.,*The Wiley/NBS Registry of Mass Spectral Data*, 4th Ed. Wiley-Interscience, New York, 1988.

98. McLafferty F.W., Stauffer D.B., *Wiley Registry of Mass Spectral Data*, 6th Ed. Mass Spectrometry Library Search System Bench-Top/PBM, version 3.10., Newfield, 1994.

99. Adams R.P., *Identification of essential oil components by gaz chromatography / quadrupole mass spectroscopy*, Allured Publishing, Carol Stream, Illinois, 2001.

100. National Institute of Standards and Technology. PC Version 1.7 of The NIST/EPA/NIH Mass Spectra Library. Perkin-Elmer Corp: Norwalk, CT, 1999.

101. Hadjieva P., Sandra P., Stoinova-Ivanova B., Verzele M., Open tubular gas chromatography-mass spectral « electron impact and chemical ionization » - analysis of bulgarian rose oil (*Rosa damascena* mill.), *Rivista italiana EPPOS*, 1980, **62**, 367-372.

102. Vernin G., Faure R., Pieribattesti J.C., Two ent-Kauranoid Diterpenes Constituents of *Cryptomeria japonica* D. Don: □-Kaurene and 16-Hydroxy-□□ -Kaurane, *J. Essent. Oil Res*., 1990, **2**, 211-214.

103. Vernin G., Metzger J., Suon K.N., Fraisse D., Ghiglione C., Hamoud A., Párkányi C., GC-MS SPECMA Bank Analysis of Essential Oils and Aromas. GC-MS-EI-PCI Data Bank Analysis of Sesquiterpenic Compounds in Juniper Needle Oil, *Lebensm.-Wiss. u-Technol*., 1990, **23**, 25-33.

104. Vernin G., Lageot C., Couplage CPG/SM pour l'analyse des arômes et des huiles essentielles, *Analysis*, 1992, **20**, 34-39.

105. Schultze W., Lange G., Schmaus G., Isobutane and ammonia chemical ionization mass spectrometry of sesquiterpene hydrocarbons, *Flav. Fragr. J.*, 1992, **7**, 55-64.

106. Lange G., Schultze W., *Studies on Terpenoid and Non-Terpenoid Esters Using Chemical Ionization Mass Spectrometry in GC/MS Coupling*, in *Bioflavour'87*, Schreier P. Ed., de Gruyter W.& Co., Berlin, New-York, 1988, 105-114.

107. Lange G., Schultze W., *Differentiation of Isopulegol Isomers by Chemical Ionization Mass Spectrometry*, in *Bioflavour'87*, P.Schreier Ed., W. de Gruyter & Co., Berlin, New-York, 1988, 115-122.

108. Lange G, Schultze W., Application of isobutane and ammonia chemical ionization mass spectrometry for the analysis of volatile terpene alcohols and esters, *Flav. Fragr. J*., 1987, **2**, 63-73.

109. Bicchi C., Frattini C., Raverdino V., Considerations and remarks on the analysis of *Anthemis nobilis* L. essential oil by capillary gas chromatography and "hyphenated" techniques, *J. Chromatogr*., 1987, **411**, 237-249.

110. Bruins A. P., Negative Ion Chemical Ionization Mass Spectrometry in the Determination of Components in Essential Oils, *Anal. Chem*., 1979, **51**, 967-972.

111. Bruins A.P., Gas Chromatography-Mass Spectrometry of essential oils. Part II. Positive ion and negative ion chemical ionization techniques, *Capillary Gas Chromatography in Essential Oils Analysis*, Ed. Bicchi C., Sandra P., Heidelberg, Huethig Verlag, 1987, 329-356.

112. Brevard H., Spectrométrie de masse et modes d'ionisations appliqués à l'étude de l'huile essentielle de *Ruscus acuelatus*, Thèse de l'Université de Nice, 1985.

113. Hendriks H., Bruins A.P., Study of three type of essential oil of *Valeriana officinalis* L. by combined gas chromatography-negative ion chemical ionization mass spectrometry, *J. Chromatogr*., 1980, **190**, 321-330.

114. Hendriks H., Bruins A.P., A tentative identification of components in the essential oil of *Cannabis sativa* L. by a combination of gas chromatography negative ion chemical ionization mass spectrometry and retention indices, *Biochem. Mass Spectrom*., 1983, **10**, 377-381.

115. Zupanc M., Prošek M., Dušan M., Combined CI and EI mass spectra in the analysis of essential oils, *J. High Res. Chromatogr.*, 1992, **15**, 510-513.

116. Arpino P.J., L'ionisation chimique une façon de modéliser les réactions de chimie organique dans un spectromètre de masse, *L'actualité Chimique*, 1982, **4**, 19-28.

117. De Hoffmann E., Charette J., Stroobant V., *Spectrométrie de masse*, Masson, 1994, Paris.

118. Harrison A.G., *Chemical ionisation mass spectrometry*, 2nd Ed. CRC Press, 1992, Boca Raton, Florida.

119. Chapman J.R., *Practical Organic Mass Spectrometry*, 2nd Ed. Wiley J. & Sons, 1998, Chichester, England.

120. Munson B., Chemical ionization mass spectrometry: ten years later, *Anal. Chem*., 1997, **49**, 772A-778A.

121. Dougherty R.C., Negative chemical ionization mass spectrometry, *Anal. Chem*., 1981, **53**, 625A-636A.

122. Bicchi C., Liberto E., Sgorbini B., Mondello L., Acampora Zellner B., Costa R., Quantitative analysis of essential oils: complex task, *Flav. Fragr. J*., 2008, **23**, 382-391.

123. Cicchetti E., Merle P., Chaintreau A., Quantitation in gas chromatography: usual practices and performances of a reponse factor database, *Flav. Fragr. J*., 2008, **23**, 450-459.

124. Costa R., Acampora Zeller B., Crupi M., De Fina M., Valentino M., GC-MS, GC-O and enatio-GC investigation of essential oil of *Trachonanthus camphorates* L., *Flav. Fragr. J*., 2008, **23**, 40-48.

125. Brereton R.G., Chemometrics: Data Analysis of the Laboratory and Chemical Plant, Wiley Interscience, New York, 2003, p. 504.

126. Ausloos P., Clifton C.L., Lias S.G., Mikaya A.I., Stein S.E., Tchekhovskoi D.V., Sparkman O.D., Zaikin V., Zhu D., The critical evaluation of a comprehensive mass spectral library, *J. Am. Soc. Mass Spectrom*., 1999, **10**, 287-299.

127. McLafferty F.W., Stauffer D.A., Loh S.Y., Wesdemiotis C., Unknown identification using reference mass spectra. Quality evaluation of databases, *J. Am. Soc. Mass Spectrom*., 1999, **10**, 1229-1240.

128. Karpa A., Kresovich S., Bhat K.V., Ayad W.G., Hodgkin T., Molecular tools in plant genetic resources conservation: a guide to the technologies. *IPGRI Technical Bulletin* N°2, 2002, 1-46.

129. Zietkiewicz E., Rafalski A., Labuda D., Genome fingerprinting by Simple Sequence Repeat (SSR) – Anchored Polymerase Chain Reaction Amplification, *Genomics*, 1994, **20**, 176-183.

130. Alvarez I., Wendel J.F., Ribosomal ITS sequences and plant phylogenetic inference, *Mol. Phylogenet. Evol.*, 2003, **29**, 417-434.

131. Petit R.J., Duminil J., Fineschi S., Hampe A., Salvini D., Vendramin G.G., Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations, *Mol. Ecol.*, 2005, **14**, 689-701.

132. Bazina E., Makris A., Vender C., Skoula M., Genetic and Chemical Relations Among Selected Clones of *Salvia officinalis*. *Journal of Herbs, Spices & Medicinal Plants*, 2002, 9, 269-273.

133. Echeverrigaray S., Agostini G., Atti-Serfini L., Paroul N., Pauletti G.F., Dos Santos A.C., Correlation between the chemical and genetic relationships among commercial thyme cultivars. *J. Agric. Food Chem*., 2001, **49**, 4220-4223.

134. Naydenov K.D., Tremblay F.M., Alexandrov A., FentoN.J., Structure of *Pinus sylvestris* L. populations in Bulgaria revealed by chloroplast microsatellites and terpenes analysis: Provenance tests. *Biochem. Syst. Ecol*., 2005, **33**, 1226-1245.

135. Skoula M., Abbes J.E., Johnson C.B., Genetic variation of volatiles and rosmarinic acid in populations of *Salvia fruticosa* mill growing in Crete. *Biochem. Syst. Ecol*., 2000, **28**, 551-561.

136. Ríos J. L., Recio M.C. Medicinal plants and antimicrobial activity,*J. Ethnopharmacol.*, 2005, **100**, 80-84.

137. Raskin I., Ribnicky D.M., Komarnytsky S., Ilic N., Poulev A., Borisjuk N., Brinker A., Moreno D. A., Ripoll C., Yakoby N., O'Neal J.M., Cornwell T., Pastor I., Fridlender B., Plants and human health in the twenty-first century, *Trends Biotechnol.*, 2002, **20**, 522-531.

138. McChesney J.D., Venkataraman S.K., Henri J.T. Plant Natural products: Back to the future or into extinction?, *Phytochem.*, 2007, **68**, 2015-2022.

139. Belaiche P., Traité de phytothérapie et d'aromathérapie, l'Aromatogramme. Ed. Maloine, Paris, 1979.

140. Benjilali B., Tantaoui-Elaraki E.A., Ismail Alaoui N., Ayadi A., Méthode d'études des propriétés antiseptiques des huiles essentielles par contact direct en milieu gélosé, *Plan. Med. Phytoth.*, 1986, **20**, 155-167.

141. Kalemba D., Kunicka A., Antibacterial and antifungal properties of essential oils, *Current Medicinal Chemistry*, 2003, **10**, 813-829.

142. Carson C.F., Hammer K.A., Riley T.V., *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiology Reviews*, 2006, **19**, 50-62.

143. Carson C.F., Mee B.J., Riley T.V., Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined par time-kill, lysis, leakage and salt tolerance assays and electron microscopy. *Antimicrobial Agents Chemotherapy*, 2002, **46**, 1914-1920.

144. Rajab M.S., Cantrell C.L., Franzblau, S. G., & Fischer, N. H. (1998). Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study. *Planta Medica*, 1998, **64**, 2-4.

145. Marquez B., Bacterial efflux systems and efflux pumps inhibitors, *Biochimie*, 2005, **87**, 1137-1147.

H. LISTE DES TRAVAUX

I. PUBLICATIONS DANS JOURNAUX INTERNATIONAUX A COMITE DE LECTURE

[**PT1**] : ***Teucrium massiliense***

Djabou, N., Paolini, J., Desjobert, J. M., Allali, H., Baldovini, N., Costa, J., Muselli, A. "Qualitative and quantitative analysis of volatile components of *Teucrium massiliense* L. Identification of 6-methyl-3-heptyl acetate as a new natural product", *Flavour and Fragrance Journal*, **2010**, 25, 475-487.

[**PT2**] : ***Teucrium marum***

Djabou, N., Andreani, S., Varesi, L., Tomi, J., Costa, J., Muselli, A. "Analysis of volatile fraction of *Teucrium marum* L." *Flavour and Fragrance Journal*, **2012. Article soumis, en revison.**

[**PT3**]: ***Teucrium scorodonia* (deux sous-espèces)**

Djabou, N., Allali, H., Battesti, M.J., Tabti, B., Costa, J., Muselli, A., Varesi, L. "Chemical and genetic differentiation of two Mediterranean subspecies of *Teucrium scorodonia* L." *Phytochemistry*, **2012**, 74, 123-132.

[**PT4**] : ***Teucrium flavum* (deux sous-espèces)**

Djabou, N., Batesti, M.J., Allali, H., Desjobert, J.M., Varesi, L., Costa, J., Muselli, A. "Chemical and genetic differentiation of Corsican subspecies of *Teucrium flavum* L." *Phytochemistry*, **2011**, 72, 1390-1399.

[**PT5**] : ***Teucrium polium* (deux sous-espèces)**

Djabou, N., Muselli, A., Allali, H., Dib, M.A., Tabti, B., Varesi, L., Costa, J. "Chemical and genetic diversity of two Mediterranean subspecies of *Teucrium polium* L." *Phytochemistry*, **2012. Article soumis, en révision.**

[**PT6**] : ***Teucrium* activités biologiques**

Djabou, N., Guinoiseau, E., Giuliani, M.C., Andreani, S., Lorenzi, V., Bolla, J.M., Costa, J., Berti, L., Luciani, A., Muselli, A. "Phytochemical composition of Corsican *Teucrium* essential oils and antibacterial activity against foodborne or toxi-infectious pathogens" *Food Control,* **2012. Article soumis.**

II. PUBLICATIONS DES SEQUENCES GENETIQUES DANS LA BANQUE DE DONNEES GENBANK

1. Djabou, N., Paolini J, Batesti, M.J., Costa, J., Varesi, L., Muselli, A."Ribosomal protein L32 (rpl32) gene and rpl32-trnL intergenic spacer of *Teucrium polium*",**2011**.*Genebank* accession numbers JQ044762 to JQ044770.

2. Djabou, N., Desjobert J.M, Batesti, M.J., Varesi, L., Muselli, A., Costa, J."tRNA-Leu (trnL) gene, trnL-trnF intergenic spacer and tRNA-PHE (trnF) gene of *Teucrium polium*",**2011**.*Genebank* accession numbers JQ044771 to JQ044782.

3. Djabou, N., Muselli, A., Desjobert J.M, Paolini J, Costa, J., Varesi, L."Internal transcriber spacer 1, 5.8S ribosomal RNA gene and internal transcriber spacer 2 of *Teucrium polium*",**2011**.*Genebank* accession numbers JN903715 to JN903736.

4. Djabou, N., Allali, H., Tabti, B., Muselli, A., Varesi, L., Costa, J. "*Teucrium scorodonia* L. trnL-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence; and trn-phe (trnF) gene, partial sequence; chloroplast", **2011**.*Genebank* accession numbers JF694842 to JF694868.

5. Djabou, N., Allali, H.,Batesti, M.J.,Varesi, L., Costa, J., Muselli, A."*Teucrium scorodonia* L. rpl32-trnL intergenic spacer: chloroplast", **2011**.*Genebank* accession numbers JF694896 to JF694922.
6. Djabou, N., Allali, H., Desjobert JM, Muselli, A., Costa, J., Varesi, L."*Teucrium scorodonia* L. internal transcriber spacer 1, partial internal transcriber spacer 2, partial sequence", **2011**.*Genebank* accession numbers JF694869 to JF694895.

7. Djabou N., Battesti MJ, Muselli A, Paolini J, Desjobert JM, Costa J, Varesi L. "*Teucrium flavum* internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence", **2010**. *Genebank* accession numbers HQ646955 to HQ646967.

8. Djabou N., Varesi L, Muselli A, Paolini J, Desjobert JM, Costa J. "*Teucrium flavum* rpl32-trnL intergenic spacer: chloroplast", **2010**. *Genebank* accession numbers HQ646958 to HQ646980.
9. Djabou N., Paolini J, Desjobert JM, Muselli A, Costa J, Varesi L. "*Teucrium flavum* tRNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence; and trn-Phe (trnF) gene, partial sequence; chloroplast", **2010**. *Genebank* accession numbers HQ646981 to HQ646993.

10. Vallascas,E., Djabou,N., Deiana,F., Costa,J., Desjobert,J.M., Paolini,J., Muselli,A. and Varesi,L. "Phylogeny of endemic Mediterranean *Teucrium marum* based on nuclear and chloroplast DNA markers. Internal transcriber spacer 1; 5.8S ribosomal RNA gene; and internal transcriber spacer 2", **2010**. *Genebank,* GU396043 to GU 396089.

11. Vallascas, E., Djabou, N., Muselli, A., Andreani, S., Varesi, L., Costa, J. "Phylogeny of endemic Mediterranean *Teucrium marum* based on nuclear and chloroplast DNA markers. tRNA-Leu (trnL) gene: chloroplast", **2009**. *Genebank,* GU331782 to GU331791.

12. Djabou, N., Vallascas, E., Costa, J., Andreani, S., Desjobert, J.M., Varesi, L., Muselli, A. "Phylogeny of endemic Mediterranean *Teucrium marum* based on nuclear and chloroplast DNA markers: trnL-rpl32 intergenic spacer: chloroplast", **2009**. *Genebank,* GU331771 to GU331781.

III. TEXTES DES PUBLICATIONS

[**PT1**] : ***Teucrium massiliense***

Djabou, N., Paolini, J., Desjobert, J. M., Allali, H., Baldovini, N., Costa, J., Muselli, A. "Qualitative and quantitative analysis of volatile components of *Teucrium massiliense* L. Identification of 6-methyl-3-heptyl acetate as a new natural product", *Flavour and Fragrance Journal*, **2010**, 25, 475-487.

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Qualitative and quantitative analysis of volatile components of *Teucrium massiliense* L. – identification of 6-methyl-3-heptyl acetate as a new natural product

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ABSTRACT: Volatile components extracted from the aerial parts and separated organs of Corsican and Sardinian *Teucrium massiliense* have been studied. The chemical composition of essential oils obtained by hydrodistillation (HD) has been investigated using GC–RI, GC–MS (EI and CI modes) and ^{13}CNMR spectroscopy. For the first time, a real concentration via calibration curves and response factor calculations has been determined for each oil component. The concentrations of the major components were 6-methyl-3-heptyl acetate (23.83–18.16 g/100 g), 3-octyl acetate (10.55–6.95 g/100 g), isobutyl isovalerate (7.67–2.91 g/100 g), germacrene D (6.13–1.01 g/100 g) and linalool (6.63–5.23 g/100 g). To the best of our knowledge, the occurrence of 6-methyl-3-heptyl acetate as a natural product is reported for the first time. Furthermore, the chemical composition of volatile fractions emitted from the aerial parts and separated organs of *T. massiliense* L. has been studied by HS–SPME/GC–RI and GC–MS after optimization of SPME parameters. Concerning the contribution of plant organs to the aromatic plant fingerprint, we noted that the flowers produced more volatiles than other organs. The volatile fractions obtained from the different organs were qualitatively quite similar to each other but differed in the percentages of their major components. Also, the influence of HS–SPME extraction parameters (equilibrium and extraction times and temperature) on the chemical composition of the plant volatile fraction is discussed. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: *Teucrium massiliense* L.; volatile components; HS–SPME; GC–MS; 6-methyl-3-heptyl acetate

Introduction

The genus *Teucrium* (family Lamiaceae), which includes 300 species widespread all around the world, consists of perennial herbs or shrubs commonly named germanders.[1,2] *Teucrium* species are generally aromatic and ornamental plants and are also valued as a pollen source. Many species of *Teucrium* are currently used in folk medicine as stimulants, tonics, stomach ache remedies and also antidiabetic agents.[3–5] The chemistry of this genus has been investigated: mainly flavonoids[6] and neoclerodane diterpenoids[7,8] were identified and their biological activities were reported. *Teucrium* essential oils have been the subject of several investigations and diverse chemical compositions were recently reviewed by Ali *et al.*[9] The oils were found to be rich in sesquiterpenes, such as α- and/or τ-cadinols, caryophyllene oxide, (*E*)-β-farnesene, δ-cadinene, α-humulene, β-caryophyllene and/or germacrene D, in association with monoterpenes such as α- and/or β-pinenes, linalool, sabinene and/or limonene.

T. massiliense L. is a low-growing sub-shrub (1 m) with grey-green leaves and rose-pink flowers which bloom on July to September. It grows wild in some areas of the Mediterranean basin, particularly in Corsica[10] and Sardinia.[11] In France, *T. massiliense* is a protected species listed under Annex I of the '*Arrêté du 20 janvier 1982 modifié*', relating to French protected plant species.[12] In Corsica, *T. massiliense* is considered as very rare and threatened species; a small number of populations occurs in a small number of separate areas, and some of them have only a few individuals.[10] Folk medicine indicates that a decoction of *T. massiliense* aerial parts was used as a febrifuge and antimalaric effect.[10,11,13] The occurrence of some pharmacologically active compounds in *T. massiliense* extracts have been reported, especially, neoclerodane diterpenoids[14,15] were reported as insect antifeedants[16,17] and antifungal, antimicrobial and moluscicidal agents.[18,19] To the best of our knowledge, only one study reported the chemical composition and antioxidant activity of *T. massiliense* essential oil from Sardinia.[13] Among the 34 identified compounds, 3,7-dimethyloctan-2-one (15.2%), butyl-2-methylbutyrate (12.1%), linalool (10.6%) and linalyl acetate (7.1%) were reported as the main components.

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Hydrodistillation (HD) is the most common sampling preparation technique used to obtain the volatile fraction from aromatic plants, but it is time-consuming and needs large amounts of sample. Headspace solid-phase micro-extraction (HS–SPME) is a rapid and simple procedure successfully used to sample the volatile components of aromatic and medicinal plants.[20–24] HS–SPME analysis allowed the fingerprint of the plant headspace to be rapidly obtained. HS sampling needs the optimization of extraction parameters. As reported in the literature,[22–24] the most effective fibre used from vegetable matrices was generally one consisting of three polymers, a liquid (PDMS) for the less polar components and two solids (DVB and CAR) for the more polar components. Concerning times and temperatures of equilibrium and extraction, various conditions have been reported, according to the plant material.[20–24] In the present study, the temperature and extraction times used for the HS analysis were optimized.

As part of our on-going chemical investigations of the essential oils of *Teucrium* species from Corsica and Sardinia,[25,26] the aim of the present study was, on the one hand, the characterization of essential oils of *T. massiliense* L. from both islands, using a combination of capillary GC–RI, GC–MS (EI and CI modes) and ^{13}CNMR spectroscopy after fractionation on column chromatography. We reported, for the first time, a real concentration determination via calibration curves and response factor calculations for each oil component. The purpose was also, on the other hand, the characterization of the volatile fraction obtained from full aerial parts and different organs of fresh *T. massiliense* L., using HS–SPME/GC–RI and HS–SPME/GC–MS. To the best of our knowledge, the components sampling from the vapour phase in equilibrium of *T. massiliense* using HS–SPME is reported here for the first time. Moreover, a comparison of both volatile components extracted by SPME and HD from the same plant material is discussed.

Experimental

Plant Material and Oil Isolation

The aerial parts of *T. massiliense* were collected at the flowering stage in July 2008 around Evisa (Col de Sevi, Zichina and Casanova; Corsica, France) and around Cagliari (Sardinia, Italy). Voucher specimens were deposited in the Herbarium of the Universities of Cagliari and of Corsica, respectively. The fresh aerial parts (300–440 g) were submitted to hydrodistillation for 5 h, using a Clevenger-type apparatus according to the *European Pharmacopoeia*[27] and yielded 0.04–0.07% w/w of oil.

Oil Fractionation

Initially, the collective oil (sample C, 2.3 g) was obtained by a mixture of all Corsican oil samples and subsequently it was submitted to flash chromatography [FC; silica gel 200–500 μm, elution with *n*-pentane (PE), then with diethyl ether (Et_2O)]. Two fractions were obtained: fraction I apolar (0.29 g, hydrocarbon compounds) and fraction II polar (1.95 g, oxygenated compounds). Fraction II (1.9 g) was separated on silica gel (200–500 μm) and 14 fractions (II.1–II.14) were eluted with a mixture of *n*-pentane and diethyl ether of increasing polarity. The main components of each fraction, their percentages and their identification numbers (corresponding to the order of elution on the apolar column) are reported in Figure 1 and Table 1.

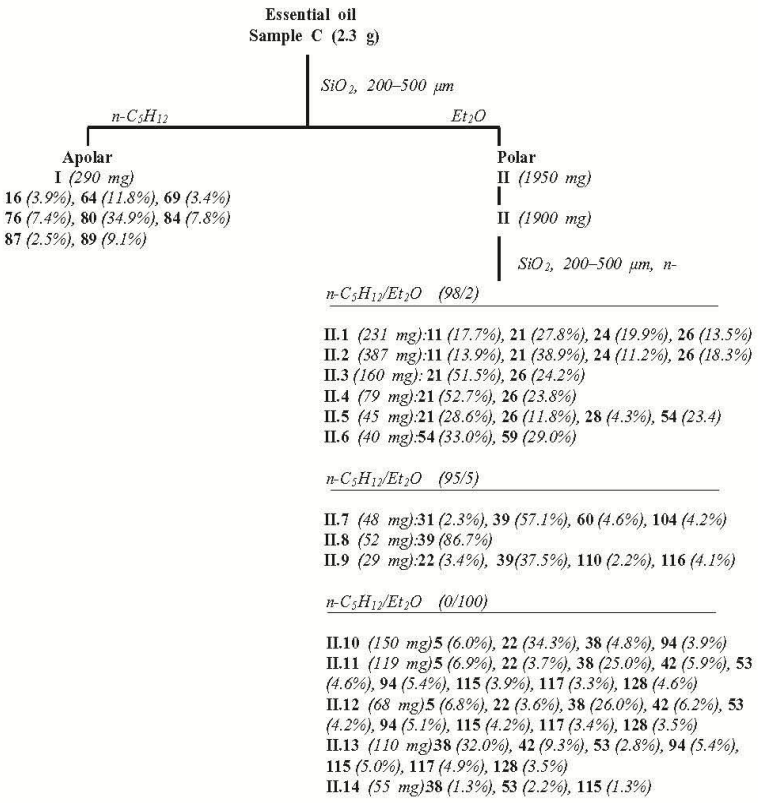

Figure 1. Fractionation of *T. massiliense* L. essential oil of Corsica

Table 1. Chemical compositions of *T. massiliense* L. essential oils from Corsica and Sardinia

No.[a]	Compounds*	IRI_a[b]	RI_a[c]	RI_p[d]	RF[e]	Corsican samples[f]										Sardinian sample[g]		Identification[i]
						C		C1		C2		C3		C4		S1		
						%[h]	g/100 g[i]	%[h]	g/100 g[i]	%[h]	g/100 g[i]	%[h]	g/100 g[i]	%[h]	g/100 g[i]	%[h]	g/100 g[i]	
1	Ethyl-2-methyl butyrate	843	829	1011	1.55	0.2	0.16	0.1	0.13	0.2	0.20	0.1	0.12	0.1	0.12	–	–	RI, MS
2	(*Z*)-hex-3-en-1-ol	851	832	1368	1.34	0.2	0.23	0.2	0.14	0.1	0.11	0.1	0.11	0.1	0.11	0.1	0.13	RI, MS
3	Isobutyl isobutyrate	899	899	1065	1.55	0.7	0.68	1.4	1.39	1	1.03	0.9	0.92	1.3	1.32	0.1	0.12	RI, MS
4	2-Hexyl acetate	920	917	1124	1.55	0.4	0.42	0.7	0.73	0.5	0.52	0.6	0.65	0.6	0.61	0.6	0.63	RI,MS,Ref.
5	6-Methyl-3-heptanol	–	945	1320	1.31	1.5	1.60	1.1	1.20	0.7	0.83	0.8	0.89	1	1.11	0.3	0.36	RI,MS,Synt.
6	Oct-1-en-3-ol	962	960	1429	1.34	tr	0.03	tr	0.03	0.1	0.16	0.1	0.11	0.1	0.11	tr	0.03	RI, MS
7	Octan-3-one	969	963	1207	1.31	tr	0.03	0.3	0.32	0.1	0.10	0.1	0.10	0.2	0.23	0.2	0.25	RI, MS
8	Myrcene	987	980	1332	1.01	tr	0.02	tr	0.02	tr	0.03	tr	0.02	tr	0.02	tr	0.02	RI, MS
9	3-Octanol	981	981	1366	1.34	0.4	0.39	0.3	0.28	0.2	0.27	0.3	0.43	0.4	0.39	0.2	0.28	RI, MS
10	Decene	988	982	1423	1.01	tr	0.02	tr	0.02	tr	0.02	tr	0.02	tr	0.02	tr	0.02	RI,MS,Ref.
11	Isobutyl isovalerate	993	989	1154	1.55	5.9	5.80	7.8	7.67	6.8	6.59	7.2	7.14	6.5	6.50	3	2.91	RI, MS
12	3-Methyl butyl isobutyrate	994	997	1167	1.55	0.2	0.21	0.2	0.21	0.2	0.20	0.1	0.11	0.2	0.21	0.1	0.11	RI, MS
13	2-Methyl butyl isobutyrate	1002	1001	1167	1.55	0.8	0.80	1.1	1.00	0.8	0.80	0.8	0.82	1	1.03	0.4	0.42	RI, MS
14	2-Heptyl acetate	1024	1014	1356	1.55	0.4	0.40	0.3	0.29	0.4	0.41	0.3	0.28	0.4	0.40	0.2	0.21	RI,MS,Ref.
15	Limonene	1025	1020	1186	1.01	0.4	0.35	0.1	0.09	0.3	0.16	0.2	0.17	0.4	0.24	0.2	0.13	RI, MS
16	(*Z*)-β-Ocimene	1029	1026	1207	1.01	3.1	2.31	1.9	1.58	2.1	1.60	2.1	1.72	2.2	1.90	1	0.87	RI, MS
17	2,6-Dimethyl-5-heptanal	1039	1034	1348	1.4	tr	0.03	0.1	0.12	0.1	0.13	tr	0.03	tr	0.03	–	–	RI, MS
18	(*E*)-β-Ocimene	1041	1036	1221	1.01	0.6	0.51	0.5	0.41	0.7	0.63	0.6	0.5	0.6	0.52	0.2	0.1	RI, MS
19	*trans*-Linalool oxide THP	1058	1054	1435	1.59	tr	0.04	0.1	0.08	tr	0.03	–	–	tr	0.39	–	–	RI, MS
20	*trans*-Linalool oxide THF	1072	1061	1440	1.59	tr	0.03	0.2	0.22	tr	0.02	–	–	tr	0.04	–	–	RI, MS
21	6-Methyl-3-heptyl acetate	–	1074	1267	1.55	20.3	19.12	25.1	23.75	23.6	22.1	22.1	20.48	24.3	23.83	19.5	18.16	RI,MS,Synt.
22	Nonanal	1086	1087	1514	1.34	0.1	0.15	tr	0.03	0.1	0.13	tr	0.03	tr	0.03	tr	0.03	RI, MS
23	Linalool	1083	1088	1394	1.34	4.8	5.23	4.7	4.94	3.1	3.44	3.1	3.43	3.3	3.43	5.8	6.63	RI, MS
24	3-Methyl butyl isovalerate	1092	1092	1257	1.55	3.3	3.40	3.8	3.99	3.1	3.03	3	2.85	3.1	3.09	1.8	1.87	RI, MS
25	2-Methyl butyl isovalerate	1094	1094	1260	1.55	0.8	0.90	0.9	0.90	0.7	0.69	0.8	0.78	0.6	0.62	0.5	0.52	RI, MS
26	3-Octanyl-acetate	1113	1111	1315	1.55	7.1	6.95	10.6	10.55	8.3	8.05	9.4	9.26	7.6	7.35	7.1	6.96	RI,MS, ^{13}C
27	*neo-allo*-Ocimene	1126	1126	1363	1.01	0.3	0.28	1	0.88	0.6	0.53	0.6	0.43	0.5	0.37	0.8	0.70	RI, MS
28	Citronellal	1129	1131	1456	1.4	0.4	0.54	0.9	1.02	0.9	1.10	0.7	0.86	1	1.04	0.3	0.49	RI, MS
29	Menthone	1136	1133	1446	1.34	0.1	0.11	tr	0.04	0.1	0.08	tr	0.05	0.1	0.11	–	–	RI, MS
30	Isomenthone	1146	1141	1452	1.31	0.1	0.11	tr	0.03	tr	0.02	tr	0.04	tr	0.04	–	–	RI, MS
31	Isopulegone	1151	1154	1621	1.31	0.2	0.22	tr	0.21	0.1	0.13	0.1	0.09	0.1	0.10	tr	0.03	RI,MS,Ref.
32	Methyl-4-acetophenone	1157	1156	1747	1.31	0.2	0.21	tr	0.02	0.1	0.06	tr	0.02	0.2	0.21	–	–	RI, MS
33	Terpinen-4-ol	1164	1160	1562	1.34	0.1	0.13	tr	0.03	0.1	0.11	0.1	0.10	0.1	0.01	–	–	RI, MS
34	2,6-Dimethyl-3-octyl acetate	1170	1174	1356	1.55	0.5	0.53	1.4	1.47	0.9	0.89	1	1.03	0.7	0.79	0.7	0.76	RI,MS,Ref.
35	α-Terpineol	1176	1176	1700	1.34	0.1	0.10	0.1	0.10	tr	0.04	tr	0.03	0.1	0.09	–	–	RI, MS
36	Decanal	1188	1186	1498	1.4	tr	0.03	0.1	0.10	tr	0.03	tr	0.03	tr	0.03	tr	0.03	RI, MS
37	Dodecane	1200	1201	1500	1.0	tr	0.02	tr	0.02	tr	0.03	tr	0.02	tr	0.02	tr	0.02	RI, MS
38	Citronellol	1213	1213	1726	1.34	3.6	4.12	2	2.28	3.9	4.12	3.6	3.87	4.1	4.34	0.6	0.73	RI, MS
39	Pulegone	1215	1216	1603	1.31	6.7	6.91	2.2	2.32	2.2	2.25	2.2	2.42	2.6	2.56	1	1.06	RI, MS
40	(*Z*)-3-Hexenyl-2-methyl butyrate	1220	1219	1457	1.55	tr	0.03	tr	0.03	tr	0.03	–	–	–	–	–	–	RI, MS
41	(*Z*)-Piperitenone oxide	1232	1230	1722	1.59	0.2	0.34	tr	0.03	0.3	0.32	0.2	0.29	0.2	0.28	–	–	RI, MS
42	Geraniol	1235	1235	1801	1.34	0.8	1.01	0.3	0.35	0.1	0.14	0.2	0.16	0.1	0.07	1.1	1.26	RI, MS
43	Linalyl acetate	1239	1241	1557	1.55	0.1	0.09	tr	0.03	tr	0.05	tr	0.03	tr	0.03	0.2	0.27	RI, MS
44	Geranial	1244	1243	1707	1.4	0.7	0.74	0.1	0.10	0.1	0.14	0.3	0.23	0.4	0.41	1.7	1.87	RI, MS
45	Citronnellyl formate	1259	1256	1583	1.55	0.4	0.42	0.7	0.59	0.5	0.51	0.5	0.53	0.3	0.34	0.1	0.12	RI, MS
46	Neryl formate	1265	1262	1649	1.55	tr	0.02	tr	0.02	tr	0.04	tr	0.03	tr	0.03	–	–	RI, MS
47	Lavandulyl acetate	1275	1268	1593	1.55	tr	0.03	0.1	0.03	tr	0.03	0.1	0.03	0.1	0.03	–	–	RI, MS

Table 1. Continued

No.[a]	Compounds*	fRI_a[b]	RI_a[c]	RI_p[d]	RF[e]	C		C1		Corsican samples[f] C2		C3		C4		Sardinian sample[g] S1		Identification[i]
						%[h]	g/100 g[j]	%[h]	g/100 g[j]	%[h]	g/100 g[j]	%[h]	g/100 g[j]	%[h]	g/100 g[j]	%[h]	g/100 g[j]	
48	Benzyl isobutanoate	1269	1271	1817	1.55	tr	0.03	tr	0.02	–	–	–	–	–	–	–	–	RI, MS
49	Geranyl formate	1284	1280	1666	1.55	0.3	0.28	0.2	0.19	0.2	0.17	0.2	0.21	0.1	0.11	0.4	0.41	RI, MS
50	(*E,E*)-2,4-Decadienal	1290	1291	1820	1.4	tr	0.02	0.1	0.11	tr	0.02	tr	0.02	tr	0.03	–	–	RI, MS
51	Theaspirane I	1299	1291	1480	1.22	–	–	tr	0.02	tr	0.03	–	0.02	–	–	0.2	0.22	RI, MS
52	Theaspirane II	1313	1306	1517	1.22	–	–	0.1	0.08	tr	0.02	tr	0.02	–	–	0.1	0.12	RI, MS
53	Piperitenone	1318	1309	1870	1.31	0.7	0.70	0.1	0.09	0.1	0.13	tr	0.02	0.2	0.14	–	–	RI, MS
54	Citronellyl acetate	1337	1334	1626	1.55	1.4	1.31	1.6	1.61	2.2	2.11	2.1	1.94	1.7	1.67	0.4	0.44	RI, MS
55	Neryl acetate	1342	1335	1694	1.55	tr	0.02	0.1	0.08	0.2	0.15	0.1	0.09	0.1	0.09	–	–	RI, MS
56	δ-Elemene	1340	1336	1535	1.0	tr	0.02	–	–	–	–	–	–	tr	0.02	tr	0.02	RI,MS,Ref.
57	*trans*-2-Undecenal	1346	1342	1726	1.4	tr	0.04	0.1	0.13	tr	0.04	tr	0.04	tr	0.04	–	–	RI, MS
58	α-Cubebene	1355	1349	1452	1.55	tr	0.03	–	–	0.1	0.12	0.1	0.08	0.1	0.09	tr	0.04	RI, MS
59	Geranyl acetate	1362	1360	1715	1.55	0.9	0.87	0.4	0.40	0.5	0.50	0.6	0.60	0.8	0.77	1.1	1.17	RI, MS
60	(*E*)-β-Damascenone	1363	1361	1770	1.31	0.3	0.33	0.2	0.19	0.1	0.10	0.1	0.10	0.2	0.20	–	–	RI, MS
61	Isoledene	1382	1366	1462	1.0	tr	0.02	–	–	tr	0.02	–	–	–	–	0.2	0.17	RI, MS
62	α-Ylangene	1376	1371	1476	1.0	tr	0.02	–	–	tr	0.02	–	–	0.1	0.08	–	–	RI, MS
63	α-Copaene	1379	1377	1488	1.0	0.1	0.08	0.1	0.07	–	–	tr	0.01	–	–	tr	0.02	RI, MS
64	β-Bourbonene	1386	1381	1483	1.0	2	1.74	1.8	1.66	3.5	3.30	3	2.89	2.9	2.74	1.1	0.71	RI, MS
65	1,5-*di*-*epi*-β-Bourbonene	1389	1384	1483	1.0	0.1	0.07	0.2	0.14	0.3	0.22	0.6	0.48	0.5	0.36	0.2	0.17	RI,MS,Ref.
66	Tetradecane	1392	1402	1402	1.0	tr	0.01	tr	0.02	tr	0.02	tr	0.02	tr	0.02	tr	0.02	RI,MS,Ref.
67	Cinnamyl acetate	1408	1411	2152	1.55	0.1	0.11	0.2	0.20	0.1	0.09	tr	0.03	tr	0.03	0.2	0.23	RI, MS
68	α-Gurjunene	1413	1413	1475	1.0	0.1	0.09	0.1	0.90	tr	0.02	tr	0.02	0.1	0.02	0.1	0.05	RI, MS
69	β-Ylangene	1420	1419	1534	1.0	0.7	0.56	0.5	0.45	0.6	0.41	0.7	0.59	0.7	0.58	0.3	0.19	RI, MS
70	β-Copaene	1430	1423	1581	1.0	0.3	0.26	0.3	0.25	0.5	0.33	0.4	0.30	0.6	0.41	0.2	0.12	RI, MS
71	Citronnellyl propionate	1427	1426	1549	1.55	tr	0.03	–	–	–	–	–	–	–	–	–	–	RI, MS
72	*trans*-α-Bergamotene	1434	1429	1547	1.0	0.2	0.17	0.1	0.09	0.2	0.12	0.2	0.13	0.2	0.13	0.1	0.06	RI, MS
73	α-Sesquisabinene	1435	1433	1626	1.0	0.1	0.09	0.1	0.08	0.1	0.06	0.1	0.08	0.1	0.08	0.1	0.07	RI, MS
74	Aromadendrene	1443	1437	1605	1.0	0.2	0.12	0.1	0.07	0.2	0.12	0.3	0.22	0.3	0.21	0.2	0.14	RI, MS
75	(*E*)-β-Farnesene	1446	1446	1628	1.0	0.4	0.31	0.1	0.08	0.3	0.12	0.3	0.25	0.3	0.21	2.3	1.77	RI, MS
76	*allo*-Aromadendrene	1462	1455	1605	1.0	1.8	1.40	1.6	1.30	1.6	1.01	1.7	1.17	1.7	1.31	1.4	0.98	RI, MS
77	β-Humulene	1460	1456	1605	1.0	tr	0.02	–	–	0.2	0.12	0.2	0.09	tr	0.02	0.1	0.05	RI, MS
78	α-Curcumene	1473	1470	1731	1.0	0.5	0.33	0.5	0.32	0.5	0.31	0.5	0.32	0.7	0.45	6	5.07	RI, MS
79	γ-Muurolene	1474	1471	1642	1.0	tr	0.02	0.1	0.06	0.1	0.05	tr	0.02	tr	0.02	tr	0.02	RI, MS
80	Germacrene-D	1479	1476	1666	1.0	7.6	6.13	6.5	5.25	4.9	3.96	5.9	5.10	6.1	5.26	1.4	1.01	RI, MS
81	γ-Humulene	1483	1477	1685	1.0	tr	0.02	–	–	0.2	0.08	0.1	0.05	tr	0.02	–	–	RI, MS
82	Zingiberene	1489	1483	1687	1.0	0.3	0.24	0.2	0.12	0.2	0.09	0.2	0.12	0.2	0.14	2	1.75	RI, MS
83	4-*epi*-Cubebol	1490	1487	1870	1.34	0.1	0.10	–	–	–	0.00	tr	0.03	–	–	tr	0.03	RI, MS
84	Bicyclogermacrene	1494	1490	1685	1.0	0.6	0.50	0.3	0.29	0.6	0.46	0.4	0.33	0.6	0.50	0.3	0.23	RI, MS
85	β-Curcumene	1503	1500	1687	1.0	0.3	0.29	tr	0.20	0.1	0.06	0.1	0.07	tr	0.02	–	–	RI, MS
86	β-Bisabolene	1503	1502	1720	1.0	–	–	0.1	0.08	tr	0.02	tr	0.02	tr	0.02	5.4	4.86	RI, MS
87	(*Z*)-γ-Bisabolene	1505	1505	1709	1.0	0.5	0.43	0.2	0.17	0.1	0.08	0.2	0.17	0.4	0.37	0.3	0.22	RI, MS
88	Cubebol	1514	1506	1924	1.34	tr	0.02	0.5	0.55	0.3	0.44	0.3	0.37	0.2	0.29	–	–	RI, MS
89	δ-Cadinene	1520	1512	1715	1.0	0.9	0.75	0.4	0.35	1.4	1.15	1.3	1.00	1.3	1.02	tr	0.02	RI, MS
90	β-Sesquiphellandrene	1516	1517	1765	1.0	–	–	0.1	0.07	tr	0.01	–	–	tr	0.02	2.3	1.87	RI, MS
91	(*E*)-γ-Bisabolene	1531	1527	1733	1.0	0.1	0.07	0.1	0.08	tr	0.02	tr	0.02	tr	0.02	–	–	RI,MS,Ref.
92	(*E*)-Nerolidol	1553	1549	2037	1.34	–	–	–	–	tr	0.03	–	–	–	–	0.2	0.22	RI, MS
93	α-Turmerol	1563	1557	2170	1.34	–	–	0.1	0.11	0.1	0.14	0.1	0.11	tr	0.03	0.3	0.32	RI, MS
94	1,5-Epoxy salvial-4(14)-ene	1554	1558	1910	1.59	0.2	0.21	0.1	0.11	tr	0.02	0.1	0.11	0.1	0.11	tr	0.03	RI, MS
95	Spathulenol	1572	1560	2065	1.34	0.9	1.16	0.7	0.83	1	1.32	1.1	1.20	1.1	1.21	1.9	2.18	RI, MS

Table 1. Continued

No.[a]	Compounds[a]	fRI[a][b]	RI[a][c]	RI[p][d]	RF[e]	Corsican samples[f]								Sardinian samples[g]				Identification[i]
						C		C1		C2		C3		C4		S1		
						% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	
96	Palustrol	1562	1562	1920	1.34	0.1	0.11	tr	0.03	tr	0.03	0.1	0.11	tr	0.03	–	–	RI, MS
97	Germacrene D-4-ol	1571	1565	2025	1.34	0.1	0.10	tr	0.03	tr	0.04	tr	0.03	tr	0.35	–	–	RI, MS
98	Caryophyllene oxide	1578	1570	1942	1.59	0.3	0.39	0.1	0.13	0.2	0.30	0.3	0.40	0.3	0.39	0.3	0.35	RI, MS
99	Viridiflorol	1592	1572	2071	1.34	0.1	0.12	tr	0.03	tr	0.03	tr	0.03	tr	0.04	0.1	0.10	RI, MS
100	β-Copaen-4α-ol	1575	1575	2141	1.34	–	–	0.1	0.13	tr	0.03	tr	0.03	–	–	0.1	0.12	RI, MS
101	Globulol	1589	1575	2074	1.34	0.2	0.25	0.1	0.12	0.1	0.06	0.1	0.14	0.1	0.11	–	–	RI, MS
102	Germacradiene-11-ol	1579	1580	2065	1.34	0.1	0.10	0.1	0.10	0.1	0.11	0.1	0.10	0.1	0.10	–	–	RI, MS
103	Iedol	1600	1589	1996	1.34	0.3	0.10	tr	0.10	0.1	0.10	0.1	0.10	0.2	0.10	0.5	0.65	RI, MS
104	β-Oplopenone	1594	1591	2023	1.31	0.6	0.60	0.3	0.31	0.4	0.56	0.3	0.31	0.5	0.51	0.5	0.49	RI, MS
105	Humulene epoxide II	1602	1599	2044	1.59	tr	0.03	tr	0.03	tr	0.04	tr	0.04	tr	0.04	tr	0.03	RI, MS
106	Hexadecane	1600	1601	1602	1.0	tr	0.02	tr	0.02	tr	0.02	tr	0.02	tr	0.02	tr	0.02	RI,MS,Ref.
107	ar-Curcumen-7-ol	1610	1611	2240	1.34	0.4	0.47	0.2	0.24	0.4	0.50	0.4	0.53	0.4	0.52	0.4	0.50	RI,MS,Ref.
108	Isospathulenol	1625	1618	2175	1.34	0.3	0.41	0.2	0.26	0.1	0.18	0.2	0.26	0.2	0.25	0.4	0.51	RI,MS,Ref.
109	*allo*-Aromadendriene epoxide	1623	1622	1948	1.59	–	–	0.2	0.30	0.1	0.11	tr	0.36	tr	0.03	0.3	0.43	RI,MS,Ref.
110	*epi*-Cubenol	1623	1623	2059	1.34	tr	0.03	tr	0.02	–	–	tr	0.02	tr	0.03	–	–	RI, MS
111	Cubenol	1630	1633	2013	1.34	tr	0.03	0.3	0.33	0.1	0.05	0.2	0.23	0.2	0.03	0.1	0.13	RI, MS
112	τ-Muurolol	1633	1634	2109	1.34	0.6	0.66	0.2	0.23	0.5	0.61	0.5	0.57	0.5	0.59	0.2	0.22	RI, MS
113	τ-Cadinol	1633	1635	2125	1.34	0.4	0.46	0.5	0.57	0.4	0.45	0.4	0.52	0.4	0.52	0.6	0.79	RI, MS
114	Caryophylla-4(14)-8(15)-dien-5α-ol	1641	1636	2292	1.34	0.1	0.14	0.2	0.30	0.1	0.19	0.1	0.14	0.1	0.15	0.2	0.26	RI,MS,Ref.
115	α-Cadinol	1643	1641	2184	1.34	0.3	0.26	0.1	0.12	0.2	0.13	0.2	0.12	0.1	0.07	–	–	RI, MS
116	(*Z,E*)-Farnesol	1635	1654	2224	1.34	0.7	0.79	0.1	0.08	0.1	0.12	0.2	0.22	0.4	0.35	1.4	1.53	RI, MS
117	Eudesma-4(15),7-dien-1β-ol	1671	1671	2348	1.34	0.7	0.80	0.4	0.46	1.2	1.36	0.8	0.89	1.1	1.12	–	–	RI, MS
118	(*Z,E*)-Farnesol	1685	1688	2311	1.34	–	–	–	–	–	–	–	–	–	–	0.8	0.94	RI, MS
119	ar-Curcumen-15-al	1681	1691	2283	1.4	–	–	tr	0.02	–	–	–	–	–	–	0.2	0.21	RI,MS,Ref.
120	Heptadecane	1700	1701	1703	1.0	tr	0.01	tr	0.01	tr	0.02	tr	0.02	tr	0.02	tr	0.02	RI,MS,Ref.
121	Xanthorrhizol	1732	1727	2664	1.34	–	–	–	–	0.1	0.07	0.1	0.08	0.1	0.10	0.2	0.26	RI, MS
122	Lepidozenal	1744	1737	2232	1.4	0.1	0.08	tr	0.02	0.1	0.06	0.1	0.07	0.1	0.10	0.4	0.50	RI,MS,Ref.
123	Diisobutyl ester	1826	1829	2522	1.55	–	–	0.2	0.20	tr	0.03	–	–	–	–	0.7	0.82	RI,MS,Ref.
124	6,10,14-Trimethyl-pentadecanone	1845	1842	2125	1.4	–	–	tr	0.02	0.1	0.07	0.1	0.14	0.1	0.14	0.6	0.66	RI,MS,Ref.
125	Hexadecyl acetate	1970	1951	2407	1.55	tr	0.02	0.1	0.09	–	0.00	–	–	–	–	–	–	RI,MS,Ref.
126	Hexadecanoic acid	1962	1969	2826	1.55	tr	0.02	tr	0.02	tr	0.04	tr	0.02	0.1	0.14	0.1	0.12	RI,MS,Ref.
127	Abietatriene	2046	2036	2218	1.0	0.1	0.06	tr	0.01	tr	0.03	tr	0.02	–	–	0.7	0.65	RI,MS,Ref.
128	Methyl heptadecanol	2030	2037	2482	1.34	0.4	0.42	0.7	0.74	1.1	1.35	0.9	1.01	0.6	0.68	0.2	0.22	RI,MS,Ref.
129	Octadecanol	2070	2073	2529	1.34	0.3	0.32	0.3	0.30	0.5	0.62	0.4	0.44	0.5	0.56	0.4	0.50	RI,MS,Ref.
130	(*E*)-Phytol	2114	2097	2559	1.34	0.2	0.25	tr	0.02	–	–	–	–	0.1	0.10	0.5	0.62	RI, MS
131	*n*-Heneicosane	2100	2101	2102	1.0	tr	0.01	0.3	0.22	0.4	0.37	0.4	0.38	0.2	0.19	tr	0.02	RI,MS,Ref.
132	*n*-Docosane	2200	2201	2204	1.0	tr	0.02	0.1	0.06	0.2	0.11	0.1	0.07	0.1	0.07	0.2	0.16	RI,MS,Ref.

Table 1. Continued

No.[a]	Compounds*	RI_a[b]	RI_s[c]	RI_p[d]	RF[e]	Corsican samples[f]								Sardinian sample[g]		Identification[i]
						C		C1		C2		C3		C4		S1		
						% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	% [h]	g/100 g[j]	
133	*n*-Tricosane	2300	2301	2302	1.0	0.1	0.06	tr	0.01	–	–	–	–	tr	0.02	0.3	0.23	RI,MS,Ref.
134	Tetracosane	2400	2402	2403	1.0	tr	0.02	–	–	–	–	–	–	tr	0.02	0.1	0.05	RI,MS,Ref.
135	Pentacosane	2498	2502	2498	1.0	tr	0.02	tr	0.01	tr	0.02	–	–	–	–	tr	0.02	RI,MS,Ref.
136	Hexacosane	2598	2584	2598	1.0	tr	0.02	–	–	–	–	–	–	–	–	0.1	0.05	RI,MS,Ref.
137	Heptacosane	2700	2703	2700	1.0	tr	0.02	0.1	0.06	–	–	0.1	–	tr	0.02	tr	0.02	RI,MS,Ref.
	Total identification (%)					93.9	92.04	93.3	92.36	90.2	87.35	89.5	87.14	92.5	90.92	87.2	85.3	
	Hydrocarbon compounds					21.4	17.89	17.5	15.71	20.0	15.97	22.4	17.04	20.9	17.79	27.6	22.73	
	Oxygenated compounds					72.5	74.15	75.8	76.65	70.2	71.38	67.1	70.1	71.6	73.13	59.6	62.57	
	Monoterpene hydrocarbons					4.4	3.47	3.5	2.98	3.7	2.95	3.5	2.84	3.7	3.05	2.2	1.82	
	Sesquiterpene hydrocarbons					16.8	14.11	13.5	12.27	15.7	12.38	18.3	13.63	16.9	14.32	24	19.61	
	Non-terpenic hydrocarbons					0.2	0.31	0.5	0.46	0.6	0.64	0.6	0.57	0.3	0.42	1.4	1.3	
	Oxygenated monoterpenes					18.8	20.33	10.6	11.84	11.1	12.07	10.5	11.59	12.3	12.9	9.5	12.07	
	Oxygenated sesquiterpenes					6.6	7.42	4.5	5.56	5.7	7.08	5.8	7.12	6.2	7.6	9.1	10.8	
	Non-terpenic oxygenated compounds					46.9	46.15	60.7	59.23	53.4	52.23	50.8	51.39	53.0	52.53	40.5	39.08	

[a] Order of elution is given on apolar column (Rtx-1).
[b] Retention indices of literature on the apolar column (RI_a) reported from references[28–31]. To our knowledge, the retention index of 21 was reported for the first time.
[c] Retention indices on the apolar Rtx-1 column (RI_a).
[d] Retention indices on the polar Rtx-Wax column (RI_p).
[e] Response factors (RF). For the calculation mode, see text.
[f] Corsican samples: C, mixture of sample oils 1–4. Localities of sampling: C1, Evisa (village); C2, Zichina; C3, Col de Sevi; C4, Casanova.
[g] Sardinian sample: Locality of sampling: S1, Cagliari.
[h] Percentages are given on the apolar column except for components with identical RI_a (percentages are given on the polar column). tr, trace (< 0.05%).
[i] Concentration in g/100 g. For the calculation mode, see text.
[j] RI, retention indices; MS, mass spectra in electronic impact mode; Synt, synthesized compounds; Ref., compounds identified from commercial data libraries: MacLafferty and Stauffer[31] (5, 10, 56, 122, 123, 124, 125, 126, 131, 132, 133, 134, 135, 136, 137); National Institute of Standards and Technology[30] (31, 34, 115, 128, 129); and Joulain and König[28] (4, 14, 65, 66, 91, 106, 107, 108, 109, 119, 120, 127).
* Among the 137 identified compounds, only 3, 8, 9, 13, 23, 28, 35, 42, 43, 44, 59, 64, 74, 79, 80, 82, 86, 89, 95, 103 were reported in the literature of *T. massiliense* oils.[13]

HS–SPME Conditions

The fresh aerial parts and the single organs (flowers, leaves and stems) of *T. massiliense* were cut roughly into pieces (1–2 cm long) with scissors before subjection to HS–SPME. The SPME device (Supelco) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 30 μm) was used for extraction of the plant volatiles. Optimization of conditions was carried out using fresh aerial parts of the plant (1 g in a 20 ml vial) and based on the number and the sum of total peak areas measured on GC–FID. Temperature, equilibration time and extraction time were selected after nine experiments combining four temperatures (30°C, 50°C, 70°C and 90°C), four equilibration times (20, 40, 60 and 80 min) and three extraction times (15, 30 and 45 min). HS–SPME and subsequent analyses were performed in triplicate. After sampling, a SPME fibre was inserted into the GC and GC–MS injection ports for desorption of volatile components (5 min), both using the splitless injection mode. Before sampling, each fibre was reconditioned for 5 min in the GC injection port at 260°C. The coefficient of variation ($9.6\% < CV < 13.4\%$), calculated on the basis of total area obtained from the FID signal for the samples, indicated that the HS–SPME method produced reliable results. In the same way, the CV of the major compounds was always <15%.

Gas Chromatography

GC analyses were carried out using a Perkin-Elmer Autosystem GC apparatus (Waltham, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). The temperature programme was: 60°C to 230°C at 2°C/min, then held isothermal at 230°C for 30 min; carrier gas, helium at 1 ml/min; injector and detector temperatures were held at 280°C; split injection was conducted with a split ratio of 1:80; injected volume, 0.1 μl. For HS–SPME–GC analysis, only the Rtx-1 (polydimethylsiloxane) column was used and volatile components were desorbed in a GC injector with a SPME inlet liner (0.75 mm i.d.; Supelco).

Gas Chromatography–Mass Spectrometry (GC–MS; Electronic Impact and Chemical Ionization)

GC–MS (EI) conditions. The oils and the fractions obtained by GC were analysed with a Perkin-Elmer Turbo mass detector (quadrupole), coupled to a Perkin-Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. Carrier gas, helium at 1 ml/min; oven temperature programmed from 60°C to 230°C at 2°C/min and then held isothermally at 230°C for 35 min; injector temperature, 280°C; split ratio, 1:80; ion source temperature, 150°C; energy ionization, 70 eV; electron ionization mass spectra were acquired over the mass range 35–350 Da; scan rate, 1/s; injection volume, 0.2 μl pure oil. GC–MS analyses of the volatile fractions sampling by HS–SPME were carried out only on a Rtx-1 capillary column; for the desorption, the GC injector was equipped with a SPME inlet liner (0.75 mm i.d.; Supelco).

GC–MS (CI) conditions. The PCI and NCI mass spectra were recorded on the same apparatus equipped with a Rtx-Wax column and specific ionization chemical source. The GC conditions were as described above. Ionizing gas, methane or ammonia; ion source temperature, 150°C; source pressure, 0.2 mbar; energy ionization, 70 eV; chemical ionization mass spectra were acquired over the mass range 60–350 Da; scan rate, 1/s; injection volume, 0.2 μL pure oil.

NMR Analysis

^{13}C-NMR spectra of the FII.4 CC fraction (Figure 1) were measured in deuterated chloroform, using a Bruker Avance 400 Fourier transform spectrometer (Wissembourg, France) operating at 100.13 MHz for ^{13}C-NMR and equipped with a 5 mm probe. All shifts were referred to the internal standard tetramethylsilane (TMS). ^{13}C-NMR spectra of the chromatographic fractions were recorded with the following parameters: pulse width, 4 μs (flip angle, 45°); acquisition time, 2.7 s for 128 K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.183 Hz/pt. The number of accumulated scans was 5000 for a sample (around 40 mg oil in 0.5 ml deuterochloroform), depending of the amount of product. Exponential line broadening multiplication (1 Hz) of the free induction decay was applied before Fourier transformation. ^{1}H- and ^{13}C-NMR spectra of the synthesized standard compounds were recorded in deuterated chloroform on a Bruker Avance DRX 500 spectrometer equipped with a 5 mm probe and operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C. ^{1}H spectra were acquired using 4.5 kHz spectral width (SW), 64 K complex data points, acquisition time (aq) of 7.28 s, and relaxation delay (D1) of 5 s. ^{13}C spectra were acquired using 22.7 kHz for SW, 64 K complex data points, 1.44 s for aq, and D1 of 2 s. ^{1}H decoupling was achieved using WALTZ 16 pulse sequence.

Component Identification

Identification of the components was based on: (a) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literature data;[28,29,32] (b) on computer matching with commercial mass spectral libraries[29–32] and comparison of spectra with those of our personal library; and (c) comparison of RI, MS and NMR spectral data with those of synthetic material prepared in our laboratory.

Component Quantification

Quantification of essential oil components was carried out using peak area normalization with response factors (RFs). Due to the complexity of the oil, together with the lack of standards, RF determination of all its components was unrealistic. We therefore used a solution introduced by Costa *et al.*,[32] based on grouping the essential oil components by their functional groups and then by their chemical class. RFs and calibration curves were determined by diluting each standard in hexane, at five concentrations, each specimen containing tridecane (final concentration 0.7 g/100 g) as internal standard; the analyses were performed in triplicate. It is worthwhile specifying that the response factors reported in Table 2 are the means of the response factors produced by each standard compound within a chemical class. The application of this analytical procedure allowed the determination of the concentration (g/100 g) of all oil components. Relative amounts of individual components of *T. massiliense* volatile fractions obtained by HS–SPME were calculated on the basis of their GC peak areas on an Rtx-1 capillary column without FID response factor correction. The use of an internal standard is not necessary for the HS–SPME technique, which does not represent the composition of a plant and where the equilibrium is strongly influenced by the matrix composition.

Chemicals

For the measurement of response factors (RFs), the chemicals used were: *neo-allo*-ocimene, α-pinene, β-pinene, γ-terpinene, limonene, β-caryophyllene, α-humulene, aromadendrene, nerol, lavandulol, (*E*)-hex-3-en-1-ol, cedrol, globulol, pentyl acetate, lavandulyl acetate, *trans*-myrtenyl acetate, cedryl acetate, artemisia ketone, camphor, jasmone, isoborneol methyl ether, carvacrol methyl ether, caryophyllene oxide, (*E*)-2-hexenal, (*E,E*)-2,4-decadienal and (*E*)-2-decenal. Authentic chemical samples were obtained from Sigma Aldrich and Fluka in the highest available purity. Chemicals for synthetic use, including propanal, 3-methylbutyl bromide, magnesium, acetic anhydride, diethyl ether, petroleum ether, triethylamine, 4-dimethylaminopyridine, dichloromethane, magnesium sulphate, were purchased from Sigma-Aldrich.

Synthesis of 6-Methylheptan-3-ol

A solution of propanal (3.88 g, 67 mM, 1 eq.) in diethyl ether (15 ml) was added dropwise to an ethereal solution of 3-methylbutyl magnesium

Table 2. Measurements of response factors (RFs) for the different chemical groups

Compounds		Mean ± STD	RFs
Monoterpene hydrocarbons			
	neo-allo-Ocimene	1.00 ± 0.01	1.01 ± 0.05
	α-Pinene	1.04 ± 0.01	
	β-Pinene	1.08 ± 0.01	
	γ-Terpinene	1.01 ± 0.01	
	Limonene	0.94 ± 0.01	
Sesquiterpene hydrocarbons			
	β-Caryophyllene	0.98 ± 0.01	1.00 ± 0.03
	α-Humulene	1.01 ± 0.01	
	Aromadendrene	1.03 ± 0.01	
Alcohols			
	Nerol	1.31 ± 0.02	1.34 ± 0.04
	Lavandulol	1.32 ± 0.02	
	(*E*)-Hex-3-en-1-ol	1.40 ± 0.02	
	Cedrol	1.31 ± 0.02	
	Globulol	1.34 ± 0.02	
Esters			
	Pentyl acetate	1.53 ± 0.01	1.55 ± 0.03
	Lavandulyl acetate	1.54 ± 0.01	
	trans-Myrtenyl acetate	1.53 ± 0.01	
	Cedryl acetate	1.59 ± 0.01	
Ketones			
	Artemisia cetone	1.30 ± 0.01	1.31 ± 0.02
	Camphor	1.31 ± 0.01	
	Jasmine	1.32 ± 0.01	
Ethers			
	Isoborneol methyl ether	1.25 ± 0.01	1.24 ± 0.01
	Carvacrol methyl ether	1.23 ± 0.01	
Oxide			
	Caryophyllene oxide	1.59 ± 0.01	1.59 ± 0.01
Aldehydes			
	(*E*)-2-Hexenal	1.39 ± 0.01	1.40 ± 0.02
	(*E,E*)-2,4-Decadienal	1.42 ± 0.01	
	(*E*)-2-Decenal	1.40 ± 0.01	

bromide prepared from 3-methylbutyl bromide (10.53 g, 70 mM, 1.05 eq) and magnesium turnings (2.06 g, 85 mM, 1.27 eq) in 100 ml diethyl ether. The reaction mixture was then stirred for 20 h and quenched by the careful addition of water. After decantation, the aqueous phase was extracted with diethyl ether, and the combined organic phases washed with brine, dried on magnesium sulphate and evaporated to give a crude oil, which was distillated with a Kugelrohr apparatus to furnish 6-methylheptan-3-ol (5.25 g, 60%) as a colourless oil. ^{1}H-NMR ($CDCl_3$); δ, 0.86 (d, 3H, J = 7 Hz), 0.87 (d, 3H, J = 7 Hz), 0.92 (t, 3H, J = 7 Hz), 1.10–1.62 (m, 8H), 3.48 (m, 1H). ^{13}C-NMR ($CDCl_3$), δ, 73.71, 34.92, 34.83, 30.20, 28.24, 22.79, 22.62, 9.96. EI–MS *m/z* (%) 41 (44), 43 (32), 55 (55), 56 (16), 57 (7), 58 (8), 59 (100), 69 (5), 82 (6), 83 (58), 101(13).

Synthesis of 6-Methyl-3-heptyl Acetate

6-Methylheptan-3-ol (2.0 g, 15.3 mM, 1 eq), triethylamine (1.85 g, 18.3 mM, 1.2 eq) and 4-dimethylaminopyridine (218 mg, 1.78 mM, 0.12 eq) were dissolved in dichloromethane (40 ml) and cooled to 0°C. Acetic anhydride (2.1 g, 20.6 mM, 1.3 eq) was then added dropwise and the resulting solution was stirred for 4 h at room temperature, washed twice with water and brine, dried on magnesium sulphate and evaporated to give a crude oil, which was purified by column chromatography on silica gel (petroleum ether:diethyl ether, 95:5) to give 6-methyl-3-heptyl acetate (1.97 g, 74%) as a colourless oil. ^{1}H-NMR ($CDCl_3$); δ, 0.85 (d, 6H, J = 7 Hz), 0.87 (t, 3H, J = 7 Hz), 1.08-1.24 (m, 2H), 1.45–1.60 (m, 5H), 2.04 (s, 3H), 4.77 (quint, 1H, J = 7 Hz). ^{13}C-NMR ($CDCl_3$), δ, 170.98, 75.78, 34.39, 31.43, 28.00, 26.94, 22.60, 22.50, 21.26, 9.59. EI–MS *m/z* (%) 39 (4), 41 (9), 43 (100), 56 (14), 57 (9), 69 (7), 83(9), 97 (2), 101 (7), 112 (2).

Results and Discussion

Essential Oil Compositions

Integrated analysis of Corsican and Sardinian oils allowed the identification of 137 compounds, accounting for 93.3% and 87.2% of the total oils, respectively (Table 1). Among them, 24 monoterpenes, 59 sesquiterpenes, one diterpene and 53 non-terpenic compounds were identified. The oils were dominated by oxygenated compounds, accounting for 75.8–67.1% and 59.6%, respectively. The identification of 103 components was performed by comparison of their EI–MS and retention indices with those in the laboratory-produced library. The majority of these compounds were commercial standard components and the few others were previously identified in large amounts in essential oils or fractions obtained by CC, by comparison with literature

spectral data and retention indices and ensured by ^{13}C-NMR. Thirty-three components were identified by comparison of their EI–MS and the apolar retention indices with those of commercial or literature libraries (see Table 1). However, the main component of Corsican and Sardinian oils (22.1–25.1% and 19.5%, respectively) remained unidentified. Its identification required the development of an analytical strategy involving the pooling of all Corsican oils in a mixture called 'collective oil' (sample C), in order to investigate CC fractions and sub-fractions by a combination of techniques including GC–RI, GC–MS (EI and CI) and ^{13}C-NMR, then its hemi-synthesis.

Successive column chromatography allowed the unknown compound **21** in the fraction II.4 (**21**: 52.7%) to be concentrated. Since its molecular ion was absent, its EI–MS showed a base peak at *m/z* 43, suggesting the occurrence of an acetate derivative. Using ammonia as the reagent gas in PCI mode, the spectra of **21** showed quasi-molecular ions at *m/z* 190 $[M + NH_4]^+$ as base peak and its PCI–CH_4 MS exhibited base peak ion at *m/z* 113 $[M + H - RCOOH]^+$ ion resulting from the decarboxylation of protonated molecule. The ^{13}C-NMR spectra of the fraction II.4 (**21**: 52.7%) exhibited 10 signals assigned from the DEPT spectra to one quaternary carbon (170.98 ppm), two methines (75.78 and 28.00 ppm), three methylenes (34.39, 31.43 and 26.94 ppm) and four methyls (22.60, 22.50, 21.26 and 9.59 ppm). These signals confirmed the presence of a ramified acetate derivative, while the $C_{10}H_{20}O_2$ formula was deduced from the DEPT spectra. The joint information from EI–MS and CI–MS and ^{13}C-NMR spectra suggested the occurrence of 6-methyl-3-heptyl acetate. To confirm its identification, we synthesized it via 6-methylheptan-3-ol (see Experimental section and Figure 2). To the best of our knowledge, this is the first report of 6-methyl-3-heptyl acetate as a natural compound. This ester was previously cited as an intermediary in the synthesis of arsenic derivatives.[33]

In order to provide the real concentration of oil constituents, we carried out a methodology based on the calculation of response factors for all chemical groups determined (Table 2). The concentrations (g/100 g) of all the 137 components identified in *T. massiliense* oil are listed in Table 1. As reported in the literature,[3,2] the best response toward FID detector is given by hydrocarbons (RF = 1), whereas a different trend occurred for oxygenated compounds, which produced higher values of the response factor. The relative concentrations of the main components in Corsican and Sardinian oils were 6-methyl-3-heptyl acetate (20.48–23.83; 18.16 g/100 g), 3-octyl acetate (7.35–10.55; 6.96 g/100 g), isobutyl isovalerate (6.50–7.67; 2.91 g/100 g), germacrene D (3.96–5.26; 1.01 g/100 g) and linalool (3.43–4.94; 6.63 g/100 g), respectively.

To our knowledge, we found 117 components never described in *T. massiliense* essential oils (see Table 1), among them both the main components, 6-methyl-3-heptyl acetate and 3-octyl acetate. All analysed samples differed significantly from the Sardinian oil reported in the literature;[13] as for the main components, linalool was the only common component. Finally, from the taxonomic point of view, *T. massiliense* essential oils seem to be original in comparison with the two other Corsican *Teucrium* species previously investigated,[25,26] in which hydrocarbon, monoterpenes and/or sesquiterpenes were identified.

HS–SPME Analysis of Volatile Constituents

Emitted volatile components from aerial parts, flowers, leaves and stems of three Corsican *T. massiliense* samples were extracted using HS–SPME under optimized parameters. Using fresh aerial parts plant material from Col de Sevi, the optimization of HS–SPME parameters was based on the number and the sum of total peak areas obtained by GC–FID. The maximum sum of total peak area was acquired for a temperature of 70°C, an equilibrium time of 40 min and an extraction time of 30 min. The GC–RI and GC–MS analysis allowed the identification of 69 components. including 10 monoterpenes, 28 oxygenated sesquiterpenes and 31 non-terpenic compounds (Table 3).

Concerning the contribution of plant organs to the aromatic plant fingerprint, we note that volatile constituents were more abundant in flowers than in the other parts of the plant. The volatile fractions obtained from the different organs were qualitatively rather similar but differed in the percentages of their major components. The major volatiles were 6-methyl-3-heptyl acetate (18.6–35.1%), germacrene D (1.6–13.7%), 3-octyl acetate (5.6–12.8%), isobutyl isovalerate (1.3–10.3%) and β-bourbonene (1.1–11.8%). We noted that 6-methyl-3-heptyl acetate was the main component in all organs (23.7–35.1%). In the HS–SPME fractions from the aerial parts and flowers, it was followed by germacrene D (8.5–13.7%) and/or 3-octyl acetate (5.6–7.6%), while 3-octyl acetate (7.2–11.3%) and β-bourbonene (4.0–11.8%) were the other main volatiles identified from the stems. Moreover, HS–SPME fractions from the leaves were dominated by non-terpenic oxygenated compounds, accounting for 78.8–80.4%: 6-methyl-3-heptyl acetate (30.5–35.1%) and 3-octyl acetate (9.5–12.8%) were identified, with isobutyl isovalerate (9.4–10.3%).

The influence of HS–SPME extraction parameters on the emitted volatiles from *T. massiliense* aerial parts is shown in Table 4. As reported in our previous studies,[23,24] the temperature was the most important parameter for the extraction of volatiles from plants. The increase of temperature allowed the different classes of volatiles to be successively extracted. Our results showed that the optimal temperature (70°C) was an analytical compromise based on the maximal amount of volatiles extracted, which ensures the extraction of a volatile fraction which is representative of the essential oil.

Concerning the comparison between the HS fraction and HD oil (sample C3) obtained from the same plant material, no qualitative differences were shown, while quantitative differences were observed for oxygenated sesquiterpenes, which were always present in higher amounts in the essential oil. It was difficult to establish a direct correlation between HS and HD extraction results, because the first technique was controlled by an equilibrium step and the latter was based on the quasi-total extraction of plant volatiles. Concerning the comparison of both techniques in terms of isolation time, HS–SPME was clearly faster (90 min), while 300 min were required for hydrodistillation. In the same way, the amount of plant material used for the headspace analysis was smaller (1 g), while the oil production by hydrodistillation needed 300–440 g of plant material. For these reasons, HS–SPME can be applied to routine control analysis of rare and threatened aromatic plants which yield low or no essential oil.

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Table 3. Volatile components extracted by HS–SPME from aerial parts, flowers, leaves and stems of *T. massiliense* L.

No[a]	Compounds*	IRI_a[b]	RI_a[c]	Percentages[d]											
				Col de Sevi				Zichina				Casanova			
				Aerial parts	Flowers	Leaves	Stems	Aerial parts	Flowers	Leaves	Stems	Aerial parts	Flowers	Leaves	Stems
1a	Hexanal	782	771	0.1 ± 0.01	tr	0.3 ± 0.01	0.1 ± 0.07	0.2 ± 0.01	–	0.5 ± 0.07	0.1 ± 0.01	0.1 ± 0.07	–	0.4 ± 0.01	0.1 ± 0.07
1b	(*E*)-2-Hexenal	832	830	0.1 ± 0.01	tr	0.3 ± 0.01	–	0.3 ± 0.07	0.1 ± 0.01	1.1 ± 0.28	0.1 ± 0.01	0.3 ± 0.01	tr	0.4 ± 0.07	0.2 ± 0.01
3	Isobutyl isobutyrate	899	899	0.8 ± 0.07	0.3 ± 0.07	0.7 ± 0.07	0.6 ± 0.14	0.9 ± 0.01	1.0 ± 0.01	0.6 ± 0.14	0.5 ± 0.01	1.1 ± 0.01	0.9 ± 0.07	0.7 ± 0.07	0.7 ± 0.01
4	2-Hexyl acetate	920	917	0.4 ± 0.07	0.2 ± 0.01	0.5 ± 0.01	0.3 ± 0.01	0.4 ± 0.01	0.3 ± 0.01	0.4 ± 0.01	0.6 ± 0.01	0.5 ± 0.01	0.5 ± 0.14	0.7 ± 0.01	0.7 ± 0.01
5	6-Methyl-3-heptanone	935	930	0.5 ± 0.01	0.3 ± 0.07	3.3 ± 0.21	1.9 ± 0.14	1.2 ± 0.07	0.5 ± 0.01	4.5 ± 0.92	0.7 ± 0.07	1.1 ± 0.14	0.8 ± 0.07	1.7 ± 0.28	1.0 ± 0.14
6	Oct-1-en-3-ol	962	960	0.2 ± 0.01	0.1 ± 0.01	0.5 ± 0.07	0.1 ± 0.07	0.4 ± 0.01	0.1 ± 0.01	1.9 ± 0.01	0.1 ± 0.01	0.3 ± 0.07	0.1 ± 0.01	0.5 ± 0.14	0.2 ± 0.01
7	Octan-3-one	969	963	0.1 ± 0.01	tr	0.2 ± 0.01	–	0.1 ± 0.01	–	0.4 ± 0.14	–	0.1 ± 0.01	tr	0.3 ± 0.07	–
7a	(*E,E*)-2,4-Heptadienal	975	976	0.4 ± 0.07	0.1 ± 0.01	1.0 ± 0.07	1.4 ± 0.07	0.7 ± 0.07	0.2 ± 0.07	1.9 ± 0.07	0.6 ± 0.01	0.3 ± 0.01	0.2 ± 0.07	0.3 ± 0.01	0.2 ± 0.07
9	3-Octanol	981	981	0.4 ± 0.01	–	–	4.6 ± 0.14	0.9 ± 0.14	–	–	4.6 ± 0.28	0.3 ± 0.07	–	tr	0.9 ± 0.07
11	Isobutyl isovalerate	993	989	6.1 ± 0.21	4.3 ± 0.01	10.3 ± 0.85	1.5 ± 0.14	7.9 ± 0.21	8.4 ± 0.28	10.2 ± 0.35	1.3 ± 0.07	7.6 ± 0.64	8.5 ± 0.35	9.4 ± 0.99	2.3 ± 0.28
12	3-Methyl butyl isobutyrate	994	997	0.4 ± 0.07	0.1 ± 0.01	0.7 ± 0.01	0.6 ± 0.01	1.0 ± 0.07	1.2 ± 0.21	0.4 ± 0.07	0.8 ± 0.01	0.3 ± 0.07	0.2 ± 0.07	0.1 ± 0.07	0.8 ± 0.01
13	2-Methyl butyl isobutyrate	1002	1001	1.0 ± 0.07	0.4 ± 0.01	3.1 ± 0.21	0.4 ± 0.07	0.8 ± 0.14	0.7 ± 0.01	1.7 ± 0.21	0.2 ± 0.07	1.1 ± 0.14	1.2 ± 0.14	1.4 ± 0.14	tr
13a	Phenyl acetaldehyde	1008	1010	0.4 ± 0.14	0.3 ± 0.07	0.7 ± 0.07	0.3 ± 0.07	0.2 ± 0.01	0.1 ± 0.01	0.5 ± 0.01	0.4 ± 0.01	0.6 ± 0.07	0.8 ± 0.21	0.4 ± 0.07	0.3 ± 0.01
15	Limonene	1025	1020	0.2 ± 0.07	0.1 ± 0.01	0.1 ± 0.01	–	tr	tr	tr	–	0.1 ± 0.07	tr	0.2 ± 0.01	tr
16	(*Z*)-β-Ocimene	1029	1026	4.2 ± 0.14	4.8 ± 0.07	2.4 ± 0.07	4.0 ± 0.07	5.7 ± 0.01	7.6 ± 0.14	1.7 ± 0.21	3.8 ± 0.01	2.9 ± 0.21	6.6 ± 0.14	0.9 ± 0.01	1.0 ± 0.01
17	2,6-Dimethyl-5-heptanal	1037	1034	0.1 ± 0.01	0.1 ± 0.01	–	tr	0.1 ± 0.01	0.1 ± 0.01	–	tr	0.1 ± 0.01	0.1 ± 0.01	–	tr
18	(*E*)-β-Ocimene	1041	1036	1.1 ± 0.14	0.8 ± 0.07	tr	1.1 ± 0.07	1.2 ± 0.07	tr	tr	1.5 ± 0.07	1.0 ± 0.07	1.2 ± 0.21	tr	0.7 ± 0.01
21	6-Methyl-3-heptyl acetate		1074	24.3 ± 1.48	23.7±1.27	30.5±0.92	18.6±1.27	26.9±0.28	25.5±0.78	30.7±0.85	21.3±0.49	28.7±1.13	26.7±0.78	35.1±0.78	22.3±0.78
22	Nonanal	1086	1087	2.4 ± 0.01	tr	4.6 ± 0.07	5.2 ± 0.21	2.6 ± 0.01	tr	4.6 ± 0.21	2.9 ± 0.14	2.1 ± 0.07	–	3.3 ± 0.01	4.1 ± 0.07
23	Linalool	1083	1088	2.8 ± 0.21	tr	3.4 ± 0.07	7.3 ± 0.35	3.5 ± 0.07	tr	3.8 ± 0.21	4.4 ± 0.42	4.1 ± 0.28	–	2.0 ± 0.07	6.9 ± 0.21
24	3-Methyl butyl isovalerate	1092	1092	2.6 ± 0.14	6.7 ± 0.71	0.4 ± 0.07	0.8 ± 0.14	4.0 ± 0.21	4.9 ± 0.21	0.8 ± 0.07	4.4 ± 0.28	3.1 ± 0.28	6.9 ± 0.28	0.7 ± 0.07	1.1 ± 0.14
25	2-Methyl butyl isovalerate	1094	1094	0.9 ± 0.14	2.2 ± 0.42	0.4 ± 0.07	0.7 ± 0.14	0.9 ± 0.07	1.3 ± 0.01	0.5 ± 0.07	1.1 ± 0.01	1.1 ± 0.07	1.9 ± 0.28	0.6 ± 0.14	1.1 ± 0.01
25a	(*Z*)-Rose oxide	1097	1095	0.2 ± 0.07	0.7 ± 0.01	–	tr	0.3 ± 0.01	0.6 ± 0.07	–	0.3 ± 0.07	0.4 ± 0.14	0.7 ± 0.07	–	tr
26	3-Octyl acetate	1113	1111	8.5 ± 0.49	7.6 ± 0.14	9.5 ± 0.85	10.5±0.49	8.1 ± 0.35	5.6 ± 0.21	10.9±0.49	11.3±0.49	8.6 ± 0.35	7.1 ± 0.49	12.8±0.78	7.2 ± 0.14
26a	(E)-Rose oxide	1111	1113	0.1 ± 0.01	0.1 ± 0.01	–	tr	0.1 ± 0.01	tr	–	0.3 ± 0.07	0.1 ± 0.01	tr	–	tr
27	*neo-allo*-Ocimene	1126	1126	0.7 ± 0.14	1.1 ± 0.01	0.5 ± 0.01	0.7 ± 0.07	0.8 ± 0.01	1.4 ± 0.01	0.7 ± 0.01	1.4 ± 0.01	0.8 ± 0.07	1.4 ± 0.14	0.5 ± 0.14	0.1 ± 0.01
27a	Methyl salicylate	1169	1168	0.2 ± 0.01	0.7 ± 0.07	0.8 ± 0.07	0.1 ± 0.01	0.4 ± 0.07	0.4 ± 0.01	0.6 ± 0.07	0.7 ± 0.01	1.2 ± 0.01	0.8 ± 0.14	1.0 ± 0.01	0.5 ± 0.07
38	Citronellol	1213	1213	1.1 ± 0.14	2 ± 0.07	1.2 ± 0.28	3.7 ± 0.07	1.7 ± 0.07	1.5 ± 0.07	0.9 ± 0.07	3.1 ± 0.21	2.3 ± 0.21	1.9 ± 0.21	1.5 ± 0.01	3.1 ± 0.01
42	Geraniol	1235	1235	0.3 ± 0.01	0.2 ± 0.07	0.2 ± 0.01	0.2 ± 0.07	0.2 ± 0.07	0.4 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.07	1.3 ± 0.07	0.2 ± 0.01	0.2 ± 0.01
43	Linalyl acetate	1239	1241	0.1 ± 0.01	0.1 ± 0.01	–	–	tr	tr	–	–	0.1 ± 0.07	0.1 ± 0.01	–	–
44	Geranial	1244	1243	0.1 ± 0.01	tr	0.6 ± 0.07	0.9 ± 0.07	0.1 ± 0.07	–	0.3 ± 0.01	0.8 ± 0.01	0.1 ± 0.01	–	0.4 ± 0.01	0.5 ± 0.01
45	Citronnellyl formate	1259	1256	0.6 ± 0.07	0.7 ± 0.01	0.1 ± 0.01	–	0.6 ± 0.01	0.8 ± 0.01	–	tr	0.6 ± 0.01	0.7 ± 0.01	0.1 ± 0.01	–
46	Neryl formate	1265	1262	tr	tr	–	–	0.1 ± 0.01	0.1 ± 0.01	–	0.1 ± 0.01	tr	tr	–	0.1 ± 0.01
48	Benzyl isobutanoate	1269	1271	0.2 ± 0.01	0.3 ± 0.07	0.4 ± 0.07	0.5 ± 0.01	0.4 ± 0.01	0.4 ± 0.07	0.3 ± 0.01	0.4 ± 0.01	0.3 ± 0.07	0.4 ± 0.01	0.5 ± 0.01	0.3 ± 0.01
49	Geranyl formate	1284	1280	0.1 ± 0.01	tr	1.2 ± 0.07	0.1 ± 0.01	tr	–	0.2 ± 0.01	–	tr	–	0.7 ± 0.07	–
51	Theaspirane I	1299	1291	0.1 ± 0.01	–	0.8 ± 0.14	0.1 ± 0.01	0.2 ± 0.01	–	0.6 ± 0.21	0.1 ± 0.01	0.1 ± 0.01	–	0.6 ± 0.14	0.1 ± 0.01
52	Theaspirane II	1313	1306	0.1 ± 0.01	–	0.45±0.07	tr	tr	–	0.2 ± 0.01	0.1 ± 0.01	tr	–	0.4 ± 0.01	0.1 ± 0.01
54	Citronellyl acetate	1337	1334	0.9 ± 0.14	2.9 ± 0.14	1.2 ± 0.07	1.8 ± 0.01	1.1 ± 0.01	1.4 ± 0.07	0.7 ± 0.14	3.7 ± 0.14	2.7 ± 0.01	2.2 ± 0.49	1.6 ± 0.07	4.2 ± 0.07
59	Geranyl acetate	1362	1360	1.2 ± 0.01	0.3 ± 0.14	0.1 ± 0.01	2.4 ± 0.07	0.2 ± 0.01	0.3 ± 0.01	tr	0.2 ± 0.01	0.5 ± 0.07	0.1 ± 0.07	0.4 ± 0.01	0.9 ± 0.01
61	Isoledene	1382	1366	0.4 ± 0.07	0.2 ± 0.01	0.1 ± 0.01	0.7 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
64	β-Bourbonene	1386	1381	3.9 ± 0.14	2.2 ± 0.21	4.7 ± 0.14	4.0 ± 0.14	4.6 ± 0.14	2.5 ± 0.07	6.7 ± 0.71	6.1 ± 0.57	4.3 ± 0.21	2.0 ± 0.14	1.1 ± 0.01	11.8±0.21
65	1,5-di-epi-β-Bourbonene	1389	1384	0.3 ± 0.07	0.1 ± 0.01	0.4 ± 0.01	0.5 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.5 ± 0.07	0.3 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	1.8 ± 0.14
68	α-Gurjunene	1413	1413	1.4 ± 0.14	1.6 ± 0.01	0.4 ± 0.07	0.8 ± 0.14	1.0 ± 0.14	1.3 ± 0.07	0.4 ± 0.01	0.9 ± 0.01	0.8 ± 0.01	1.1 ± 0.01	0.1 ± 0.01	1.6 ± 0.07
69	β-Tangene	1420	1419	1.1 ± 0.07	1.0 ± 0.07	0.5 ± 0.01	0.2 ± 0.01	0.5 ± 0.01	0.9 ± 0.01	0.4 ± 0.14	0.1 ± 0.01	0.6 ± 0.07	0.6 ± 0.07	0.4 ± 0.01	0.1 ± 0.01
70	β-Copaene	1430	1423	0.3 ± 0.01	tr	–	1.1 ± 0.28	0.2 ± 0.01	0.1 ± 0.01	–	0.7 ± 0.01	0.4 ± 0.01	0.1 ± 0.01	–	1.5 ± 0.01

Table 3. Continued

No[a]	Compounds*	RI_a[b]	RI_a[c]	Percentages[d]											
				Col de Sevi			Zichina			Casanova					
				Aerial parts	Flowers	Leaves	Stems	Aerial parts	Flowers	Leaves	Stems	Aerial parts	Flowers	Leaves	Stems
70a	Geranyl acetate	1428	1427	0.2 ± 0.01	0.2 ± 0.14	0.3 ± 0.07	–	0.1 ± 0.01	0.1 ± 0.01	0.4 ± 0.01	tr	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.01
72	*trans*-α-Bergamotene	1434	1429	0.6 ± 0.07	0.2 ± 0.07	0.4 ± 0.01	0.3 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.3 ± 0.07	0.4 ± 0.01	0.4 ± 0.01	0.1 ± 0.07	0.4 ± 0.01	0.2 ± 0.01
73	α-Sesquisabinene	1435	1433	0.7 ± 0.01	0.5 ± 0.07	0.3 ± 0.01	0.7 ± 0.14	0.4 ± 0.07	0.5 ± 0.01	0.3 ± 0.14	0.7 ± 0.14	0.8 ± 0.14	0.4 ± 0.07	0.5 ± 0.01	1.1 ± 0.01
75	(*E*)-β-Farnesene	1446	1446	2.3 ± 0.21	2.4 ± 0.07	1.3 ± 0.21	2.7 ± 0.21	0.8 ± 0.01	0.6 ± 0.01	1.7 ± 0.57	0.4 ± 0.01	1.8 ± 0.14	1.7 ± 0.21	1.3 ± 0.21	2.2 ± 0.14
76	*allo*-Aromadendrene	1462	1455	2.5 ± 0.07	1.0 ± 0.07	0.1 ± 0.07	1.7 ± 0.21	1.6 ± 0.14	2.1 ± 0.01	0.2 ± 0.01	2.2 ± 0.14	1.2 ± 0.07	0.5 ± 0.01	tr	3.1 ± 0.01
77	β-Humulene	1460	1456	0.2 ± 0.07	0.6 ± 0.14	–	0.8 ± 0.14	0.2 ± 0.01	0.2 ± 0.01	–	0.4 ± 0.07	0.2 ± 0.01	–	–	0.7 ± 0.01
78	α-Curcumene	1473	1470	0.1 ± 0.01	tr	–	0.1 ± 0.01	tr	–	–	tr	0.1 ± 0.01	–	–	0.3 ± 0.01
79	γ-Muurolene	1474	1471	0.2 ± 0.01	tr	0.3 ± 0.01		0.1 ± 0.01	tr	1.0 ± 0.35	tr	1.2 ± 0.14	tr	2.1 ± 0.01	1.3 ± 0.21
80	Germacrene D	1479	1476	10.9 ± 1.06	13.7 ± 1.27	3.5 ± 0.14	3.3 ± 0.28	8.5 ± 0.28	13.4 ± 0.42	3.8 ± 0.01	3.5 ± 0.28	5.2 ± 0.28	9.9 ± 1.13	3.1 ± 0.78	1.6 ± 0.01
81	γ-Humulene	1483	1477	1.3 ± 0.21	3.2 ± 0.07	0.2 ± 0.01	0.4 ± 0.07	1.0 ± 0.07	2.0 ± 0.28	0.1 ± 0.01	0.3 ± 0.01	0.5 ± 0.01	0.5 ± 0.01	0.3 ± 0.01	0.4 ± 0.01
82	Zingiberene	1489	1483	0.7 ± 0.07	0.6 ± 0.01	0.1 ± 0.01	0.8 ± 0.07	0.2 ± 0.07	0.4 ± 0.07	0.1 ± 0.01	0.1 ± 0.01	0.7 ± 0.01	0.7 ± 0.01	0.2 ± 0.07	0.9 ± 0.01
84	Bicyclogermacrene	1494	1490	1.6 ± 0.01	2.3 ± 0.07	0.4 ± 0.07	1.5 ± 0.07	0.9 ± 0.07	1.9 ± 0.21	0.1 ± 0.01	0.2 ± 0.01	0.5 ± 0.07	1.4 ± 0.07	0.1 ± 0.01	0.2 ± 0.07
84a	γ-Cadinene	1507	1503	0.4 ± 0.01	0.6 ± 0.01	–	0.3 ± 0.07	0.2 ± 0.01	0.3 ± 0.01	–	0.4 ± 0.07	tr	tr	–	0.1 ± 0.01
85	β-Curcumene	1503	1504	1.1 ± 0.07	1.5 ± 0.07	–	0.7 ± 0.07	0.7 ± 0.14	1.2 ± 0.07	–	0.01 ± 0.01	1.2 ± 0.01	1.1 ± 0.07	–	1.0 ± 0.14
87	(*Z*)-γ-Bisabolene	1505	1505	2.6 ± 0.28	3.6 ± 0.14	0.2 ± 0.01	–	0.7 ± 0.01	1.5 ± 0.07	0.2 ± 0.01	tr	0.9 ± 0.07	1.2 ± 0.14	1.0 ± 0.21	0.5 ± 0.01
89	δ-Cadinene	1520	1512	0.6 ± 0.07	0.9 ± 0.07	tr	0.1 ± 0.01	0.2 ± 0.07	0.8 ± 0.01	tr	tr	0.1 ± 0.01	0.6 ± 0.01	–	0.1 ± 0.01
89a	α-Cadinene	1536	1534	0.1 ± 0.01	tr	0.1 ± 0.01	tr	tr	tr	tr	0.2 ± 0.01	tr	–	tr	0.2 ± 0.01
94	1,5-Epoxy salvial-4(14)-ene	1554	1556	0.1 ± 0.01	0.1 ± 0.01	0.7 ± 0.07	1.2 ± 0.14	0.2 ± 0.07	0.2 ± 0.01	tr	1.0 ± 0.28	0.1 ± 0.01	0.1 ± 0.01	tr	0.2 ± 0.01
95	Spathulenol	1572	1557	0.1 ± 0.01	0.1 ± 0.07	0.2 ± 0.07	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	tr	0.4 ± 0.01	0.3 ± 0.07	0.1 ± 0.01	tr	0.6 ± 0.01
96	Palustrol	1562	1562	tr	0.1 ± 0.01	–	0.4 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	–	0.5 ± 0.07	0.1 ± 0.01	0.1 ± 0.01	–	0.3 ± 0.01
103	Ledol	1600	1589	tr	0.1 ± 0.01	–	–	0.1 ± 0.07	0.1 ± 0.01	–	0.4 ± 0.21	0.1 ± 0.07	0.1 ± 0.01	–	0.5 ± 0.07
112	τ-Muurolol	1633	1634	tr	tr	–	0.2 ± 0.01	tr	tr	–	0.2 ± 0.01	tr	tr	–	0.1 ± 0.01
115	α-Cadinol	1643	1641	–	–	–	–	tr	–	–	0.1 ± 0.01	tr	–	–	0.1 ± 0.01
120	Heptadecane	1700	1701	–	–	–	–	tr	–	–	0.1 ± 0.01	0.1 ± 0.01	–	–	0.2 ± 0.07
	Total identification (%)			96.5 ± 0.07	99 ± 0.14	95.7 ± 0.07	94.1 ± 0.42	98.1 ± 0.77	97.6 ± 0.64	98.4 ± 0.21	92.7 ± 0.85	98.4 ± 0.78	96.0 ± 1.27	91.5 ± 0.35	95.5 ± 0.57
	Total area 10^3[e]			1267	1920	162	110	1053	1437	176	266	975	1281	109	329
	Hydrocarbon compounds			38.7	44.3	16.3	27.0	30.3	40.7	18.0	24.5	26.8	31.7	12.7	32.8
	Oxygenated compounds			57.8	54.7	79.4	67.1	67.8	56.9	80.4	68.2	71.6	64.3	78.8	62.7
	Non-terpenic oxygenated compounds			53.2	51.2	71.7	52.8	61.0	53.8	74.4	56.3	63.6	60.9	73.6	50.0
	Monoterpene hydrocarbons			6.1	7.0	3.1	5.9	7.7	10.3	2.4	6.7	5	9.2	1.6	1.8
	Oxygenated monoterpenes			4.3	3.1	6.8	12.4	6.3	2.6	6.0	9.3	7.4	3.0	5.2	10.9
	Sesquiterpene hydrocarbons			32.6	37.3	13.2	21.1	22.6	30.4	15.6	17.7	21.7	22.5	11.1	30.8
	Oxygenated sesquiterpenes			0.2	0.4	0.9	1.9	0.5	0.5	Tr	2.6	0.6	0.4	tr	1.8

[a] Order of elution is given on apolar column (Rtx-1). Numbers corresponding to those in Table 1.
[b] Retention indices of literature on the apolar column (RI_a) reported from references.[28–31]
[c] Retention indices on the apolar Rtx-1 column (RI_a).
[d] Percentages (means of three analyses) obtained by GC–FID (on RTX-1: apolar column) with peak-area normalization under optimized HS–SPME parameters: temperature, 70°C; equilibrium time, 40 min; extraction time, 30 min. 'tr, trace (<0.05%).'
[e] Total area is expressed in arbitrary units.
* The components identified only in the HS-fraction were assigned by a number followed by a letter.

Table 4. Influence of HS–SPME parameters (temperature, equilibrium and extraction times) on the volatiles of *T. massiliense* L.

Class of compounds (%)	HS–SPME parameters: Equilibrium time t_{eq} min (T_{ext} = 70°C; t_{ext} = 15 min)				HS–SPME parameters: Extraction time t_{ext} min (T_{ext} = 70°C; t_{eq} = 40 min)			HS–SPME parameters: Extraction temperature T_{ext} °C (t_{ext} = 30 min; t_{eq} = 40 min)			
	20	40	60	80	30	45	30	50	70	90
Hydrocarbon compounds	33.5	46.9	48.5	36.3	38.7	27.6	19.9	23.7	38.7	33.0
Oxygenated compounds	57.7	49.3	49.0	61.3	57.8	64.1	76.0	72.7	57.8	59.3
Non-terpenic oxygenated compounds	53.0	43.6	42.6	53.0	53.2	55.5	70.5	66.7	53.2	53.2
Monoterpene hydrocarbons	6.5	5.7	5.8	6.6	6.1	5.5	8.9	7.2	6.1	4.4
Oxygenated monoterpenes	7.3	5.4	5.1	7.1	4.3	7.2	5.5	5.8	4.3	5.2
Sesquiterpene hydrocarbons	27	41.2	42.7	29.7	32.6	22.1	11.0	16.5	32.6	28.6
Oxygenated sesquiterpenes	0.9	0.9	1.3	1.1	0.2	1.4	–	0.2	0.2	0.9
Total identification (%)	91.2	96.2	97.5	97.6	96.5	91.7	95.9	96.4	96.5	92.3
Total area 10^{3a}	586	885	802	745	1267	1062	516	706	1267	1192

[a] Total area is expressed in arbitrary units.
Fresh aerial parts of *T. massiliense* used for this study were harvested in Col de Sevi.

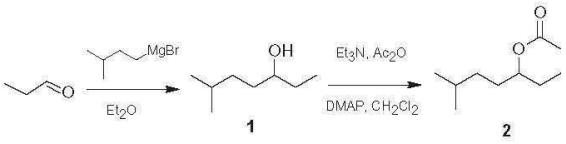

Figure 2. Synthesis of 6-methylhept-3-yl acetate **2** from propanal via 6-methylheptan-3-ol **1**

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References

1. L. Bini Maleci, O. Servettaz. *Plant. Syst. Evol.* **1991**, *174*, 83.
2. T. Autunes, I. Sevinate-Pinto. *Flora Morphol. Oekol.* **1991**, *185*, 207.
3. A. Hisham, N. Pathare, S. Al-Saidi, A. Al-Salmi. *J. Essent. Oil Res.* **2006**, *18*, 465.
4. A. Kabouche, O. Taoufek, A. Nacer, Z. Kabouche, C. Bruneau. *J. Essent. Oil. Res.* **2006**, *18*, 175.
5. A. Ulubelen, G. Topcu, U. Sonmez. *Bioactive Nat. Prod.* **2000**, *D*, 591.
6. J. B. Harborne, F. A. Tomas-Barberan, C. A. Williams, M. I. Gil. *Phytochemistry* **1986**, *25*, 2811.
7. M. Bruno, S. Rosselli, A. Maggio, F. Piozzi, L. Scaglioni, N. A. Arnold, M. Monique. *Chem. Pharm. Bull.* **2004**, *52*, 1497.
8. J. Coll, Y. Tandron. *Phytochemistry* **2005**, *66*, 2298.
9. N. A. Ali, M. Wurster, N. Arnold, U. Lindequist, L. Wessjohan. *Rec. Nat. Prod.* **2008**, *2*, 25.
10. D. Jeanmonod, J. Gamisans. *Flora Corsica*. Ed. Edisud: Aix-en-Provence, **2007**; 638.
11. I. Camarda. *Boll. Soc. Sarda Sci. Nat.* **1990**, *27*, 147.
12. Arrêté du 20 janvier 1982 modifié relatif à la liste des espèces végétales protégées sur l'ensemble du territoire. Liste de portée nationale. Articles L411-1 et 2 du code de l'environnement. Journal officiel du 13 mai **1982**.
13. L. Giamperi, A. Bucchini, D. Fraternale, P. Cara, D. Ricci. *J. Essent. Oil Res.* **2008**, *20*, 446.
14. M. Bruno, F. Piozzi, B. Rodriguez, G. Savona, M. C. de la Torre, O. Servettaz. *Phytochemistry* **1992**, *31*, 4366.
15. G. Fontana, M. P. Paternostro, G. Savona, B. Rodriguez, M. C. de la Torre. *J. Nat. Prod.* **1998**, *61*, 1242.
16. G. M. Krishna Kumari, S. Aravind, J. Balachandran, M. R. Ganesh, S. Soundarya Devi, S. S. Rajan, R. Malathi, K. Ravilkumar. *Phytochemistry* **2003**, *64*, 1119.
17. M. Bruno, A. M. Maggio, F. Piozzi, S. Puech, S. Rosselli, M. S. J. Simmonds. *Biochem. Syst. Ecol.* **2003**, *31*, 1051.
18. A. T. Merritt, S. V. Ley. *Nat. Prod. Rep.* **1992**, *9*, 243.
19. M. Marthanda Murthy, M. Subramanyam, M. Hima Bindu, J. Annapurna. *Fitoterapia* **2005**, *76*, 336.
20. C. Bicchi, C. Cordero, E. Liberto, B. Sgorbini, P. Rubiolo. *J. Chromatogr. A* **2007**, *1152*, 138.
21. F. Belliardo, C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini. *J. Chromatogr. A* **2006**, *44*, 416.
22. C. Bicchi, S. Drigo, P. Rubiolo. *J. Chromatogr. A* **2000**, *892*, 469.
23. J. Paolini, E. Nasica, J. M. Desjobert, A. Muselli, A. F. Bernardini, J. Costa. *J. Phytochem. Anal.* **2007**, *19*, 266.
24. A. Muselli, M. Pau, J. M. Desjobert, M. Foddai, M. Usai, J. Costa. *Chromatographia* **2009**, *69*, 575.
25. S. Cozzani, A. Muselli, J. M. Desjobert, A-F. Bernardini, F. Tomi, J. Casanova. *Flavour Fragr. J.* **2005**, *20*, 436.
26. A. Muselli, L. M. Desjobert, J. Paolini, A-M. Bernardini, J. Costa, A. Rosa, M. Assunta Dessi. *J. Essent. Oil Res.* **2009**, *21*, 138.
27. Conseil de l'Europe. *Pharmacopée Européenne*. Maisonneuve S.A.: Sainte Ruffine, **1996**.

28. W. Jennings, T. Shibamoto. In *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass-capillary Gas Chromatography*. Academic Press: New York, **1980**.
29. MassFinder (version 3.0). Hochmunth D. Scientific Consulting, Germany, **2006**.
30. National Institute of Standards and Technology. PC version 1.7 of the NIST/EPA/NIH Mass Spectral Library, Perkin-Elmer Corp., Norwalk, CT, **1999**.
31. F.W. Mc Lafferty, D. B. Stauffer. *Wiley Registry of Mass Spectral Data*, 6th edn. Mass Spectrometry Library Search System Bench-Top/PBM, Version 3.10d. Palisade: Newfield, **1994**.
32. R. Costa, B. A. Zellner, M. L. Crupi, M. Fina, M. Valentino, P. Dugo, G. Dugo, L. Mondello. *Flavour Fragr. J.* **2008**, *23*, 40.
33. G. K. Kamai, M. M. Ugulava, R. D. Gigauri, M. M. Injia, G. N. Chachava. *Bull. Acad. Sci. Georgian SSR* **1971**, *1*, 61.

[**PT2**] : ***Teucrium marum***

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Analysis of volatile fraction of *Teucrium marum* L.

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Abstract

Volatile components of *T. marum* from Corsica were investigated using a combination of analytical techniques. On the one hand, analysis of essential oils obtained by hydrodistillation (HD) was performed using capillary GC-RI, GC/MS and ^{13}C-NMR spectroscopy after fractionation on column chromatography. On the other hand, the emitted volatile fractions from full aerial parts and separated organs of fresh *T. marum* were investigated using HS-SPME/GC-RI and HS-SPME/GC/MS. The intraspecies variations of the chemical compositions of essential oils and volatiles fractions from 25 Corsican sample locations were investigated using statistical analysis. The main essential oil components were caryophyllene oxide (0.4-20.5 %), (E)-β-caryophyllene (0.9-18.9 %), (E)-α-bergamotene (1.0-21.5 %), (3Z, 6E, 10E)-α-springene (1.1-17.8 %), β-bisabolene (0.4-14.7 %), β-sesquiphellandrene (0.9-10.9 %) and estragole (0.5-7.0 %), while dolichodial (20.0 - 83.4%), (E)-α-bergamotene (0.2 - 20.6 %), (E)-β-caryophyllene (0.3 - 23.1 %), dolicholactone (0.4 - 20.9 %), β-bisabolene (0.2 - 10.0 %), estragole (1.1 - 10.5 %) and β-sesquiphellandrene (0.2 - 8.3%) were the main volatiles emitted by plant. The C-13 NMR data of (3Z,6E,10E)-α-springene and its occurrence in *T. marum* essential oils were reported for the first time. The importance of sample preparation step was discussed through the absence/occurrence of dolichodial in both liquid and gaseous matrices studied, respectively.

Keywords: *Teucrium marum* L.; essential oil; volatile components; HS-SPME; GC-MS; dolichodial; (3Z,6E,10E)-α-springene.

Introduction

The genus *Teucrium* belongs to the *Lamiaceae* family and comprises more than 300 species widespread all around the world. *Teucrium* is a genus of perennial plants, commonly named germanders which are valued as ornamental plants and pollen source.[1,2] *Teucrium* species are herbs, shrubs or subshrubs[1,2] known for a long time for their medicinal properties: they are used as diuretic, antiseptic, antipyretic and antispasmodic agents for the treatment of digestive and pulmonary disorders.[3] The biological activities of these species are mainly based on the presence of diterpenoids, flavonoids and phenolic acids.[4-6] Recently, some neo-clerodane diterpenoids have been shown to be potent hepatotoxic agents,[7] these compounds have been also mainly studied for their insect antifeedant activities.[8]

Teucrium marum L. grows in some areas of Mediterranean basin, particularly in Corsica and Sardinia where it is described as an endemic plant. [1,9,10] The plant aerial parts were used as antibacterial, anti-inflammatory and antipyretic agents in folk medicine.[11] *T. marum* is a perennial little suffrutic, bushy and evergreen plant that grows up to 30-40 cm high. Thin branches are covered with a thick white down and the plant is highly aromatic.[12]

The chemical composition of *T. marum* has been the subject of numerous studies[1,5,9,11-18] in which phenylpropanoid,[12] neo-clerodane diterpenoids[5,14,15] and iridoid cyclopentane monoterpenes [1,9,11-13,16-18] has been reported. Iridoids like dolichodial, teucrein and dolicholactone are volatile components of *T. marum* identified in the organic solvent extracts obtained by soxhlet[9,13] and simultaneous distillation-extraction.[1] In addition, dolichodial (9.3 %) was also identified in *T. marum* essential oils obtained by hydrodistillation with sesquiterpene hydrocarbons such as isocaryophyllene (20.2 %), (E)-β-bisabolene (14.7 %), β-sesquiphellandrene (11.2 %), α-santalene (10.9 %).[11] The ecological role of these iridoids were well described; they are known to be produced for protective purpose in response to injury by a number of insect[15] and to be aphid sex pheromones.[12] Moreover, the plant is so

phase in equilibrium of *T. marum* using HS-SPME are reported here for the first time. The intraspecies variations of volatile fractions obtained from the 25 Corsican sample locations using statistical analysis were discussed.

Experimental

Plant Material and Essential Oil Isolation

The aerial parts of *T. marum* were collected before flowering from January to May 2009 in 25 Corsican stations (Table 1). Voucher specimens were deposited in the Herbarium of the University of Corsica. The fresh aerial parts (400 - 530 g) were submitted to Hydrodistillation (HD) for 5 h using a Clevenger-type apparatus according to the *European Pharmacopoeia*[28] and yielded 0.016 - 0.027 % w/w of the essential oil.

Essential Oil Fractionation

The collective essential oil (2.4 g) obtained by the mixture of all Corsican sample essential oils was submitted to flash chromatography (FC, silica gel 200-500 µm, elution with pentane (PE), then diethyl ether (Et_2O)) and was separated into two fractions: fraction I apolar (0.57 g: hydrocarbon compounds) and fraction II polar (1.95 g: oxygenated compounds). Fraction I (0.5 g) was further chromatographed on silica gel (63-200 µm) impregnated with silver nitrate (20%), leading to seven fractions (I.1-I.7), by elution with n-pentane. Fraction II (1.68 g) was separated on silica gel (200-500 µm) and 19 fractions (II.1-II.19) were eluted with mixture of n-pentane: diethyl ether of increasing polarity. The main components of each fraction, their percentages and their numbers (corresponding to the order of elution on an apolar column) are reported in Figure 1.

Solvent extract

Dried aerial parts of *T. marum* samples (100 g) were extracted under reflux conditions (Soxhlet process, 6 h) with hexane (200 mL). The extract was filtered on charcoal and concentrated under vacuum. The organic solvent extract (61 mg) was dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4–5 °C.

Liquid-Liquid Extraction of Hydrolate

The Hydrolate was obtained according to processes used by industry during the hydrodistillation of fresh aerial parts of *T. marum*. The first 500 mL of water of hydrodistillation were recovered, then they were subjected to an liquid-liquid extraction (LLE) consisting in treating 3 times 250 mL of sample with 50 ml of diethyl ether (Et_2O) at ambient temperature. The Hydrolate extract obtained (100 mg for a 1L) was dried after evaporation on Na_2SO_4.

HS-SPME conditions

The fresh aerial parts and the separated organs (flowers, calyxes, leaves and stems) from a selected station of *T. marum* (Sermano, T1) were cut roughly with scissors (1-2 cm long) before subjection to HS-SPME. The SPME device (Supelco) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 30 µm) was used for extraction of the plant volatiles. Optimization of SPME conditions was carried out using fresh aerial parts of the plant (1 g in a 20 mL vial) and based on the number and the sum of total peak areas measured on GC-FID. Temperature, equilibration time and extraction time were selected after fourteen experiments combining four temperatures (30, 50, 70 and 90 °C), six equilibration times (30, 60, 90, 120, 240 and 360 min) and four extraction times (5, 15, 30 and 45 min). HS-SPME and subsequent analyses were performed in triplicate. After sampling, SPME fibre was inserted into the GC and GC-MS injection ports for desorption of volatile

components (5 min), both using the splitless injection mode. Before sampling, each fibre was reconditioned for 5 min in the GC injection port at 260 °C. Sample coefficients of variation (CV) calculated on the basis of total area obtained from the FID-signal, were comprised between 9.6 % and 13.4 % and suggested that the HS-SPME method produced reliable results. In the same way, the major compound CVs were always less than 15 %.

Gas Chromatography

GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60 to 230 °C at 2 °C.min^{-1} and then held isothermal 230 °C (30 min). Carrier gas: helium (1 mL.min^{-1}). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 µL. For HS-SPME-GC analysis, only Rtx-1 (polydimethylsiloxane) column was used and volatile components were desorbed in a GC injector with a SPME inlet liner (0.75 mm. i.d., Supelco).

Gas Chromatography-Mass Spectrometry

The essential oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. Ion source temperature: 150 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired with a mass range of 35 – 350 Uma; scan mass: 1s.

Essential Oil injected volume: 0.1 µL, fraction injected volume: 0.2 µL. GC-MS analysis of the volatile fractions sampling by HS-SPME were carried out only on a Rtx-1 capillary column, for the desorption GC injector was equipped with a SPME inlet liner (0.75 mm. i.d., Supelco).

NMR analysis

^{13}C-NMR spectra of the **FI.7** CC-fraction (Figure 1) was measured in deuterated chloroform using a Bruker Avance 400 Fourier Transform spectrometer (Wissembourg, France) operating at 100.13 MHz for ^{13}C-NMR and equipped with a 5 mm probe. All shifts were referred to the internal standard tetramethylsilane (TMS). ^{13}C-NMR spectra of the chromatographic fractions were recorded with the following parameters: pulse width, 4 µs (flip angle, 45°); acquisition time, 2.7 s for 128 K Data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution, 0.183 Hz/pt. The number of accumulated scans was 5000 for a sample (around 40 mg of the essential oil in 0.5 mL of deuterochloroform) depending of the amount of product. Exponential line broadening multiplication (1 Hz) of the free induction decay was applied before Fourier Transformation. The ^{1}H-NMR spectra were recorded in $D_2$0/Hydrolate (10/90) with the following parameters, pulse width (PW), 4.3 µs; relaxation delay 1 s, acquisition time, 2.6 s, 128 K data table with a spectral width (SW) of 6 000 Hz.

Component Identification

Identification of the components was based (i) on the comparison of their GC retention indices (RI) on apolar and polar columns, determined to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literatures data;[29-31] (ii) on computer matching with commercial mass spectral libraries[30-33] and comparison of spectra with those of our personal library.

Component Quantification

The quantification was performed using the methodology reported by Bicchi et al.[34]. The normalized % abundances were calculated integrating FID response factors calculated from tridecane (0.7 g/100g) used as internal standard. Relative amounts of individual components obtained during HS-SPME analysis, were calculated on the basis of their GC peak areas on the Rtx-1 capillary column, without FID response factor correction.

Statistical analysis

Data analyses were performed using Principal Component Analysis (PCA) and Cluster Analysis (CA).[35] Both methods aim at reducing the multivariate space in which objects (essential oil samples) are distributed but are complementary in their ability to present results.[36] Indeed, PCA provides the data for diagrams in which both objects (essential oil samples) and variables (essential oil components) are plotted while canonical analysis informs a classification tree in which objects (sample locations) are gathered. PCA was carried out using function 'PCA' from the statistical R software. The variables (volatile components) have been selected using function from the statistical software. The Cluster Analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of essential oil samples.

Results and Discussion

1. Essential oil components

Preliminary analysis of the 25 *T. marum* essential oil samples allowed identifying only 46 compounds. Essential oil chromatographic profiles displayed a wide variety of various chemical classes of compounds in low proportion. Consequently, all the *T. marum* sample

essential oils were pooled in order to produce "Collective essential oil" and all CC-fractions and sub-fractions were investigated by GC-RI, GC/MS and ^{13}C-NMR (Figure 1). Integrated analysis of "Collective essential oil" allowed the identification of 93 components which accounting for 95.5 % of the sample essential oil (Table 2). The main essential oil components were caryophyllene oxide **74** (9.8 %), (E)-α-bergamotene **58** (8.2 %), (E)-β-bisabolene **68** (7.5 %), (E)-β-caryophyllene **56** (5.3 %), β-sesquiphellandrene **70** (3.7 %), estragole **21** (3.5 %) and compound **87** (3.2 %). All components were identified by comparison of their EI-MS and GC-retention indices with those of our laboratory-produced "Arômes" library, excepted for 17 components which were identified by comparison from literature spectral data and retention indices (see Table 2).

Moreover, compound **87** which amounted 89.5 % in the hydrocarbon CC-fraction **I.7** remained unidentified. Computer matching with MS-spectral data from the Wiley library suggested the (3E,6E,10E)-α-springene. The identification of **87** was carried out by joint information of EI-MS and ^{13}C-NMR data. IE mass spectra of **87**, exhibited a weak signal at m/z 272 (1.8 %) corresponding likely to the molecular ion and a base peak at m/z 69. The ^{13}C-NMR spectra of fraction **I.7** (**87**: 89.5 %) exhibited 20 higher carbon signals, which were assigned from the DEPT spectra to 10 olefin carbons (4 quaternary carbons, 5 methines and one methylene), 5 methylenes and 5 methyls. The chemical shift values confirmed the presence of a hydrocarbon linear diterpene while the $C_{20}H_{32}$ formula was deduced from the DEPT spectra. The identification of (3Z,6E,10E)-α-springene was unambiguously established by comparison of its ^{13}C-NMR spectral data with those of (Z,E)-α-farnesene and (E)-β-farnesene **60** chosen as references noted P' and P'' in Figure 2. To our knowledge, we reported for the first time the occurrence of **87** in *Teucrium* genus. This hydrocarbon diterpene was previously reported in mammalian like peccary [37] and springbok antelope.[38] To our knowledge, we reported here for the first time its ^{13}C-NMR data. It is noticeable that Corsican

T. marum essential oil differed by the absence of dolichodial to the Sardinian sample essential oil cited in literature. [11]

1.1. Chemical variability of T. marum essential oils

The 25 sample essential oils obtained from aerials parts of *T. marum* harvested at the same vegetative stage (before flowering) were investigated in order to obtain a better insight into the essential oil chemical variability. Normalized percentage abundances of all identified components were used for statistical analysis. Table 2 reported the minimal and maximal normalized % abundances of all constituents measured from the 25 sample essential oils, for the total results, see supplementary material (Table 4). Although the 25 essential oil samples contained similar types of compounds, there were differences in the % abundances of the main components: caryophyllene oxide **74** (0.4-20.5 %), (E)-β-caryophyllene **56** (0.9-18.9 %), (E)-α-bergamotene **58** (1.0-21.5 %), (3Z, 6E, 10E)-α-springene **87** (1.1-17.8 %), β-bisabolene **68** (0.4-14.7 %), β-sesquiphellandrene **70** (0.9-10.9 %) and estragole **21** (0.5-7.0 %). To identify the relationship between the chemical composition of essential oil and environmental factors, Principal Component Analysis (PCA: Figures 3) and Cluster Analysis (CA: Figure 4) were applied to a matrix linking essential oil composition to sample location from discriminate family compounds. The principal factorial plane constructed using axes 1 and 2 accounts for 73.67 % of the variance. The F1 axis (52.23 %) shows the positive correlation with oxygenated compounds (**OC**), as well as, oxygenated mono and sesquiterpenes (**OM** and **OS**) and oxygenated non-terpenic compounds (**ONC**) and negative correlation with hydrocarbon compounds (**HC**), as well as, hydrocarbon sesquiterpenes (**HS**) and hydrocarbon diterpene compounds (**HD**) (Figure 3). The plot established using the first two axes suggests that we have two groups (Figures 3 and 4). Group I included 20 samples and was characterized by higher amounts of hydrocarbon compounds (33.5–56.1 %) than group II

which included five samples (T1, T7, T8, T14 and T15) and the collective essential oil (HE) in which the hydrocarbon compounds accounted for 21.6–34.9 %. Conversely, the oxygenated compounds were higher in the sample essential oils of group II (49.5–62.1 %) than in the group I (27.7–49.9 %). As seen in Figure 3, the normalized % abundances of hydrocarbon sesquiterpenes (HS) and oxygenated sesquiterpenes (OS) were discriminating variables. Relative to the Sardinian sample essential oil reported in the literature,[11] Corsican *T. marum* essential oils were close similar except for dolichodial which was never detected in the 25 sample essential oils evaluated.

2. HS-SPME analysis of volatiles constituents

The volatiles emitted from the *T. marum* aerial parts and separated organs (calyxes, leaves, stems, and flowers) harvested in one location (T1, Sermano) were investigated using HS-SPME under optimized parameters. The optimization of HS-SPME sampling parameters was carried out using fresh aerial parts and based on the sum of total peak areas obtained by GC-FID. The maximum sum of total peak area was acquired for a temperature of 70 °C, an equilibrium time of 120 min and an extraction time of 30 min.

The GC-RI and GC/MS analysis identified 34 components which amounted for 81.6 - 95.4 % of the chemical composition of the volatile fractions extracted by HS-SPME (Table 3). Among them, we identified 12 oxygenated monoterpenes, 11 sesquiterpenes and 11 non-terpenic compounds. All volatiles were identified by comparison of their EI-MS and retention indices with those in the laboratory-produced library except for 3 identified by comparison of their EI-MS and the apolar retention indices with those of literature library. It is noteworthy that all the volatiles sampled by HS-SPME were also identified in the *T. marum* essential oil, excepted for 6-methylhept-5-en-2-one **5a**, dolichodial **31a**, epi-dolichodial **31b**, eugenol **31c**, and (Z)-β-farnesene **49a**

Regarding the organ contribution to the aromatic plant fingerprint, it should be noted that the volatile constituents were more abundant in the leaves than in the other parts of the plant. Our analysis showed that the chemical composition of the HS fractions obtained from different organs was qualitatively similar, but differed by the relative percentage abundances of the main components: dolichodial **31a** (3.8-51.5 %), (E)-α-bergamotene **58** (5.1-20.7 %), (E)-β-caryophyllene **56** (11.1-21.5 %), dolicholactone **49** (0.4-6.0 %), β-bisabolene **68** (1.5-5.0 %), estragole **21** (4.0-6.6 %) and β-sesquiphellandrene **70** (1.2-4.2%). Oxygenated compounds were preferentially produced in calyxes and leaves (70.1 and 63.6 %, respectively) in which dolichodial amounted for 51.5 and 49.5 % respectively, while it was lower in stems and flowers (3.8 and 5.1 %, respectively). Conversely, hydrocarbon constituents were highly metabolized in stems and flowers (54.4 and 46.9 %, respectively) instead of 25.4 and 29.8 % in calyxes and leaves. Relative to *T. marum* essential oils, dolichodial **31a** was the main emitted volatile component sampled by HS-SPME while it was never detected in the essential oil obtained by hydrodistillation.

Concerning the occurrence of dolichodial in the HS-SPME fraction and its absence in the essential oil obtained by hydrodistillation, we hypothesis the probable lost of dolichodial by chemical transformation under the thermal condition and the acid pH of hydrodistillation. During hydrodistillation, the aldehyde groups of dolichodial were hydrated and the di-hydrate form obtained was transferred in the Hydrolate aqueous phase due to its greater water affinity. This hypothesis was supported by (i) the calculation of Log P using ACD/Labs Software V11.02 (© 1994-2012 ACD/Labs) for dolichodial (+1.45±0.37) and its di-hydrate form (-1.27±0.56) which ensure the lipophilic character of the first and the water affinity of the latter; (ii) the presence of dolichodial **31a**, dolicholactone **49** and epi-dolichodial **31b** identified as main components in the Hydrolate extract obtained by LLE from the Hydrolate, (iii) the presence of dolichodial **31a** and dolicholactone **49** identified as main components in the

hexane extract obtained by Soxhlet extraction from the same plant material, (iv) the absence of aldehydic protons in ^{1}H-NMR spectrum of the hydrolate acquired in D_2O confirms the absence of the dialdehyde dolichodial form.

2.1. Chemical variability of the volatile fractions extracted from T. marum.

In addition, volatile fractions emitted from *T. marum* aerial parts harvested at the same vegetative stage (before flowering) in 25 locations were investigated by HS-SPME/GC and HS-SPME/GC-MS. Table 3 reported the minimal and maximal percentages of all constituents measured from the 25 HS-SPME volatile fractions, for the total results, see supplementary material (Table 5). Although the 25 volatiles fractions exhibited similar types of compounds, there were significant differences in the relative abundances of the major components. For the main components, the relative abundance ranged as follow: dolichodial **31a** (20.0 to 83.4%), (E)-α-bergamotene **58** (0.2 to 20.6 %), (E)-β-caryophyllene **56** (0.3 to 23.1 %), dolicholactone **49** (0.4 to 20.9 %), β-bisabolene **68** (0.2 to 10.0 %), estragole **21** (1.1 to 10.5 %) and β-sesquiphellandrene **70** (0.2 to 8.3%). In order to study the chemical variability of the volatile fractions, PCA (Figures 5) and CA (Figure 6) were performed using chemical class compounds as discriminating variables. The principal factorial plane (constructed using axes 1 and 2) accounts for 95.87% of the variance. The F1 axis (80.07%) was positively correlated with hydrocarbon compounds (**HC**), hydrocarbon sesquiterpenes (**HS**) and oxygenated non-terpenic compounds (**ONC**) and negatively correlated with oxygenated compounds (**OC**) and oxygenated monoterpenes (**OM**) (Figure 5). Statistical analysis clearly showed the presence of two groups (Figures 5 and 6). Group I included 18 samples characterized by higher amounts of monoterpene oxygenated compounds (45.0-87.2 %), than group II (7 samples) in which monoterpenoids were lower (15.9-66.7 %). Group I could be divided in two subgroup; subgroup Ia was dominated by higher amounts of dolichodial **31a** (55.1-83.4 %) than in the

subgroup Ib in which dolichodial **31a** accounted for 32.0-65.7 %. Group II was clearly characterized by most important occurrence of hydrocarbon compounds (22.0-46.3 %) such as than group I (4.9–31.5 %). We noted that dolichodial **31a** was the major compound in all Corsican samples, while it was never detected in the 25 sample essential oils evaluated.

Several conclusions could be drawn for this study:

i) Corsican *T. marum* essential oils were characterized by the occurrence of some hydrocarbon sesquiterpenes as main components.

ii) Dolichodial was the major volatile component extracted by HS-SPME from the aerial parts of the plant. More precisely, dolichodial was higher in leaves and calyxes and lower in flowers and stems.

iii) The statistical analysis of the intraspecies variations of the volatile components of *T. marum*, have showed an double clustering for both, sample essential oils obtained by HD and volatile fractions extracted by HS-SPME from 25 Corsican sample locations. Therefore, it is not possible to establish correlations between the volatiles of *T. marum* and environmental parameters.

iv) This work is a good example to demonstrate the need to adapt the sample preparation strategy for the complete characterization of the volatiles of plant material. Moreover, in order to determine novel chemotypes or characterize the volatiles of plant material not yet studied, it could be interesting to carry out at least two sample preparation techniques.

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References

[1] J. Sanz, M. Mus, J. A. Rossello, *Bot. J. Linn. Soc.* **2000**, *132*, 253.

[2] H. Coste, *Flore descriptive et illustrée de la France de la Corse et contrées limitrophes,* 3 volumes, Librairie Blanchard, Paris, **1909**, pp. 138-142.

[3] B. F. Puntero, I. I. Peinadd, A. M. Villar di Fresno, *J. Ethnopharmac*. **1997**, *55*, 93.

[4] J. B. Harborne, F. A. Tomas-Barberan, C. A. Williams, M. I., Gil, *Phytochemistry* **1986**, *25*, 2811.

[5] G. Savona, F. Piozzi, O. Servettaz, B. Rodriguez, F. Fernandez-Gadea, *Phytochemistry* **1984**, *23*, 611.

[6] G. Savona, M. P. Paternostro, F. Piozzi, J. R. Hanson, P. B. Hitchcock, S. A. Thomas, *J. Chem. Soc. Perkin Trans.* **1978**, *1*, 1080.

[7] F. Piozzi, M. Bruno, R. Ciriminna, C. Fazio, N. Vassallo, N. A. Arnold, M. C. de la Torre, B. Rodriguez, *Planta Med*. **1997**, *63*, 483.

[8] M. Bruno, R. Cirimanna, F. Piozzi, S. Rosselli, M. S. J. Simmonds, *Phytochemistry* **1999**, *52*, 1055.

[9] C. Beaupin, R. Granger, J. C. Rossi, J. Passet, L. Peyron, *Rivista Ital*. **1977**, *59*, 369.

[10] S. Pignatti, *Flora d'Italia,* Edagricole, Bologna, Italy, **1982**, pp. 243-244.

[11] D. Ricci, D. Fraternale, L. Giamperi, A. Bucchini, F. Epifano, G. Burini, M. Curini, *J. Ethnopharmacol.* **2005**, *98*, 195.

[12] F. Poli, A. M. Srrilli, P. Scartezzini, M. Muzzoli, A. Maxia, M. Ballero, M. Serafini, A. Bianco, *Nat. Prod. Res.* **2007**, **21**, 1061.

[13] O. Servettaz, L. Bini Maleci, A. Pinetti, *Pl. Syst. Evol*. **1992**, *179*, 129.

[14] F. Bellesia, U. M. Pagnoni, A. Pinetti, R. Trave, *J. Chem. Res. (S)* **1983**, 328.

[15] T. Eisner, M. Eisner, D. J. Aneshansley, C-L. Wu, J. Meinwald, *Chemoecology* **2000**, *10*, 211.

[16] F. Bellesia, U. M. Pagnoni, A. Pinetti, R. Trave, *Phytochemistry* **1983**, *22*, 2201.

[17] R. Grandi, U. M. Pagnoni, A. Pinetti, R. Trave, *Phytochemistry* **1983**, *22*, 2726.

[18] U. M. Pagnoni, A. Pinetti, R. Trave, L. Garanti, *Aust. J. Chem.* **1976**, *29*, 1375.

[19] C. Bicchi, C. Cordero, E. Liberto, B. Sgorbini, P. Rubiolo, *J. Chromatogr. A*. **2007**, *1152*, 138.

[20] F. Belliardo, C. Bicchi, C. Cordero, E. Liberto, P. Rubiolo, B. Sgorbini, *J. Chromatogr. A*. **2006**, *44*, 416.

[21] C. Bicchi, S. Drigo, P. Rubiolo, *J. Chromatogr. A*. **2000**, *892*, 469.

[22] J. Paolini, E. Nasica, J. M. Desjobert, A. Muselli, A. F. Bernardini, J. Costa, *J. Phytochem. Anal*. **2007**, *19*, 266.

[23] A. Muselli, M. Pau, J. M. Desjobert, M. Foddai, M. Usai, J. Costa, *Chromatographia*. **2009**, *69*, 575.

[24] S. Cozzani, A. Muselli, J.M. Desjobert, A. Bernardini, F. Tomi, J. Casanova, *Flavour Fragr. J.*, **2005**, *20*, 436.

[25] A. Muselli, J. Costa, J.M. Desjobert, M.A. Dessi, J. Paolini, A. J. Bernardini, *Essent. Oil Res.* **2009**, *21*, 138.

[26] N. Djabou, J. Paolini, J.M. Desjobert, H. Allali, N. Baldovini, J. Costa, M. Muselli, *Flavour Fragr. J.* **2010**, *25*, 475

[27] N. Djabou, M.J. Battesti, H. Allali, J.M. Desjobert, L. Varesi, J. Costa, A. Muselli, *Phytochemistry*, **2011**, DOI : 10.1016/j.phytochem.2011.03.024.

[28] Conseil de l'Europe, *Pharmacopée Européenne*, Maisonneuve S. A. : Sainte Ruffine, **1996**.

[29] W. Jennings, T. Shibamoto, *In: Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography*. Academic Press, New-York, **1980**.

[30] MassFinder (version 3.0), In: Hochmunth D., Scientific Consulting, Germany, **2006**.

[31] W.A. Konig, D.H. Hochmuth, D. Joulain, *Terpenoids and Related Constituents of Essential oils*. Library of Mass Finder 2.1, Institute of Organic Chemistry, Hambourg, **2001**.

[32] National Institute of Standards and Technology, PC version 1.5a of the NIST/EPA/NIH Mass Spectral Library, Perkin Elmer Corporation, **1996**.

[33] F. W. Mc Lafferty, D. B. Stauffer, *The Wiley/NBS registry of mass spectra data, vol. 1-7*. Wiley-Interscience publication, New-York, **1989**.

[34] C. Bicchi, E. Liberto, M. Matteodo, B. Sgorbini, L. Mondello, B. A. Zellner, R. Costa, P. Rubiolo, *Flavour Fragr. J.* **2008**, *23*, 382.

[35] R.G. Brereton, *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*. Wiley Interscience, New York, **2003**.

[36] D.L. Massart, *Chemometrics: A textbook*. Elsevier Sciences Ltd. New York, 1998.

[37] J. S. Waterhouse, J. Ke, J. A. Pickett, P. J. Weldon. *J. Chem. Ecol.* **1996**, *22*, 1307.

[38] B. V. Burger, M. Le Roux, H. S. C. Spies, V. Truter, R. C. Bigalke. *Z. Naturforsch.* **1981**, *36c*, 340.

Table 1: Data relative to the plant material harvested in Corsica.

No[a]	Localities[b]	GPS Coordinates[c]	Alt.[d]
T1	Sermano	42°18'39"N; 9°16'01"E	684
T2	Corte	42°18'08"N; 9°08'59"E	407
T3	Chisa	41°55'17"N; 9°15'44"E	394
T4	Minervio	42°53'12"N; 9°20'07"E	214
T5	Nonza	42°47'00"N; 9°20'27"E	27
T6	Teghime	42°40'34"N; 9°23'05"E	513
T7	Ponte Leccia	42°07'50"N; 9°08'15"E	253
T8	Santa Lucia di Mercurio	42°19'22"N; 9°13'07"E	636
T9	Porto1	42°15'50"N; 9°42'11"E	14
T10	Porto2	42°16'13"N; 8°41'37"E	84
T11	Galeria	42°24'22"N; 8°38'55"E	80
T12	Ospedale	41°39'01"N; 9°11'26"E	887
T13	Tralonca	42°20'23"N; 9°12'29"E	693
T14	Scala di Santa Regina	42°21'56"N; 9°03'56"E	604
T15	Moltifao	42°29'01"N; 9°06'57"E	405
T16	Venaco	42°13'54"N; 9°10'09"E	700
T17	Oletta	42°37'50"N; 9°20'52"E	172
T18	Lancone	42°36'14"N; 9°23'04"E	250
T19	Bonifacio	41°23'12"N; 9°10'01"E	47
T20	Bastia (Sierra di Pignu)	42°41'11"N; 9°24'34"E	373
T21	Ersa	42°58'20"N; 9°22'53"E	459
T22	Piana	42°13'31"N; 8°37'08"E	317
T23	Restonica 1	42°17'48"N; 9°08'50"E	429
T24	Restonica 2	42°17'76"N; 9°06'59"E	553
T25	Roccapina	41°29'53"N; 8°56'51"E	131

[a] Sample codes
[b] Harvest localities
[c] Sample positioning
[d] Sample altitudes

Table 2. Chemical compositions of *T. marum* essential oils

No.[a]	Compounds	*I*RI$_a$[b]	RI$_a$[c]	RI$_p$[d]	Collective Oil[e] %	Sample oils[f] % Min	Sample oils[f] % Max	Identification[g]
1	hexanal	780	772	1100	0.1	nd	0.2	RI, MS
2	(E)-2-hexenal	832	832	1206	0.6	nd	1.4	RI, MS, Ref.
3	hexanol	837	841	1331	0.2	nd	0.6	RI, MS, Ref.
4	pentyl acetate	881	881	1137	tr	nd	0.2	RI, MS
5	benzaldehyde	941	937	1532	tr	nd	0.8	RI, MS
6	1-octen-3-ol	959	962	1446	0.7	0.1	2.4	RI, MS
7	octanal	980	981	1310	0.1	nd	0.4	RI, MS
8	hexyl acetate	1006	1002	1265	0.1	nd	1.4	RI, MS
9	phenylacetaldehyde	1012	1010	1646	1.0	tr	1.1	RI, MS
10	2,6-dimethyl-5-heptenal	1034	1033	1247	0.2	nd	0.2	RI, MS
11	2-methylbutyl butyrate	1041	1040	1258	1.0	0.1	2.7	RI, MS
12	(Z)-linalool oxide THP	1058	1051	1410	0.3	nd	0.4	RI, MS
13	fenchone	1069	1068	1412	0.2	tr	2.8	RI, MS
14	nonanal	1083	1083	1368	0.6	0.1	1.6	RI, MS
15	linalool	1086	1088	1550	0.7	0.1	3.3	RI, MS
16	1-oct-3-enyl acetate	1093	1094	1390	1.0	0.1	3.1	RI, MS
17	2-methylbutyl isovalerate	1095	1095	1250	0.5	nd	0.6	RI, MS
18	(Z)-rose oxide	1100	1098	1362	0.1	nd	0.6	RI, MS
19	1,2-limonene epoxide	1117	1120	1148	tr	nd	0.8	RI, MS
20	citonnellal	1132	1130	1514	1.6	0.2	3.4	RI, MS
21	estragole	1175	1176	1641	3.5	0.5	7.0	RI, MS
22	hexyl butanoate	1177	1178	1403	2.3	0.1	4.5	RI, MS
23	myrtenol	1178	1178	1790	0.1	nd	0.3	RI, MS
24	decanal	1183	1183	1500	0.3	0.1	0.6	RI, MS
25	decanol	1197	1194	1732	tr	nd	0.2	RI, MS
26	citronellol	1210	1208	1763	0.2	0.1	1.4	RI, MS
27	nerol	1213	1215	1685	0.8	tr	2.3	RI, MS
28	hexyl-2-methyl butyrate	1220	1220	1258	0.2	0.3	2.6	RI, MS
29	2-methylbutyl hexanoate	1235	1235	1475	tr	nd	1.2	RI, MS
30	geraniol	1235	1238	1727	1.5	tr	1.8	RI, MS
31	(Z)-myrtanol	1242	1240	1869	tr	nd	1.2	RI, MS
32	geranial	1244	1244	1727	1.0	0.1	2.1	RI, MS
33	(E)-2-decenal	1240	1248	1541	tr	nd	0.6	RI, MS
34	citronellyl formiate	1259	1256	1613	0.4	tr	1.2	RI, MS
35	nonanoic acid	1263	1257	2106	0.4	nd	1.1	RI, MS, Ref.
36	undecan-2-one	1273	1273	1592	1	nd	0.6	RI, MS
37	menthyl acetate	1278	1274	1553	0.4	0.2	0.6	RI, MS
38	geranyl formiate	1284	1283	1605	tr	nd	0.3	RI, MS
39	undecan-2-ol	1284	1285	1719	tr	nd	0.4	RI, MS
40	(E,E)-2,4-decadienal	1291	1290	1820	0.4	0.1	2.1	RI, MS

41	hexyl tiglate	1333	1314	1578	0.3	0.1	0.4	RI, MS
42	myrcenyl acetate	1328	1329	1725	0.3	tr	0.5	RI, MS
43	citronellyl acetate	1337	1335	1651	1.0	0.2	2.6	RI, MS
44	geranyl acetate	1362	1360	1773	3.0	1.2	5.4	RI, MS
45	benzyl isovalerate	1374	1362	1851	0.3	nd	0.4	RI, MS, Ref.
46	(E)-β-damascenone	1363	1365	1770	0.3	nd	0.3	RI, MS
47	methyleugenol	1369	1367	2113	0.4	nd	0.9	RI, MS
48	hexyl hexanoate	1363	1372	1599	0.3	0.1	3.0	RI, MS
49	dolicholactone	1385	1386	2134	0.7	0.1	0.7	RI, MS
50	dodecanal	1389	1389	1402	0.3	nd	0.4	RI, MS
51	dodecan-2-one	1381	1391	1711	tr	nd	0.3	RI, MS
52	iridomyrmecine	1397	1397	2112	0.5	0.1	0.3	RI, MS
53	neryl acetone	1412	1409	1851	0.4	nd	0.3	RI, MS
54	2-undecanyl acetate	1419	1419	1654	0.5	nd	0.3	RI, MS
55	α-barbatene	1414	1422	1563	1.2	nd	1.1	RI, MS, Ref.
56	(E)-β-caryophyllene	1421	1423	1604	5.3	0.9	18.9	RI, MS
57	geranyl acetone	1430	1428	1825	tr	tr	0.5	RI, MS
58	(E)-α-bergamotene	1434	1432	1577	8.2	1.0	21.5	RI, MS
59	β-barbatene	1445	1441	1593	1.1	0.2	1.5	RI, MS, Ref.
60	(E)-β-farnesene	1446	1446	1676	0.4	nd	1.1	RI, MS
61	α-humulene	1455	1451	1687	1.3	0.5	3.8	RI, MS
62	neryl propionate	1451	1456	1782	0.2	nd	0.2	RI, MS
63	β-ionone	1468	1462	1936	0.3	nd	0.3	RI, MS
64	γ-curcumene	1475	1471	1730	0.4	nd	0.7	RI, MS
65	(E)-β-bergamotene	1480	1480	1675	0.4	0.1	2.0	RI, MS
66	zingiberene	1489	1486	1723	0.3	nd	0.5	RI, MS
67	α-cuparene	1498	1497	1840	0.5	nd	0.5	RI, MS
68	β-bisabolene	1503	1501	1718	7.5	0.4	14.7	RI, MS
69	apofarnesal	1522	1508	1760	0.4	nd	0.7	RI, MS, Ref.
70	β-sesquiphellandrene	1516	1515	1785	3.7	0.9	10.9	RI, MS
71	(E)-α-bisabolene	1530	1535	1753	0.4	nd	1.0	RI, MS
72	(E)-nerolidol	1546	1548	2053	0.8	0.1	4.1	RI, MS
73	dodecanoic acid	1567	1553	2462	0.5	0.2	2.4	RI, MS, Ref.
74	caryophyllene oxide	1576	1572	1996	9.8	0.4	20.5	RI, MS
75	fokienol	1582	1582	2180	0.7	0.2	1.7	RI, MS
76	dodecyl acetate	1585	1585	1960	0.7	tr	1.7	RI, MS, Ref.
77	humulene epoxide II	1601	1596	2073	3.2	0.4	4.7	RI, MS
78	α-acorenol	1623	1617	2130	0.7	tr	1.2	RI, MS
79	caryophylla-4(14),8(15)diene-5-α-ol	1635	1632	2284	0.7	0.2	2.5	RI, MS
80	α-bisabolol	1673	1672	2175	1.5	0.2	2.8	RI, MS
81	pentadecanal	1702	1693	2085	1.2	tr	2.8	RI, MS, Ref.
82	(Z,E)-farnesol	1694	1696	2330	0.5	0.1	0.6	RI, MS
83	tetradecanoic acid	1748	1755	2164	0.2	tr	0.7	RI, MS, Ref.
84	hexadecanal	1782	1796	2320	0.6	tr	0.8	RI, MS, Ref.
85	hexahydrofarnesyl acetone	1817	1824	2120	0.6	tr	1.0	RI, MS, Ref.
86	2-heptadecanone	1870	1888	2427	0.2	tr	0.5	RI, MS, Ref.

87	(3Z,6E,10E)-α -springene	-	1941	2190	3.2	1.1	17.8	RI, MS, ^{13}C
88	hexadecyl acetate	2004	2000	2290	0.6	tr	2.3	RI, MS, Ref.
89	octadecanol	2070	2069	2530	1.7	0.5	5.1	RI, MS
90	(E)-phytol	2107	2101	2630	2.5	0.5	4.5	RI, MS
91	octadecyl acetate	2190	2195	2465	3.3	0.8	5.6	RI, MS
92	2-eicosanone	2200	2213	2730	0.3	tr	0.5	RI, MS, Ref.
93	2-heneicosanone	2300	2280	2836	0.6	0.1	1.5	RI, MS, Ref.
	Total identification				95.5			
	Yields % (w/w)					0.016	0.027	
	Hydrocarbon compounds				33.9			
	Hydrocarbon sesquiterpenes				30.7			
	Hydrocarbon diterpenes				3.2			
	Oxygenated compounds				61.6			
	Oxygenated monoterpenes				17.6			
	Oxygenated sesquiterpenes				18.6			
	Oxygenated diterpenes				2.5			
	Oxygenated no-terpenic compounds				22.9			

[a] Order of elution is given on apolar column (Rtx-1).

[b] Retention indices of literature on the apolar column (*l*RI$_a$) reported from [29,31,32]. To our knowledge, Retention Indices of **87** was reported for the first time.

[c] Retention indices on the apolar Rtx-1 column (RI$_a$).

[d] Retention indices on the polar Rtx-Wax column (RI$_p$).

[e] Collective oil: Mixture of all Corsican *T. marum* oils. Quantification was carried out using RFs relative to tridecane as internal standard. %: Normalized Percentages are given on the apolar column except for components with identical RI$_a$ (percentages are given on the polar column), tr = trace (<0.05%).

[f] Sample oils: Minimal and maximal normalized percentages (%) from the 25 sample oils are given on the apolar column except for components with identical RI$_a$ (percentages are given on the polar column), tr = trace (<0.05%).

[g] RI: Retention Indices; MS: Mass Spectrometry in electronic impact mode; ^{13}C: Carbon-13 Nuclear Magnetic Resonance; Ref.: compounds identified from literature data: [31] (**2**, **3**, **35**, **55**, **59**, **73**, **76**, **81**, **83**, **84** and **85**) and [29] (**45**); and commercial data libraries: [32] (**69**, **86**, **88**, **92** and **93**). The identification of **87** was performed by ^{13}C-NMR analysis after CC fraction.

Table 3. Volatile compounds extracted by HS-SPME from aerial parts and separated organs of *T. marum*.

N°[a]		IRI_a[b]	RI_a[c]	Aerial parts (T1)	Separated organs (T1)[d]				Aerial parts (T1-T25)[e]	
					Calyxes	Leaves	Stems	Flowers	Min	Max
1	Hexanal	780	772	0.3±0.00	0.3±0.07	0.4±0.00	0.5±0.14	0.6±0.07	0.1±0.00	2.2±0.27
2	(E)-2-hexenal	832	832	0.4±0.07	0.2±0.00	0.5±0.00	0.4±0.28	0.3±0.07	0.1±0.00	3.4±0.07
4	isopentyl acetate	881	881	0.2±0.00	0.2±0.07	0.3±0.00	0.2±0.00	0.4±0.28	nd	1.0±0.14
5	benzaldehyde	941	937	0.3±0.21	0.2±0.07	0.1±0.00	0.4±0.14	0.4±0.14	nd	0.6±0.14
5a	6-methylhept-5-en-2-one*	978	960	0.2±0.00	0.2±0.07	0.2±0.07	0.6±0.21	0.8±0.07	0.1±0.00	1.9±0.07
8	hexyl acetate	1006	1002	0.3±0.14	0.6±0.07	1.1±0.28	0.5±0.28	0.8±0.07	0.1±0.06	1.5±0.30
11	2-methylbutyl butyrate	1041	1040	0.4±0.07	0.5±0.14	0.2±0.14	0.4±0.21	0.8±0.04	0.1±0.04	5.6±0.71
14	nonanal	1083	1083	0.4±0.00	0.2±0.00	0.1±0.00	0.1±0.00	7.9±0.07	0.1±0.00	1.2±0.21
15	linalool	1086	1088	0.4±0.00	1.2±0.21	0.1±0.00	1.0±0.71	0.6±0.00	nd	3.1±0.42
16	1-oct-3-enyl acetate	1093	1094	0.3±0.00	0.6±0.00	0.5±0.07	0.5±0.07	0.6±0.00	0.1±0.00	1.6±0.07
20	citronellal	1132	1130	1.1±0.07	1.4±0.28	0.9±0.07	2.0±0.99	0.5±0.14	0.2±0.11	2.7±0.07
21	estragole	1175	1176	**5.3±0.78**	**6.6±0.35**	**4.0±1.06**	**4.4±0.42**	**4.0±0.07**	**1.1±0.21**	**10.5±0.71**
24	decanal	1180	1183	0.2±0.00	0.2±0.07	0.2±0.00	0.7±0.00	0.3±0.07	0.1±0.00	0.5±0.14
26	citronellol	1210	1208	0.1±0.00	0.1±0.00	0.1±0.00	0.1±0.00	0.3±0.07	nd	1±0.04
27	neral	1213	1215	0.2±0.00	0.5±0.07	0.1±0.00	0.1±0.00	0.2±0.00	nd	2.1±0.14
28	2-methylhexyl butyrate	1220	1220	1.1±0.21	1.4±0.57	0.6±0.07	0.8±0.42	2.3±0.00	nd	2.1±0.07
31	(Z)-myrtanol	1242	1240	0.5±0.07	0.3±0.14	0.2±0.07	1.1±0.14	0.5±0.00	0.1±0.00	2.8±0.14
31a	dolichodial*	1164	1264	**31.0±2.83**	**51.5±0.71**	**49.5±2.12**	**3.8±0.35**	**5.1±0.14**	**20.0±1.00**	**83.4±4.33**
31b	epi-dolichodial*	1279	1290	0.7±0.71	0.7±0.00	0.3±0.00	0.4±0.07	2.8±0.28	0.2±0.00	5.0±0.14
31c	eugenol*	1331	1327	0.4±0.00	0.3±0.07	0.3±0.00	0.2±0.07	1.1±0.07	0.1±0.01	1.1±0.23
43	citronellyl acetate	1337	1335	0.3±0.14	0.3±0.14	0.2±0.00	0.2±0.07	1.1±0.07	0.1±0.00	1±0.14
44	geranyl acetate	1362	1360	2.3±0.42	2.3±0.57	2.9±0.07	0.7±0.28	5.2±0.85	0.2±0.07	2.1±0.07
49	dolicholactone	1385	1386	**6.0±0.21**	**0.4±0.07**	**0.7±0.00**	**6.0±1.98**	**1.1±0.21**	**0.4±0.10**	**20.9±1.41**
49a	(Z)-β-farnesene*	1420	1417	0.1±0.00	0.3±0.00	0.1±0.00	0.2±0.00	0.2±0.00	tr	4.6±0.21
56	(E)-β-caryophyllene	1421	1423	**16.9±1.41**	**13.7±0.78**	**11.1±0.92**	**18.2±0.49**	**21.5±1.77**	**0.3±0.40**	**23.1±0.89**
58	(E)-α-bergamotene	1434	1432	**9.3±1.06**	**5.1±0.71**	**9.1±0.28**	**20.7±1.34**	**9.4±0.28**	**0.2±0.06**	**20.6±3.00**
59	β-barbatene	1445	1441	-	1.0±0.00	-	0.1±0.00	2.8±0.07	0.1±0.00	6.7±0.42
60	(E)-β-farnesene	1446	1446	1.7±0.49	1.2±0.00	1.5±0.07	1.6±0.64	-	0.1±0.00	1.3±0.12
61	α-Humulene	1455	1451	3.1±0.14	0.9±0.57	2.0±0.28	3.4±0.21	4.6±0.42	0.2±0.00	4.5±0.00
65	(E)-β-bergamotene	1480	1480	1.1±0.28	0.6±0.14	1.1±0.07	1.2±0.71	1.6±0.00	0.1±0.03	1.7±0.12
68	β-bisabolene	1503	1501	**2.8±0.57**	**1.5±0.21**	**2.8±0.07**	**5.0±1.34**	**3.8±0.14**	**0.2±0.07**	**10.0±1.41**
70	β-sesquiphellandrene	1516	1515	**2.3±0.42**	**1.2±0.21**	**2.3±0.07**	**4.2±1.13**	**3.1±0.07**	**0.2±0.00**	**8.3±1.77**
74	caryophyllene oxide	1576	1572	0.8±0.00	0.1±0.00	0.4±0.07	2.1±0.21	1.4±0.07	0.1±0.00	2.0±0.07
77	humulene epoxide II	1601	1596	0.5±0.07	0.3±0.14	0.1±0.00	0.4±0.28	0.4±0.07	nd	1.1±0.21
	Total identification (%)			90.5	95.4	93.4	81.6	85.7		
	Total area 10^5[f]			590	690	850	240	480		
	Hydrocarbon compounds			37.2	25.4	29.8	54.4	46.9		

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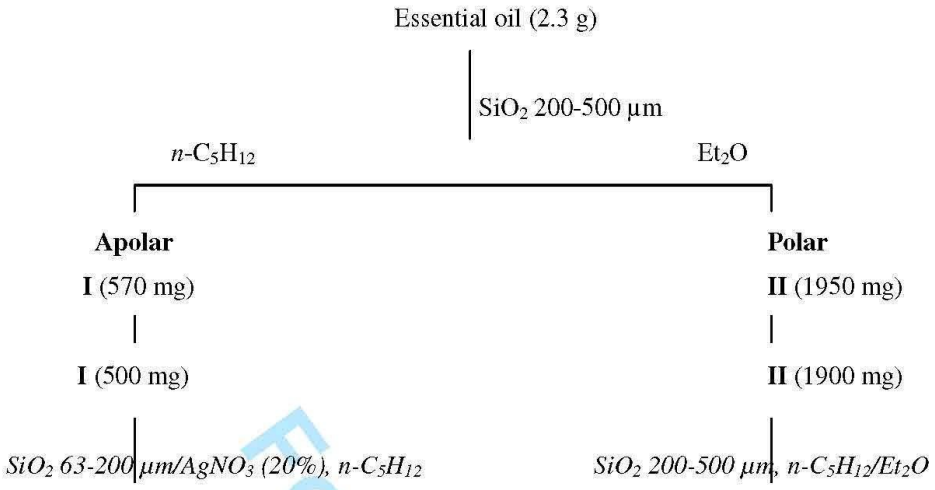


n-C_5H_{12}/Et_2O (98/2)

I.1 (109 mg) **56**(7.1%), **58**(3.9%)
I.2 (65 mg) **56**(26.3%), **58**(35.3%), **68**(3.1%), **70**(7.4%)
I.3 (63 mg) **56**(21.8%), **58**(19.3%), **68**(29.1%), **70**(15.5%)
I.4 (82 mg) **56**(9.3%), **58**(4.5%), **68**(48.3%), **70**(13.6%)
I.5 (63 mg) **61**(10.9%), **68**(35.1%), **87**(29.7%)
I.6 (40 mg) **68**(2.5%), **87**(57.1%)
I.7 (34 mg) **87**(89.5%)

II.1 (65 mg) **22**(25.5%), **28**(10%), **91**(6.6%)
II.2 (66 mg) **16**(5%), **22**(22.3), **28**(9.3%), **91**(11.6%)
II.3 (45 mg) **22**(19.1%), **28**(7.5%), **91**(16.2%)
II.4 (63 mg) **22**(15.5%), **28**(5.5%), **91**(21.1%)
II.5 (63 mg) **91**(21.7%), **43**(4.3%)
II.6 (50 mg) **43**(13.2%), **44**(22.8%), **91**(5.3%)
II.7 (62 mg) **43**(5%), **44**(29.3%)
II.8 (39 mg) **54**(8.5%), **74**(11.5%)

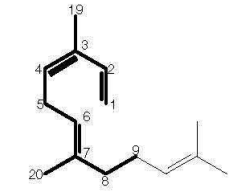
n-C_5H_{12}/Et_2O (95/5)

II.9 (55 mg) **54**(12.7%), **74**(30.2%), **77**(7.6%)
II.10 (120 mg) **74**(66.1%), **77**(13.3%)
II.11 (56 mg) **33**(4.7%), **27**(4.6%), **47**(4.6%), **74**(55.2%), **77**(6.8%)
II.12 (51 mg) **15**(4.6%), **27**(5.1%), **33**(14.3%), **72**(12.7%)

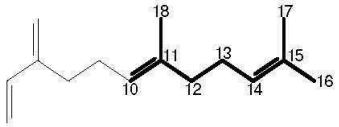
n-C_5H_{12}/Et_2O (0/100)

II.13 (50 mg) **15**(8.1%), **20**(5.7%), **80**(16.1%)
II.14 (54 mg) **80**(4.9%), **90**(7.1%)
II.15 (184 mg) **75**(3.7%), **79**(6.1%), **89**(7.7%), **90**(9.5%)
II.16 (102 mg) **75**(4%), **79**(6.6%), **89**(9.8%), **90**(7.4%)
II.17 (134 mg) **49**(5.3%), **52**(1.7%), **79**(4.2%), **89**(6.1%)
II.18 (86 mg) **49**(9.4%), **53**(3.2%)
II.19 (106 mg) **49**(4.7%), **53**(3.1%)

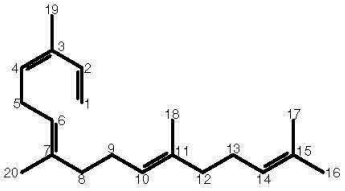
Figure 1: Fractionation of *T. marum* essential oil



(Z, E)-α-farnesene (P')



(E)-β-farnesene (P'')



(3Z, 6E, 10E)-α-springene (P)

C	δ ppm (P')	δ ppm (P)	δ ppm (P'')
1	113.45	113.46	
2	133.72	133.68	
3	131.94	131.93	
4	129.74	129.75	
5	26.53	26.56	
6	122.41	122.39	
7	135.65	135.67	
8	39.69	39.72**	
9	26.65	26.86***	
10		124.12*	124.09
11		135.04	135.36
12		39.66**	39.75
13		26.76***	26.28
14		124.41*	124.42
15		131.27	131.23
16		25.70	25.68
17		17.69	17.68
18		16.02	16.02
19	16.11	16.11	
20	19.75	19.75	

*, **, *** : interchangeable chemical shifts

Figure 2: Structure and ^{13}C-NMR data of (3Z, 6E, 10E)-α-springene

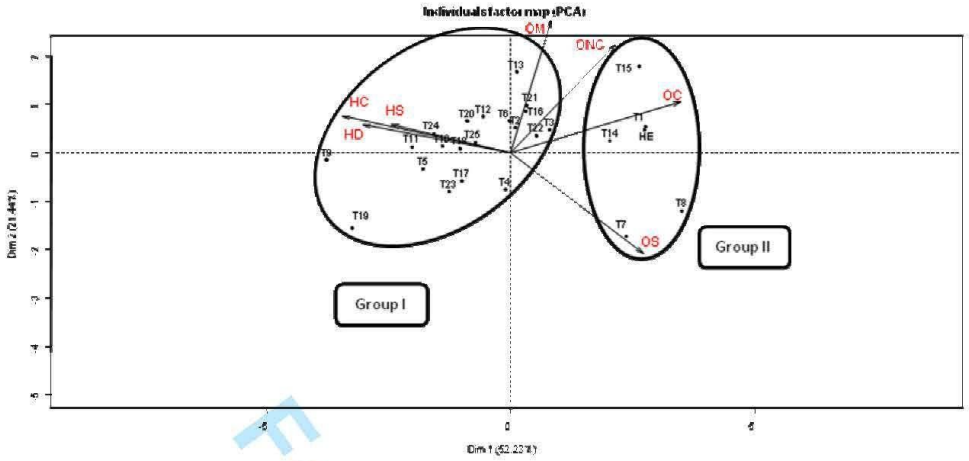


Figure 3: PCA of chemical compositions of *T.marum* essential oils: Distribution of variables corresponding to those of Table 2 and distribution of samples (coding numbers of locations). **OC:** oxygenated compounds, **OM:** oxygenated monoterpenes, **OS:** oxygenated sesquiterpenes, **ONC** oxygenated non-terpenic compounds, **HC:** hydrocarbon compounds, **HS:** hydrocarbon sesquiterpenes, **HD:** hydrocarbon diterpenes.

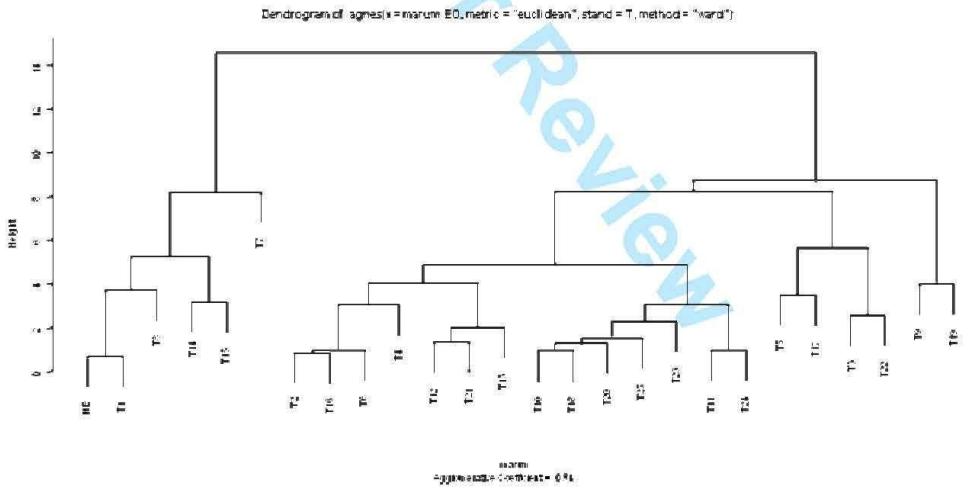


Figure 4**:** CA of chemical compositions of *T. marum* oils

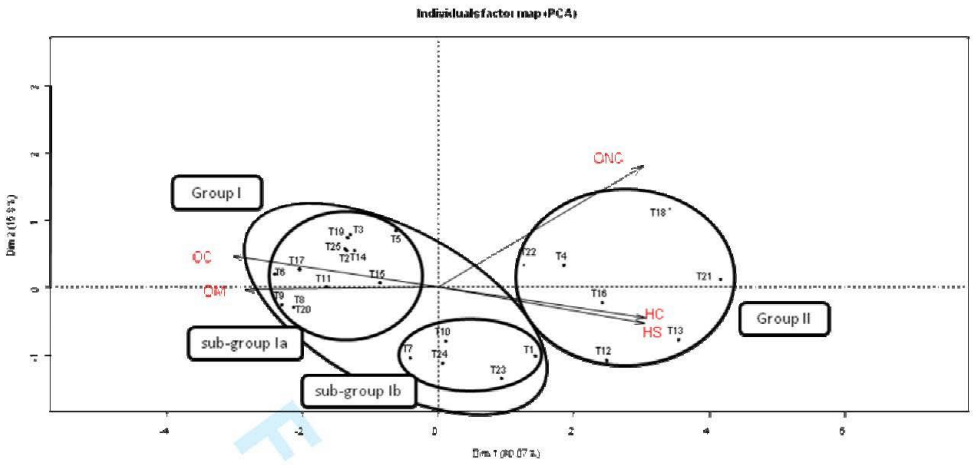


Figure 5: PCA of chemical compositions of HS-SPME volatile fractions of *T. marum*: Distribution of variables corresponding to those of Table 3 and distribution of samples (coding numbers of locations). **OC:** oxygenated compounds, **OM:** oxygenated monoterpenes, **ONC** oxygenated non-terpenic compounds, **HC:** hydrocarbon compounds, **HS:** hydrocarbon sesquiterpenes.

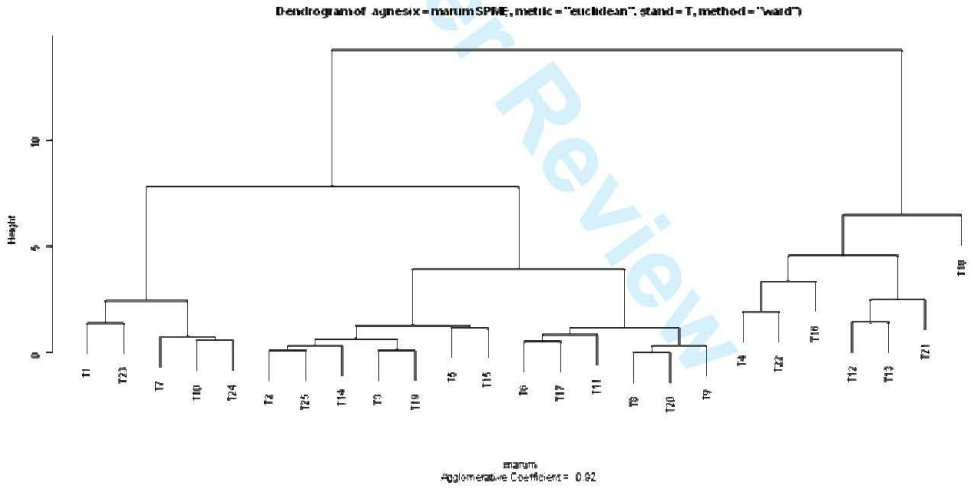


Figure 6. CA of chemical compositions of HS-SPME volatile fractions of *T. marum*.

[**PT3**]: ***Teucrium scorodonia*** **(deux sous-espèces)**

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Chemical and genetic differentiation of two Mediterranean subspecies of *Teucrium scorodonia* L.

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ABSTRACT

Chemical and genetic diversity of *Teucrium scorodonia* L. subsp. *scorodonia* from Corsica and *T. scorodonia* L. subsp. *baeticum* from western Algeria were investigated. Diversity within and among the two populations of subspecies was assessed according to the chemical composition of their essential oils, and genetic diversity was evaluated using three polymorphic genetic markers. Chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Genetic structures were mapped using two chloroplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region). The statistical analysis showed that the two subspecies were clearly distinguished by these chemical and genetic markers. The chemical composition of oil differed qualitatively and quantitatively between the subspecies. Corsican oil samples contained germacrene B (4.2–8.8%) and γ-elemene (2.6–5.7%), which were not detected in Algerian oil samples. The oils of the *scorodonia* and *baeticum* subspecies were dominated by sesquiterpene hydrocarbon compounds (75.6–82.9% and 69.6–79.4%, respectively), but they differed in oxygenated sesquiterpene content (3.1–8.9% and 8.4–20.3%, respectively). Neighbor-joining trees constructed from chloroplast DNA and ITS region sequences showed the existence of two groups associated with taxonomic and chemical characteristics. One group consisted of *T. scorodonia* subsp. *scorodonia* and the other of *T. scorodonia* subsp. *baeticum*, indicating that variation in the essential oil composition of *T. scorodonia* subspecies depends more on genetic background than environmental characteristics.

1. Introduction

The genus *Teucrium* (Lamiaceae family) includes 300 species that occur all over the world, with most concentrated around the Mediterranean basin (Beni Maleci et al., 1995). Many species of *Teucrium* are used in traditional folk medicine and it is claimed they have hypoglycemic, antipyretic, antiulcer and antibacterial properties (Menichini et al., 2009). Some *Teucrium* species are valuable as alimentary plants; hydroalcoholic extracts of wild germander (*Teucrium chamaedrys*) are currently used in the preparation of flavored wines, bitters and liqueurs (Bosisio et al., 2004).

The genus *Teucrium* is one of the richest sources of neoclerodane diterpenes: more than 220 diterpenes have been described, many of which are of interest because of their insect-repellent and medicinal properties (Coll and Tandrón, 2005; Menichini et al., 2009). *Teucrium* essential oils have been the subject of several investigations and their diverse chemical compositions were recently reviewed (Awadh Ali et al., 2008). It appears that *Teucrium* essential oils are characterized by mono- and sesquiterpene hydrocarbon compounds.

Teucrium scorodonia L. is a pubescent rhizomatous dwarf shrub 15–50 cm in height. The leaves are triangular-ovate, crenate and cordate at the base and are petiolate. The inflorescence, which may be up to 15 cm, is simple or branched with ovate to lanceolate bracts and may be much shorter than the calyx. The calyx is villous and more or less glandular and it is strongly veined in fruit. The corolla (9 mm) is villous and pale greenish-yellow; white or reddish corollas occur, although rarely (Jeanmonod and Gamisans, 2007; Quezel and Santa, 1963; Tutin et al., 1972).

From a taxonomic point of view, *T. scorodonia* L. has three subspecies according to *Flora Europaea* (Tutin et al., 1972): (i) *T. scorodonia* subsp. *scorodonia*, which is the most common in Europe; (ii) *T. scorodonia* subsp. *baeticum* (Boiss and Reuter), commonly named *Teucrium pseudoscorodonia* Desf., which occurs in southwestern Spain and North Africa (Quezel and Santa, 1963); and (iii) *T. scorodonia* subsp. *euganeum* (Vis.) Arcangeli, which is localized to mainland Italy and Sicily. Therefore, Pignatti (1982) describes

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for Italy, the species *T. scorodonia* L., without subspecies and raise to species rank *T. euganeum* (Vis.) Arcangeli, at present included within the endemic *T. siculum* rafin (Maccioni et al., 2007). In Corsica, only *T. scorodonia* subsp. *scorodonia* is present. It is widely distributed in broadleaf forests and generally grows in acid soil from sea level up to 1800 m (Jeanmonod and Gamisans, 2007). Similarly, only *T. scorodonia* subsp. *baeticum* is reported in Algeria, it is widely distributed in forests near the Mediterranean Sea (Quezel and Santa, 1963).

In addition to their unequal geographical distribution, the both subspecies exhibit the following morphological differences (Tutin et al., 1972; Quezel and Santa, 1963; Jeanmonod and Gamisans, 2007). (i) *T. scorodonia* subsp. *scorodonia* has stems with petioles and leaves that are more or less appressed pubescent. The leaves are acute and the inflorescence axis and calyx are covered with short hairs. The calyx is 4.5–5.5 mm in length and the corolla tube is about twice as long as the calyx. (ii) *T. scorodonia* subsp. *baeticum* has stems with petioles and leaves covered in patent and somewhat crispate hairs. The leaves are rounded at the apex and the inflorescence axis and calyx are covered with abundant short glandular hairs. The calyx is 6–7 mm in length and the length of the corolla tube scarcely exceeds that of the calyx.

There are only two reports on the chemical composition of essential oils of *T. scorodonia* L., one from Italy (Maccioni et al., 2007) and one from Spain (Velasco-Negueruela and Perez-Alonso, 1990). The former report describes the chemical composition of *T. scorodonia* subsp. *scorodonia*, which was characterized by the following hydrocarbon sesquiterpene compounds: germacrene B (26.2%), (*E*)-β-caryophyllene (25.2%), α-humulene (8.0%), α-cubebene (8.0%) and germacrene D (6.3%). The latter report describes the chemical compositions of Spanish *T. scorodonia* subsp. *scorodonia* and *T. scorodonia* subsp. *baeticum* from north and southwest Spain, respectively. The two oils were qualitatively similar but differed in the relative amounts of monoterpene compounds, the concentration of which was greater in the *scorodonia* subspecies (10.1–20.1%) than in the *baeticum* subspecies (3.5–6.9%). The major compounds in *T. scorodonia* subsp. *scorodonia* and *T. scorodonia* subsp. *baeticum* subspecies were sesquiterpene compounds: an unresolved mixture of aristolene and (*E*)-β-caryophyllene (12.3–21.0% and 35.1–39.7%, respectively), germacrene D (6.4–13.4% and 4.9–11.3%, respectively), aromadendrene (0.9–6.5% and 10.6–14.0%, respectively) and an unresolved mixture of caryophyllene oxide and spathulenol (4.9–13.0% and 5.0–9.1%, respectively).

Very few studies have been conducted on the genetic structure of *Teucrium* species. El Oualidi et al. (1999) were able to distinguish between several species of *Teucrium* using ITS sequences. Boulila et al. (2010) used random amplified polymorphic DNA markers to study genetic diversity among and within seven Tunisian *T. polium* L. populations from five bioclimatic areas. Their results showed that the polymorphisms coincided with the bioclimatic zones. Chemical composition often varies between plant populations (Southwell et al., 2010; Zhang et al., 2010). Such variation has been attributed to environmental or genetic factors (Adams et al., 2003). Some authors have reported correlations between genetic markers and chemical composition within populations (Anamika et al., 2010; Bezic et al., 2009; De Pasquale et al., 2006; Khanuja et al., 2005; Morone-Fortunato et al., 2010; Paolini et al., 2009), but others have not detected any correlation between these two factors (Hadian et al., 2011). Maintenance of a minimum level of intra- and interpopulation genetic diversity is important for the genetic fitness of a species (Barret and Kohn, 1991). Therefore, quantification of the diversity of genetic and chemical factors is important for understanding the adaptive ability of a species and for developing conservation programs.

In the course of our characterization of Mediterranean *Teucrium* species (Cozzani et al., 2005; Djabou et al., 2010, 2011; Muselli et al., 2009), we investigated the chemical and genetic diversity of *T. scorodonia* L. subsp. *scorodonia* from Corsica and *T. scorodonia* L. subsp. *baeticum* from western Algeria (Fig. 1, Supplementary material). In the one hand, possible environmental effects were assessed according to the chemical composition of essential oils which was studied using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. On the other hand, genetic diversity of two subspecies of *T. scorodonia* was quantified using chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) sequence data. cpDNA is maternally inherited and is dispersed through seeds, whereas nrDNA is inherited from both parents and is dispersed through pollen. Genetic structure was mapped using three polymorphic genetic markers: two chloroplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region). These markers are heterogeneous at the inter- and intraspecies levels (Wares and Cunningham, 2001; Petit et al., 2003). To our knowledge, our study is the first on the genetic structure of *T. scorodonia* L.

2. Results and discussion

2.1. Essential oil composition

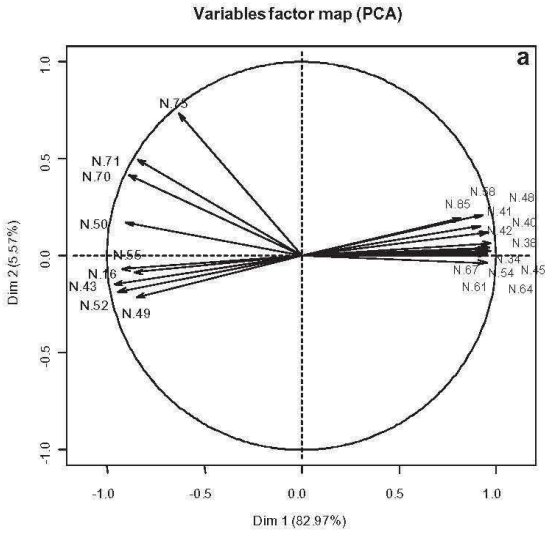
Preliminary analysis showed that the ten GC chromatograms of Corsican *T. scorodonia* subsp. *scorodonia* oil samples were similar. In the same way, the 17 GC chromatograms of Algerian *T. scorodonia* subsp. *baeticum* oil samples exhibited the same patterns. In addition, a few differences were observed between the GC chromatograms of both subspecies (Fig. 2, Supplementary material). Therefore, all Corsican *T. scorodonia* subsp. *scorodonia* oils were pooled and all Algerian *T. scorodonia* subsp. *baeticum* oils were pooled. They were then fractionated using column chromatography and reanalyzed using GC and GC/MS.

Integrated analysis of both total oils (HE1 and HE2) from Corsican *T. scorodonia* subsp. *scorodonia* oil samples and Algerian *T. scorodonia* subsp. *baeticum*, allowed the identification of 84 compounds, accounting for 98.2 and 98.8 g/100 g of the total (Table 1). Seventy four of which were verified by comparing their EI-MS and retention indices with those in our laboratory library. Ten components were identified by comparing their EI-MS and apolar retention indices with those of commercial or literature libraries. Of these, 18 were monoterpenes (nine hydrocarbon and nine oxygenated), 46 were sesquiterpenes (29 hydrocarbon and 17 oxygenated), two were oxygenated diterpenes, 14 were nonterpenic compounds (10 acyclic and four aromatic), three C_{13}-norisoprenoid compounds and one phenylpropanoid compound. The main components of *T. scorodonia* subsp. *scorodonia* (HE1) were (*E*)-β-caryophyllene **43** (20.5 g/100 g), germacrene B **67** (8.3 g/100 g), α-humulene **49** (6.9 g/100 g), germacrene D **52** (6.7 g/100 g), α-cubebene **34** (6.2 g/100 g) and γ-elemene **45** (3.9 g/100 g). The main components of *T. scorodonia* subsp. *baeticum* (HE2) were (*E*)-β-caryophyllene **43** (35.4 g/100 g), germacrene D **52** (22.1 g/100 g), α-humulene **49** (9.3 g/100 g), caryophyllene oxide **71** (4.3 g/100 g) and linalool **16** (3.5 g/100 g). All compounds present in the Algerian HE2 total oil sample were also present in the Corsican HE1 oil, except for styrene **3**, *allo*-ocimene **20**, β-copaene **44** and 4-epicubenol **53**.

It is noteworthy that the *T. scorodonia* oils contained three unusual C_{13}-norisoprenoid compounds referred to as megastigmanes: (*E*)-β-damascenone and theaspirane I and II. (*E*)-β-Damascenone is present in low amounts (<0.3%) in Corsican *T. chamaedrys* oils (Muselli et al., 2009), *T. massiliense* oils (Djabou et al., 2010) and *T. flavum* oils (Djabou et al., 2011) and could be considered as taxonomic marker.

To identify the relationship between the chemical composition of oil and taxonomic classification, principal component analysis and

PCA: Distribution of variables (component codes corresponding to those of Table 1)



compounds	N°
linalool	16
α-cubebene	34
α-copaene	38
β-cubebene	40
β-elemene	41
α-gurjunene	42
(E)-β-caryophyllene	43
γ-elemene	45
(E)-β-farnesene	48
α-humulene	49
alloaromadendrene	50
germacrene D	52
zingiberene	54
bicyclogermacrene	55
β-bisabolene	58
δ-cadinene	61
(E)-α-bisabolene	64
germacrene B	67
spathulenol	70
caryophyllene oxyde	71
humulene epoxide II	75
(E)-phytol	85

PCA: Distribution of samples (coding numbers of locations).

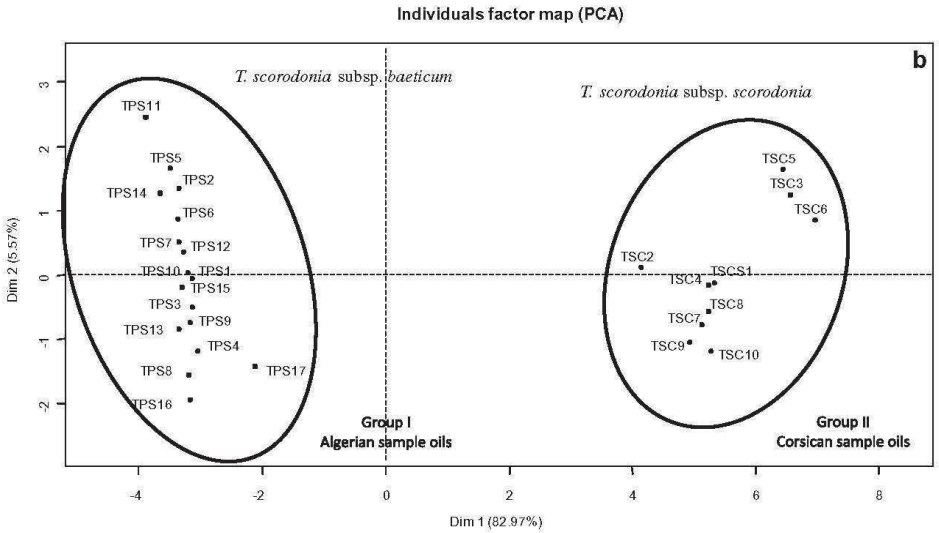


Fig. 1. PCA of chemical compositions of *T. scorodonia* subsp. *scorodonia* (TSC) and *T. scorodonia* subsp. *baeticum* (TPS) oils. (a) Distribution of variables (component codes corresponding to those of Table 1). (b) Distribution of samples (coding numbers of locations).

cluster analysis were applied to a matrix linking essential oil composition to subspecies identity. Normalized percentage abundances of all identified components were used for statistical analysis (Table 1, Supplementary material). Oils produced from both the ten Corsican *T. scorodonia* subsp. *scorodonia* and the 17 *T. scorodonia* subsp. *baeticum* samples were dominated by sesquiterpene hydrocarbon compounds (75.6–82.9% and 69.6–79.4%, respectively), but they differed in the amount of oxygenated sesquiterpene compounds (8.4–20.3% for *T. scorodonia* subsp. *baeticum* oils and 3.1–8.9% for *T. scorodonia* subsp. *scorodonia* oils). The data presented in Figs. 1 and 2 were obtained from the correlation matrix and the standardized matrix. Figure 1a shows the distributions of the volatile compounds **16**, **34**, **38**, **40**, **41**, **42**, **43**, **45**, **48**, **49**, **50**, **52**, **54**, **55**, **58**, **61**, **64**, **67**, **70**, **71**, **75** and **85** choose as statistical variables. The selected compounds were at least once higher than 2.5%. As shown in Fig. 1a and b, the principal component analysis (constructed using

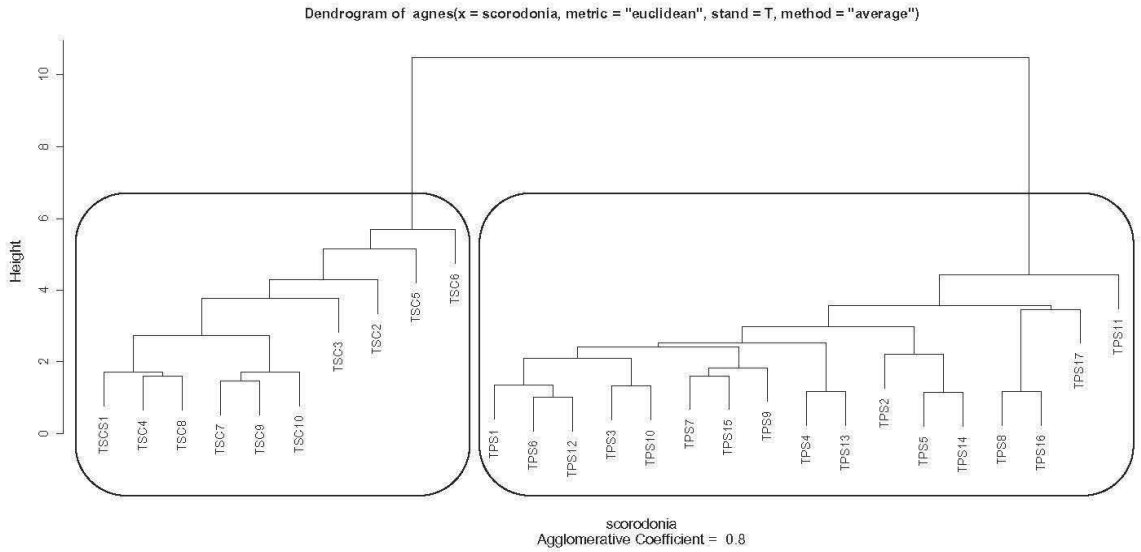


Fig. 2. CA of chemical compositions of *T. scorodonia* subsp. *scorodonia* (TSC) from Corsica and *T. scorodonia* subsp. *baeticum* (TPS) oils from Algeria.

axes 1 and 2) accounted for 88.54% of all oils. Concerning the hydrocarbon sesquiterpenes, the F1 axis (82.97%) were positively correlated with **34**, **38**, **40**, **41**, **42**, **45**, **48**, **54**, **58**, **61**, **64** and **67** and negatively correlated with **43**, **49**, **50**, **52** and **55** (Fig. 1a).

The plot established using the first two axes suggests that there are two main groups (Fig. 1b). This is in accordance with the general structure of the dendrogram obtained from the CHA (Fig. 2). Group I included 17 *T. scorodonia* subsp. *baeticum* oil samples from Algeria (TPS1–17) and group II included 10 *T. scorodonia* subsp. *scorodonia* oil samples from Corsica (TSC1–10). Group I was characterized by a higher amount of (*E*)-β-caryophyllene **43** (31.5–37.1%) and germacrene D **52** (18.1–26.8%) than group II (17.3–25.2% and 4.6–10.1%, respectively). Corsican oil samples in group II contained germacrene B **67** (4.2–8.8%) and γ-elemene **45** (2.6–5.7%), which were not detected in the Algerian oil samples of group I. The presence of γ-elemene in *T. scorodonia* subsp. *scorodonia* oils and its absence from *T. scorodonia* subsp. *baeticum* oils is a result of Cope rearrangement: γ-elemene is considered an artifact of germacrene B (Adio, 2009). The two compounds are structurally related because germacranes are formed by ring closure at carbons 1 and 10 of farnesanes. The presence of sesquiterpenes belonging to the germacrene group (germacrene A, B, C, D and E) in essential oils could be associated with environmental variables. Soil pH and the presence of divalent metal ions such as Mg^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+} affect the production of these compounds by plants (Duarte et al., 2010). It appears that differences in oil composition between the Corsican and Algerian samples could be caused by environmental conditions or could be related to their taxonomic classifications.

T. scorodonia subsp. *scorodonia* oil from Corsica was similar to that from Italy (Maccioni et al., 2007) in that germacrene B was the major compound (26.2%). *T. scorodonia* subsp. *scorodonia* and *T. scorodonia* subsp. *baeticum* oils from Spain (Velasco-Negueruela and Perez-Alonso, 1990) were similar to Algerian *T. scorodonia* subsp. *baeticum* oils in that germacrene B was not present.

2.2. DNA analysis

Nucleotide sequences of the ITS region and the two cpDNA noncoding regions were determined for 30 leaf samples of *T. scorodonia* subsp. *scorodonia* from 10 sites in Corsica and for 51 leaf samples of *T. scorodonia* subsp. *baeticum* from 17 sites in Algeria (Fig. 1, Supplementary material).

The total lengths of the noncoding regions were 864 bp for *TRNL-F* and 562 bp for *RPL32-TRNL* and the total length of the ITS region was 592 bp (230, 163 and 199 bp for ITS1, 5.8s and ITS2, respectively). The sequences were deposited in the GenBank database (Accession Nos. JF694842–JF694868 for *TRNL-F*, JF694896–JF694922 for *RPL32-TRNL* and JF694869–JF694895 for the ITS region). Individuals from the same location had identical sequences for each marker.

Based on the similarity of the genetic diversity parameters for the two cpDNA markers for each subspecies, we concatenated the two sequences for later analysis. The haplotype diversity (H) for the concatenated sequences of subspecies *scorodonia* and subspecies *baeticum* was 0.378 and 0.118, respectively, and the nucleotide diversity (π) was 0.00043 and 0.00008, respectively. Genetic parameters are presented in Table 2.

The ITS region showed greater diversity than cpDNA irrespective of the subspecies (Table 2). This is probably because of the difference in the mode of transmission of these markers, i.e., via pollen for ITS and via seed for cpDNA. Variations in the ITS region were present exclusively in noncoding regions (ITS1 and ITS2); the 5.8S gene was highly conserved in *T. scorodonia* L.

cpDNA regions were characterized by few mutations, but also by many gaps between the two subspecies: positions 255–265 for *T. scorodonia* subsp. *scorodonia*, and positions 554–563, 1158–1167 and 1185–1192 for *T. scorodonia* subsp. *baeticum*.

The unweighted pair group method with arithmetic mean (UPGMA) and neighbor-joining (NJ) trees constructed from the cpDNA and ITS region sequences showed the same partitioning, which indicates the existence of two groups correlated with taxonomic classification, one consisting of *T. scorodonia* subsp. *scorodonia* and the other consisting of *T. scorodonia* subsp. *baeticum*. NJ trees are presented in Fig. 3. Differentiation between these two groups was consistently indicated by high bootstrap values (>50%). The differentiation between subspecies was illustrated by the significantly higher Fst values: 0.5520 for ITS and 0.8315 for cpDNA (Table 2). Differentiation was also confirmed by AMOVA

Table 1
Chemical compositions of Corsican *T. scorodonia* subsp. *scorodonia* and Algerian *T. scorodonia* subsp. *baeticum* essential oils.

No.[a]	Compounds				Corsica					Algeria					Identification[g]
		/RI$_l$[b]	RI$_a$[c]	RI$_p$[d]	HE1[e]	Mean[f]	SD[f]	Min[f]	Max[f]	HE2[e]	Mean[f]	SD[f]	Min[f]	Max[f]	
1	(*E*)-2-Hexenal	832	834	1211	0.1	0.1	0.0	tr	0.1	tr	0.1	0.0	tr	0.1	RI, MS, Ref.
2	(*Z*)-Hex-3-en-1-ol	851	843	1368	0.1	0.1	0.0	tr	0.1						RI, MS
3	Styrene	873	874	1248						0.1	0.1	0.0	tr	0.2	RI, MS
4	Benzaldehyde	941	931	1512	0.3	0.4	0.5	tr	1.4						RI, MS
5	Oct-1-en-3-ol	962	963	1429	0.9	1.1	0.5	0.4	1.9	0.7	0.7	0.4	0.1	1.6	RI, MS
6	Sabinene	973	966	1110	1.1	0.9	0.5	tr	1.5						RI, MS
7	3-Octanol	981	982	1385	0.3	0.3	0.2	tr	0.5	0.1	0.1	0.0	tr	0.1	RI, MS
8	Myrcene	987	986	1145	0.1	0.1	0.0	tr	0.1	tr	0.1		tr	0.1	RI, MS
9	α-Terpinene	1013	1010	1170	0.1	0.1	0.0	tr	0.1	tr	0.1		tr	0.1	RI, MS
10	Limonene	1025	1023	1186	1.2	0.9	0.6	0.1	1.6						RI, MS
11	(*Z*)-β-Ocimene	1029	1027	1207	0.9	0.8	0.5	0.2	1.5	0.6	0.8	0.5	0.2	1.9	RI, MS
12	(*E*)-β-Ocimene	1041	1037	1221	0.3	0.4	0.2	0.1	0.7	0.3	0.2	0.1	tr	0.5	RI, MS
13	γ-Terpinene	1051	1049	1230	0.1	0.1	0.0	tr	0.1						RI, MS
14	Terpinolene	1082	1080	1263	0.8	0.8	0.5	0.1	1.3						RI, MS
15	Nonanal	1086	1083	1394	0.4	0.3	0.2	0.1	0.5	0.1	0.1	0.0	tr	0.1	RI, MS
16	Linalool	1083	1085	1514	0.5	0.7	0.9	0.3	0.9	3.5	4.0	1.3	2.1	6.2	RI, MS
17	*cis*-Sabinene hydrate	1087	1090	1528	0.1	0.1	0.0	tr	0.1						RI, MS
18	1-Oct-3-enyl acetate	1093	1094	1363	0.1	0.1	0.0	tr	0.1						RI, MS, Ref.
19	3-Octanyl-acetate	1113	1108	1315	0.1	0.1	0.0	tr	0.1						RI, MS
20	*allo*-Ocimene	1118	1119	1364						0.1	0.1	0.0	tr	0.2	RI, MS
21	2-Methyl benzofurane	1149	1146	1570	0.1	0.1	0.0	tr	0.1						RI, MS, Ref.
22	Terpinen-4-ol	1164	1163	1562	0.3	0.3	0.1	0.1	0.4						RI, MS
23	Methyl salicylate	1171	1170	1720	0.2	0.1	0.0	tr	0.2	0.2	0.1	0.1	tr	0.4	RI, MS
24	α-Terpineol	1176	1173	1700	0.2	0.1	0.1	tr	0.3	0.2	0.2	0.1	tr	0.3	RI, MS
25	Estragole	1175	1176	1645	0.1	0.1	0.0	tr	0.1						RI, MS
26	Myrtenol	1178	1187	1764	0.1	0.1	0.0	tr	0.1						RI, MS
27	Decanal	1188	1197	1498	0.1	0.1	0.0	tr	0.1						RI, MS
28	Nerol	1210	1211	1787	0.1	0.1	0.0	tr	0.2	0.1	0.1	0.2	tr	0.1	RI, MS
29	Geraniol	1235	1235	1801	0.1	0.1	0.0	tr	0.2	0.2	0.2	0.1	tr	0.4	RI, MS
30	Decanol	1268	1269	1749	0.1	0.1	0.0	tr	0.1	0.1	0.1	0.0	tr	0.2	RI, MS, Ref.
31	Theaspirane I	1299	1291	1480	0.1	0.1	0.0	tr	0.1	0.1	0.1	0.0	tr	0.1	RI, MS
32	Theaspirane II	1313	1306	1517	0.1	0.1	0.0	tr	0.1	0.1	0.1	0.0	tr	0.1	RI, MS
33	Neryl acetate	1342	1344	1701	0.1	0.1	0.1	tr	0.3						RI, MS
34	α-Cubebene	1355	1351	1442	6.2	7.8	2.7	5.6	11.3	0.1	0.2	0.2	tr	0.6	RI, MS
35	Geranyl acetate	1362	1362	1768	0.1	0.2	0.2	0.1	0.6	0.2	0.1	0.0	tr	0.2	RI, MS
36	(*E*)-β-Damascenone	1363	1366	1770	0.1	0.1	0.0	tr	0.1						RI, MS
37	α-Ylangene	1376	1371	1470	0.5	0.6	0.1	0.5	0.8	0.4	0.4	0.2	0.2	1.1	RI, MS
38	α-Copaene	1379	1377	1475	2.1	2.3	0.6	1.3	3.5						RI, MS
39	*β*-Bourbonene	1386	1383	1500	0.1	0.2	0.2	tr	0.3	0.9	0.8	0.4	0.2	1.3	RI, MS
40	*β*-Cubebene	1390	1388	1513	2.3	3.0	0.7	2.0	4.5						RI, MS
41	*β*-Elemene	1389	1388	1571	1.9	1.7	0.5	0.8	2.8						RI, MS
42	α-Gurjunene	1413	1411	1510	3.5	2.9	1.0	0.4	4.4						RI, MS
43	(*E*)-β-Caryophyllene	1421	1423	1568	21.1	20.8	5.1	17.3	25.2	35.4	33.8	1.7	30.8	37.1	RI, MS
44	β-Copaene	1430	1431	1572						0.4	0.3	0.2	0.2	1.0	RI, MS
45	γ-Elemene	1429	1432	1616	3.9	4.3	1.2	2.6	5.7						RI, MS, Ref.
46	α-Guaiene	1440	1437	1538	0.4	0.5	0.1	0.3	0.7						RI, MS
47	Isogermacrene D	1445	1446	1681	0.1	0.1	0.1	0.1	0.2						RI, MS, Ref.
48	(*E*)-β-Farnesene	1446	1455	1661	1.8	1.8	0.8	0.9	3.1						RI, MS
49	α-Humulene	1455	1455	1665	6.9	6.4	1.4	4.6	8.3	9.3	9.1	0.6	7.2	10.2	RI, MS
50	Alloaromadendrene	1462	1459	1638	0.3	0.3	0.4	0.2	0.5	1.7	1.9	0.5	1.3	2.9	RI, MS
51	α-Curcumene	1473	1473	1769	0.9	0.7	0.5	0.2	1.6						RI, MS
52	Germacrene-D	1479	1479	1666	6.7	6.2	5.0	4.6	10.1	22.1	22.2	3.1	18.2	25.8	RI, MS
53	4-Epicubenol	1485	1486	1863						0.2	0.2	0.1	0.1	0.3	RI, MS

(*continued on next page*)

Table 1 (*continued*)

No.[a]	Compounds				Corsica					Algeria					Identification[g]
		$/RI_l$[b]	RI_a[c]	RI_p[d]	HE1[e]	Mean[f]	SD[f]	Min[f]	Max[f]	HE2[e]	Mean[f]	SD[f]	Min[f]	Max[f]	
54	Zingiberene	1489	1490	1717	3.8	3.4	1.2	2.1	4.5	0.4	0.3	0.1	0.2	0.4	RI, MS
55	Bicyclogermacrene	1494	1492	1727	0.4	0.3	1.1	0.2	0.9	3.8	3.1	0.6	1.9	4.2	RI, MS
56	α-Muurolene	1496	1496	1719	0.3	0.4	0.1	0.3	0.7	0.1	0.2	0.1	tr	0.5	RI, MS
57	(*E*,*E*)-α-Farnesene	1498	1499	1744	0.4	0.4	0.2	0.1	0.8						RI, MS
58	β-Bisabolene	1503	1504	1720	3.3	2.8	0.9	1.7	4.2	0.6	0.5	0.2	0.1	0.8	RI, MS
59	γ-Cadinene	1507	1508	1749	0.5	0.6	0.2	0.3	0.9	0.1	0.2	0.2	tr	0.8	RI, MS
60	*trans*-Calamenene	1517	1512	1816	0.2	0.2	0.1	0.1	0.3	0.1	0.1	0.0	tr	0.2	RI, MS
61	δ-Cadinene	1520	1518	1752	2.5	2.4	0.4	2.1	2.9	1.2	1.0	0.3	0.6	1.8	RI, MS
62	β-Sesquiphellandrene	1516	1518	1765	0.7	0.6	0.2	0.4	0.8	0.3	0.2	0.1	tr	0.4	RI, MS
63	α-Cadinene	1534	1531	1743	0.3	0.2	0.1	0.2	0.3	0.6	0.5	0.1	0.3	0.7	RI, MS
64	(*E*)-α-Bisabolene	1530	1537	1733	1.3	1.4	0.6	0.8	2.2						RI, MS
65	β-Caryophyllene oxide	1546	1540	1964	0.2	0.3	0.1	0.2	0.4						RI, MS, Ref.
66	Dodecanoic acid	1554	1546	1874	0.2	0.2	0.1	0.1	0.3						RI, MS, Ref.
67	Germacrene B	1552	1556	1820	8.3	6.5	1.7	4.2	8.8						RI, MS, Ref.
68	(*E*)-Nerolidol	1553	1556	2037	0.1	0.1	0.0	tr	0.1						RI, MS
69	Palustrol	1569	1562	1920	0.1	0.1	0.0	0.1	0.1						RI, MS
70	Spathulenol	1572	1566	2119	0.3	0.3	0.9	0.1	0.9	3.2	2.4	0.7	0.8	3.6	RI, MS
71	Caryophyllene oxide	1578	1571	1980	1.0	1.0	1.1	0.3	1.6	4.3	4.5	1.4	2.1	6.8	RI, MS
72	Globulol	1589	1580	2074	0.1	0.1	0.0	0.1	0.2	0.2	0.2	0.1	0.1	0.4	RI, MS
73	Viridiflorol	1592	1586	2089	0.1	0.1	0.1	0.1	0.3	0.2	0.2	0.1	0.1	0.3	RI, MS
74	Ledol	1600	1595	2029	0.2	0.2	0.1	0.1	0.4	0.2	0.1	0.1	tr	0.4	RI, MS
75	Humulene epoxide II	1602	1598	2044	0.9	0.6	0.3	0.2	1.1	1.3	1.3	0.5	0.6	2.6	RI, MS
76	1,10-Diepicubenol	1615	1610	2031	0.2	0.2	0.1	tr	0.3	0.3	0.3	0.1	0.1	0.6	RI, MS
77	Epicubenol	1623	1617	2039	0.3	0.4	0.1	0.2	0.5	0.3	0.2	0.1	0.1	0.4	RI, MS
78	Cubenol	1630	1623	2003	0.2	0.2	0.1	tr	0.5	0.2	0.3	0.1	0.1	0.4	RI, MS
79	Caryophylla-4(14),8(15)-dien-5α-ol	1626	1629	2275	0.8	0.7	0.4	0.4	1.6	0.3	0.3	0.2	tr	0.8	RI, MS
80	τ-Muurolol	1633	1633	2153	0.6	0.4	0.4	0.1	1.1	1.4	1.2	0.3	0.6	1.8	RI, MS
81	τ-Cadinol	1633	1641	2159	0.7	0.8	0.3	0.4	1.4	0.9	0.6	0.2	0.4	0.9	RI, MS
82	α-Cadinol	1643	1645	2211	0.2	0.2	0.4	0.1	0.4	1.3	1.7	0.4	1.2	2.6	RI, MS
83	Hexahydrofarnesyl acetone	1817	1831	2125	0.5	0.4	0.4	0.1	1.2						RI, MS, Ref.
84	(*E*)-Phytol	2114	2104	2588	1.4	1.9	1.2	1.1	4.6	0.3	0.3	0.2	tr	0.8	RI, MS
	Total identification				98.2					98.8					
	Yields (v/w)					0.068	0.010	0.053	0.084		0.056	0.008	0.041	0.084	
	% Hydrocarbon compounds				85.3					78.6					
	% Monoterpene hydrocarbons				4.6					1.1					
	% Sesquiterpene hydrocarbons				80.7					77.5					
	% Oxygenated compounds				12.9					20.2					
	% Oxygenated monoterpenes				2.0					4.4					
	% Oxygenated sesquiterpenes				6.0					14.3					
	% Non-terpenic oxygenated compounds				3.5					1.2					
	% Diterpene oxygenated compounds				1.4					0.3					

[a] Order of elution is given on apolar column (Rtx-1).
[b] Retention indices of literature on the apolar column ($/RI_l$) reported from Joulain and Konig (1998).
[c] Retention indices on the apolar Rtx-1 column (RI_a).
[d] Retention indices on the polar Rtx-Wax column (RI_p).
[e] HE1: Mixture of all *T. scorodonia* subsp. *scorodonia* sample oils from Corsica, concentrations in g/100 g. HE2: Mixture of all *T. scorodonia* subsp. *baeticum* sample oils from Algeria, concentrations in g/100 g.
[f] Normalized percentage abundances obtained from all sample oils from Corsica and Algeria respectively, given on the apolar column except for components with identical RI_a (% on the polar column), tr = trace (<0.05%).
[g] RI: retention indices; MS: mass spectra in electronic impact mode; Ref.: compounds identified from literature data: Konig et al. (2001) (**1**, **19**, **22**, **31**, **46**, **48**, **66**, **67**, **68** and **83**).

Table 2
Genetic parameters on ITS region and cpDNA markers of *T. scorodonia* L.

Markers	*Teucrium scorodonia* subspecies	*N*	Polymorphic sites *N*	Haplotypes or ribotypes *N*	*H*	*p*	*k*	Fst
ITS	*Baeticum*	51	3	4	0.551	0.00122	0.721	0.552
	Scorodonia	30	8	5	0.756	0.00461	2.733	
cpDNA	*Baeticum*	51	1	2	0.118	0.00008	0.118	0.831
	Scorodonia	30	3	3	0.378	0.00043	0.6	

H = haplotype or ribotype diversity; *p* = nucleotide diversity; *k* = average number of nucleotide differences.

(Table 3), which showed that the largest proportion of the variation was accounted for by variation between the two subspecies (87% for ITS and 96% for cpDNA).

The genetic structures of these subspecies do not appear to be correlated with altitude. Mantel tests performed using both types of markers and altitude produced nonsignificant results (*T. scorodonia* subsp. *scorodonia*: $r = -0.121$, $P = 0.823$ for ITS and $r = 0.101$, $P = 0.712$ for cpDNA; *T. scorodonia* subsp. *baeticum*: $r = -0.124$, $P = 0.703$ for ITS and $r = -0.023$, $P = 0.956$ for cpDNA).

Analysis of various genetic parameters showed that *T. scorodonia subsp. scorodonia* exhibited more variability than *T. scorodonia* subsp. *baeticum*, irrespective of the type of marker. This variability could be explained by the sizes of the harvest areas; *T. scorodonia* subsp. *scorodonia* was collected in Corsica from an area of 4700 km^2, whereas *T. scorodonia* subsp. *baeticum* was harvested in Algeria from an area of only 600 km^2. This difference could explain the lower variability observed in *T. scorodonia* subsp. *baeticum* with both types of markers (Table 3).

Table 3
Analysis of molecular variance (AMOVA) for populations of *T. scorodonia* L. based on cpDNA and nrDNA.

Source of variation	*df*	Percentage of variation	
		ITS	cpDNA
Among groups	1	87.09233	95.93745
Among populations within groups	3	3.19890	0.82530
Within populations	18	9.70878	3.23725

Two groups: *T. scorodonia* subsp. *scorodonia* and *T. scorodonia* subsp. *baeticum*.

2.3. Concluding remarks

The molecular techniques used in this study proved to be powerful tools for studying inter- and intraspecific variation and for elucidating the influence of genotype on chemical profile (Anamika et al., 2010; De Pasquale et al., 2006; Morone-Fortunato et al., 2010; Paolini et al., 2009). At group level, the population structure mapped using genetic markers was similar to that observed using chemical composition (Figs. 1–3). The concordance between the two data sets is in accordance with the findings of others who used terpenoids and genetic markers (Zaouali and Boussaid, 2008). Taken together, these data suggest that variation in the essential oil composition of *T. scorodonia* subspecies depends more on genetic background than environmental characteristics.

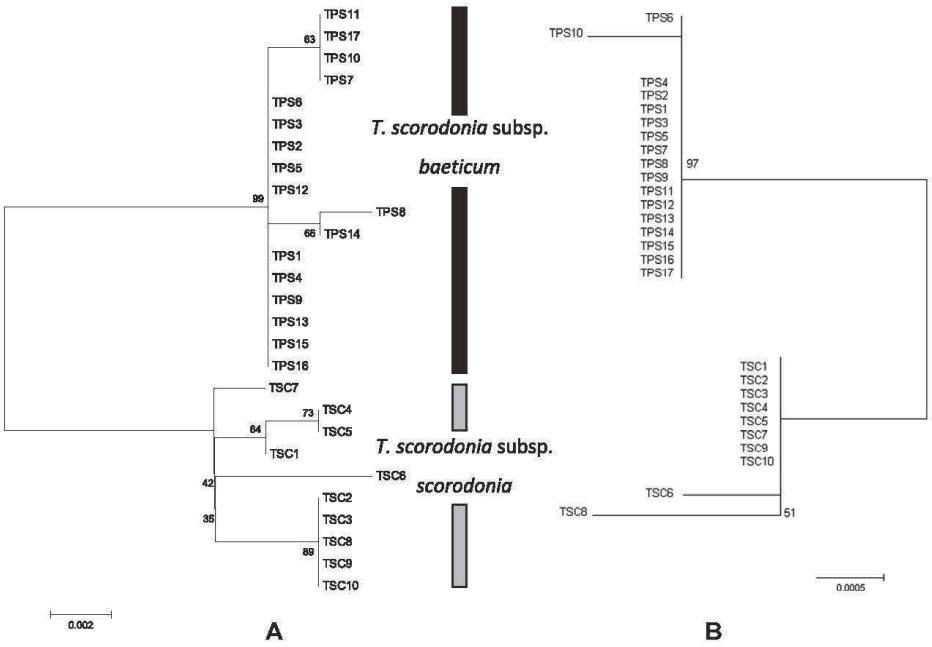


Fig. 3. Neighbor joining trees of *Teucrium scorodonia* subspecies (numbers at the node indicate bootstrap values). (A) ITS region sequences. (B) cpDNA sequences.

The present study revealed a significant difference in diversity between subspecies of *T. scorodonia* according to chemical and DNA analyses. As the nuclear markers used in this study are characterized by their neutrality, they were not linked to the biosynthesis of a specific chemical compound. Further studies on polymorphisms and the expression of genes involved in the biosynthesis of terpenes could provide additional information on the structures of plant populations.

The study suggests that the structures of nuclear and chloroplast markers are linked to the characteristics of essential oils in both subspecies. As altitude had no influence on the genetic structure of either population of *T. scorodonia* subspecies, it is possible that the observed phenotypic differences were genetically based. This study indicates that conservation strategies are needed because of the threat of overgrazing and excessive harvesting.

3. Experimental

3.1. Plant material and oil isolation

The aerial parts of *T. scorodonia* L. subsp. *scorodonia* from Corsica were collected at the flowering stage (June to July 2009) at 10 Corsican locations (TSC1–10). Voucher specimens were deposited with the herbarium of the University of Corsica. The aerial parts of *T. scorodonia* L. subsp. *baeticum* (= *T. pseudoscorodonia* Desf.) from western Algeria were collected at the flowering stage (May to June 2010) from 17 locations (TPS1–17). Voucher specimens were deposited with the herbarium of the University of Tlemcen. The altitude of the sites from which samples were obtained ranged from 50 to 1100 m in Corsica and from 40 to 700 m in Algeria (Fig. 1, Supplementary material).

To obtain essential oils, the fresh aerial parts of both subspecies (400–500 g) were subjected to hydrodistillation for 5 h using a Clevenger-type apparatus according to the *European Pharmacopoeia* (Council of Europe, 1997), yielding 0.053–0.084% and 0.041–0.068% of yellow oil for *T. scorodonia* subsp. *scorodonia* and *T. scorodonia* subsp. *baeticum*, respectively. For genetic studies, leaves from both subspecies were sampled from all populations described above. In total, 81 individual plants were sampled (three plants per population); all samples were obtained from plants 1 to 50 m from each other and were collected randomly.

3.2. Oil fractionation

Two collective oils called HE1 and HE2 were obtained by the mixture of all Corsican and all Algerian samples, respectively. Both oils (1 g for each oil) were consecutively submitted to chromatography on a normal phase silica column (12 g, 15–40 mm, 60 Å) using an automatized Combi Flash apparatus (Teledyne Isco, Inc. Lincoln, NE, USA) equipped with a fraction collector monitored by an UV-detector. For Corsican (HE1) and Algerian (HE2) oils, the apolar fractions (hydrocarbon compounds, 0.82 and 0.79 g, respectively) were eluted with hexane (n-C_6H_{14}) and the polar fractions (oxygenated compounds, 0.15 and 0.18 g, respectively) were eluted with diisopropyloxide ($(C_3H_7)_2O$). All fractions were analyzed by GC and GC–MS. We checked that the compounds identified in the column chromatography fractions were present in the individual oil samples by comparing their retention indices (RI_a, RI_p) and Electronic Impact mass spectra (EI-MS) with those of the total oil samples.

3.3. Gas chromatography

GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60–230 °C at 2 °C min^{-1} and then held isothermal 230 °C (30 min). Carrier gas: helium (1 mL min^{-1}). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 μL.

3.4. Gas chromatography–mass spectrometry

The oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. Ion source temperature: 150 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired with a mass range of 35–350 Da; scan mass: 1 s. Oil injected volume: 0.1 μL, fraction injected volume: 0.2 μL.

3.5. Component identification

Identification of the components was based (i) on the comparison of their GC retention indices (RI) on non-polar and polar columns, determined to the retention time of a series of n-alkanes with linear interpolation, with those of authentic compounds or literatures data (Mass Finder, 2006); (ii) on computer matching with commercial mass spectral libraries (McLafferty and Stauffer, 2006; NIST/EPA/NIH, 1999; Adams, 2001; Mass Finder, 2006) and comparison of spectra with those of our laboratory-made library.

3.6. Component quantification

The quantification of the collective essential oil components was carried out using the methodology reported by Costa et al. (2008) and modified in our laboratory (Djabou et al., 2010). To estimate the concentrations of the constituents of the oils, we calculated response factors for all chemical groups relative to tridecane used as internal standard. The concentrations of the oil components are listed in Table 1. The normalized% abundances were calculated, using the methodology reported by (Bicchi et al., 2008) in order to perform the statistical analysis of the oil samples. Tridecane was introduced in all sample oils at same concentration (0.7 g/100 g) as internal standard.

3.7. Nuclear and chloroplast DNA markers

Total DNA extraction, primers, conditions of Polymerase Chain Reaction (PCR) and sequencing of cpDNA markers, *TRNL-F* and the *RPL32-TRNL* regions; have been described by Falchi et al. (2009). The ITS region, were amplified with primers ITS1, and ITS4, referring to the protocol described by White et al. (1990). The amplified products were run in a 1.2% agarose gel, stained with ethidium bromide, and visualized and photographed under UV light (Kodak Gel Logic, Sciencetech, Les Ulis, France). The purified PCR products were sequenced in both directions in an ABI 3700 automated sequencer (Applied Biosystems; PerkinElmer, Courtaboeuf, France), following standard methods and using the primers used for amplification. Sequences were aligned using the CLUSTALX program (Thompson et al., 1997).

3.8. Statistical analysis

Data analyses were performed using Principal Component Analysis (PCA) and Cluster Analysis (CA) (Brereton, 2003). Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results (Massart, 1998). Indeed, PCA provides the data for diagrams in which both objects (oil samples) and variables (oil components) are plotted while canonical analysis informs a classification tree in which objects (sample locations) are gathered. PCA was carried out using function 'PCA' from the statistical R software. The variables (volatile components) have been selected using function from the statistical software. The Cluster Analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of oil samples.

Haplotype and nucleotide diversity were determined using the DNASP 4.0 program (Rozas et al., 2003). For each marker, two analyses were performed using MEGA 4 program (Kumar et al., 2008). Neighbor Joining (NJ) and Unweighted Pair Group Method with Arithmetic mean (UPGMA) trees were built by calculating genetic distance based on Kimura's two parameters model (1980) with 1000 replicates. To reveal the geographic structure of each locus, analyses of molecular variance (AMOVA) were performed to assess the genetic differentiation among groups of populations, using Arlequin version 3.0 (Excoffier et al., 2005).

Genetic diversity (Fst) and Mantel tests were performed using ARLEQUIN version 3.0 software with 1000 replicates (Excoffier et al., 2005). The Mantel tests were conducted to try to correlate genetic distances to the altitude. These tests were conducted with the two subspecies of *T. scorodonia* and for each marker.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2011.09.002.

References

Adams, R., 2001. Identification of Essential Oils by Capillary Gas Chromatography/Mass Spectroscopy. Allured Publishing, Carol stream.

Adams, R., Schwarzbach, A., Pandey, R., 2003. The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from *Juniperus*. Biochem. Syst. Ecol. 31, 375–387.

Adio, A., 2009. Germacrene A–E and related compounds: thermal, photochemical and acid induced transannular cyclizations. Tetrahedron 65, 1533–1552.

Anamika, P., Thapa, G., Basu, A., Mazumdar, P., Kalita, M.C., Sahoo, L., 2010. Rapid plant regeneration, analysis of genetic fidelity and essential aromatic oil content of micropropagated plants of Patchouli, Pogostemon cablin (Blanco) Benth. – an industrially important aromatic plant. Ind. Crops Prod. 32, 366–374.

Awadh Ali, N.A., Wurster, M., Arnold, N., Lindequist, U., Wessjohan, L., 2008. Chemical composition of the essential oil of *Teucrium yemense* Deflers. Rec. Nat. Prod. 2, 25–32.

Barret, S., Kohn, J., 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, New York.

Beni Maleci, L., Pinetti, A., Servettaz, O., 1995. Micromorphological and phytochemical characters of two subspecies of *Teucrium flavum* (Labiatae) from Italian flora. Flora 190, 237–242.

Bezic, N., Samanic, I., Dunkic, V., Besendorfer, V., Puizina, J., 2009. Essential oil composition and internal transcribed spacer (ITS) sequence variability of four South-Croatian *Satureja* species (Lamiaceae). Molecules 14, 925–938.

Bicchi, C., Liberto, E., Matteodo, M., Sgorbini, B., Mondello, L., Zellner, B.A., Costa, R., Rubiolo, P., 2008. Quantitative analysis of essential oils: a complex task. Flav. Fragr. J. 23, 382–391.

Bosisio, E., Giavarini, F., Dell'Agli, M., Galli, G., Galli, C., 2004. Analysis by high-performance liquid chromatography of teucrin A in beverages flavoured with an extract of *Teucrium chamaedrys* L.. Food Addit. Contam. 21, 407–414.

Boulila, A., Béjaoui, A., Messaoud, C., Boussaid, M., 2010. Genetic diversity and population structure of *Teucrium polium* (Lamiaceae) in Tunisia. Biochem. Genet. 48, 57–70.

Brereton, R., 2003. Chemometrics: Data Analysis for the Laboratory and Chemical Plant. Wiley Interscience, New York.

Coll, J., Tandrón, Y.A., 2005. Isolation and structure elucidation of three neo-clerodane diterpenes from *Teucrium fruticans* L. (LABIATAE). Phytochemistry 66, 2298–2303.

Costa, R., Zellner, B.A., Crupi, M.L., Fina, M.R., Valentino, M.R., Dugo, P., Dugo, G., Mondello, L., 2008. GC–MS, GC–O and enantio–GC investigation of the essential oil of *Tarchonanthus camphoratus* L. Flav. Fragr. J. 23, 40–48.

Council of Europe, 1997. European Pharmacopoeia, third ed. Council of Europe, Strasbourg.

Cozzani, S., Muselli, A., Desjobert, J., Bernardini, A., Tomi, F., Casanova, 2005. Chemical composition of essential oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica. Flav. Fragr. J. 20, 436–441.

De Pasquale, F., Siragusa, M., Abbate, L., Tusa, N., De Pasquale, C., Alonzo, G., 2006. Characterization of five sour orange clones through molecular markers and leaf essential oils analysis. Hort. Sci. 109, 54–59.

Djabou, N., Paolini, J., Desjobert, J.M., Allali, H., Baldovini, N., Costa, J., Muselli, A., 2010. Quantitative and qualitative analysis of volatile components of *Teucrium massiliense* L. – identification of 6-methyl-3-heptyl acetate as a new natural product. Flav. Fragr. J. 25, 475–487.

Djabou, N., Batesti, M.J., Allali, H., Desjobert, J.M., Varesi, L., Costa, J., Muselli, A., 2011. Chemical and genetic differentiation of Corsican subspecies of *Teucrium flavum* L.. Phytochemistry 72, 1390–1399.

Duarte, A., Santos, S., Seraphin, J., Ferri, P., 2010. Environmental influence on phenols and essential oils of *Myrciaria cauliflora* leaves. J. Braz. Chem. Soc. 21, 1672–1680.

El Oualidi, J., Verneau, O., Puech, S., Dubuisson, J., 1999. Utility of rDNA ITS sequences in the systematics of *Teucrium* section *Polium* (Lamiaceae). Pl. Syst. Evol. 215, 49–70.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (Version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1, 47–50.

Falchi, A., Paolini, J., Desjobert, J.M., Melis, A., Costa, J., Varesi, L., 2009. Phylogeography of *Cistus creticus* L. on Corsica and Sardinia inferred by the TRNL-F and RPL32-TRNL sequences of cpDNA. Mol. Phylogenet. Evol. 52, 538–543.

Hadian, J., Ebrahimi, S., Mirjalili, M., Azizi, A., Ranjbar, H., Friedt, W., 2011. Chemical and genetic diversity of *Zataria multiflora* Boiss. accessions growing wild in Iran. Chem. Biodiversity 8, 176–188.

Jeanmonod, D., Gamisans, J., 2007. Flora Corsica, Edisud. La compagnie des éditions de la Lesse, Aix-en-Provence, pp. 630–640.

Khanuja, S., Shasany, A., Pawar, A., Lal, R., Darokar, M., Naqvi, A., Rajkumar, S., Sundaresan, V., Lal, N., Kumar, S., 2005. Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon Spreng* (Poaceae). Biochem. Syst. Ecol. 33, 171–186.

Konig, W.A., Hochmuth, D.H., Joulain, D., 2001. Terpenoids and Related Constituents of Essential oils., Library of Mass Finder 2.1 ed. Institute of Organic Chemistry, Hamburg.

Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: a biologist-centric software for evolutionary of DNA and protein sequences. Brief. Bioinformatics 9, 299–306.

Maccioni, S., Baldini, R., Tebano, M., Cioni, P.L., 2007. Essential oil of *Teucrium scorodonia* L. ssp. *scorodonia* from Italy. Food Chem. 104, 1393–1395.

Mass Finder, 2006. In: Hochmunth, D., Version 3.0, Scientific Consulting, Germany.

Massart, D., 1998. Chemometrics: A Textbook. Elsevier Science Ltd., New York.

McLafferty, F.W., Stauffer, D.B., 2006. Wiley Registry of Mass Spectral Data, eighth ed. Mass Spectrometry Library Search System Bench-Top/PBM. Palisade, Newfield.

Menichini, F., Conforti, F., Rigano, D., Formisano, C., Piozzi, F., Senatore, F., 2009. Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. Food Chem. 115, 679–686.

Morone-Fortunato, I., Montemurro, C., Ruta, C., Perrini, R., Sabetta, W., Blanco, A., Lorusso, E., Avato, P., 2010. Essential oils, genetic relationships and in vitro establishment of *Helichrysum italicum* (Roth) G. Don ssp. *italicum* from wild Mediterranean germplasm. Ind. Crops Prod. 32, 639–649.

Muselli, A., Costa, J., Desjobert, J., Dessi, M.A., Paolini, J., Bernardini, A., 2009. Chemical composition of the essential oils of *Teucrium chamaedrys* L. from Corsica and Sardinia. J. Essent. Oil Res. 21, 138–143.

National Institute of Standards and Technology, 1999. NIST/EPA/NIH Mass Spectral Library. PC Version 1.7. Perkin-Elmer Corp., Norwalk, CT, USA.

Paolini, J., Falchi, A., Quilichini, Y., Desjobert, J.M., De Cian, M.C., Varesi, L., Costa, J., 2009. Morphological, chemical and genetic differentiation of two subspecies of *Cistus creticus* L. (*C. creticus* subsp. *eriocephalus* and *C. creticus* subsp. *corsicus*). Phytochemistry 70, 1146–1160.

Petit, R., Aguinagalde, I., De Beaulieu, J., Bittkau, C., 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300, 1563–1565.

Pignatti, S., 1982. Flora d'Italia. Edagricole, Bologna.

Quezel, P., Santa, S., 1963. Nouvelle Flore de l'Algérie. Centre National de la Recherche Scientifique, Paris.

Rozas, J., Sanchez-Del Barrio, J., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19, 2496–2497.

Southwell, I., Russell, M., Tate, H., Tungon, J., Sam, C., Dickinson, G., Robson, K., Leakey, R., 2010. Geographic and phenotypic variation in heartwood and essential oil characters in natural populations of *Santalum austrocaledonicum* in Vanuatu. Chem. Biodiversity 7, 1990–2006.

Thompson, J., Gibson, T., Plewniak, F., Jeanmougin, F., Higgins, D., 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Tutin, T., Heywood, V.H., Burges, N., Moore, D., Valentie, D., Webb, D., 1972. Flora Europaea. Cambridge University Press, Cambridge.
Velasco-Negueruela, A., Perez-Alonso, M., 1990. The volatiles of six *Teucrium* species from the Iberian Peninsula and the Balearic Islands. Phytochemistry 29, 1165–1169.
Wares, J., Cunningham, C., 2001. Phylogeography and historical ecology of the North Atlantic intertidal. Evolution 55, 2455–2469.
White, T., Bruns, T., Lee, S., Taylor, J., 1990. In: Innes, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic press, San Diego, California.
Zaouali, Y., Boussaid, M., 2008. Isozyme markers and volatiles in Tunisian *Rosmarinus officinalis* L. (Lamiaceae): a comparative analysis of population structure. Bioch. Syst. Ecol. 36, 11–21.
Zhang, C., Qin, M., Shu, P., Hong, J., Lu, L., He, D., 2010. Chemical variations of the essential oils in flower heads of *Chrysanthemum indicum* L. from China. Chem. Biodiversity 7, 2951–2962.

[**PT4**] : ***Teucrium flavum*** **(deux sous-espèces)**

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Chemical and genetic differentiation of Corsican subspecies of *Teucrium flavum* L.

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ABSTRACT

Corsica Island exhibited the particularity to display *Teucrium flavum* subsp. *glaucum* and subsp. *flavum* on the same territory with the same bioclimatic conditions. For the first time, volatile components extracted from aerial parts and genetic diversity of both Corsican *T. flavum* L. subspecies have been investigated through (i) the characterization of the chemical composition of essential oils and (ii) the study of three polymorphic genetic markers. Chemical analysis were performed using combination of capillary GC/RI, GC-MS after fractionation on column chromatography and the definition of the genetic structure were carried out using two chlororoplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region). According to statistical analysis, both subspecies were clearly distinguished by the chemical and genetic studies. Chemical compositions of oils from both subspecies were qualitatively similar but they differed by the normalized% abundances of their major components; oils from subsp. *flavum* were dominated by large amounts of hydrocarbon monoterpenes while oils obtained from subsp. *glaucum* were characterized by higher amounts of oxygenated compounds. The genetic analysis divided *T. flavum* L. populations in two groups, the first displayed subsp. *glaucum* populations and the latter group exhibited subsp. *flavum* populations. The presence of two groups is weakly consistent with chemical differentiation. These data suggest that the differences in the volatile composition of the two *T. flavum* subspecies depends more on the genetic background and less on environmental factors.

1. Introduction

The genus *Teucrium* (Lamiaceae family) includes 300 species widespread all around the world which are perennial herbs or shrubs commonly named germanders (Beni Maleci and Servettaz, 1991). *Teucrium* species are generally aromatic and ornamental plants as well as are valued as pollen source. They have been used in folk medicine since ancient Greek times for coughs and asthma. Most of *Teucrium* species are bitter, astringent and anti-rheumatic herbs that were used as antispasmodic for gastric ulcer and intestinal inflammation, to stimulate the digestion and as diuretic, antiseptic, antipyretic and antihelmintic agents (Barrachina et al., 1995; Ulubelen et al., 2000; Abdollahi et al., 2003; Menichini et al., 2009) *Teucrium* species were also used as alimentary plants and some of them are currently used in the preparation of flavoured wines, herbal teas, bitters and liqueurs, as well as leaf and flower infusions are used for flavoring beer in some countries (Maccioni et al., 2007).

The genus *Teucrium* is one of the richest sources of neoclerodane diterpenes: more than 220 diterpenes have been described up to now, and many of these are interesting because of their ecological role as antifeedants against different species of insects and for their role in the plant medicinal properties (Bruno et al., 2004; Coll and Tandrón, 2005; Piozzi et al., 2005; Menichini et al., 2009). *Teucrium* essential oils have been the subject of several investigations and diverse chemical compositions were recently reviewed; it appears that *Teucrium* essential oils were characterized by mono and sesquiterpenes hydrocarbon compounds (Awadh Ali et al., 2008).

Teucrium flavum L. is an evergreen perennial shrub possessing pubescent stems up to 60 cm, triangular–ovate leaves and yellow flowers, which appear from May to August (Presti et al., 2010; Jeanmonod and Gamisans, 2007). The plant can be found in the cracks of lime rocks from sea level up to 1000 m (Presti et al., 2010). *T. flavum* L. was previously classified in *Chamaedrys* (Miller) Schreber section and on the basis of investigation of flowers and inflorescences, a new classification of *Teucrium* genus was proposed in which the species *T. flavum* was included in Sect. *Polium* Schreber (Lakusic et al., 2006). *T. flavum* L. is represented in the Mediterranean flora by four subspecies: *flavum*, *glaucum*, *gymnoca-*

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lyx and *hellenicum* (Presti et al., 2010). Relative to Italy which displays *glaucum* and *flavum* subspecies as islander and continental species respectively, Corsica Island exhibited the particularity to display both subspecies on the same territory with the same bioclimatic conditions. The first one is widely diffused, while the latter is considered as rare and localized subspecies; a small number of few individuals occur in a unique area of South-East Corsica near Porto-Vecchio (Jeanmonod and Gamisans, 2007). In addition, both *flavum* and *glaucum* subspecies exhibited phenotypic differences according to the plant size (30–70 cm high for *flavum* subsp. instead of 25–50 cm for *glaucum* subsp.), the leaf length (2–3 cm long for *flavum* subsp. instead of 1.5–2 cm for *glaucum* subsp.) and the leaf and stem are completely covered by peltate hairs in subsp. *flavum* while it are incompletely covered for subsp. *glaucum* (Jeanmonod and Gamisans, 2007). Same observations were reported for both subspecies *flavum* from Italy mainland and *glaucum* from Sardinia Island (Beni Maleci et al., 1993), however; the authors noted minor differences in the micromorphological studies of trichromes distribution between *glaucum* and *flavum* subspecies.

The chemical composition of *T. flavum* essential oils have been subject of some studies. The data of previous chemical investigations were including in Table 1, in which the main components, their abundances and the corresponding references has been reported. Whatever the subspecies, the organs studied; the plant origin and the extraction modes, the volatile components of *T. flavum* were hydrocarbon monoterpenes and sesquiterpenes. The literature showed that volatile chemical compositions of *T. flavum* were qualitatively quite similar and only quantitative differences occur in the relative amounts of the volatiles components. These differences were presumably due to environmental conditions and it might be explained by ambiguous taxonomic determination of subspecies. Moreover, no chemical variability was observed between both Italian subspecies originated from differenced habitats i.e. continental and islander areas for *flavum* and *glaucum* subspecies, respectively (Beni Maleci et al., 1993). The present study below the advantage to investigate both subspecies of *T. flavum* originated from the same geographical area and influenced by same bioclimatic parameters.

In the last decade, new valuable tools, based on DNA analysis, have been made available for taxonomic studies. DNA sequence data have allowed for major advances in plant systematic, and constitute a considerable challenge to identify regions with sufficient variation to differentiate closely related species. The detection of DNA polymorphism at random or specific *loci* in the genome has been instrumental in solving controversial taxon attributions by comparing genotypes independently from phenotypes. By identifying polymorphic sequences in the genomic DNA, these tools allow phylogenetic and taxonomic studies.

The nuclear ITS region is one of the most extensively sequenced markers, and the region is a component of an rDNA cistron, which consists of 18S, ITS1, 5.8S, ITS2 and 28s. ITS1 and ITS2 are non coding regions have proven useful in resolving phylogenetic relationships at the genus and species levels due to their relatively rapid evolution rate. Recently there has been much progress in the identification of rapidly evolving cpDNA regions, with many of these regions advocated for use in DNA barcoding (Gao et al., 2010; Sui et al., 2010).

The genetic of *Teucrium* genus has been scarcely described and to our knowledge, only two studies deals with the genetic diversity of *Teucrium polium*. El Oualidi et al. (1999) tested the utility of ITS sequences analysis for resolving relationships in *Teucrium* sect. *Polium*. The resultant phylogeny analysis showed a low correlation with traditional taxonomic classifications. Boulila et al. (2010) studied the genetic diversity among Tunisian population of *Teucrium polium* based on RAPD markers. The population genetic structure was in accordance with that revealed using terpenes and cluster analysis demonstrated that populations clustered in accordance with chemical variability and bioclimatic characteristics.

As part of our ongoing chemical investigation of the essential oils of Corsican *Teucrium* genus (Cozzani et al., 2005; Muselli et al., 2009; Djabou et al., 2010), we investigated for the first time the chemical and the genetic diversity of Corsican *Teucrium flavum* L. through the study (i) of the chemical composition of essential oils and (ii) of three various genetic markers. Chemical analysis were performed using combination of capillary GC/RI, GC-MS after fractionation on column chromatography and the definition of the genetic structure were carried out using two chlororoplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region).

2. Results and discussion

2.1. Essential oil compositions

The analytical strategy developed for the identification of the *T. flavum* oil components, involve the pool of all *T. flavum* subsp. *glaucum* sample oils in order to obtain an collective oil called "HE" which was analysed by GC and GC/MS after CC. Moreover, to provide real concentration of the oil constituents, we carried out a methodology based on the calculation of response factors for all chemicals groups determined (Table 2). The concentrations (g/100 g) of all the components identified are listed in (Table 3). In addition, we have checked that compounds identified in CC-fractions were also presents in *T. flavum* subspecies *glaucum* oil samples by comparison of its retention indices ($\mathbf{RI_A}$, $\mathbf{RI_P}$) and EI-mass spectra with those of total oil. We noted that all compounds present in subsp. *glaucum* collective oil were also identified in subsp. *flavum* oils. In order to perform the statistical analysis, the normalized% abundances of all identified components were calculated (Table 3). The analysis of *T. flavum* sample oils allowed the identification of 81 compounds, accounting for 92.7–95.7% of the total amount. Among them, 36 monoterpenes (10 hydrocarbons and 26 oxygenated), 13 sesquiterpenes (10 hydrocarbons and 3 oxygenated), 1 oxygenated diterpene and 31 oxygenated non terpenic compounds were identified. The identification of 69 components was performed by comparison of their EI-MS and retention indices with those in our laboratory produced library. Twelve components were identified by comparison of their EI-MS and the apolar retention indices with those of commercial or literature libraries (see Table 3). Both chemical oil compositions of *T. flavum* subspecies were qualitatively similar but they differed by the normalized% abundances of their major components. The main components of subsp. *glaucum* were limonene (21.1–31.8%), α-pinene (8.5–17.6%), β-pinene (9.7–12.4%), (*Z*)-β-ocimene (2.4–8.1%) and (E)-phytol (2.4–5.5%). The main components of subsp. *flavum* were α-pinene (19.8–21.5%), limonene (21.9–22.3%), β-pinene (16.4–18.1%) and (*Z*)-β-ocimene (14.5–17.0%). We noted that *T. flavum* oils were dominated by hydrocarbon compounds (60.5–83.7%), however both oils differed by the amounts of oxygenated compounds which accounting for 19.9–35.2% in *glaucum* subspecies oils instead of 11.2–12.4% in *flavum* subspecies oils.

To identify possible differentiation between the chemical oil compositions and the taxonomic classification, principal component analysis (PCA) and cluster analysis (CA; dendrograms) were applied to a matrix linking essential oil compositions to subspecies identities. The data presented in Figs. 1 and 2 were obtained from the correlation matrix and the standardized matrix. The distribution of the volatile compounds: α-pinene (**7**), β-pinene (**12**), myrcene (**13**), limonene (**18**), (*Z*)-β-ocimene (**19**), (*E*)-β-ocimene (**20**), (*E*)-β-farnesene (**65**), (*E,E*)-α-farnesene (**70**), octadecanol (**78**) and (*E*)-phytol (**79**) is shown in Fig. 1a and the distribution of oil sam-

Table 1
Data of previous chemical investigations of volatile components from *T. flavum* and its subspecies.

References		Kovacevic et al., 2001	Stanic et al., 1993	Baher and Mirza, 2003	Menichini et al., 2009	Bellomaria et al., 1998		Presti et al., 2010			Maggi et al., 2009	Beni Maleci et al., 1993						Pelissier et al., 1996
Subspecies		–	–	–	–	*hellenicum*		*flavum*			*flavum*	*flavum*			*glaucum*			*flavum*
Plant material		A.P.	A.P.	A.P.	A.P.	Fl.	Lv.	Lv.	Fl.	Fr.	Fr.	Lv.	Br.	Ca.	Lv.	Br.	Ca.	A.P.
Plant origin		Serbia	Croatia	Iran	Greece	Greece		Sicilia (Italy)			Italy	Italy			Sardinia (Italy)			Corsica
Extraction modes		HD	HD	HD	HD	HD		HD			MW	SE						SE
N°	Components	%	%	%	%	%		g/100 g			%	%						%
7	α-Pinene	17.1	17.3	nd	nd	6.8	3.4	0.1–0.2	0.1	tr	4.5–5.3	60.1	38.5	36.1	25.6	51.7	48.3	18.0
12	β-Pinene	11.5	11.2	nd	nd	5.2	3.1	0.1–0.3	0.2	tr	3.1–4.5	21.2	18.7	18.3	14.8	26.5	23.1	15.1
18	Limonene	6.4	6.2	nd	nd	1.5	2.8	0.2–0.6	0.2	tr	3.0–3.5	tr	2.3	8.2	7.8	11.8	22.5	39.0
26	Linalool	1.9	1.2	nd	3.4	1.4	1.2	tr	0.2	nd	3.4–7.8	nd	nd	nd	nd	nd	nd	nd
60	β-Caryophyllene	5.4	15.8	30.6	12.2	18.1	22.2	3.1–4.6	4.7	3.2	5.1–5.7	nd	nd	nd	nd	nd	nd	nd
65	(*E*)-β-Farnesene	nd	nd	2.2	nd	0.2	7.9	0.2–0.4	0.3	0.5	5.7–7.3	nd	nd	nd	nd	nd	nd	nd
67	*Trans*-β-bergamotene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
68	Germacrene D	2.1	1.1	4.8	2.8	21.9	22.2	9.6–14.0	7.7	4.3	3.1–13.2	nd	nd	nd	nd	nd	nd	nd
69	β-Bisabolene	35.0	nd	21.3	nd	7.6	7.1	31.5–47.0	48.2	48.7	5.0–7.5	nd	6.1	6.9	nd	nd	nd	nd
	α-Humulene	3.6	6.8	8.4	6.0	nd	11.8	2.2–2.4	0.3	1.6	2.5–2.8	nd	nd	nd	nd	nd	nd	nd
	Allo-aromadendrene	nd	9.2	1.3	1.5	nd	nd	nd	0.2	nd	tr	nd	nd	nd	nd	nd	nd	nd
	(*Z*,*E*)-α-farnesene	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.5–14.9	nd	nd	nd	nd	nd	nd	nd
	τ-Cadinol	nd	nd	6.9	nd	0.5	0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Caryophyllene oxide	0.8	1.7	3.8	7.9	2.6	0.8	0.5–1.9	0.8	2.6	nd	nd	nd	nd	nd	nd	nd	nd
	Spathulenol	0.2	nd	4.5	nd	17.9	2.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Only the main components were reported; only one sample was studied, except for leaves (3 samples) in Presti et al. (2010) and Fruits-like (2 samples) in Maggi et al. (2009). Plant material: A.P.: Aerial parts, Fl.: Flowers, Lv.: Leaves, Fr.: Fruits, Br.: Bracts, Ca.: Calyces; Extraction modes: HD: Hydrodistillation, MW: Extraction assisted by Microwaves, SE: Solvent extraction; tr: <0.05; nd: compounds not detected.

Table 2
Measurements of response factors (RFs) for the different chemical groups.

Compounds	RF mean ± STD
Monoterpene hydrocarbons	**1.01 ± 0.05**
Neo-alloocimene	1.00 ± 0.01
α-Pinene	1.04 ± 0.01
β-Pinene	1.08 ± 0.01
γ-Terpinene	1.01 ± 0.01
Limonene	0.94 ± 0.01
Aromatic	**0.93 ± 0.04**
p-Cymene	0.93 ± 0.04
Sesquiterpene hydrocarbons	**1.00 ± 0.03**
β-Caryophyllene	0.98 ± 0.01
α-Humulene	1.01 ± 0.01
Aromadendrene	1.03 ± 0.01
Alcohols	**1.34 ± 0.04**
Nerol	1.31 ± 0.02
Lavandulol	1.32 ± 0.02
Trans-3-hexen-1-ol	1.40 ± 0.02
Cedrol	1.31 ± 0.02
Globulol	1.34 ± 0.02
Esters	**1.55 ± 0.03**
Pentyl acetate	1.53 ± 0.01
Lavandulyl acetate	1.54 ± 0.01
Trans-myrtenyl acetate	1.53 ± 0.01
Cedryl acetate	1.59 ± 0.01
Ketones	**1.30 ± 0.02**
Artemisia ketone	1.30 ± 0.01
Decan-2-one	1.28 ± 0.03
Camphor	1.31 ± 0.01
Jasmone	1.32 ± 0.01
Ethers	**1.24 ± 0.01**
Isobornyl methyl ether	1.25 ± 0.01
1,8-Cineole	1.25 ± 0.03
Carvacrol methyl ether	1.23 ± 0.01
Oxides	1.59 ± 0.01
Caryophyllene oxide	1.59 ± 0.01
Aldehydes	**1.40 ± 0.02**
(*E*)-2-hexenal	1.39 ± 0.01
(*E*,*E*)-2,4-decadienal	1.42 ± 0.01
(*E*)-2-decenal	1.40 ± 0.01

ples is presented in Fig. 1b. As shown in Fig. 1 the principal component analysis (constructed using axes 1 and 2) accounts for 77.2% of all oils. The F1 axis (64.52%) shows that hydrocarbon monoterpene compounds (60.5–83.7% of total composition) are positively correlated with α-pinene (**7**), β-pinene (**12**) and (*Z*)-β-ocimene (**19**) and negatively correlated with limonene (**18**) (Fig. 1a).

The plot established using the first two axes suggests that there are two main groups (Fig. 1b). The group **I** includes 10 samples (TFG51–55, TFG57–60, TFG64); they were characterized by moderate amounts of hydrocarbon monoterpenes (50.9–70.3%) and higher amount of oxygenated compounds (19.9–35.2%) while the group **II** includes 3 samples (TFF56, TFF73 and TFF74), in which the amount of hydrocarbon monoterpenes were higher (more than 80%) and the amount of oxygenated compounds was lower 11.2–12.4%. It is noticeable that the clustering obtained by statistical analysis was in accordance with the repartition of both subspecies in Corsica. The wide repartition of the samples belonging to group I was in accordance with the repartition of *glaucum* subspecies which widespread in Corsica, while the restricted localization of samples of group II originated from Porto-Vecchio, was in accordance with the localized status of the *flavum* subspecies in Corsica (Jeanmonod and Gamisans, 2007). This clustering ensured our botanical determination of both subspecies.

2.2. DNA analysis

All sequences of ITS region and cpDNA markers were deposited in GenBank data base. The accessions numbers are HQ646955 to HQ646967 for ITS region, HQ646958 to HQ646980 for *RPL32-TRNL* intergenic spacer and HQ646981 to HQ646993 for *TRNL-F*. No differences were observed between the three individuals of each same station. The sequences are reported in Table 4 for ITS region and in Table 5 for *RPL32-TRNL* and *TRNL-F*.

The sequences of ITS region from the 13 *T. flavum* accessions contained 595 base pairs (bp) (234, 162 and 197 bp for ITS1, 5.8s and ITS2, respectively). Characteristics of the ITS sequences from the accessions analyzed are summarized in Table 6. ITS1 showed more variation than ITS2, 19 and 12 polymorphic sites respectively and the highest values of ribotype (*H*) and nucleotide diversities (π). The 5.8S gene was highly conserved and showed only one transition and the lowest values of ribotype and nucleotide diversities (Table 6).

The majority of these substitutions differentiate the *glaucum* and *flavum* subspecies: 14 for ITS1, one for 5.8S gene and height for ITS2.

Tajima, Fu and Li tests presented a neutral selection pattern ($P > 0.10$) (Table 6).The UPGMA and NJ trees using Kimura 2P distances were built with the 13 ITS region sequences and showed the same partition with bootstrap values exceeding 50% (UPGMA tree, Fig. 3A).

The total length of the cpDNA regions, with alignment gap, was 857 bp for *TRNL-F* and 565 bp for *RPL32-TRN L*. The sequences of subsp. *glaucum* are characterized by indels located in noncoding regions (intergenic spacers), in position 315 to 320 for *RPL32-TRN L* and 740 and 845 for *TRNL-F*. Based on the similarity of the genetic diversity parameters for the two cpDNA regions, excluding alignment gap (Table 6), we concatenated the two sequences for later analysis (1422 base pairs). The haplotype diversity *H* was 0.500 and the nucleotide diversity π was 0.00093 for the concatenated sequences. Genetic parameters were reported in Table 6.

Sequence variation demonstrated non significant deviation for expectations of neutrality by both the Tajima test ($D = 0.04929$; $P > 0.10$) and the Fu and Li test ($F_S = 0.3345$; $P > 0.10$).

The ITS region showed the greatest genetic diversity than cpDNA region with 9 and 3 haplotypes, respectively. This is probably due at their inheritance conditions, biparental for ITS region and maternal for cpDNA.

The UPGMA and NJ trees constructed from *cpDNA* (Fig. 3B) and ITS region sequences (Fig. 3A) suggested the existence of two groups correlated with the taxonomic classification, one with *T. flavum* subsp. *glaucum* and the other with *T. flavum* subsp. *flavum*. The differentiation between these two groups was consistently indicated by high bootstrap values (>50%).

2.3. Combined analysis

The patterns of relatedness observed in chemical profiles appear to correspond exactly with the genetic profiles generated by ITS and cpDNA sequences, suggesting that there may be a genetic basis for chemical profiles observed. In the other hand, this partition and correlation was in accordance with taxonomic classification.

The concordance between the two data sets was in accordance with the findings from other studies using chemical and genetic markers (Skoula et al., 1999; Echeverrigaray et al., 2001; Naydenov et al., 2005; Zaouli and Boussaid, 2008). Both chemical oil compositions of *T. flavum* subspecies were qualitatively similar but they differed by the normalized% abundances of their major components; oils from *flavum* subspecies were dominated by large amounts of hydrocarbon monoterpenes while oils obtained from *glaucum* subspecies were characterized by higher amounts of oxygenated compounds. These data suggest that the differences in the volatile composition of the two *T. flavum* subspecies depends more on the genetic background and less on environmental factors.

Table 3
Chemical compositions of *T. flavum* subsp. *glaucum* (TFG) and subsp. *flavum* (TFF) essential oils from Corsica.

No.[a]	Components	lRI^b	RI_a^c	RI_e^d	HE[f] g/100 g	TFG53[e]	TFG54	TFG57	TFG60	TFG59	TFG55	TFG58	TFG52	TFG51	TFG64	TFF73	TFF74	TFF56	Identification[g]
1	(*E*)-Hex-3-en-1-ol	812	823	1330	0.1	0.1	0.1	0.1	tr	tr	tr	tr	0.1	tr	0.2	0.2	tr	tr	RI, MS
2	*Trans*-2-hexenal	832	834	1216	0.1	0.1	0.1	0.1	0.1	0.3	tr	0.3	tr	0.1	tr	0.2	tr	0.1	RI, MS Ref
3	(*Z*)-Hex-3-en-1-ol	851	843	1368	0.1	0.2	0.1	tr	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.3	RI, MS
4	(*E*)-Hex-2-en-1-ol	851	854	1389	0.2	0.3	0.2	0.1	tr	0.4	tr	tr	tr	-	-	0.1	tr	-	RI, MS
5	2-Heptanol	880	884	1307	0.1	0.2	0.3	0.1	0.1	0.1	0.2	0.1	0.1	0.1	-	0.2	0.1	0.3	RI, MS Ref
6	α-Thujene	932	923	1021	0.1	0.2	tr	0.1	0.1	tr	0.2	tr	0.1	0.1	tr	0.2	0.2	0.1	RI, MS
7	α-Pinene	936	932	1023	12.2	15.8	10.1	8.5	14.0	12.1	17.2	11.9	13.5	13.1	17.6	21.9	21.9	22.3	RI, MS
8	Camphene	950	944	1067	0.2	0.3	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.3	tr	0.3	0.2	tr	RI, MS
9	Oct-1-en-3-ol	962	960	1429	0.2	0.2	0.1	tr	0.2	0.1	0.3	0.3	0.1	0.2	0.5	tr	0.1	0.7	RI, MS
10	Octan-3-one	969	963	1207	0.4	0.4	0.1	0.7	0.3	1.6	0.4	1.6	0.4	-	-	0.2	0.3	0.7	RI, MS
11	Sabinene	973	966	1121	0.4	0.5	0.2	0.4	0.4	0.1	0.4	0.3	0.4	0.4	0.6	0.5	0.5	0.3	RI, MS
12	β-Pinene	978	973	1112	10.3	12.1	10.4	10.5	11.4	12.4	11.6	10.6	10.4	11.7	9.7	18.1	16.4	17.9	RI, MS
13	Myrcene	987	981	1332	1.9	2.0	0.9	2.1	2.0	1.9	2.0	2.4	2.2	2.1	2.3	2.0	2.1	3.2	RI, MS
14	3-Octanol	981	981	1366	0.5	0.5	0.7	0.4	0.4	0.3	0.5	0.4	0.4	0.2	0.2	0.7	0.6	0.1	RI, MS
15	Yomogi alcohol	991	996	1376	0.1	0.1	0.1	0.1	tr	tr	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	RI, MS Ref
16	Phenyl acetaldehyde	1012	1007	1608	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr	tr	tr	tr	0.1	RI, MS
17	p-Cymene	1015	1014	1267	0.2	0.2	0.2	0.3	0.2	0.4	0.2	0.3	0.2	0.1	0.2	tr	0.2	tr	RI, MS
18	Limonene	1025	1026	1186	27.4	29.3	23.9	28.6	29.4	22.3	29.3	21.1	31.8	28.0	26.2	20.0	21.5	19.8	RI, MS
19	(*Z*)-β-Ocimene	1029	1026	1207	6.0	4.8	2.4	6.2	6.2	6.8	7.4	7.2	7.1	8.1	7.0	15.5	17.0	14.5	RI, MS
20	(*E*)-β-Ocimene	1041	1038	1221	1.2	1.3	0.7	1.6	1.5	0.9	1.8	2.0	1.6	0.7	0.1	2.2	1.5	2.3	RI, MS
21	Non-1-en-3-ol	1058	1050	1514	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	tr	0.1	0.1	0.1	tr	RI, MS Ref
22	1-Octanol	1063	1053	1524	0.2	0.5	0.2	0.1	0.1	0.2	0.1	0.2	tr	tr	0.4	0.1	tr	0.1	RI, MS
23	6-Methyl-3-heptyl acetate	1074	1072	1270	0.7	0.7	0.5	0.8	0.4	0.3	0.9	0.4	0.3	0.8	0.7	0.3	0.2	0.4	RI, MS
24	Rose furane	1091	1080	1563	0.3	0.2	0.3	1.2	0.2	0.3	0.2	0.5	0.3	0.3	0.2	0.4	0.7	0.4	RI, MS Ref
25	Nonanal	1086	1082	1394	0.3	0.2	0.3	0.3	0.2	0.4	0.5	0.5	0.5	0.1	0.5	0.3	0.2	tr	RI, MS
26	Linalool	1083	1084	1514	0.5	0.2	0.5	0.5	0.4	1.0	0.3	1.0	0.5	0.2	0.3	0.2	0.2	tr	RI, MS
27	*Trans*-p-mentha-2,8-dien-1-ol	1113	1104	1614	0.4	0.2	0.6	0.6	0.6	0.3	0.1	0.3	0.6	0.2	0.3	0.7	0.8	1.6	RI, MS
28	3-Octanyl acetate	1113	1108	1315	1.1	0.6	0.2	0.4	1.5	1.9	0.4	1.8	0.4	1.3	1.2	0.2	0.2	0.1	RI, MS
29	cis-p-Mentha-2,8-dien-1-ol	1125	1117	1651	0.7	0.1	0.6	2.5	0.3	0.3	0.5	0.3	0.3	0.1	0.1	0.7	0.9	0.3	RI, MS
30	Pinocarvone	1137	1130	1557	1.5	1.4	1.3	1.2	1.3	1.7	1.2	1.7	1.5	0.3	0.3	1.3	1.1	0.1	RI, MS
31	*p*-Mentha-1,5-dien-8-ol	1148	1140	1689	0.6	0.2	1.4	1.9	0.3	0.4	0.1	0.2	0.5	0.1	0.1	0.6	0.2	0.1	RI, MS Ref
32	2-Methyl benzofurane	1149	1146	1573	0.3	0.2	0.7	0.7	0.1	0.2	0.1	0.4	0.3	0.1		0.2	0.2	0.1	RI, MS Ref
33	Cryptone	1160	1156	1655	0.1	tr	0.2	0.2	0.1	0.2	0.1	0.1	tr	0.1	0.1	0.2	0.1	0.1	RI, MS
34	Terpinen-4-ol	1164	1162	1562	0.7	0.3	0.5	0.3	0.3	1.0	0.1	0.9	0.3	0.1	0.5	0.2	0.1	0.5	RI, MS
35	α-Terpineol	1176	1172	1700	1.0	0.2	1.5	0.3	0.5	1.2	0.3	1.1	0.7	0.9	0.4	0.2	0.2	0.5	RI, MS
36	Myrtenol	1178	1173	1780	1.2	0.2	0.7	0.7	0.6	2.4	0.1	2.3	0.3	0.4	0.6	0.3	0.3	0.4	RI, MS
37	*Trans*-isoperitenol	1182	1179	1720	1.1	0.4	1.2	1.4	0.1	1.3	0.1	1.2	0.8	1.2	0.5	0.4	0.2	0.1	RI, MS
38	Decanal	1188	1186	1498	0.6	0.2	0.8	0.9	0.9	0.3	0.1	0.2	0.8	-	0.1	0.4	0.3	tr	RI, MS
39	*Trans*-carveol	1200	1199	1821	0.4	0.2	0.7	0.1	0.3	0.5	0.1	0.5	0.6	0.2	0.2	0.2	0.1	0.1	RI, MS
40	Cis-p-mentha-1(7),8-dien-2-ol	1206	1206	1871	0.2	0.1	0.4	0.3	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	RI, MS
41	Nerol	1210	1210	1675	0.2	0.2	tr	0.2	0.2	0.3	0.2	0.3	0.3	0.1	0.1	0.1	0.1	0.2	RI, MS
42	Carvone	1214	1216	1726	0.4	0.2	tr	0.5	0.3	0.2	0.1	0.5	0.6	0.1	0.3	0.1	0.2	0.1	RI, MS
43	Geraniol	1235	1235	1801	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.2	0.1	-	0.1	tr	0.1	0.1	RI, MS
44	(*E*)-2-decenal	1240	1238	1644	0.3	0.1	0.4	0.2	0.2	0.2	0.2	0.3	0.2	0.1	0.2	0.1	tr	0.1	RI, MS
45	Perilla aldehyde	1260	1243	1774	0.1	tr	0.1	0.2	tr	0.2	tr	0.1	tr	tr	0.2	tr	0.1	tr	RI, MS
46	Nonanoic acid	1263	1266	2098	0.1	tr	0.2	0.1	0.1	0.1	tr	0.1	0.1	-	0.2	tr	tr	tr	RI, MS
47	p-Mentha-1-en-9-ol	1283	1269	1929	0.5	0.3	0.9	0.1	0.4	0.5	0.2	0.6	0.3	tr	0.1	0.1	tr	tr	RI, MS
48	Perillyl alcohol	1280	1273	1980	0.1	0.1	0.1	0.4	0.1	0.2	tr	0.2	0.2	0.1	tr	0.1	0.2		RI, MS
49	Isobornyl acetate	1276	1280	1580	0.1	0.1	0.2	tr	0.1	0.1	tr	0.1	0.1	0.1	tr	tr	tr	tr	RI, MS
50	Theaspirane I	1299	1291	1480	0.5	0.2	0.8	0.5	0.4	0.5	0.5	0.5	0.3	0.1	0.8	0.1	0.3	0.1	RI, MS
51	Theaspirane II	1313	1305	1517	0.3	0.1	0.4	0.4	0.2	0.3	0.3	0.3	0.2	0.1	0.7	0.1	0.2	0.1	RI, MS
52	(*Z*)-carvyl acetate	1318	1316	1720	0.2	0.2	0.4	0.1	0.2	0.1	0.2	0.1	0.7	0.2	0.1	0.1	0.1	tr	RI, MS
53	α-terpinyl acetate	1335	1332	1693	0.4	0.2	0.5	0.1	0.2	0.5	0.3	0.5	0.7	0.3	0.3	0.2	0.2	0.4	RI, MS
54	Eugenol	1331	1334	2157	0.7	0.4	1.7	1.2	0.6	0.6	0.4	0.7	0.5	0.9	1.3	0.2	0.1	0.3	RI, MS
55	Citronellyl acetate	1337	1337	1644	0.1	0.1	0.1	0.1	0.7	0.1	0.1	0.1	tr	0.1	0.1	tr	0.3	tr	RI, MS

56	(*E*)-2-Undecenal	1346	1342	1726	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.2	0.1	tr	0.1	tr	0.1	0.2	RI, MS
57	(*E*)-β-Damascenone	1363	1362	1770	0.9	0.5	1.1	0.5	0.9	1.2	1,0	1.3	0.3	0.7	0.6	0.2	0.3	0.5	RI, MS
58	Dodecanoic acid	1369	1369	2469	0.2	0.1	1.5	0.2	0.1	0.1	0.1	0.2	0.1	-	0.1	tr	0.1	tr	RI, MS Ref
59	α-Bourbonene	1389	1383	1521	0.3	0.1	0.1	0.4	0.1	0.2	0.2	0.2	0.5	0.4	0.5	0.3	0.1	0.2	RI, MS Ref
60	*β*-Caryophyllene	1421	1418	1580	0.3	0.1	1,0	0.1	0.2	0.4	0.1	0.2	0.6	0.1	0.1	0.1	0.1	tr	RI, MS
61	2-Undecanyl acetate	1425	1421	1632	0.1	tr	0.2	0.1	tr	0.1	tr	0.1	tr	tr	0.1	0.1	0.1	tr	RI, MS
62	Geranyl acetone	1430	1431	1834	0.1	0.1	0.2	tr	0.1	0.1	0.1	tr	tr	-	0.1	tr	tr	tr	RI, MS
63	Veratraldehyde	1432	1435	2370	0.1	0.1	0.2	0.1	tr	0.1	tr	0.1	0.1	-	0.3	tr	0.1	-	RI, MS
64	α-Sesquisabinene	1435	1439	1626	0.1	0.1	0.1	0.1	tr	tr	tr	0.1	0.1	0.5	0.2	tr	0.1	-	RI, MS
65	(*E*)-β-Farnesene	1446	1447	1628	2.3	2.9	4.4	2.3	2.5	1.3	1.9	2.4	2.6	3,0	1.8	0.6	0.4	0.9	RI, MS
66	*β*-ionone	1468	1465	1918	0.1	0.1	0.6	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	tr	0.1	RI, MS
67	*Trans*-β-Bergamotene	1480	1474	1608	0.1	0.1	0.3	0.1	tr	0.4	0.2	0.3	0.1	tr		tr	tr	tr	RI, MS
68	Germacrene-D	1479	1477	1666	0.7	tr	0.3	0.1	tr	0.9	0.2	1.1	0.8	0.6	0.5	tr	0.4	tr	RI, MS
69	*β*-bisabolene	1503	1493	1720	0.4	0.4	0.7	0.3	0.1	0.2	0.2	0.2	0.2	1.4	0.7	0.2	0.2	0.1	RI, MS
70	(*E,E*)-α-farnesene	1498	1500	1693	2,0	2.1	2.2	2.6	2.2	2.1	1.5	1.1	1.6	2.9	3,0	0.9	0.7	0.4	RI, MS
71	β-sesquiphellandrene	1516	1514	1765	0.2	0.3	0.3	0.2	tr	0.1	0.1	0.1	0.2	0.9	tr	0.1	0.1	0.2	RI, MS
72	(*E*)-α-Bisabolene	1530	1524	1733	0.2	0.2	0.2	0.2	tr	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	RI, MS
73	Guaiol	1591	1584	2090	0.3	0.4	0.3	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.1	0.2	0.3	tr	RI, MS
74	Bulnesol	1665	1651	2194	0.1	0.3	0.2	0.1	tr	0.1	tr	0.1	tr	0.1	0.1	0.1	tr		RI, MS
75	α-Bisabolol	1673	1679	2201	0.9	0.8	1,0	0.7	0.8	0.9	1,0	1.1	0.7	0.8	1.6	0.5	0.3	0.2	RI, MS
76	Diisobutyl phthalate	1826	1829	2522	0.5	0.2	1.4	tr	0.5	0.7	0.5	0.6	0.2	0.1		0.1	tr	0.1	RI, MS Ref
77	Hexahydrofarnesyl acetone	1845	1842	2125	0.1	0.2	0.1	0.2	tr	tr	tr	tr	0.1	tr	tr	tr	0.1	tr	RI, MS Ref
78	Octadecanol	2070	2070	2529	1.4	1.6	1.6	0.6	1.6	1.4	2.3	2.3	1.2	1.5	0.7	0.1	tr	0.6	RI, MS
79	(*E*)-Phytol	2114	2097	2559	4.5	3.9	3.7	5.1	4.9	4.2	2.4	3.2	2.9	5.4	5.5	0.5	0.5	0.3	RI, MS
80	Nonadecanol	2150	2144	2605	0.5	0.4	1.6	0.2	0.6	0.6	0.7	0.5	0.6	0.2	0.1	0.1	tr	0.3	RI, MS Ref
81	Octadecyl acetate	2198	2194	2499	0.4	0.5	0.6	0.2	0.3	0.4	0.5	0.3	0.2	0.1	0.1	0.1	tr	0.1	RI, MS
	%				94.9	92.7	95.7	93.7	94.5	94.4	93.8	93.9	95.7	93.1	92.7	95.4	95.1	93.5	
	% Hydrocarbons				66.5	72.8	60.5	64.8	70.5	62.8	74.8	61.9	74.2	72.5	70.7	83,0	83.7	82.3	
	% Monoterpene hydrocarbons				59.9	66.5	50.9	58.4	65.4	57.1	70.3	56,0	67.4	62.6	63.7	80.7	81.5	80.4	
	% Sesquiterpene hydrocarbons				6.6	6.3	9.6	6.4	5.1	5.7	4.5	5.9	6.8	9.9	7,0	2.3	2.2	1.9	
	% Oxygenated				35.0	19.9	35.2	28.9	24,0	31.6	19,0	32,0	21.5	20.6	22,0	12.4	11.4	11.2	
	% Oxygenated monoterpenes				12.7	6.2	15.8	14.3	9.2	14.8	6.2	14.9	10.2	6.8	6.9	6.7	6.5	6,0	
	% Oxygenated sesquiterpenes				1.3	1.5	1.5	1,0	1.1	1.2	1.2	1.4	1,0	1.1	1.8	0.8	0.6	0.2	
	% No-terpenic oxygenated				16.5	8.3	14.2	8.5	8.8	11.4	9.2	12.5	7.4	7.3	7.8	4.4	3.8	4.7	
	% Diterpene oxygenated				4.5	3.9	3.7	5.1	4.9	4.2	2.4	3.2	2.9	5.4	5.5	0.5	0.5	0.3	

[a] Order of elution is given on apolar column (Rtx-1).
[b] Retention indices of literature on the apolar column (RI_a) reported from Konig et al., 2001 and National Institute of Standards and Technology, 1999.
[c] Retention indices on the apolar Rtx-1 column (RI_a).
[d] Retention indices on the polar Rtx-Wax column (RI_p).
[e] Normalized Percentages are given on the apolar column except for components with identical RI_a (percentages are given on the polar column), tr = trace (<0.05%).
[f] Corsican samples: HE: Mixture of all subsp. *glaucum* sample oils. Concentration in g/100 g.
[g] RI: Retention Indices; MS: Mass Spectra in electronic impact mode; Ref.: compounds identified from commercial data libraries: National Institute of Standards and Technology, 1999 (**21, 31, 32, 59, 76, 77, 80**); and literature data: Joulain and Konig (1998) (**2, 5, 15, 24, 58**).

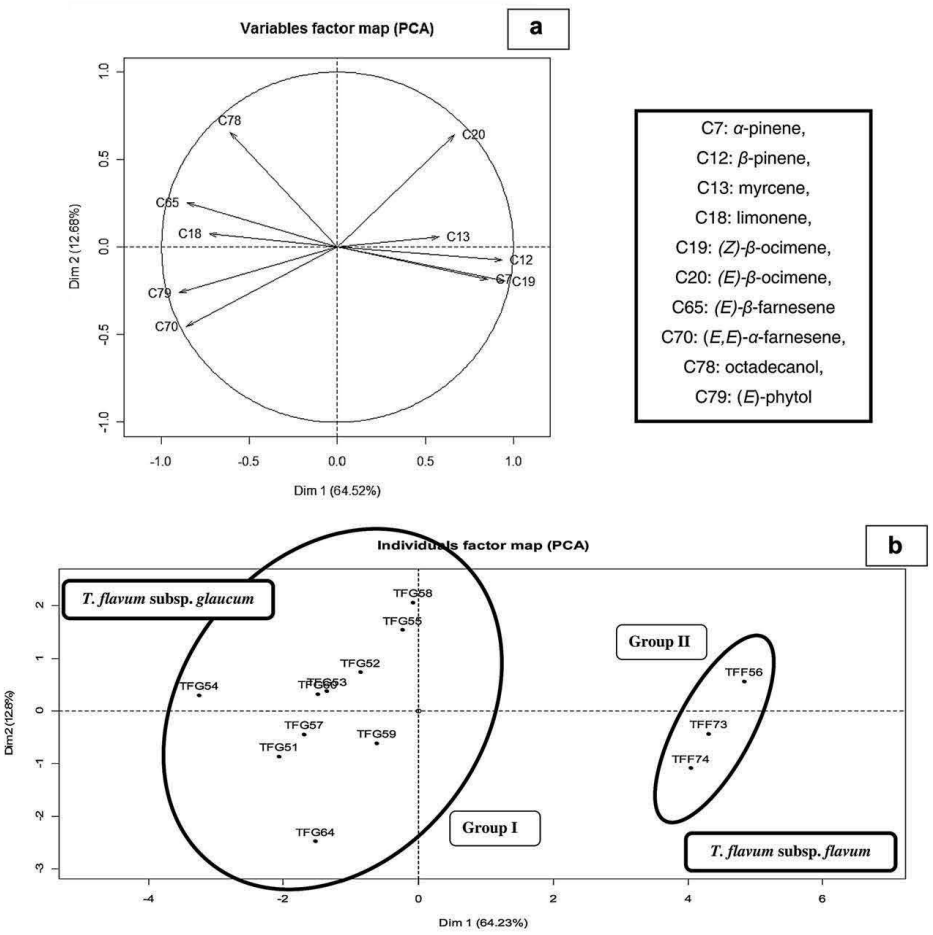

Fig. 1. PCA of chemical compositions of T. *flavum* subsp. *glaucum* (TFG) and subsp. *flavum* (TFF) oils from Corsica. (a) PCA distribution of variables (component codes corresponding to those of Table 2). (b) PCA Distribution of samples (coding numbers of locations).

3. Experimental

3.1. Plant material and oil isolation

The aerial parts of *T. flavum* were collected at the flowering stage from May to June in 13 Corsican locations: ten locations for subsp. *glaucum*, TFG51: Alzi, TFG52: Maccinaggio, TFG53: Erbalunga, TFG54: Cagnano, TFG55: Pozzo, TFG57: Porto Vecchio, TFG58: Bustanico, TFG59: Santa Lucia di Mercurio, TFG60: Saint Andrea di Bozzio, TFG64: Antisanti, and three stations for subsp. *flavum* TFG56: Porto Vecchio1, TFF73: Porto Vecchio2, TFF74: Porto Vecchio3. Voucher specimens were deposited in the Herbarium of the University of Corsica. For chemical investigations, the fresh aerial parts (400–530 g) were submitted to hydrodistillation for 5 h using a Clevenger-type apparatus according to the European Pharmacopoeia (Council of Europe, 1997) and yielded 0.020–0.041% of an yellow oil. For genetic studies, leaves from of *T. flavum* were sampled from the same 13 populations reported above. In total, 39 individuals were sampled (three individuals per population); all individuals sampled within each site were separated by about 1–50 m from each other and were collected randomly.

3.2. Oil Fractionation

The collective oil (2 g) obtained by the mixture of all Corsican *T. flavum* subsp. *glaucum* was submitted to flash chromatography (FC, silica gel 200–500 μm). The apolar fraction I (hydrocarbon compounds, 1.36 g) was eluted with pentane (n-C_5H_{12}) and the polar fraction II (oxygenated compounds, 0.56 g) with diethyl ether (Et_2O). Both fractions were analyzed by GC and GC-MS.

3.3. Gas chromatography

GC analyses were carried out using a Perkin Elmer Autosystem GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used

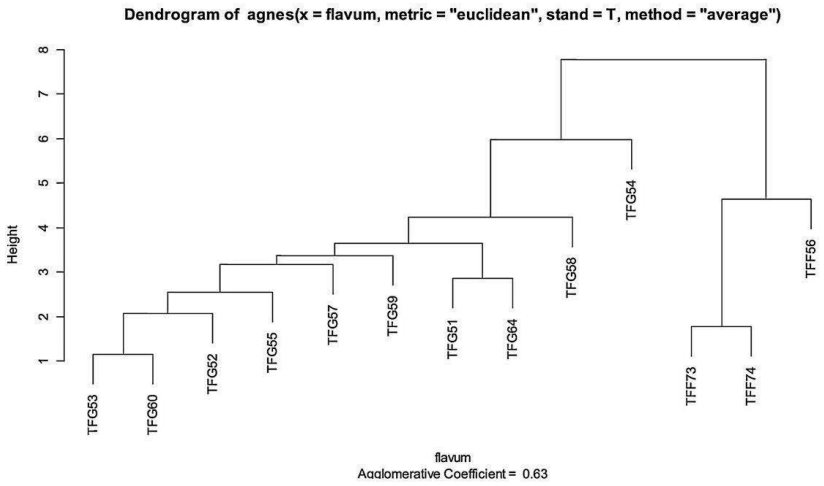

Fig. 2. CA of chemical compositions of T. *flavum* subsp. *glaucum* (TFG) and subsp. *flavum* (TFF) oils from Corsica.

Table 4
ITS region sequences for *Teucrium flavum* subsp. *glaucum* (TFG) and subsp. *flavum* (TFF).

	8	1	1	3	3	4	5	6	7	7	1	1	1	1	1	1	1	2	2	3	4	4	4	4	4	5	5	5	5	5	5	5
		6	7	1	8	0	8	4	2	8	1	2	4	4	5	7	9	0	3	8	1	6	7	7	9	1	1	3	4	6	6	6
											5	6	7	9	9	6	9	8	2	9	1	1	2	9	5	4	7	6	9	5	6	6	9
	ITS1																			5,8S				ITS2									
TFG59	C	C	A	C	A	A	A	C	C	C	G	C	T	C	A	T	G	G	C	C	C	G	A	T	T	A	C	G	T	G	G	T
TFG51																															A	
TFG52, TFG57, TFG64				T																				C							A	
TFG60, TFG53				T	G																			C							A	
TFG58, TFG54																								C							A	
TFG55				T																				C								
TFF56	T		G	T		G	G	T	T	T	A	T	C	.	G	C	.	A	T	T	T	A	G	C	C		T	A		A	A	C
TFF74	T	T	G	T		G	G	T	T	T	A	T	C	T	.	C	A	A	T	T	T	A	G	C	C			A		A	A	C
TFF73	T		G	T		G	G	T	T	T	A	T	C	.	.	C	.	A	T	T	T	A	G	C	C			A		A	A	C

Table 5
RPL32 and TRNL region sequences for *Teucrium flavum* subsp. *glaucum* (TFG) and subsp. *flavum* (TFF).

	2	9	3	3	3	3	3	3	3	3	6	1	1
		3	1	1	1	1	1	2	2	6	0	3	4
			5	6	7	8	9	0	9	2	1	0	1
												5	0
	RPL32-TRNL										*TRNL-F*		
TFG52, TFG57	G	C	_	_	_	_	_	_	G	A	T	_	C
TFG59, TFG60, TFG58, TFG54, TFG55, TFG51, TFG53			_	_	_	_	_	_	_			_	
TFG64	A		_	_	_	_	_	_	_			_	_
TFF73, TFF74, TFF56		G	T	T	G	G	G	G		G	C	T	

for simultaneous sampling to two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60–230 °C at 2 °C.min^{-1} and then held isothermal 230 °C (30 min). Carrier gas: helium (1 mL min^{-1}). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 μL.

3.4. Gas chromatography-mass spectrometry

The oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole detector, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. Ion source

Table 6
Genetic diversity and neutrality parameters on ITS region and cpDNA of *Teucrium flavum*.

	Length bp	N polymorphic sites	N haplotype or ribotypes	Nucleotide diversity π	Haplotype or ribotype diversity H	Tajima test D	Fu and Li test $F*$
ITS 1	234	19	6	0.02718	0.833	0.1644**	0.6132**
5.8 sRNA	162	1	2	0.00236	0.385	0.4256**	0.7323**
ITS2	198	12	7	0.01878	0.731	−0.1573**	0.4159**
ITS region	595	32	9	0.01758	0.936	0.0638**	0.6006**
TRNL-F	867	1	2	0.00045	0.385	0.4256**	0.7323**
RPL32-TRNL	565	3	3	0.00165	0.500	0.1428**	0.0491**
cpDNA region	1422	4	3	0.00093	0.500	0.0493**	0.3345**

bp = base pairs.
** $P > 0.10$.

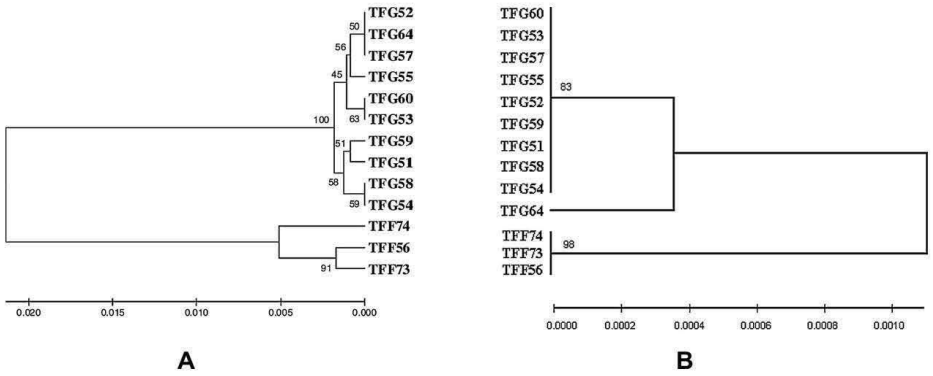

Fig. 3. (A) UPGMA tree of ITS region sequences (numbers at the node indicate bootstrap values). (B) UPGMA tree of cpDNA sequences (numbers at the node indicate bootstrap values). TFG: *Teucrium flavum* subsp. *glaucum*; TFF: *Teucrium flavum* subsp. *flavum*.

temperature: 150 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired with a mass range of 35–350 Da; scan mass: 1 s. Oil injected volume: 0.1 μL, fraction injected volume: 0.2 μL.

3.5. Component identification

Identification of the components was based (i) on the comparison of their GC retention indices (RI) on non polar and polar columns, determined to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literatures data (Jennings and Shibamoto, 1980; Konig et al., 2001; MassFinder, 2006), (ii) on computer matching with commercial mass spectral libraries (MassFinder, 2006; NIST/EPA/NIH, 1996; McLafferty and Stauffer, 1988; Adams, 2001) and comparison of spectra with those of our laboratory-made library.

3.6. Component quantification

The quantification of the collective essential oil components was carried out using the methodology reported by Costa et al. (2008) and modified as follows. Response factors (RF) of 29 standard compounds grouped into nine chemical groups (monoterpene hydrocarbons, aromatic hydrocarbons, sesquiterpene hydrocarbons, alcohols, ketones, aldehydes, esters, ethers and oxides) were measured by GC (Table 2). RFs and calibration curves were determined by diluting each standard in dichloromethane, at seven concentrations, containing tridecane (final concentration 0.7/100 g) as internal standard. Analysis of each standard was performed in triplicate. The normalized% abundances were calculated, using the methodology reported by Bicchi et al. (2008) in order to perform the statistical analysis of the oil samples. Tridecane was introduced in all sample oils at same concentration (0.7/100 g) as internal standard.

3.7. Nuclear and chloroplast DNA markers

Total DNA extraction, primers, conditions of Polymerase Chain Reaction (PCR) and sequencing of cpDNA markers, *TRNL-F* and the *RPL32-TRNL* regions; have been described by Falchi et al. (2009). The ITS region, were amplified with primers ITS1, and ITS4, referring to the protocol described by White et al. (1990). The amplified products were run in a 1.2% agarose gel, stained with ethidium bromide, and visualized and photographed under UV light (Kodak Gel Logic, Sciencetech, Les Ulis, France). The purified PCR products were sequenced in both directions in an ABI 3700 automated sequencer (Applied Biosystems; PerkinElmer, Courtaboeuf, France), following standard methods and using the primers used for amplification. Sequences were aligned using the CLUSTAL X program (Thompson et al., 1997).

3.8. Statistical analysis

Data analyses were performed using Principal Component Analysis (PCA) and Cluster Analysis (CA) (Brereton, 2003). Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results (Massart, 1998). Indeed, PCA provides the data

for diagrams in which both objects (oil samples) and variables (oil components) are plotted while canonical analysis informs a classification tree in which objects (sample locations) are gathered. PCA was carried out using function 'PCA' from the statistical R software. The variables (volatile components) have been selected using function from the statistical software. The Cluster Analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of oil samples.

Haplotype (or ribotype) and nucleotide diversities were determined using the DNASP 4.0 program (Rozas et al., 2003). Neutrality was assessed using the Tajima D test, and the Fu and Li test implemented in Arlequin version 3.0 (Excoffier et al., 2005). For each marker, two analyses were performed using MEGA 4 program (Kumar et al. 2008). Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Neighbor-Joining (NJ) trees were built by calculating genetic distance based on Kimura's two parameters model (1980) with 1000 replicates.

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References

Abdollahi, M., Karimpour, H., Monsef-Esfehani, H.R., 2003. Antinociceptive effects of *Teucrium polium* L. total extract and essential oil in mouse writhing test. Pharmacol. Res. 43, 31–35.

Adams, R.P., 2001. Identification of essential oils by capillary gas chromatography/mass spectroscopy. Allured Publishing, Carol stream.

Awadh Ali, N.A., Wurster, M., Arnold, N., Lindequist, U., Wessjohan, L., 2008. Chemical composition of the essential oil of *Teucrium yemense* Deflers. Rec. Nat. Prod. 2, 25–32.

Baher, Z.F., Mirza, M., 2003. Volatile Constituents of *Teucrium flavum* L. from Iran. J. Essent. Oil Res. 15, 106–107.

Barrachina, M.D., Bello, R., Martínez-Cuesta, M.A., Esplugues, J., Primo-Yúfera, E., 1995. Anti-inflammatory activity and effects on isolated smooth muscle of extract from different *Teucrium* species. Phytother. Res. 9, 368–371.

Bellomaria, B., Arnold, N., Valentini, G., 1998. Essential Oil of *Teucrium flavum* subsp. *Hellenicum* from Greece. J. Essent. Oil Res. 10, 131–133.

Beni Maleci, L., Servettaz, O., 1991. Morphology and distribution of trichomes in Italian species of *Teucrium* sect *Chamaedrys* (Labiatae) – a taxonomical evaluation. Pl. Syst. Evol. 174, 83–91.

Beni Maleci, L., Servettaz, O., Pinetti, A., 1993. Volatile compounds and micromorphological characters of the two Italian subspecies of *Teucrium flavum*. Riv. Ital. EPPOS 11, 664–671.

Bicchi, C., Liberto, E., Matteodo, M., Sgorbini, B., Mondello, L., Zellner, B.A., Costa, R., Rubiolo, P., 2008. Quantitative analysis of essential oils: a complex task. Flav. Fragr. J. 23, 382–391.

Boulila, A., Bějaoui, A., Messaoud, C., Boussaid, et M., 2010. Genetic diversity and population structure of *Teucrium polium (Lamiaceae)* in Tunisia. Biochem. Genet. 48 (1–2), 57–70.

Brereton, R.G., 2003. Chemometrics: Data Analysis for the Laboratory and Chemical Plant. Wiley Interscience, New-York.

Bruno, M., Rosselli, S., Maggio, A., Piozzi, F., Scaglioni, L., Arnold, N.A., Simmonds, M.S., 2004. Neoclerodanes from *Teucrium orientale*. Chem. Pharm. Bull. 52, 1497–1500.

Coll, J., Tandrón, Y.A., 2005. Isolation and structure elucidation of three neoclerodane diterpenes from *Teucrium fruticans* L. (LABIATAE). Phytochemistry 66, 2298–2303.

Costa, R., Zellner, B.A., Crupi, M.L., Fina, D.E., Valentino, M.R., Dugo, M.R., Dugo, G., Mondello, L., 2008. GC–MS, GC–O and enantio–GC investigation of the essential oil of *Tarchonanthus camphoratus* L. Flav. Fragr. J. 23, 40–48.

Council of Europe, 1997. European Pharmacopoeia, third ed. Council of Europe, Strasbourg. .

Cozzani, S., Muselli, A., Desjobert, J., Bernardini, A., Tomi Casanova, F., 2005. Chemical composition of essential oil of *Teucrium polium* subsp. *Capitatum* (L.) from Corsica. Flav. Fragr. J. 20, 436–441.

Djabou, N., Paolini, J., Desjobert, J., Allali, H., Baldovini, N., Costa, J., Muselli, A., 2010. Quantitative and qualitative analysis of volatile components of *Teucrium massiliense* L. – identification of 6-methyl-3-heptyl acetate as a new natural product. Flav. Fragr. J. 25, 475–487.

Echeverrigaray, S., Agostini, G., Atti-Serfini, L., Paroul, N., Pauletti, G.F., dos Santos, et A.C., 2001. Correlation between the chemical and genetic relationships among commercial thyme cultivars. J. Agric. Food Chem. 49 (9), 4220–4223.

El Oualidi, J.E., Verneau, O., Puech, S., Dubuisson, J.Y., 1999. Utility of rDNA ITS sequence in the systematics of *Teucrium* section *Polium (Lamiaceae)*. Pl. Syst. Evol. 215, 49–70.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis Evolutionary. Bioinform. Online 1, 47–50.

Falchi, A., Paolini, J., Desjobert, J.M., Melis, A., Costa, J., Varesi, L., 2009. Phylogeography of *Cistus creticus* L. on Corsica and Sardinia inferred by the TRNL-F and RPL32-TRNL sequences of cpDNA. Mol. Phylogenet. Evol. 52, 538–543.

Gao, T., Yao, H., Song, J., Zhu, Y., Liu, C., Chen, S., 2010. Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family. BMC Evol. Biol. 10, 324–331.

Jeanmonod, D., Gamisans, J. 2007. Flora Corsica (Ed.), Edisud : Aix-en-provence, pp. 638–640.

Jennings, W., Shibamoto, T., 1980. In: Jovanovitch, H.B. (Ed.), Qualitative Analysis of Flavour and Fragrance Volatiles by Glass-capillary Gas Chromatography, Academic Press, New-York.

Konig, W.A., Hochmuth, D.H., Joulain, D., 2001. Terpenoids and Related Constituents of Essential oils. Library of Mass Finder 2.1, Institute of Organic Chemistry, Hamburg.

Kovacevic, N.N., Lakusic, B.S., Ristic, M., 2001. Composition of essential oils of seven *Teucrium* species from Serbia and Montenegro. J. Essent. Oil Res. 13, 163–165.

Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief. Bioinform. 9, 299–306.

Lakusic, B., Lakusic, D., Jancic, R., Stevanovic, B., 2006. Morpho-anatomical differentiation of the Balkan populations of the species *Teucrium flavum* L. (Lamiaceae). Flora –Morphol. Distrib. Funct. Ecol. Plants 201, 108–119.

Maccioni, S., Baldini, R., Tebano, M., Cioni, P.L., 2007. Essential oil of *Teucrium scorodonia* L. Ssp. *scorodonia* from Italy. Food Chem. 104, 1393–1395.

McLafferty, F.W., Stauffer, D.B., 1988. The Wiley/NBS Registry of Mass Spectra Data, fourth ed. Wiley-Interscience, New-York.

Maggi, F., Bilek, T., Cristalli, G., Papa, F., Sagratini, G., Vittori, S., 2009. Comparison of the characterisation of the fruit-like aroma of *Teucrium flavum* L. subsp. *flavum* by hydrodistillation and solid-phase micro-extraction. J. Sci. Food Agric. 89, 2505–2518.

Massart, D.L., 1998. Chemometrics: A Textbook. Elsevier Sciences Ltd., New York.

MassFinder, 2006. In: Hochmunth, D. (Ed.), Version 3.0, Scientific Consulting, Germany.

Menichini, F., Conforti, F., Rigano, D., Formisano, C., Piozzi, F., Senatore, F., 2009. Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. Food Chem. 115, 679–686.

Muselli, A., Costa, J., Desjobert, J., Dessi, M.A., Paolini, J., Bernardini, A., 2009. Chemical Composition of the Essential Oils of *Teucrium chamaedrys* L. from Corsica and Sardinia. J. Essent. Oil Res. 21, 138–143.

National Institute of Standards and Technology, 1999. NIST/EPA/NIH Mass Spectral Library.PC Version 1.7. Perkin-Elmer Corp., Norwalk, CT, USA.

Naydenov, K.D., Tremblay, M., Alexandrov, A., Fenton, N.J., 2005. Structure of *Pinus sylvestris* L. populations in Bulgaria revealed by chloroplast microsatellites and terpenes analysis: provenance tests. Biochem. Syst. Ecol. 33, 1226–1245.

Pelissier, Y., Marion, C., Malan, A., Milhau, M., Quastana, C., Bessiere, J.M., 1996. Volatile components of *Lamiaceae* from Corsica. Riv. Ital. EPPOS 15, 520–535.

Piozzi, F., Bruno, M., Rosselli, S., Maggio, A., 2005. Advances on the chemistry of furano-diterpenoids from *Teucrium* genus. Heterocycles 65, 1221–1234.

Presti, M.L., Crupi, M.L., Costa, R., Dugo, G., Mondello, L., 2010. Seasonal variation of *Teucrium flavum* L. essential oil. J. Essent. Oil Res. 22, 211–216.

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19, 2496–2497.

Skoula, M., Hilali, I.E., Makris, M., 1999. Evaluation of the genetic diversity of *Salvia fruticosa* Mill clones using RAPD markers and comparison with essential oil profiles. Biochem. Syst. Ecol. 27, 559–568.

Stanic, G., Petricic, J., Blazevic, N., Plazibat, M., 1993. Essential oil of *Teucrium flavum* L. from Croatia. J. Essent. Oil Res. 5, 625–627.

Sui, X., Huang, Y., Tan, Y., Guo, Y., Long, C., 2010. Molecular Authentication of the Ethnomedicinal Plant *Sabia parviflora* and Its Adulterants by DNA Barcoding Technique Planta Medica. doi: 10.1055/s-0030-2150468.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL X windows interface. Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Ulubelen, A., Topu, G., Sönmez, U., 2000. Chemical and biological evaluation of genus *Teucrium*. Bioactive natural Products (Part D), Elsevier, pp. 591–648.

White, T., Bruns, T., Lee, S., Taylor, J., 1990. In: Innes, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic press, San Diego, California, pp. 315–322.

Zaouli, Y., Boussaid, M., 2008. Isozymes markers and volatiles in Tunisian *Rosmarinus officinalis* L. (Lamiaceae): a comparative analysis of population structure. Biochem. Syst. Ecol. 36, 11–21.

[**PT5**] : ***Teucrium polium*** **(deux sous-espèces)**

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Chemical and Genetic Diversity of two Mediterranean subspecies of *Teucrium polium* L.

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ABSTRACT

Chemical and genetic diversity of *Teucrium polium* L. subsp. *polium* from western Algeria and *Teucrium polium* L. subsp. *capitatum* from Corsica were investigated. Diversity within and among the two populations of subspecies was assessed according to the chemical composition of their essential oils, and the genetic diversity evaluated using three polymorphic genetic markers. Chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Genetic structures were mapped using two chloroplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region). The statistical analysis showed that the two subspecies were clearly distinguished by these chemical and genetic markers. The oil chemical compositions differed qualitatively and quantitatively between the subspecies. Both collective oils were dominated by hydrocarbon compounds however the Algerian sample oils exhibited higher amounts of hydrocarbon sesquiterpenes than those of Corsica (31.2 g/100g vs. 4.4 g/100g) while the latter displayed higher amounts of hydrocarbon monoterpenes than the first (59.3 g/100g vs. 34.3 g/100).Neighbor-joining, Maximum likelihood and Bayesian trees constructed from chloroplast markers and nuclear ITS region sequences showed the existence of two groups associated with taxonomic and chemical characteristics. The first is consisted by *T. polium* subsp. *capitatum* samples, and the second is constituted by *T. polium* subsp. *polium*, indicating that variation in the essential oil composition within subspecies depends on genetic background. The samples of subsp. *capitatum* from Corsica are a homogeneous group, in contrast to samples of subsp. *polium* from Algeria. This structure is obtained with both genetic and chemical data. Chemical and genetic variations between *T. polium* subsp. *polium* were discussed and environmental characteristics were compared.

Keywords: *Teucrium polium* L. subsp. *polium*, *Teucrium polium* L. subsp. *capitatum*, Chemical and genetic diversity, Essential oil, Chloroplast and ribosomal nuclear markers, environmental factors

1. Introduction

Teucrium is a polymorphic and cosmopolitan genus of perennial plants, the largest of the Lamiaceae family in the Mediterranean area, which comprise more than 300 species (Tutin et al., 1972). From a taxonomical point of view, the genus *Teucrium* has been divided into nine sections identifiable through the calyx shape and the inflorescence structure (Navarro and El Oualidi, 2000). Among them, *Polium* section, which includes about half of the species, has been subdivided into four subsections: *Polium*, *Simplicipilosa*, *Pumilium* and *Rotundifolia*. *Polium* section was considered as complex section owing to considerable morphological variations within and between populations and the instability of morphological characters. So it includes many poorly differentiated taxa with probable recent origin through hybridization followed by polyploidization (El oualidi et al., 1999, 2002). In North Algeria, 12 subspecies of *T. polium* were described, among them *T. polium* subsp. *polium* was the most common and was widespread in Tell mountains (Baba Aissa, 2000; Quezel and Santa, 1963). In Corsica, only the subspecies *capitatum* was reported (Jeanmonod and Gamisans, 2007).

From a botanical point of view, an unusual feature of *Teucrium* genus compared with other members of Lamiaceae is that the flowers completely lack the upper lip of the corolla (De Martino et al., 2010). *Teucrium polium* is a perennial and out-crossing species with prostrate stems, white to bright pink flowers, crenate leaves, and ramified indumentums. It shows a high variation both in morphological and ploidal levels (El Oualidi et al., 2002; Navarro and El Oualidi, 2000; Tutin et al., 1972). *T. polium* subsp. *polium* is most present in mountains of west Mediterranean region, with white to bright pink flowers, lateral corolla-lobes can be glabrous or hairy with leaves less than 15 mm and flowering stems 10-25 cm, contrarily to subsp. *capitatum* who its lateral corolla-lobes are always hairy with white or grey hairs present on stems (Tutin et al., 1972). In north Algeria, we noted the presence of *T. polium* subsp. *polium* with two flower colors; specimens with pink flowers were localized in

littoral and mountain regions while specimens with white flowers were only widespread in mountain regions (Baba Aissa, 2000; Quezel and Santa, 1963). *T. polium* subsp. *capitatum* grows wild in southern Europe, central and south-west Asia and North Africa. This plant is scarcely found in continental France, but grows in stony ground in Corsica. It is a perennial, pubescent, aromatic plant, 20-50 cm high, with green-grayish leaves and white flowers, which appear from June to August.

Many species of *Teucrium* are used in traditional folk medicine and it is claimed they have hypoglycemic, antipyretic, antiulcer and antibacterial properties (Couladis et al., 2003; Fernandez et al., 1997; Menichini et al., 2009). *Teucrium polium* was traditionally used for different therapeutic purpose such as gastrointestinal disorders (Parsaee and Shafiee-Nick, 2006), hypertension, convulsion, infection, inflammation, rheumatism and diabetes mellitus (Ansari Asl et al., 2003; Hudaib et al., 2008). The plant was also used to relative pains during pregnancy (Vahdani et al., 2011).

From the chemical point of view, the genus *Teucrium* is one of the richest sources of neoclerodane diterpenes: more than 220 diterpenes have been described, many of which are of interest because of their insect-repellent and medicinal properties (Bruno et al., 2003, 2004; Piozzi et al., 2005). It was demonstrated that neo-clerodane diterpenes are useful chemotaxonomic markers (Servettaz et al., 1989). Some neoclerodane diterpenoids with fungicide and insecticide activities (Bruno et al., 2003) and abeo-abietanes diterpenes as protective factors against oxidative stress (Fiorentino et al., 2010) were reported from *Teucrium polium*. Essential oils of *Teucrium* genus have been the subject of many studies. We noted that according to the species studied, essential oil yields ranged from 0.05% to 1.5% and the amounts of main constituents (mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes) differed notably (Awadh Ali et al., 2008; Saracoglu et al., 2007). Most of these essential oils are known for their anti-inflammatory, antibacterial, antimicrobial,

antihypertensive, hypoglycemic, hypolipidemic, antiphytovial, and antioxidant properties (Aburjai et al., 2006; Bezic et al., 2011; Chizzola, 2006; Couladis et al., 2003; Kabouche et al., 2007; Menichini et al., 2009; Vahdani et al., 2011). Concerning *Teucrium polium*, 22 studies established the chemical composition of oils extracted by hydrodistillation (Aburjai et al., 2006; Al-Qudah et al., 2011; Antunes et al., 2004; Ashnagar et al., 2007; Bezic et al., 2011; Cakir et al., 1998; Cozzani et al., 2005; De Martino et al., 2010; Eikani et al., 1999; Hammoudi and Hadj Mahammed, 2010; Hassan et al., 1979; Kabouche et al., 2007; Kamel and Sandra, 1994; Kovacevic et al., 2001; Menichini et al., 2009; Moghtader, 2009; Pérez-Alonso et al., 1993; Sarer and Konuklugil, 1987; Stanciu et al., 2006; Tomi and Casanova, 2006; Vahdani et al., 2011; Vokou and Bessiere, 1985). Table 1 reviewed the data of previous chemical investigations with the main components, their abundances and the corresponding references grouped by same geographical origin. Whatever to the origin of the plant, the main components were hydrocarbon monoterpenes such as limonene, α- and β-pinene; hydrocarbon sesquiterpenes such as β-caryophyllene, γ-muurolene, γ-cadinene and germacrene D; and oxygenated sesquiterpenes such as α- and τ-cadinol, patchouli alcohol, caryophyllene oxide and 8-cedren-13-ol. To our knowledge essential oil from *Teucrium polium* subsp. *polium* was never reported in literature.

Very few studies have been conducted on the genetic structure of *Teucrium* species and to our knowledge, only two studies deals with the genetic diversity of *Teucrium polium*. El Oualidi et al. (1999) tested the utility of ITS sequences analysis for resolving relationships in *Teucrium* sect. *Polium*. The resultant phylogeny analysis showed a low correlation with traditional taxonomic classifications. Boulila et al. (2010) studied the genetic diversity among Tunisian population of *Teucrium polium* based on RAPD markers. The population genetic structure was in accordance with that revealed using terpenes and cluster analysis

demonstrated that populations clustered in accordance with chemical variability and bioclimatic characteristics.

Maintenance of a minimum level of intra- and interpopulation genetic diversity is important for the genetic fitness of a species (Barret and Kohn, 1991). Therefore, quantification of the diversity of genetic and chemical factors is important for understanding the adaptive ability of a species and for developing conservation programs. We quantified the genetic diversity of two subspecies of *T. polium* using chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) sequence data. cpDNA is maternally inherited and is dispersed through seeds, whereas nrDNA is inherited from both parents and is dispersed through pollen. These markers are heterogeneous at inter- and intraspecies levels (Petit et al., 2003; Wares and Cunningham, 2001).

In the course of our characterization of Mediterranean *Teucrium* species (Cozzani et al., 2005; Djabou et al., 2010, 2011a, 2011b; Muselli et al., 2009), we investigated for the first time the chemical composition and genetic diversity of *T. polium* L. subsp. *polium* harvested in 3 area of Tlemcen province (western Algeria). Owing to the morphological variations of *Teucrium polium*, it was interesting to investigate specimens with white flowers (only localized in the mountain area 1) and specimens with pink flowers (localized in the littoral area 2 and in the mountain area 3) (Fig. 1). In the same time we reinvestigated the chemical composition of Corsican *T. polium* L. subsp. *capitatum* and we established its genetic diversity for the first time. Corsican samples were considered as out-group for comparison purpose (Fig. 1). Environmental effects were assessed according to the chemical composition of essential oils and genetic diversity was evaluated using three polymorphic genetic markers. Chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Genetic structure was mapped using two chloroplast markers (*RPL32-TRNL* and *TRNL-F*) and ribosomal nuclear markers (ITS region).

2. Results and discussion

2.1. Essential oil composition

Preliminary analysis of 20 Algerian *T. polium* subsp. *polium* oil samples showed that their GC chromatograms were qualitatively similar but differed by the abundances of their main components. In the same way, preliminary analysis of Corsican *T. polium* subsp. *capitatum* oil samples showed GC chromatograms rather similar. Therefore, in order to carry out a detailed study of oils, all Algerian and all Corsican oil samples were pooled in two Collective oils called HE1 and HE2, respectively. Then they were fractionated using column chromatography (CC) and reanalyzed using GC and GC/MS. The concentrations of the oil components expressed in g/100g of essential oils are listed in Table 2. We checked that the compounds identified in the CC fractions were present in the individual oil samples by comparing their retention indices ($\mathbf{RI_A}$, $\mathbf{RI_P}$) and Electronic Impact mass spectra (EI-MS) with those of the total oil samples. The complete results obtained from all individual samples were tabulated in Table 3 (*supplementary materiel*).

GC and GC/MS analysis of both collective essential oils allowed the identification of 107 compounds accounting for 99.6 g/100g in *T. polium* subsp. *polium* collective oil from western Algeria (HE1) and 86 compounds accounting for 98.9 g/100g in *T. polium* subsp. *capitatum* collective oil from Corsica (HE2). More of them were identified in both oils and in total, 146 components were identified; 127 of which were ensured by comparing their EI-MS and retention indices with those in our laboratory library and 21 components were identified by comparing their EI-MS and apolar retention indices with those of commercial or literature libraries (see Table 2). The main components of *T. polium* subsp. *polium* collective oil were *β*-pinene **12** (16.6 g/100g), germacrene D **99** (14.8 g/100g), *α*-pinene **6** (7.2 g/100g), spathulenol **118** (6.4 g/100g), limonene **19** (5.6 g/100g), bicyclogemacrene **103** (5.5 g/100g)

and myrcene **14** (2.9 g/100g). The main components of *T. polium* subsp. *capitatum* were α-pinene **6** (24.1 g/100g), β-pinene **12** (9.2 g/100g), α-thujene **5** (8.1 g/100g), terpinen-4-ol **57** (6.2 g/100g), limonene **19** (5.2 g/100g), sabinene **11** (4.1 g/100g) and p-cymene **17** (4.0 g/100g). Both collective oils were dominated by hydrocarbon compounds: 65.7 g/100g and 63.7 g/100g in *T. polium* subsp. *polium* and in *T. polium* subsp. *capitatum*, respectively; however the Algerian sample oils exhibited higher amounts of hydrocarbon sesquiterpenes than those of Corsica (31.2 g/100g vs. 4.4 g/100g) while the latter displayed higher amounts of hydrocarbon monoterpenes than the first (59.3 g/100g vs. 34.3 g/100).

Normalized percentage abundances of all identified components were used for statistical analysis (Table 3, *supplementary materiel*). To identify the relationship between the chemical compositions of oils, taxonomic classification and environmental parameters, principal component analysis (PCA) and cluster analysis (CA) were applied to a matrix linking essential oil composition to subspecies identity. The data presented in Figs 2 and 3 were obtained from the correlation matrix and the standardized matrix. Fig. 2 shows the distributions of the volatile compounds **5**, **6**, **11**, **12**, **14**, **17**, **19**, **57**, **99**, **103** and **118**; and the oil samples. As shown in Fig. 2, the principal component analysis (constructed using axes 1 and 2) accounted for 85.45% of all oils. It appears that F1 axis (55.1%) was positively correlated with monoterpene compounds (**5**, **6**, **11**, **17** and **57**) and negatively correlated with sesquiterpene compounds (**99**, **103** and **118**) (Fig. 2). The plot established using the first two axes suggests that there are two main groups correlated with the geographic origin of the oil samples (Fig. 2). This is in accordance with the general structure of the dendrogram obtained from the CA (Fig. 3). Group I included 7 *T. polium* subsp. *capitatum* oil samples from Corsica (TPC1–7) and group II included 20 *T. polium* subsp. *polium* oil samples from western Algeria (TP1–20). Group I was characterized by the occurrence of monoterpene compounds such as α-pinene **6** (20.2-28.5 %), α-thujene **5** (6.1-10.3 %), terpinen-4-ol **57**

(5.1-6.3%), sabinene **11** (3.7-5.2 %) and *p*-cymene **17** (2.6-4.7 %) as main components. Group II was subdivided to two sub-groups. Sub-group IIa included 10 sample oils characterized by high amounts of sesquiterpene compounds such as germacrene D **99** (21.8-35.8 %), bicyclogermacrene **103** (1.4-17.9 %) and spathulenol **118** (4.4-16.7 %); and low amounts of hydrocarbon monoterpenes such as *β*-pinene **12** (1.1-7.6 %), limonene **19** (1.1-6.6 %) and myrcene **14** (0.1-6.6 %). Sub-group IIb included 10 sample oils, relative to sub-group IIa, they were characterized by higher amounts of hydrocarbon monoterpenes like *β*-pinene **12** (20.8-40.5 %), limonene **19** (6.4-14.2 %) and myrcene **14** (2.4-9.1 %) and lower amounts of sesquiterpene compounds such as germacrene D **99** (1.3-15.9 %), spathulenol **118** (1.8-3.5 %) and bicyclogermacrene **103** (0.1-3.5 %) (See Table 3 supplementary material). It is noticeable that the chemical composition of Corsican *T. polium* subsp. *capitatum* studied here was in accordance with those previously reported in literature (Cozzani et al., 2005; Tomi and Casanova, 2006). Moreover, the chemical composition of *T. polium* subsp. *polium* oil from Algeria seems to be similar to those of Iranian *T. polium* (Eikani et al., 1999) except for (*E*)-β-caryophyllene (18.0%) which was never detected in Algerian sample oils studied.

Concerning the correlation between oil chemical compositions, morphological characters and environmental parameters, it could be interesting to observe that the chemistry did not show direct correlations. Concerning Algerian oil samples PCA and CHA indicated two cluster linked to (i) *T. polium* subsp. *polium* samples harvested from area 1 and area 2, i.e. specimens with white flowers from mountain zone and specimens with pink flowers from littoral zone (sub-group IIb) and (ii) *T. polium* subsp. *polium* samples harvested from area 3 i.e. specimens with pink flowers from mountain zone (sub-group IIa). Some information concerning the environmental parameters of the harvest area (type of rock, soil, altitudes, temperatures and rainfall) were tabulated in Table 4 (Supplementary material). Area 1 is characterized by sandstone sequanian rock with brown fersiallitic soil originated on limestone

rock, under the influence of a cold climate with dry season less pronounced. This soil is rich in bases including Ca^{++}, Mg^{++} and K^{+}, existing in a climate vegetation (Holm oak, Aleppo pine) with a dense undergrowth, but wetter and more porous. Area 2 is calcareous marl and dune sediments based on alkaline limestone. This area is only characterized by calcareous soilhumic rich in organic matter because they are developed at the expense of former marshy (swampy) ground. From area 3, the rock is origin from the Quaternary (recent series) composed of old travertine and recent alluvium occupying the "Oueds" funds by silt sometimes clay. The soil is red fersiallitic with vertic character. It is heavier (rich of swelling clays) than the red fersiallitic soil and has high water content. It starts on limestone rocks (karsts) (Algerian Minister of Agriculture and Rural Development, 2010; Bonneau and Souchier, 1994; Bouaoune and Dahmani-Megrerouche, 2010; Collignon, 1986). Soil pH and the presence of divalent metal ions such as Mg^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+} affect the production of hydrocarbon sesquiterpenes by plants (Duarte et al., 2010). It appears that differences in oil compositions between the oil samples from the three harvest area could be caused by environmental conditions.

2.2. DNA analysis

The sequences of the ITS region and cpDNA markers were deposited in the GenBank database. The accessions numbers are JN903715 to JN903731 for ITS region, JQ044762 to JQ044765 for *RPL32-TRN L* and JQ044774 to JQ044782 for *TRNL-F*. The sequences of *T. polium* subsp. *capitatum* are used as outgroup: JN903732 for ITS region, JQ044766 to JQ044770 for *RPL32-TRN L* and JQ044771 to JQ044773 for *TRNL-F*.

The three individuals from each population, as well in Corsica and Algeria, showed identical sequences for the two cpDNA spacers and ITS region.

The sequences of the ITS region from the 20 *T. polium subsp. polium* accessions contained

597 base pairs (bp) (236, 163, and 198 bp for ITS1, 5.8S, and ITS2, respectively, excluding sites with gaps). The total lengths of the cpDNA regions were 860 bp for *TRNL-F* and 543 bp for *RPL32-TRN L* (excluding sites with gaps). On the basis of the similarity of the genetic diversity parameters for the two cpDNA regions (Table 5), we concatenated the two cpDNA sequences for later analysis (1403 base pairs). Characteristics of the ITS and cpDNA sequences from the Algerian samples are summarized in Table 5.

The 5.8S gene of ITS region was highly conserved and showed only one substitution and the lowest values of ribotype diversity (*H*) and nucleotide diversity (π). The intergenic spacers ITS1 and ITS2 showed almost the same variability with respectively 12 and 10 polymorphic sites (Table 5). The twenty *T. polium* samples were characterized by seventeen ribotypes.

The sequences of cpDNA (TRN L-F and RPL32-TRNL) showed a lower genetic diversity, with only four (RPL32-TRNL) and nine (TRNL-F) haplotypes, compared to that of nuclear DNA (Table 5). This is probably due to the difference in transmission of both types of markers, by pollen dissemination for nuclear ITS and by dissemination of seeds for cpDNA.

The trees constructed from *cpDNA* and ITS region sequences with the three methods (NJ, ML and Bayesian analyses) showed the same partition and suggested the existence of two main groups, the first with pink flower samples from mountains (area 3) and the second with two subgroups, white flower samples from mountains (area 1) and pink flower samples from littoral (area 2). These differences were supported by high bootstrap values (> 50% for NJ and ML) and high values of posterior probability (>0.95 for Bayesian analyses). Bayesian analyses only are reported in Fig. 4.

SAMOVA analysis confirmed the existence of these two main groups with maximum values of FCT (0.61306 for ITS region and 0.776215 for cpDNA). The majority of variation

was observed between these two groups (61.31% for ITS region and 77.62% for cpDNA) (Table 6 and 7).

The similarity between the specimens from area 1 (white flower mountains) and area 2 (pink flower littoral) is also confirmed by high values of gene flow observed with both types of markers (Table 8). These two areas are connected by wide valleys that can explain these values of gene flow and thus their genetic similarity, while area 3 is characterized by isolation of the mountain barriers (Canerot et al., 1986).

The flowers colour difference of samples from area 1 (white flowers) and area 2 (pink flowers) appears due to a difference in the nature of the soils. The area 1 has a rich soil of sandstone characterized by an acidic pH, while area 2 is characterized by a calcareous soil with a basic pH. The third area consists mainly of fertile alluvial soil has a pH close to 7.00 (Piqué et al., 2002). This difference of physicochemical properties could lead to epigenetic phenomena, very common in plants of *T. polium* subsp. *polium* (Table 4 supplementary material) (Kalisz and Kramer, 2008). Conversely, the basis of variation in the volatile composition of *T. polium* subsp. *polium* from the three area studied depends more on the genetic background and less on environmental factors.

2.3. Concluding remarks

The concordance between chemical and genetic data sets is in accordance with the findings from other studies (Bazina et al., 2002; Echeverrigaray et al., 2001; Naydenov et al., 2005; Skoula et al., 2000; Zaouali and Boussaid, 2008). Our studies on the genetic diversity of *Teucrium polium* may also provide a base for future basic and applied research related to many species of aromatic and medicinal plants. In further studies, we plan to compare the essential oil content and composition of various *Teucrium* populations and reveal if these findings can be correlated with genetic diversity based on nuclear and chloroplastic markers.

This information could play a vital role in the selection of appropriate genetic material to be used in future essential oil selection and breeding programs.

3. Experimental

3.1. Plant material and oil isolation

The aerial parts of *Teucrium polium* L. subsp. *polium* from western Algeria were collected at the flowering stage (May to June 2010) from 20 populations ~~locations~~ (TP1–20) widespread in 3 areas from Tlemcen department: two in Tell mountain and one in littoral (Fig. 1). Voucher specimens were deposited with the herbarium of the University of Tlemcen. The aerial parts of *Teucrium polium* L. subsp. *capitatum* from Corsica were collected at the flowering stage (June 2011) from 7 Corsican populations ~~locations~~ (TPC1–7) (Fig. 1). Voucher specimens were deposited with the herbarium of the University of Corsica. The altitudes of the harvest population ~~location~~ ranged from 70 m to 1250 m in Algeria and from 400 m to 990 m in Corsica (Table 3).

To obtain essential oils, the fresh aerial parts of both subspecies (400–500 g) were subjected to hydrodistillation for 5 h using a Clevenger-type apparatus according to the *European Pharmacopoeia* (Council of Europe, 1997), yielding (w/w) 0.082-0.105% and 0.490-0.620% of yellow oil for *T. polium* subsp. *polium* and *T. polium* subsp. *capitatum*, respectively. For genetic studies, leaves from both subspecies were sampled from all populations described above. In total, 81 individual plants were sampled (three plants per population); all samples were obtained from plants 1–50 m from each other and were collected randomly.

3.2.Oil Fractionation

Two collective oils called HE1 and HE2 were obtained by the mixture of all Algerian and all Corsican samples, respectively. Both oils (1 g for each oil) were consecutively submitted to chromatography on a normal phase silica column (12 g, 15–40 mm, 60 Å) using an automatized Combi Flash apparatus (Teledyne Isco, Inc. Lincoln, NE, USA) equipped with a fraction collector monitored by an UV-detector. For Algerian (HE1) and Corsican (HE2) oils, the apolar fractions (hydrocarbon compounds, 0.63 g and 0.60 g, respectively) were eluted with hexane (n-C_6H_{14}) and the polar fractions (oxygenated compounds, 0.31 g and 0.35 g, respectively) were eluted with diisopropyloxide ($(C_3H_7)_2O$). All fractions were analyzed by GC and GC-MS.

3.3. *Gas Chromatography*

GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60 to 230 °C at 2 °C.min^{-1} and then held isothermal 230 °C (30 min). Carrier gas: helium (1 mL.min^{-1}). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 µL.

3.4. *Gas Chromatography-Mass Spectrometry*

The oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the

same as described above. Ion source temperature: 150 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired with a mass range of 35 – 350 Da; scan mass: 1s. Oil injected volume: 0.1 µL; fraction injected volume: 0.2 µL.

3.5. *Component Identification*

Identification of the components was based (i) on the comparison of their GC retention indices (RI) on non polar and polar columns, determined to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literatures data (Jennings and Shibamoto, 1980; Konig et al., 2001; Mass Finder, 2006); (ii) on computer matching with commercial mass spectral libraries (McLafferty and Stauffer, 1988; NIST/EPA/NIH, 1999) and comparison of spectra with those of our laboratory-made library.

3.6. *Component Quantification*

The quantification of the collective essential oil components was carried out using peak normalization including response factors (RFs) with internal standard using the methodology reported by (Costa et al., 2008) and modified in our laboratory (Djabou et al., 2010). The normalized % abundances were calculated, using the methodology reported by (Bicchi et al., 2008) in order to perform the statistical analysis of the oil samples. Tridecane was introduced in all sample oils at same concentration (0.7 g/100 g) as internal standard.

3.7. *Nuclear and chloroplast DNA markers*

We sampled seven populations of *T. polium* subsp. *capitatum* growing wild in Corsica and twenty populations of *T. polium* subsp. *polium* from western Algeria (Fig. 1). For each population the leaves of three individual shrubs, take at random, was collected during spring season 2010. Genetic analysis was performed on all 81 individuals.

Total DNA was extracted from approximately 0.1 g of fresh leaf material using the DNeasy plant mini kit (Qiagen S.A., Courtaboeuf, France), according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) and sequencing of cpDNA markers have been described by (Falchi et al., 2009). The ITS region, were amplified with primers ITS1, and ITS4, referring to the protocol described by (White et al., 1990). The amplified products were run in a 1.2% agarose gel, stained with ethidium bromide, and visualized and photographed under UV light (Kodak Gel Logic, Sciencetech, Les Ulis, France). The purified PCR products were sequenced in both directions in an ABI 3700 automated sequencer (Applied Biosystems; PerkinElmer, Courtaboeuf, France), following standard methods and using the primers used for amplification. Sequences were aligned using the CLUSTAL X program (Thompson et al., 1997).

3.8. Statistical analysis

Chemical data analyses were performed using Principal Component Analysis (PCA) and Cluster Analysis (CA) (Brereton, 2003). Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results (Massart, 1998). Indeed, PCA provides the data for diagrams in which both objects (oil samples) and variables (oil components) are plotted while canonical analysis informs a classification tree in which objects (sample populations) are gathered. PCA was carried out using function 'PCA' from the statistical R software. The variables (volatile components) have been selected using function from the statistical software. The Cluster Analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of oil samples.

Genetic analysis: Haplotype and nucleotide diversity were determined using the DNASP 4.0 program (Rozas et al., 2003). For each marker, three analyses were performed using

MEGA 4 program (Kumar et al., 2008). Neighbor Joining (NJ) tree was built by calculating genetic distance based on Kimura's two parameters model (1980) with 1000 replicates. In addition, a maximum likelihood analysis (ML) was conducted. The robustness of nodes was inferred from a bootstrap analysis of 1000 replicates.

Bayesian analysis was performed using the program Mr. Bayes 3.1.1 (Huelsenbeck and Ronquist, 2001). Four Markov chains were run simultaneously for 1 000 000 generations and these were sampled every 100 generations. Data from the first 1000 generations were discarded as the `burn-in `period, after confirming that likelihood values had stabilized prior to the 1000^{th} generations. Posterior probabilities of nodes were calculated from the remaining sample.

To reveal the geographic structure of each locus, spatial analyses of molecular variance (SAMOVA) were performed to assess the genetic differentiation among groups of adjacent populations, using SAMOVA version 1.0 with 100 initial conditions (Dupanloup et al., 2002). Given the number of groups (K), the highest differentiation among groups (F_{CT}) and the population configuration were calculated using a simulated annealing procedure.

Gene flow (Nm) was performed using ARLEQUIN version 3.0 software (Excoffier et al., 2005). For cpDNA, haploid and uniparentally inherited, Nm was estimated using the expression $F_{ST} = 1/(1 + Nm)$, and for nrDNA $F_{ST} = 1/(1 + 2Nm)$, where N is the number of individuals in each population and m is the fraction of migrants in each population in each generation (Hudson et al., 1992).

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References

Aburjai, T., Hudaib, M., Cavrini, V., 2006. Composition of the Essential Oil from Jordanian Germander (*Teucrium polium* L.). J. Essent.Oil. Res. 18, 97-99.

Algerian Minister of Agriculture and Rural Development, 2010. Phase A du plan de gestion II 2006-2010. Phase descriptive et analytique. Parc National de Tlemcen.

Al-Qudah, T.S., Shibi, R.A., Alali, F.Q., 2011. In vitro propagation and secondary metabolites production in wild germander (*Teucrium polium* L.). In Vitro Cell. Dev. Biol. Plant 47, 496-505.

Ansari Asl, A., Soveid, M., Azadbakht, M., Omrani, G.H., Solimani, S.M., Samani, M., 2003. The effect of extract of *Teucrium polium* on blood sugar and insulin levels of type 2 diabetic patients. Shiraz E-Med. J. 4.1-6.

Antunes, T., Sevinate-Pinto, I., Barroso, J.G., Cavaleiro, C., Salgueiro, L.R., 2004. Micromorphology of trichomes and composition of essential oil of *Teucrium capitatum*. Flav. Fragr. J. 19, 336-340.

Ashnagar, A., Gharib Naseri, N., Foroozanfar, S., 2007. Isolation and Identification of the Major Chemical Components Found in the Upper Parts of *Teucrium polium* Plants Grown Khuzestan Province of Iran. Chin. J. Chem. 25, 1171-1173.

Awadh Ali, N.A., Wurster, M., Arnold, N., Lindequist, U., Wessjohan, L., 2008. Chemical composition of the essential oil of *Teucrium yemense* Deflers. Rec. Nat. Prod. 2, 25-32.

Baba Aissa, F., 2000. Encyclopedie des Plantes Utiles. Flore d'Algérie et du Maghreb, EDAS. ed. Librairie Moderne, Rouiba, Algérie.

Barret, S.C.H., Kohn, J.R., 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants, Oxford University Press. ed. Oxford University Press, New-York.

Bazina, E., Makris, A., Vender, C., Skoula, M., 2002. Genetic and Chemical Relations Among Selected Clones of Salvia officinalis. J. Herbs Spices Med. Plants 9, 269-273.

Bezic, N., Vuko, E., Dunkic, V., Ruscic, M., Blazevic, I., Burcul, F., 2011. Antiphytoviral Activity od Sesquiterpene-Rich Essential Oils from Four Croatian Species. Molecules 16, 8119-8129.

Bicchi, C., Liberto, E., Matteodo, M., Sgorbini, B., Mondello, L., Zellner, B.A., Costa, R., Rubiolo, P., 2008. Quantitative analysis of essential oils: a complex task. Flav. Fragr. J. 23, 382-391.

Bonneau, M., Souchier, B., 1994. Pedologie 2, Contribution et Propriétés du sol, Masson. ed. Paris.

Bouaoune, D., Dahmani-Megrerouche, M., 2010. Climatic data reconstitution of North Algeria: Application of neural network method. CR. Geosci. 342, 815-822.

Brereton, R.G., 2003. Chemometrics: Data Analysis for the Laboratory and Chemical Plant, Wiley Interscience. ed. Wiley Interscience, New-York.

Bruno, M., Maggio, A.M., Piozzi, F., Puech, S., Rosselli, S., Simmonds, M.S.., 2003. Neoclerodane diterpenoids from *Teucrium polium* subsp. *polium* and their antifeedant activity. Biochem. Syst. Ecol. 31, 1051-1056.

Bruno, M., Rosselli, S., Maggio, A., Piozzi, F., Scaglioni, L., Arnold, N.A., Simmonds, M.S.., 2004. Neoclerodanes from *Teucrium orientale*. Chem. Pharm. Bull. 52, 1497-1500.

Cakir, A., Emin, M., Harmandar, M., Ciriminna, R., Passannanti, S., 1998. Volatile Constituents of *Teucrium polium* L. from Turkey. J. Essent.Oil. Res. 10, 113-115.

Canerot, J., Cugny, P., Peybernès, B., Rahhali, I., Rey, J., Thieuloy, J.P., 1986. Comparative study of the Lower and Mid-Cretaceous sequences on different Maghrebian shelves and basins-their place in the evolution of the North African Atlantic and Neotethysian margins. Palaeogeogr. Palaeoclimatol. Palaeoecol. 55, 213-232.

Chizzola, R., 2006. Volatile compounds from some wild growing aromatic herbs of the Lamiaceae from southern France. Plant. Biosyst. 40, 206-210.

Collignon, B., 1986. Hydrogéologie appliquée des aquifères karstiques des Monts de Tlemcen (Algérie). Université d'Avignon.

Costa, R., Zellner, B.A., Crupi, M.L., DE Fina, M.R., Valentino, M.R., Dugo, P., Dugo, G., Mondello, L., 2008. GC–MS, GC–O and enantio–GC investigation of the essential oil of *Tarchonanthus camphoratus* L. Flav. Fragr. J. 23, 40-48.

Couladis, M., Tzakou, O., Verykokidou, E., Harvala, C., 2003. Screening of some Greek aromatic plants for antioxidant activity. Phytother. Res. 17, 472-476.

Council of Europe, 1997. European Pharmacopoeia, Council of Europe. ed. Strasbourg.

Cozzani, S., Muselli, A., Desjobert, J.-M., Bernardini, A.-F., Tomi, F., Casanova, 2005. Chemical composition of essential oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica. Flav. Fragr. J. 20, 436-441.

Djabou, N., Allali, H., Battesti, M.-J., Tabti, B., Costa, J., Muselli, A., Varesi, L., 2011. Chemical and genetic differentiation of two Mediterranean subspecies of *Teucrium scorodonia* L. DOI: 10.1016/j.phytochem.2011.09.002.

Djabou, N., Battesti, M.-J., Allali, H., Desjobert, J.-M., Varesi, L., Costa, J., Muselli, A., 2011. Chemical and genetic differentiation of Corsican subspecies of *Teucrium flavum* L. Phytochemistry 72, 1390-1399.

Djabou, N., Paolini, J., Desjobert, J.-M., Allali, H., Baldovini, N., Costa, J., Muselli, A., 2010. Quantitative and qualitative analysis of volatile components of *Teucrium massiliense* L. identification of 6-methyl-3-heptyl acetate as a new natural product. Flav. Fragr. J. 25, 475-487.

Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11, 2571-2581.

Echeverrigaray, S., Agostini, G., Atti-Serfini, L., Paroul, N., Pauletti, G.F., Dos Santos, A.C., 2001. Correlation between the chemical and genetic relationships among commercial thyme cultivars. J. Agric. Food Chem. 49, 4220-4223.

Eikani, M.H., Goodarznia, I., Mirza, M., 1999. Comparison between the Essential Oil and Supercritical Carbon Dioxide Exteact of *Teucrium polium* L. J. Essent.Oil. Res. 11, 470-472.

El Oualidi, J., Puech, S., Navarro, T., 2002. Geographical variation and successive adaptive of yellow-flowered *Teucrium* (Labiateae) in the Mediterranean region. Bot. Rev. 68, 209-234.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (Version 3.0): An integrated software package for population genetics data analysis Evolutionary, 1st ed.

Falchi, A., Paolini, J., Desjobert, J.M., Melis, A., Costa, J., Varesi, L., 2009. Phylogeography of *Cistus creticus* L. on Corsica and Sardinia inferred by the TRNL-F and RPL32-TRNL sequences of cpDNA. Mol. Phylogenet. Evol. 52, 538-543.

Fernandez, P.B., Iglesias, P.I., Villar del Fresno, A.M., 1997. Anti-inflammatory and antiulcer activity of *Teucrium buxifolium*. J. Ethnopharmacol. 55, 93-98.

Fiorentino, A., D'Abrosca, B., Pacifico, S., Scognamiglio, M., D'Angelo, G., Monaco, P., 2010. abeo-Abietanes from *Teucrium polium* roots as protective factors against oxidative stress. Bioorg. Medicinal Chem. 18, 8530-8536.

Hammoudi, R., Hadj Mahammed, M., 2010. Contribution à l'Etude de la Composition Chimique des Huiles Essentielles de la Plante *Teucrium polium* ssp. *geyrii* (Lamiaceae). Annales des Sciences et Technologie 2, 1-5.

Hassan, M.M., Muhtadi, F.J., AL-Badr, A.A., 1979. GLC-mass spectrometry of *Teucrium polium* oil. *Journal of pharmaceutical sciences* 68, 800-801.

Hudaib, M., Mohammad, M., Bustanji, Y., Tayyem, R., Yousef, M., Abuirjeie, M., Aburjai, T., 2008. Ethnopharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. J. Ethnopharmacol. 120, 63-71.

Hudson, R.R., Slatkin, M., Maddison, W.P., 1992. Estimation of Levels of Gene Flow From DNA Sequence Data. Genetics 132, 583-589.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17, 754-755.

Jeanmonod, D., Gamisans, J., 2007. Flora Corsica, Edisud. ed. la compagnie des éditions de la Lesse, Aix-en-Provence.

Jennings, W., Shibamoto, T., 1980. Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography, Academic Press. ed. In: Jovanovich, H.B (Ed), New-York.

Kabouche, A., Kabouche, Z., Ghannadi, A., Sajjadi, S.E., 2007. Analysis of the Essential Oil of *Teucrium polium* ssp. *aurasiacum* from Algeria. J. Essent.Oil. Res. 19, 44-46.

Kalisz, S., Kramer, E.M., 2008. Variation and constraint in plant evolution and development. Heredity 100, 171-177.

Kamel, A., Sandra, P., 1994. Gas Chromatography-Mass Spectrometry Analysis of the Volatile Oils of Two *Teucrium polium* Varieties. Biochem. Syst. Ecol. 22, 529-532.

Konig, W.A., Hochmuth, D.H., Joulain, D., 2001. Terpenoids and Related Constituents of Essential oils., Library of Mass Finder 2.1 ed. Institute of Organic Chemistry, Hamburg.

Kovacevic, N.N., Lakusic, B.S., Ristic, M., 2001. Composition of essential oils of seven Teucrium species from Serbia and Montenegro. J. Essent.Oil. Res. 13, 163-165.

Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9, 299-306.

De Martino, L., Formisano, C., Mancini, E., De Feo, V., Piozzi, F., Rigano, D., Senatore, F., 2010. Chemical Composition and Phytotoxic Effects of Essential Oils from Four *Teucrium* Species. Nat. Prod. Com. 5, 1969-1976.

Massart, D.L., 1998. Chemometrics: A Textbook, Elsevier Science Ltd. ed. Elsevier, New-

York.

Mass Finder, 2006. In: Hochmunth D., version 3.0, Scientific Consulting, Germany.

McLafferty, F.W., Stauffer, D.B., 1988. The Wiley/NBS Registry of Mass Spectra Data, Wiley-Interscience. ed. New-York.

Menichini, F., Conforti, F., Rigano, D., Formisano, C., Piozzi, F., Senatore, F., 2009. Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. Food Chem. 115, 679-686.

Moghtader, M., 2009. Chemical Composition of the Essential Oil of *Teucrium polium* L. from Iran. Am-Euras. J. Agric. & Environ. Sci. 5, 843-846.

Muselli, A., Costa, J., Desjobert, J.-M., Dessi, M.A., Paolini, J., Bernardini, A.-F., 2009. Chemical Composition of the Essential Oils of *Teucrium chamaedrys* L. from Corsica and Sardinia. J. Essent. Oil Res. 21, 138-143.

National Institute of Standards and Technology, 1999. NIST/EPA/NIH Mass Spectral Library. PC Version 1.7. Perkin-Elmer Corp., Norwalk, CT, USA.

Navarro, T., El Oualidi, J., 2000. Sinopsis of *Teucrium* L. (Lamiaceae) in the Mediterranean region and surrounding areas. Flora Med. 10, 349-363.

Naydenov, K.D., Tremblay, F.M., Alexandrov, A., Fenton, N.J., 2005. Structure of *Pinus sylvestris* L. populations in Bulgaria revealed by chloroplast microsatellites and terpenes analysis: Provenance tests. Biochem. Syst. Ecol. 33, 1226-1245.

Parsaee, H., Shafiee-Nick, R., 2006. Anti-Spasmodic ans anti-nociceptive effects of *Teucrium polium* aqueous extract. Iranian Biomed. J. 10, 145-149.

Pérez-Alonso, M.J., Velasco-Negueruela, A., Lopez-Saez, J.A., 1993. The Essential Oils of Two Iberian *Teucrium* Species. J. Essent. Oil Res. 5, 397-402.

Petit, R.J., Aguinagalde, I., De Beaulieu, J., Bittkau, C., 2003. Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity. Science 300, 1563-1565.

Piozzi, F., Bruno, M., Rosselli, S., Maggio, A., 2005. Advances on the chemistry of furano-diterpenoids from *Teucrium* genus. Heterocycles 65, 1221-1234.

Piqué, A., Tricart, P., Guiraud, R., Laville, E., Bouaziz, S., Amrhar, M., Ait Ouali, R., 2002. The Mesozoic–Cenozoic Atlas belt (North Africa): an overview. Geodin. Acta 15, 185-208.

Quezel, P., Santa, S., 1963. Nouvelle Flore de l'Algérie, Centre National de la Recherche Scientifique. ed. Paris.

Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., Rozas, R., 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19, 2496-2497.

Saracoglu, V., Arfan, M., Shabir, A., Hadjipavlou-Litina, D., Skaltsa, H., 2007. Composition and antioxidant activity of the essential oil of *Teucrium royleanum* Wall. ex Benth growing in Pakistan. Flav. Fragr. J. 22, 154-157.

Sarer, E., Konuklugil, B., 1987. Investigation of the volatile oil of *Teucrium polium* L. Doga: Turk Tip ve Eczacilik Dergisi 11, 317-325.

Skoula, M., Abbes, J.E., Johnson, C.B., 2000. Genetic variation of volatiles and rosmarinic acid in populations of *Salvia fruticosa* mill growing in Crete. Biochem. Syst. Ecol. 28, 551-561.

Stanciu, G., Mititelu, M., Popescu, M., 2006. The GC-MS characterization of the volatile oil from *Teucrium polium* L. Ovidius Univ. Anal. Chem. 17, 119-122.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876-4882.

Tomi, F., Casanova, J., 2006. 13C NMR as a tool for identification of individual components of essential oils from Labiatae - a review. Acta Hort. 723, 185-192.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentie, D.H., Webb, D.A., 1972. Flora Europaea. Cambridge University Press, Cambridge.

Vahdani, M., Faridi, P., Zarshenas, M.M., Javadpour, S., Abolhassanzadeh, Z., Moradi, N., Bakzadeh, Z., Karmostaji, A., Mohagheghzadeh, A., Ghasemi, Y., 2011. Major Compounds and Antimicrobial Avtivity of Essential Oils from Five Iranian Endemic Medicinal Plants. Pharmcog. J. 3, 48-53.

Vokou, D., Bessiere, J.M., 1985. Volatile Constituents of *Teucrium polium*. J. Nat. Prod. 48, 498-499.

Wares, J.P., Cunningham, C.W., 2001. Phylogeography and historical ecology of the North Atlantic intertidal. Int. J. Org. Evolution 55, 2455-2469.

White, T., Bruns, T., Lee, S., Taylor, J., 1990. PCR protocols: a guide to methods and applications, Academic press. ed. Innes, M., Gelfand, D., Sninsky, J., White, T., San Diego, California.

Zaouali, Y., Boussaid, M., 2008. Isozyme markers and volatiles in Tunisian *Rosmarinus officinalis* L. (Lamiaceae): a comparative analysis of population structure. Biochem. Syst. Ecol. 36, 11-21.

Figure(s)

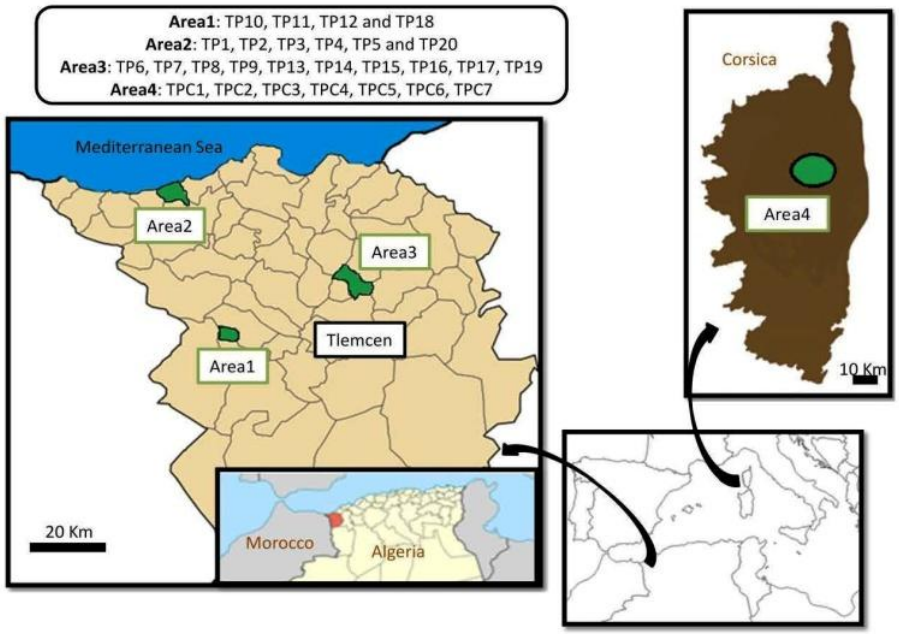

Fig. 1. Geographical Distribution of *Teucrium polium* subsp. *polium* (TP1-20) from western Algeria and *Teucrium polium* subsp. *capitatum* (TPC1-7) from Corsica

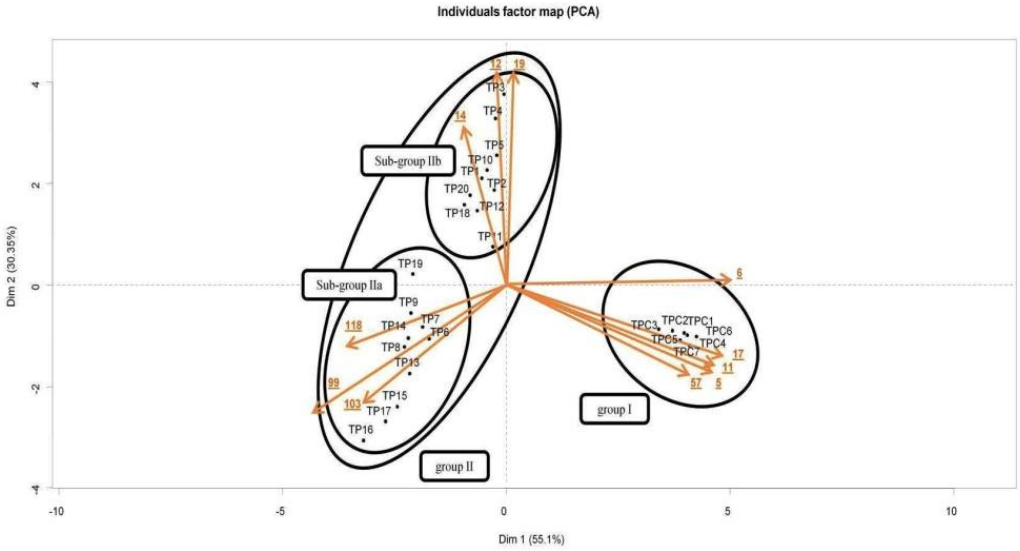

Fig. 2. PCA of chemical compositions of *T. polium* subsp. *polium* (TP) and *T. polium* subsp. *capitatum* (TPC) oils. Distribution of variables (component codes corresponding to those of Table 2) and distribution of samples (coding numbers of locations)

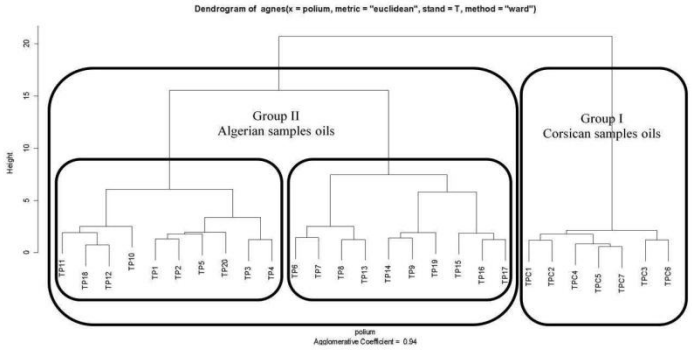

Fig. 3. CA of chemical compositions of *T. polium* subsp. *polium* (TP) from western Algeria and *T. polium* subsp. *capitatum* (TPC) oils from Corsica

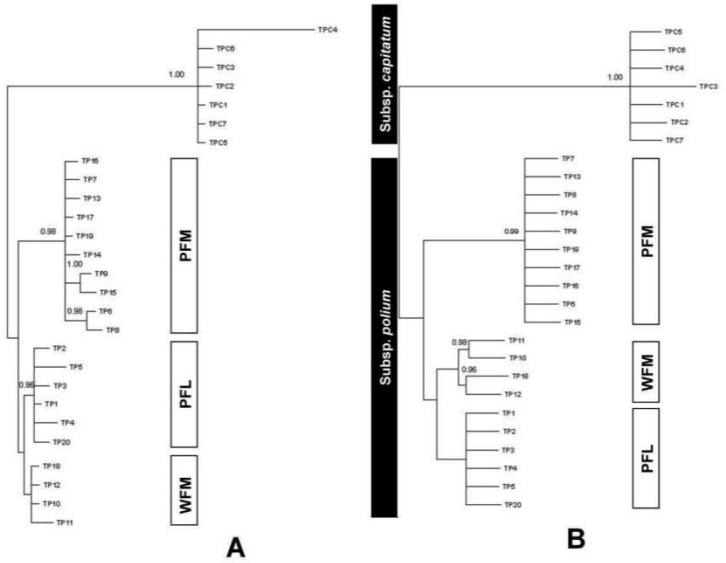

Fig. 4: Consensus trees obtained from the Bayesian analyses of ITS region (A) and cpDNA (B). Bayesian posterior probabilities >0.95 are show above branches. PFM: pink flower mountain (area 3); PFL: pink flower littoral (area 2); WFM: white flower mountain (area 1).

Table(s)
Click here to download Table(s): T_polium_tables.xlsx

Table 1: Data of previous chemical investigations of volatile components from *Teucrium polium* and its subspecies

	Plant origin	Iran					Algeria		Greece		Jordan		Turkey		Egypt		Espagne		Corse	Croatia
	Subspecies	np					*aurasiacum*	*geyrii*	*capitatum*	np	np		np		*album*	*pilosum*	*valentinum*	*gnaphalodes*	*capitatum*	np
	References	(A)	(B)	(C)	(D)		(E)	(F)	(G)	(H)	(I)	(J)	(K)	(L)	(M)		(N)		(O) (P)	(Q)
	Extraction modes	HD	HD	HD	HD	CO2	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD
N°	Components	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
5	*α*-thujene	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5	nd	nd	nd	0.3	nd	1.4	0.9	5.0	nd
6	*α*-pinene	4.3	12.5	8.0	9.0	9.8	9.5	0.2	0.2	0.4	0.7	nd	12.0	7.7	4.1	nd	15.8	1.6	28.8	tr
11	sabinene	nd	0.8	nd	0.1	0.2	tr	nd	0.5	nd	5.2	nd	nd	nd	1.7	nd	7.2	8.8	1.6	nd
12	*β*-pinene	3.7	7.1	5.8	9.7	7.2	8.3	0.3	0.2	0.5	1.5	nd	18.0	10.2	4.9	0.3	11.7	7.1	7.2	0.3
14	myrcene	nd	1.5	nd	1.6	2.1	0.5	0.1	nd	0.4	0.5	nd	6.8	nd	1.6	nd	2.9	1.5	1.2	0.1
17	*p*-cymene	8.2	0.5	nd	0.3	0.2	nd	nd	0.3	0.4	0.6	nd	nd	nd	0.4	0.1	3.8	0.3	7.0	nd
19	limonene	37.7	1.9	9.2	4.6	3.7	0.6	11.2	tr	0.4	0.7	nd	3.5	nd	0.6	0.4	3.2	3.1	3.0	5.9
34	linalool	nd	10.6	nd	1.6	tr	0.3	nd	0.8	1.3	nd	4.2	0.8	nd	nd	0.1	2.3	1.4	1.9	1.9
39	*β*-thujone	nd	nd	nd	nd	nd	nd	0.2	nd	nd	nd	nd	nd	nd	0.1	0.1	0.2	nd	0.5	5.7
	cis-sabinol	nd	nd	nd	nd	nd	nd	nd	1.3	nd	nd	nd	nd	6.2	nd	nd	nd	nd	nd	nd
44	*trans*-pinocarveol	nd	nd	nd	1.1	1.8	0.5	nd	nd	1.6	nd	nd	1.6	nd	0.4	0.6	4.3	7.8	2.7	nd
45	*cis*-verbenol	nd	nd	nd	nd	nd	nd	nd	2.1	nd	nd	nd	nd	nd	nd	nd	0.5	2.6	0.7	nd
46	*trans*-verbenol	nd	nd	nd	0.6	2.2	nd	nd	nd	3.0	nd	4.7	nd	nd	nd	nd	nd	nd	1.8	nd
57	terpinen-4-ol	nd	0.2	nd	nd	nd	tr	0.1	0.7	0.8	nd	5.0	nd	nd	0.9	1.0	4.5	0.5	4.6	0.2
61	verbenone	1.7	nd	nd	1.2	2.6	nd	tr	3.7	0.7	nd	5.3	nd	nd	nd	nd	0.4	nd	2.7	nd
77	carvacrol	nd	5.2	nd	nd	nd	nd	nd	10.1	0.5	nd	nd	nd	nd	0.8	0.3	0.5	0.1	0.1	nd
	(*E*)-*β*-caryophyllene	nd	7.0	9.2	18.0	16.5	0.4	9.2	9.8	7.7	8.7	nd	17.8	nd	1.6	1.7	0.7	12.1	nd	52.0
91	*γ*-elemene	nd	nd	16.8	nd	nd	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
94	aromadendrene	nd	nd	nd	nd	nd	nd	0.4	nd	nd	nd	8.7	nd	6.6	nd	nd	nd	nd	nd	nd
96	*α*-humulene	nd	2.8	nd	1.9	1.6	0.3	3.2	3.8	3.7	4.3	nd	nd	nd	0.3	2.3	nd	nd	nd	4.6
97	alloaromadendrene	1.6	nd	nd	nd	nd	0.1	0.6	0.4	0.6	4.5	nd	nd	7.3	3.6	1.4	0.1	3.4	nd	nd
98	*γ*-muurolene	nd	nd	23.2	nd	nd	nd	nd	nd	1.6	nd	nd	1.9	nd	nd	nd	0.1	nd	nd	nd
99	germacrene D	nd	5.0	3.4	13.2	23.6	nd	0.3	3.1	nd	6.8	nd	5.3	nd	1.4	4.4	0.8	0.3	nd	8.7
103	bicyclogermacrene	nd	nd	nd	9.0	11.9	nd	nd	0.7	nd	nd	nd	2.4	nd	nd	nd	0.9	0.3	nd	nd
106	*γ*-cadinene	1.5	3.7	nd	nd	nd	nd	1.4	nd	3.0	2.1	nd	nd	nd	2.6	18.0	1.0	0.4	nd	nd
108	*δ*-cadinene	nd	nd	nd	1.8	tr	nd	10.0	2.9	1.7	3.5	nd	nd	nd	4.1	8.3	0.1	nd	0.6	tr
	2,4di-tert-butylphenol	10.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
115	(*E*)-nerolidol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.5	nd	nd	0.2	0.1	nd	nd
118	spathulenol	3.6	0.2	11.7	10.4	4.0	nd	0.3	nd	nd	1.2	8.6	3.3	nd	nd	11.6	0.4	0.1	nd	tr
119	caryophyllene oxide	nd	9.7	nd	7.1	2.6	0.2	4.8	nd	5.9	1.1	nd	10.0	nd	nd	nd	0.1	nd	tr	tr
	Torreyol	nd	nd	nd	nd	nd	nd	2.8	7.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
131	*τ*-cadinol	nd	nd	nd	nd	nd	nd	4.3	nd	9.3	2.3	9.2	1.8	nd	nd	nd	0.6	0.4	nd	nd
135	*β*-eudesmol	nd	nd	7.5	nd	nd	nd	nd	nd	nd	0.3	nd	nd	nd	3.3	19.1	nd	nd	0.3	nd
137	*α*-cadinol	nd	1.7	nd	nd	nd	46.8	2.0	4.5	5.4	2.6	9.4	nd	nd	5.9	5.4	0.3	0.5	nd	tr
	patchouli alcohol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	33.3	2.8	nd	nd	nd	nd
	8-cedren-13-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	24.8	nd	nd	nd	nd	nd	nd	nd	nd	nd
	3*β*-hydroxy-*α*-muurolene	nd	nd	nd	nd	nd	22.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Only the main components were reported; main components are classified by number corresponding to the Table2; only one sample was studied, except for *T. polium* subsp. *capitatum* from Portugal (4 samples).
Extraction mode: HD: Hydrodistillation; CO2: Supercritic Carbone dioxide extract; tr: <0.05; np: subspecies not precized; nd: compounds not detetcted; present: % are not reported.
(A): Vahdani et al., 2011. (B): Morghtader, 2009. (C): Ashnagar et al., 2007. (D): Eikani et al., 1999. (E): Kabouche et al., 2007. (F): Hammoudi et al., 2010. (G): Meichini et al., 2009. (H): Vokou and Bessiere, 1985. (I): Aburjai et al., 200

[**PT6**] : ***Teucrium* activités biologiques**

Djabou, N., Guinoiseau, E., Giuliani, M.C., Andreani, S., Lorenzi, V., Bolla, J.M., Costa, J., Berti, L., Luciani, A., Muselli, A. "Phytochemical composition and antibacterial activity of *Teucrium* essential oils from Corsica" *Food Chemistry*, **2012**.

Phytochemical composition and antibacterial activity of *Teucrium* essential oils from Corsica

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Abstract

The chemical composition of the essential oils of Corsican *Teucrium* species were studied using capillary GC and GC/MS while the antibacterial activities were determinate by paper disc diffusion method and minimum inhibitory concentration (MIC) assays. We performed the chemical data compilation with previous in-house studies and the comparative data analysis between the six *Teucrium* species investigated: *T. marum*, *T. massiliense*, *T. chamaedrys*, *T. scorodonia* subsp. *scorodonia*, *T. polium* subsp. *capitatum* and *T. flavum* with two subspecies (*T. flavum* subsp. *glaucum* and *T. flavum* subsp. *flavum*). In total, 314 oil components were detected. With the aid of statistical tools, the 64 sample oils were clustered in two main groups. *Teucrium* essential oils were dominated by mono and/or sesquiterpene hydrocarbon compounds except *T. massiliense* and *T. marum* oils which exhibited oxygenated compounds as main components. In addition, we reported for the first time, the antibacterial activities of Corsican *Teucrium* essential oils against six bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria innocua*, *Campylobacter jejuni*, *Enterobacter aerogenes* wild type (CIP 60.86) and a multi-drug resistant bacterium *Enterobacter aerogenes* (EAEP289). With exception of *T. scorodonia* subsp. *scorodonia* and *T. chamaedrys* oils, all *Teucrium* essential oils inhibit the bacterial growth of the tested strains: *S. aureus*, *S. epidermidis*, *L. innocua*, *C. jejuni*, *E. aerogenes* and the multi-drug resistant (MDR) strain *E. aerogenes* EAEP289. The foodborne pathogen *C. jejuni* was found to be extremely sensitive to all the Corsican *Teucrium* oils. Our study suggests that Corsican *Teucrium* essential oils have the potential to be used as food preservatives and to prevent the growth of nosocomial bacteria.

Keywords: Corsican *Teucrium* species, Essential oils, Chemical composition, Antibacterial activities.

1. Introduction

Many new drugs derived from plant secondary metabolites have been applied for the treatment and/or prevention of various diseases (Menichini, Conforti, Rigano, Formisano, Piozzi & Senatore, 2009). Aromatic plants are frequently used in traditional medicine. Essential oils and volatile constituents extracted from them are widely used as antioxidants or antidiabetic agents and for prevention and treatment of different human diseases, such as cancer, cardiovascular diseases, including atherosclerosis, thrombosis, bacterial and viral infections (Edris, 2007). Due to the increasing consumer demand for more natural foods, the abuse of toxic synthetic food substances and the increasing microbial resistance of pathogenic microorganisms against antibiotics, natural substances isolated from plants are considered as promising sources of food preservatives (Burt, 2004).

The genus *Teucrium* (Lamiaceae family) includes 300 species widespread all over the world, but most concentrated around the Mediterranean basin (Beni Maleci, Pinetti & Servettaz, 1995). In Corsica, *Teucrium* genus comprises 8 species: *T. marum*, *T. massiliense*, *T. chamaedrys*, *T. scorodonia* subsp. *scorodonia*, *T. polium* subsp. *capitatum*, *T. flavum* with two subspecies (*T. flavum* subsp. *glaucum* and *T. flavum* subsp. *flavum*), *T. fruticans* and *T. scordium* (Jeanmonod & Gamisans, 2007). Among them, *T. fruticans*, *T. scordium* and *T. flavum* subsp. *flavum* were very rare and probably decimated. Many species of *Teucrium* are used in traditional folk medicine and it is claimed they have hypoglycemic, antipyretic, antiulcer, anti-inflammatory, antitumor and antibacterial properties (Couladis, Tzakou, Verykokidou & Harvala, 2003; Fernandez, Iglesias & Villar del Fresno, 1997; Menichini et al., 2009; Ozkan, Kuleasan, Celik, Gokturk & Unal, 2007). Maccioni, Baldini, Tebano & Cioni (2007) noted the importance of *Teucrium* species as alimentary plants; some of them are currently used in the preparation of flavoured wines and beer, herbal teas, bitters and liqueurs (Bosisio, Giavarini, Dell'Agli, Galli & Galli, 2004). The importance of this genus in

food industries lies also in the fact that many species show antimicrobial, antioxidant and antifungal activities, rendering them useful as natural perspective ingredients (Bosisio et al., 2004; Ozkan et al., 2007; Saracoglu, Arfan, Shabir, Hadjipavlou-Litina & Skaltsa, 2007; Ulubelen, Topu & Sönmez, 2000).

The genus *Teucrium* is one of the richest sources of neoclerodane diterpenes: more than 220 diterpenes have been described, many of them are of interest due to their insect-repellent and medicinal properties (Bruno et al., 2004; Coll & Tandrón, 2005; Piozzi, Bruno, Rosselli & Maggio, 2005). From its indumentums of glandular and non-glandular trichomes, the aerial parts of *Teucrium* species were well-known to produce essential oils. Essential oils of *Teucrium* genus have been the subject of many studies. According to the species studied and the plant origin, essential oil yields ranged from 0.05% to 1.5% and the amounts of main constituents (mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes) differed notably (Awadh Ali, Wurster, Arnold, Lindequist & Wessjohan, 2008; Saracoglu et al., 2007).

In previous work, we have studied the essential oil compositions of seven taxa of *Teucrium* from Corsica: *T. polium* subsp. *capitatum* (Cozzani, Muselli, Desjobert, Bernardini, Tomi & Casanova, 2005), *T. chamaedrys* (Muselli, Costa, Desjobert, Dessi, Paolini & Bernardini, 2009), *T. massiliense*, (Djabou et al., 2010), both subspecies of *T. flavum* (*T. flavum* subsp. *glaucum* and *T. flavum* subsp. *flavum*) (Djabou et al., 2011), *T. scorodonia* subsp. *scorodonia* (Djabou et al., 2012), and *T. marum* (Djabou et al., submitted manuscript) and showing intraspecific variations in the chemical composition of these essential oils according environment and/or genetic parameters. It is interesting to report a global analysis of these chemical data. So, the objectives of this work have been to (i) compile the chemical components isolated from Corsican *Teucrium* essential oils recently analyzed in our laboratory and (ii) perform a comparative analysis between chemical compositions of each

species. For *T. chamaedrys* and *T .polium* subsp *capitatum*, we supplemented the sampling in order to analyze more than one sample oil. Chemical analysis was performed using a combination of capillary GC-RI and GC/MS after fractionation using column chromatography. Furthermore, a survey of the literature revealed that there is no study on the antibacterial activity of *Teucrium* essential oils harvested in Corsica, so we investigated their antibacterial properties by means of paper disc diffusion method and minimum inhibitory concentration (MIC) assays.

1. Material and methods

1.1. Plant material and Oil isolation

Aerial parts of plants were collected in Corsica at the flowering stage in 7 localities from *T. polium* subsp. *capitatum* (**TP1**: 42° 19' 11" N, 09° 18' 03" E, **TP2**: 42° 19' 02" N, 09° 15' 51" E, **TP3**: 42° 17' 50" N, 09° 18' 33" E, **TP4**: 42° 17' 58" N, 09° 09' 00" E, **TP5**: 42° 19' 37" N, 09° 18' 01" E, **TP6**: 42° 18' 39" N, 09° 16' 01" E, **TP7**: 42° 18' 08" N, 09° 08' 59" E) and in 5 localities from *T. chamaedrys* (**TCH1**: 42° 17' 58" N, 09° 09' 00" E, **TCH2**: 42° 16' 32" N, 09° 17' 42" E, **TCH3**: 42° 17' 40" N, 09° 17' 57" E, **TCH4**: 42° 18' 22" N, 09° 17' 28" E, **TCH5**: 42° 17' 50" N, 09° 18' 33" E). In previous works, we have been analyzed 25 sample oils of *T. marum* (**TM1-25**), 10 sample oils of *T. scorodonia* subsp. *scorodonia* (**TSC1-10**), 10 sample oils of *T. flavum* subsp. *glaucum* (**TFG1-10**), 4 sample oils of *T. massiliense* (**TMC1-4**) and 3 sample oils of *T. flavum* subsp. *flavum* (**TFF1-3**). Voucher specimens were deposited in the Herbarium of the University of Corsica. The fresh aerial parts of **TP1-7** and **TCH1-5** (350-550 g) were submitted to hydrodistillation from 5 h using a Clevenger-type apparatus according to the European Pharmacopoeia (Council of Europe, 1997) and yielded (w/w) 0.490-0.620 % for *T. polium* subsp. *capitatum* oils (**TP**) and 0.235-0.350 % for *T. chamaedrys* oils (**TCH**).

1.2. Oil fractionation

From *T. polium* subsp. *capitatum* sample oils and *T. chamaedrys* sample oils, two "collective oils", called **TP** and **TCH**, were constituted by the mixture of all individual sample oils from each location. Both mixtures were submitted to column chromatography (CC, silica gel 200-500 µm and/or 63-200 µm/$AgNO_3$ (20%)). The fractions and sub-fractions, eluded using an increased gradient of pentane (n-C_5H_{12})/diethyl ether (Et_2O), were analyzed by GC-RI, GC/MS.

1.3. Gas chromatography

GC analyses were carried out using a Perkin Elmer Clarus 600 GC apparatus (Walhton, MA, USA) equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60 to 230 °C at 2 °C.min^{-1} and then held isothermal 230 °C (30 min). Carrier gas: helium (1 mL.min^{-1}). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 µL.

1.4. Gas chromatography-mass spectrometry

The oils and the fractions obtained by CC were investigated using a Perkin Elmer TurboMass quadrupole analyzer, directly coupled to a Perkin Elmer Autosystem XL equipped with two fused-silica capillary columns (60 m x 0.22 mm, film thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. Ion source temperature: 150 °C; energy ionization: 70 eV; electron

ionization mass spectra were acquired with a mass range of 35 – 350 Da; scan mass: 1s. Oil injected volume: 0.1 µL, fraction injected volume: 0.2 µL.

1.5. Component identification

Identification of the components was based (i) on the comparison of their GC retention indices (RI_A and RI_P) on non polar and polar columns, respectively determined the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literatures data (Jennings & Shibamoto, 1980; Konig et al., 2001; Mass Finder, 2006); (ii) on computer matching with commercial mass spectral libraries (McLafferty & Stauffer, 1988; NIST/EPA/NIH, 1999) and comparison of spectra with those of our laboratory-made library.

1.6. Component quantification

The quantification of the collective essential oil components was carried out using peak normalization including response factors (RFs) with internal standard using the methodology reported by Costa et al. 2008 and modified in our laboratory (Djabou et al., 2010). Concerning the seven "Collective oils", component quantification was expressed in g/100g of essential oil, while in order to perform the statistical analysis of the oil samples, the normalized % abundances were calculated, using the methodology reported by Bicchi et al. 2008. Tridecane was introduced in all sample oils at same concentration (0.7 g/100 g) as internal standard.

1.7. Antibacterial screening

1.7.1. Bacterial strains and growth conditions

The *in vitro* antibacterial activity of essential oils of Corsican *Teucrium* species was tested against six laboratory control strains: *Staphylococcus aureus* (CIP 53.156), *Staphylococcus*

epidermidis (CIP 53124), *Listeria innocua* (CIP 80.11), *Campylobacter jejuni* (CIP 107370), *Enterobacter aerogenes* wild type (CIP 60.86) and the multi-drug resistant (MDR) strain *Enterobacter aerogenes* EAEP289. All the CIP strains were provided by the Biological Resource Center of Institut Pasteur (Paris, France). EAEP289 is a kanamycin sensitive derivative from the clinical isolate EA27 (Mallea et al., 1998) and was provided by the UMR-MD1 of the School of Medicine from Marseille (France). All strains were routinely grown at 37°C on Mueller-Hinton 2 Agar (bioMérieux). The only exception concerns *C. jejuni*, which was grown on Mueller-Hinton 2 Agar supplemented with 5% of sheep blood and Campylosel mixture (bioMérieux) under microaerophilic conditions by using the Genbag microaer system (bioMérieux).

1.7.2. Disc diffusion assays

Inocula were prepared by diluting overnight cultures in Mueller-Hinton Broth (MHB, Oxoid) medium to approximately 10^6 CFU/mL. Filter paper discs (Whatman disc, 6 mm diameter) were impregnated with 15 µL of the essential oil and placed onto the inoculated Petri dishes containing Mueller-Hinton 2 Agar. After being held at room temperature for 1 h, plates were incubated at 37°C for 24 h. Following incubation, zones of inhibition were measured (mm) and recorded as the mean ± standard deviation (SD). Each test was performed in triplicate on at least three separate experiments. Positive control discs of antibiotics (Penicillin G and Ciprofloxacine, bioMérieux) were included in each assay.

1.7.3. Minimum inhibitory concentration assays

The minimum inhibitory concentration (MIC) assays were performed by a rapid INT (*p*-iodonitrotétrazolium chloride, Sigma-Aldrich) colorimetric assay, except for *C. jejuni*. The essential oils were serially twofold diluted in dimethylsulfoxide (DMSO, Sigma-Aldrich).

The DMSO was previously tested for antibacterial activity and no detrimental effect on bacterial growth was observed at the concentration used. The solutions obtained were then added (10 µL) to a 96-well microplate containing 200 µL of MHB inoculated with 10^6 CFU/mL. The microplates were incubated at 37°C for 18 h. The MICs of the samples were then detected following addition (50 µL) of INT (0.2 mg/mL). Viable bacteria reduced the yellow dye to pink. The MIC is defined as the lowest sample concentration that prevents this change and that results in the complete inhibition of bacterial growth. All determinations were performed in triplicate and a negative control, consisting of MHB with DMSO (5%, v/v), was systematically included.

For microaerophilic bacteria such as *C. jejuni*, MIC determinations were performed by a standard agar dilution macromethod (CLSI, 2007). All tests were performed on Mueller-Hinton 2 Agar. 30 µL of twofold serial dilutions in DMSO were added to 15 mL of agar to obtain concentrations ranging from 30 to 1000 µg/mL tested product. The resulting agar solutions were mixed at high speed for 15 sec, immediately poured into sterile Petri dishes, and then allowed to set for 30 min. The plates were then spot inoculated by pipetting 10^5 CFU of the strain on the plates. A negative control was prepared without essential oil, using only DMSO. Inoculated plates were incubated at 37 °C for 24 h. After the incubation period, they were observed and recorded for the presence or absence of bacterial growth. Each test was repeated at least three times.

1.8. **Statistical analysis**

Data analyses were performed using Principal Component Analysis (PCA) and Cluster Analysis (CA) (Brereton, 2003). Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed but are complementary in their ability to present results (Massart, 1998). Indeed, PCA provides the data for diagrams in which both objects (oil

samples) and variables (oil classes components and/or antibacterial inhibition) are plotted while canonical analysis informs a classification tree in which objects (sample locations) are gathered. PCA was carried out using function 'PCA' from the statistical R software. The variables (classes of volatile compounds and/or antibacterial inhibition) have been selected using function from the statistical software. The Cluster Analysis produced a dendrogram (tree) using the Ward's method of hierarchical clustering, based on the Euclidean distance between pairs of oil samples.

2. Results and discussion

2.1. Composition of the essential oils

First, we have been analyzed *T. polium* subsp. *capitatum* oils (**TPi**) and *T. chamaedrys* oils (**TCHi**) obtained from 7 and 5 individuals specimens harvested in different Corsican locations, respectively. Then, we analyzed both "collective oils" called **TP** and **TCH** by CC, GC/RI and GC/MS. It is noticeable that the individual samples of *T. polium* subsp. *capitatum* oils (**TPi**) and *T. chamaedrys* oils (**TCHi**) investigated here were similar to those previously studied in our laboratory (Cozzani et al., 2005, Muselli et al., 2009). Finally, for comparison purpose, we re-acquired GC chromatograms of the *Teucrium* collective oils (**TMC**, **TM**, **TSC**, **TFG** and **TFF**) previously in-house studied (Djabou et al., 2010, 2011, 2012) and we express the component quantification in g/100g of essential oil (Table 1, supplementary material). Data compilation of chemical compositions of Corsican *Teucrium* oils with previous in-house works, allowed the tabulation of 314 components which accounted for always more than 93.6 g/100g of the oils (Table 1, supplementary material). All these components were identified by comparison of their $RI_{(A \text{ and } P)}$ and EI-MS with those in our laboratory library excepted 64 of them which were identified by comparing their EI-MS and RI_A with those of commercial or literature libraries (Table 1, supplementary material).

Teucrium essential oils exhibited two classes of compounds: 93 non terpene aliphatic compounds and 221 terpene compounds, distributed as 107 monoterpenes, 11 norterpenes, 98 sesquiterpenes and 5 diterpenes. The oils were characterized by a large variety of (i) monoterpene skeletons: acyclic, cyclic (pinane, p-menthane) and bi-cyclic and (ii) sesquiterpene skeletons: acyclic (farnesane), cyclic (bisabolane, bergamotane, germacrane and humulane), bi-cyclic (bicyclogermacrane, caryophyllane, cadinane, muurolane and eudesmane) and tri-cyclic (cubebane, copaane, bourbonane and aromadendrane).

Concerning the main components, we choose to present a simplified table in which the components with concentration greater than 2 g/100g were reported (Table 2). It is noteworthy that the main components of Corsican *Teucrium* essential oils differed according to the taxa studied. *T. massiliense* oils (**TMC**) seems originals because of the occurrence of acyclic non terpene oxygenated compounds as main components: 6-methyl-3-heptyl acetate (19.1 g/100g), 3-octanyl acetate (7.0 g/100g), while all other *Teucrium* oils displayed terpene compounds as main components. *T. polium* oils (**TP**) and both subspecies of *T. flavum* oils (**TFG** and **TFF**) were characterized by hydrocarbon monoterpenes as main components: (i) α-pinene (24.1 g/100g), β-pinene (9.2 g/100g), α-thujene (8.1 g/100g) for **TP** oils, (ii) limonene (27.4 g/100g), α-pinene (12.2 g/100g), β-pinene (10.3 g/100g) and (*Z*)-β-ocimene (6.0 g/100g) for **TFG** oils and (iii) α-pinene (21.9 g/100g), limonene (20.0 g/100g), β-pinene (18.1 g/100g) and (*Z*)-β-ocimene (15.5 g/100g) for **TFF** oils. In the same way, *T. marum*, *T. scorodonia* and *T. chamaedrys* oils (**TM, TSC, TCH**, respectively) were dominated by sesquiterpenes compounds: (i) caryophyllene oxide (9.8 g/100g), (*E*)-α-bergamotene (8.2 g/100g), β-bisabolene (7.5 g/100g) and (*E*)-β-caryophyllene (5.3 g/100g) for **TM** oils (ii) (*E*)-β-caryophyllene (21.1 g/100g), germacrene B (8.3 g/100g), α-humulene (6.9 g/100g) and germacrene D (6.7 g/100g) for **TSC** and (iii) (*E*)-β-caryophyllene (33.9 g/100g), germacrene

D (18.5 g/100g), α-humulene (7.5 g/100g), *(E)*-β-farnesene (5.1 g/100g) and δ-cadinene (4.6 g/100g) for **TCH**.

Relative to the literature, it appears that Corsican *T. massiliense* (**TMC**) and *T. marum* (**TM**) essential oils exhibited original chemical compositions; they were differed to those originated to Sardinia (Giamperi et al., 2008; Ricci et al., 2005). Contrarily, the composition of *T. chamaedrys* essential oils was rather similar in comparison with those reported in the literature from Iran, Serbia and Italy (Chialva, Gabri, Liddle, & Ulian, 1981; Martonfi & Cernaj, 1989; Kovacevic, Lakusic & Ristic, 2001; Morteza-Semnani, Akbarzadeh & Rostami, 2005). The composition *T. polium* subsp. *capitatum* essential oil from Corsica is similar to the chemical compositions of several *T. polium* oils (subspecies not reported) from Turkey (Cakir, Emin, Harmandar, Ciriminna & Passannanti, 1998; Sarer & Konuklugil, 1987) and Serbia-Montenegro (Kovacevic et al., 2001). *T. scorodonia* oil from Corsica was close to those of Italy (Maccioni et al., 2007) and both Corsican subspecies of *T. flavum* oils exhibited chemical composition close to those of Serbia, Croatia, Iran, Greece and Sicilia (Baher & Mirza, 2003; Kovacevic et al., 2001; Menichini et al., 2009; Presti, Crupi, Costa, Dugo & Mondello, 2010; Stanic, Petricic, Blazevic & Plazibat, 1993). Finally, it is noteworthy that Corsican *Teucrium* oils contained some unusual C_{13}-norisoprenoid compounds referred to as megastigmanes: (*E*)-β-damascenone, vitispirane, dihydroedulan I and II and theaspirane I and II. The α,β-unsaturated ketone ((*E*)-β-damascenone), and the C_{13}-spiroether (vitispirane), are volatile components that have an important sensorial effect on wine aroma as it has a very low olfactory perception threshold (Silva Ferreira & Guendes de Pinho, 2004). The two former epoxides, dihydroedulan I and II were identified as important trace components in the flavor of purple passion fruit with intense rose-like aroma (Honda, Yamauchi, Tsubuki & Matsumoto, 1997). The two epoxides, theaspirane I and II, contribute to the flavor of black tea from *Camellia sinensis* (Breitmaier, 2008). In the same way, we noted that Corsican

Teucrium oils were characterized by the presence of many acetate compounds, among them 6-methyl-3-heptyl acetate was detected in all Corsican *Teucrium* oils investigated. This acyclic oxygenated compound was identified for the first time in *T. massiliense* oil as a new natural product (Djabou et al., 2010).

To identify possible correlation between the chemical oil compositions of Corsican *Teucrium* species, principal component analysis (PCA) and cluster analysis (CA; dendrograms) were applied to matrix linking essential oil components classes to species identities. For this, we acquired the GC chromatograms of individuals **TP** and **TCH** sample oils obtained from individual specimens harvested in different Corsican locations and we calculated the normalized % abundances of each oil components. The statistical matrix (Table 3) was carried out by compilation of these chemical data to those previously reported for the others *Teucrium* species ie. (i) *T. marum*, 25 sample oils (**TM1-25**), (ii) *T. scorodonia* subsp. *scorodonia,* 10 sample oils (**TSC1-10**), (iii) *T. flavum* subsp. *glaucum,* 10 sample oils (**TFG1-10**), (iv) *T. massiliense,* 4 sample oils (**TMC1-4**) and (v) *T. flavum* subsp. *flavum,* 3 sample oils (**TFF1-3**). In total, PCA and CA allowed the statistical study of 64 sample oils. The distribution of the discriminating volatile compounds classes: hydrocarbon compounds (**HC**), monoterpene hydrocarbons (**MH**), sesquiterpene hydrocarbons (**SH**), diterpene hydrocarbons (**DH**), non-terpenic hydrocarbons (**NTH**), oxygenated compounds (**OC**), monoterpenes oxygenated (**MO**), sesquiterpenes oxygenated (**SO**), diterpene oxygenated (**DO**) and non-terpenic oxygenated (**NTO**) and the distribution of sample oils for each species are shown in Figure 1. The principal compound classes analysis (constructed using axes 1 and 2) accounts for 66.3% of all oils. The F1 axis (42.79%) is positively correlated with oxygenated compounds (**OC**) and negatively correlated with hydrocarbon compounds (**HC**), monoterpene hydrocarbons (**MH**) and sesquiterpene hydrocarbons (**SH**). The plot established using the first two axes suggests the existence of two main groups (Figures 1 and 2). The group I includes

29 sample oils: all *T. marum* sample oils (**TM1-25**) and all *T. massiliense* sample oils (**TMC1-4**). **TMC** and **TM** essential oils were characterized by higher amounts of oxygenated compounds (67.1-75.8% and 27.7-62.1%, respectively) and appreciable amounts of sesquiterpene hydrocarbons (13.5-18.3% and 19.9-40.7%, respectively). *T. massiliense* sample oils were singularized by the occurrence of non-terpenic oxygenated which accounted for 50.8-60.7% of the oils. Group II includes all other 35 sample oils *i.e.* 10 *T. scorodonia* subsp. *scorodonia* (**TSC1-10**), 10 *T. flavum* subsp. *glaucum* (**TFG1-10**), 3 *T. flavum* subsp. *flavum* (**TFF1-3**), 7 *T. polium* subsp. *capitatum* (**TP1-7**) and 5 *T. chamaedrys* (**TCH1-5**). These essential oils were characterized by higher amounts of hydrocarbon compounds (60.1%-87.1%) therefore, according to the amounts of monoterpenes and sesquiterpenes, it is possible to distinguish two subgroups (see Figures 1 and 2). The subgroup IIA includes all sample oils of *T. scorodonia* subsp. *scorodonia* (**TSC-10**) and *T. chamaedrys* (**TCH1-5**); these oils were dominated by sesquiterpene hydrocarbons which accounted for 72.7-82.1% and 76.5-81.8 %, respectively, *vs.* 1.9-9.9 % for those of the subgroup IIB. The subgroup IIB includes of all samples oils of *T. polium* subsp. *capitatum* (**TP1-7**), *T. flavum* subsp. *glaucum* (**TFG1-10**) and *T. flavum* subsp. *flavum* (**TFF1-3**). These essential oils were characterized by higher amounts of hydrocarbon monoterpenes which accounted for always more than 50.9 % *vs.* 0.5-6.0 % for those of the subgroup IIA.

2.2. Effects of the essential oils on bacterial

The antibacterial activity of essential oils of Corsican *Teucrium* species were tested against six bacteria. Because of the low yield in the production of *Teucrium flavum* subsp. *flavum* essential oil and the scarcity of the plant itself, **TFF** oil was not included in the antibacterial activity testing. Results are summarized in Table 4. According to the width of the inhibition zone diameter expressed in mm, results were appreciated as follows: not sensitive (-) for

diameter equal to or below 8.0 mm, moderately sensitive (+) for diameter between 8.0 and 14.0 mm, sensitive (++) for diameter between 14.0 and 20.0 mm and extremely sensitive (+++) for diameter equal to or longer than 20.0 mm.

In the case of *S. aureus*, inhibition zone value for Penicillin G was 46.0 mm. The most active oils on this strain were **TP** and **TFG** essential oils while **TM**, **TMC**, **TSC** and **TCH** essential oils showed a moderate activity. The same antibiotic was used on *S. epidermidis* strain (29.0 mm). **TMC**, **TP** and **TFG** essential oils were extremely active on this strain, whereas **TM** and **TSC** oils were less active. Only **TCH** essential oil showed no activity on *S. epidermidis*. The antibiotic control (Ciprofloxacine) used against *L. innocua*, displayed an inhibition zone diameter of 24.0 mm. **TMC** and **TFG** essential oils were extremely active. **TP** and **TSC** oils also showed a significant inhibition on *L. innocua* growth while **TM** exerted a moderate activity and **TCH** oil was not active. Kanamycin was used as control for EAEP289 strain and the inhibition zone value was 22.0 mm. The MDR strain was not sensitive to **TMC**, **TSC** and **TCH** essential oils. A moderate activity was observed in presence of **TM** essential oil. The most active essential oils on EAEP289 strain were **TP** and **TFG**. Erythromycine inhibition zones varied from 31.0 to 42.0 mm for *E. aerogenes* and *C. jejuni*. On *E. aerogenes*, the activity of **TMC** and **TP** oils was moderate compared to **TFG** oil, which displayed the strongest activity. **TM**, **TSC** and **TCH** essential oils were not active against *E. aerogenes*. With inhibition zone diameters ranging from 22.0 to 44.0 mm, the essential oils isolated from all the *Teucrium* species showed an extremely high activity against *C. jejuni*.

The inhibition zones of the essential oils displayed wide variations among the different *Teucrium* species but stayed generally much lower than that of the standard controls (Table 4) except on *C. jejuni* (**TM**, **TMC**, **TP** and **TFG**). The qualitative agar diffusion method is commonly used as a preliminary method prior to more detailed studies. Thus, MIC assays were also performed for the different essential oils. This method is generally more sensitive

and allows quantitative determination of essential oils antibacterial efficiency.

According to the MIC values expressed in mg/mL, results were appreciated as follows: not sensitive (-) for value between 50.0 or above to 25.0 mg/mL, moderately sensitive (+) for value between 12.5 and 3.0 mg/mL, sensitive (++) for value between 2 and 0.4 mg/mL and extremely sensitive (+++) for value equal to or below 0.2 mg/mL.

The MICs determined for the *Teucrium* essential oils are presented in Table 5. The strain *S. aureus* was sensitive to **TM**, **TMC**, **TP** and **TFG** essential oils. **TSC** and **TCH** oils were moderately active on this strain. On the *S. epidermidis* strain, the most active oils were **TMC** and **TFG**. This strain was only moderately sensitive to **TP** oil but not sensitive to the last three essential oils: **TM**, **TSC** and **TCH**. *L. innocua*, was sensitive to **TMC** and **TFG** essential oils and moderately sensitive to **TM**, **TP** and **TCH** essential oils. Only **TSC** oil was not active on this strain. The MDR strain EAEP289 was found to be particularly sensitive to the *Teucrium* essential oils tested, especially to **TSC** oil followed by **TM**, **TP**, **TFG** and **TMC** oils. Only **TCH** essential oil was not active on this strain. The same profile was observed for the *E. aerogenes* wild type strain. Indeed, the whole essential oils were active (**TM**, **TP**, **TFG** and **TSC**) or moderately active (**TMC**) on this strain except **TCH** oil. At last, the foodborne pathogen *C. jejuni* strain was very sensitive to the *Teucrium* essential oils ranging from extremely sensitive (**TP** and **TFG**) to sensitive (**TM**, **TMC**, **TSC** and **TCH**).

The MIC values displayed wide variations among the different *Teucrium* species (Table 5). This variability could be attributed to the chemical composition of the tested essential oils.

To evaluate correlations between the essential oils isolated from the different Corsican *Teucrium* species and their antibacterial effects, the MIC values were subjected to PCA and CA analysis. The CA based on the Euclidean distances between groups indicated 2 clusters, called A and B, identified by their antibacterial efficiency with a dissimilarity of about 2.5 (Figure 3). The total variance was explained at 46.56% by the PCA horizontal axis (F1) and at

38.97% by the vertical axis (F2) (Figure 4). Group A, included **TM**, **TSC** and **TCH** essential oils, seems negatively correlated to the antibacterial efficiency meaning that these three oils can be considered as globally not or moderately active in this study. Conversely, group B, represented by **TMC**, **TP** and **TFG** essential oils, seems positively correlated to the antibacterial efficiency against the six strains. Thus, these three oils can be considered as globally active. However, as shown in Figures 3 and 4, the antibacterial CA and PCA analysis didn't fit to the chemical PCA and CA analysis (Figures 1 and 2). Indeed, **TM** and **TMC** essential oils which belong to the statistic chemical group dominated by oxygenated compounds (group I), were clustered in two different groups in the antibacterial statistical analysis (A and B, respectively). Also, the other *Teucrium* essential oils, included in the chemical group dominated by hydrocarbons (group II), were not assigned to the same groups in the antibacterial PCA and CA analysis. Thus, **TCH** and **TSC** essential oils (subgroup IIA) were not significantly active (group A), while **TP** and **TFG** essential oils (subgroup IIB) were significantly active (group B) against the six tested strains.

Indeed, the highest activity was observed for **TMC**, **TP** and **TFG** essential oils (group B). In contrast, **TM**, **TSC** and **TCH** essential oils were found to have weak or no activity (group A). The antibacterial activity of essential oils is directly connected to their chemical composition and conditioned by the activity of their components. The weak activity of **TSC** and **TCH** essential oils on all the tested strains may be explained by their high amounts of hydrocarbons since these compounds are known to be weakly active against bacteria (Burt, 2004). Generally, essential oils possessing the strongest antibacterial properties contain a high percentage of oxygenated compounds (Burt, 2004). Nevertheless, **TM** essential oil, containing 61.6% of oxygenated compounds (Table 1), was classified as weakly active on the tested strains. Moreover, **TP** and **TFG** essential oils were considered as active while their contents in oxygenated compounds were lower (respectively 35.2 and 35.0%) than in **TM**

essential oil (Table 1). This difference activity could be explained by the analysis of the oxygenated fraction itself. In fact, we can notice that the oxygenated part of **TM** essential oil is dominated by oxides (16.9%, see Table 1) which are known to be among the least active oxygenated compounds (Kalemba & Kunicka, 2003). In contrast, the oxygenated fractions of **TP** and **TFG** essential oils are dominated by alcohols (respectively 22.2% and 17.8%, see Table 1) known to display high activity against a wide range of bacteria (Kalemba & Kunicka, 2003). More precisely, the main oxygenated compound of **TP** essential oil, terpinen-4-ol (6.6%), is an effective antibacterial agent. Indeed, the antibacterial activity of *Melaleuca alternifolia* essential oil has been associated to the high content of this component (Carson, Hammer & Riley, 2006; Carson, Mee & Riley, 2002). This essential oil is extremely active against a wide range of bacteria included *S. aureus* and its MDR strains, which are particularly sensitive to terpinen-4-ol (Carson et al., 2002, 2006). In the same way, the main oxygenated compound of **TFG** essential oil is *(E)*-phytol (4.5%), already described in literature as a strong inhibitory compound of the bacterial growth of *Mycobacterium tuberculosis* (Rajab, Cantrell, Franzblau & Fischer, 1998). It is also interesting to notice that **TP** and **TFG** essential oils contain hydrocarbons, *i.e. p*-cymene and limonene, which can act in synergy with oxygenated compounds and thus potentiate their biological activity. Indeed, the advantageous synergistic effect of the essential oils constituents has often been observed. For example, *p*-cymene (4.0% in **TP** essential oil) is not effective when used alone, but when combined with the oxygenic compound carvacrol, synergism has been observed against *Bacillus cereus* (Dorman & Deans, 2000). The ability of *p*-cymene at being incorporated in the lipid bilayer of *B. cereus* facilitates transport of carvacrol across the cytoplasmic membrane (Ultee, Bennik & Moezelaar, 2002). Contrary to *p*-cymene, limonene (5.2% and 27.4% in **TP** and **TFG** essential oil, respectively) can exert antibacterial activity when used alone (Dorman & Deans, 2000) and can potentiate the antibacterial effect according to its

concentration in the essential oil (Van Vuuren & Viljoen, 2007). At last, **TMC** essential oil is essentially constituted of oxygenated compounds (74.2%) that seem to be directly connected to its antibacterial activity, such as pulegone (6.9%) (Duru, Oztürk, Ugur & Ceylan, 2004), linalool (5.2%) and citronellol (4.1%) (Burt, 2004; Kalemba & Kunicka, 2003). However, the antibacterial activity of 6-methyl-3-heptyl acetate (19.1%, main component of **TMC** essential oil) has never been described in the literature. For this reason, an in-depth study is required to fully understand the behavior of **TMC** essential oil against the six tested strains.

3. Concluding remarks

The aerial parts of the Corsican *Teucrium* species produce two types of essential oils clarified by statistical tools. *T. massiliense* and *T. marum* essential oils were dominated by oxygenated compounds while *T. scorodonia*, *T. chamaedrys*, *T. polium* and both subspecies of *T. flavum* essential oils were characterized by hydrocarbon compounds. More precisely, *T. massiliense* oils seem original because of the occurrence of acyclic non terpene oxygenated compounds as main components while all other *Teucrium* oils displayed terpene compounds as main components. *T. polium* oils (**TP**) and both subspecies of *T. flavum* oils (**TFG** and **TFF**) were characterized by hydrocarbon monoterpenes and *T. marum*, *T. scorodonia* and *T. chamaedrys* oils (**TM, TSC, TCH**) were dominated by sesquiterpene compounds.

The *Teucrium* essential oils activity varied significantly within species and within strains. In general, **TP** and **TFG** essential oils were the most active on the different strains due to their high level of active oxygenated compounds acting probably synergistically with some hydrocarbon compounds. The activity of **TMC** oil and particularly of 6-methyl-3-heptyl acetate could be further investigated. In contrast, **TSC** and **TCH** essential oils were the least active due to their chemotype strongly based on hydrocarbons. The weak activity of **TM** oil could be explained by a majority of barely active oxides.

While the two *E. aerogenes* strains for the disc diffusion assay and *S. epidermidis* for the MIC assay were the most resistant, *C. jejuni* was found to be the most sensitive strain for both methods. Thus, the *Teucrium* essential oils could be considered as a good way to investigate in order to struggle against the well-known foodborne pathogen *C. jejuni*.

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References

Awadh Ali, N. A., Wurster, M., Arnold, N., Lindequist, U., & Wessjohan, L. (2008). Chemical composition of the essential oil of *Teucrium yemense* Deflers. *Records of Natural Products*, *2*(2), 25-32.

Baher, Z. F., & Mirza, M. (2003). Volatile Constituents of *Teucrium flavum* L. from Iran. *Journal of Essential Oil Research*, *15*, 106-107.

Beni Maleci, L., Pinetti, A., & Servettaz, O. (1995). Micromorphological and phytochemical characters of two subspecies of *Teucrium flavum* (Labiatae) from Italian flora. *Flora*, *190*, 237-242.

Bicchi, C., Liberto, E., Matteodo, M., Sgorbini, B., Mondello, L., Zellner, B. A., & Costa, R. (2008). Quantitative analysis of essential oils: a complex task. *Flavour and Fragrance Journal*, *23*(6), 382-391.

Bosisio, E., Giavarini, F., Dell'Agli, M., Galli, G., & Galli, C. L. (2004). Analysis by high-performance liquid chromatography of teucrin A in beverages flavoured with an extract of *Teucrium chamaedrys* L. *Food Additives and Contaminants*, *21*, 407-414.

Breitmaier, E. (2008). *Terpenes* (Wiley-VCH V erlag GmbH & Co. KGaA.). Weinheim, Germany: Wiley-VCH.

Brereton, R. G. (2003). *Chemometrics: Data Analysis for the Laboratory and Chemical Plant* (Wiley Interscience.). New-York: Wiley Interscience.

Bruno, M., Rosselli, S., Maggio, A., Piozzi, F., Scaglioni, L., Arnold, N. A., & Simmonds, M. S. (2004). Neoclerodanes from *Teucrium orientale*. *Chemical and Pharmaceutical Bulletin*, *52*(12), 1497-1500.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods. *International Journal of Food Microbiology*, *94*, 223-253.

Cakir, A., Emin, M., Harmandar, M., Ciriminna, R., & Passannanti, S. (1998). Volatile Constituents of *Teucrium polium* L. from Turkey. *Journal of Essential Oil Research*, *10*, 113-115.

Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiology Reviews*, *19*, 50-62.

Carson, C. F., Mee, B. J., & Riley, T. V. (2002). Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined par time-kill, lysis, leakage and salt tolerance assays and electron microscopy. *Antimicrobial Agents Chemotherapy*, *46*, 1914-1920.

Chialva, F., Gabri, G., Liddle, P. A. P., & Ulian, F. (1981). Indagine sulla composizione dell'olio essenziale di *Hypericum perforatum* L. e di *Teucrium chamaedrys* L. *Rivista Italiana EPPOS*, *63*, 286-288.

CLSI. (2007). Clinical and Laboratory Standards Institute-Performance standards for antimicrobial disk and dilution susceptibility tests methods for antimicrobial susceptibility testing for bacteria isolated from animals-Approved standard- Third edition – CLSI document M11-A7-. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Coll, J., & Tandrón, Y. A. (2005). Isolation and structure elucidation of three neo-clerodane diterpenes from *Teucrium fruticans* L. (Labiatae). *Phytochemistry*, *66*, 2298–2303.

Costa, R., Zellner, B. A., Crupi, M. L., De Fina, M. R., Valentino, M. R., Dugo, P., & Dugo, G. (2008). GC–MS, GC–O and enantio–GC investigation of the essential oil of *Tarchonanthus camphoratus* L. *Flavour and Fragrance Journal*, *23*(1), 40-48.

Couladis, M., Tzakou, O., Verykokidou, E., & Harvala, C. (2003). Screening of some Greek aromatic plants for antioxidant activity. *Phytotherapy Research*, *17*, 472-476.

Council of Europe. (1997). *European Pharmacopoeia* (Council of Europe., Vol. third ed.). Strasbourg.

Cozzani, S., Muselli, A., Desjobert, J. M., Bernardini, A. F., Tomi, F., & Casanova, J. (2005). Chemical composition of essential oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica. *Flavour and Fragrance Journal*, *20*, 436-441.

Djabou, N., Paolini, J., Desjobert, J. M., Allali, H., Baldovini, N., Costa, J., & Muselli, A. (2010). Quantitative and qualitative analysis of volatile components of *Teucrium massiliense* L. - identification of 6-methyl-3-heptyl acetate as a new natural product. *Flavour and Fragrance Journal*, *25*, 475-487.

Djabou, N., Battesti, M. J., Allali, H., Desjobert, J. M., Varesi, L., Costa, J., & Muselli, A. (2011). Chemical and genetic differentiation of Corsican subspecies of *Teucrium flavum* L. *Phytochemistry*, *72*, 1390-1399.

Djabou, N., Allali, H., Battesti, M.-J., Tabti, B., Costa, J., Muselli, A., & Varesi, L. (2012). Chemical and genetic differentiation of two Mediterranean subspecies of *Teucrium scorodonia* L. *Phytochemistry*, *74*, 123-132.

Djabou, N., Andreani S., Varesi L., Costa J., & Muselli, A. Analysis of volatile fraction of *Teucrium marum* L. Submitted to *Flavour and Fragrance Journal.*

Dorman, H. J., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, *88*, 308-316.

Duru, M. E., Oztürk, M., Ugur, A., & Ceylan, O. (2004). The constituents of essential oil and in vitro antimicrobial activity of *Micromeria cilicia* from Turkey. *Journal of Ethnopharmacology*, *94*, 43-48.

Edris, A. E. (2007). Pharmaceutical and therapeutical potentials of essential oils and their individual volatile constituents. *Phytotherapy Research*, *21*, 308-323.

Fernandez, P. B., Iglesias, P. I., & Villar del Fresno, A. M. (1997). Anti-inflammatory and antiulcer activity of *Teucrium buxifolium*. *Journal of Ethnopharmacology*, *55*, 93-98.

Giamperi, L., Bucchini, A., Fraternale, D., Cara, P., Ricci, D., Epifano, F., Genovese, S., & Curini, M. (2008). Chemical composition and antioxidant activity of the essential oil of *Teucrium massiliense* L. *Journal of Essential Oil Research*, *20*(5), 446-449.

Honda, T., Yamauchi, A., Tsubuki, M., & Matsumoto, T. (1997). Chiral Syntheses of Edulans I, II and dihydroedulans I, II and Absolute Configurations of Edulans I, II. *Tetrahedron Asymmetry*, *8*, 837-839.

Jeanmonod, D., & Gamisans, J. (2007). *Flora Corsica* (Edisud.). Aix-en-Provence: la compagnie des éditions de la Lesse.

Jennings, W., & Shibamoto, T. (1980). *Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography* (Academic Press.). New-York: In: Jovanovich, H.B (Ed).

Kalemba, D., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, *10*, 813-829.

Konig, W. A., Hochmuth, D. H., & Joulain, D. (2001). *Terpenoids and Related Constituents of Essential oils.* (Library of Mass Finder 2.1 ed.). Institute of Organic Chemistry, Hamburg.

Kovacevic, N. N., Lakusic, B. S., & Ristic, M. (2001). Composition of essential oils of seven *Teucrium* species from Serbia and Montenegro. *Journal of Essential Oil Research*, *13*, 163-165.

Maccioni, S., Baldini, R., Tebano, M., & Cioni, P. L. (2007). Essential oil of *Teucrium scorodonia* L. ssp. *scorodonia* from Italy. *Food Chemistry*, *104*, 1393-1395.

Fernandez, P. B., Iglesias, P. I., & Villar del Fresno, A. M. (1997). Anti-inflammatory and antiulcer activity of *Teucrium buxifolium*. *Journal of Ethnopharmacology*, *55*, 93-98.

Giamperi, L., Bucchini, A., Fraternale, D., Cara, P., Ricci, D., Epifano, F., Genovese, S., & Curini, M. (2008). Chemical composition and antioxidant activity of the essential oil of *Teucrium massiliense* L. *Journal of Essential Oil Research*, *20*(5), 446-449.

Honda, T., Yamauchi, A., Tsubuki, M., & Matsumoto, T. (1997). Chiral Syntheses of Edulans I, II and dihydroedulans I, II and Absolute Configurations of Edulans I, II. *Tetrahedron Asymmetry*, *8*, 837-839.

Jeanmonod, D., & Gamisans, J. (2007). *Flora Corsica* (Edisud.). Aix-en-Provence: la compagnie des éditions de la Lesse.

Jennings, W., & Shibamoto, T. (1980). *Qualitative analysis of flavour and fragrance volatiles by glass-capillary gas chromatography* (Academic Press.). New-York: In: Jovanovich, H.B (Ed).

Kalemba, D., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, *10*, 813-829.

Konig, W. A., Hochmuth, D. H., & Joulain, D. (2001). *Terpenoids and Related Constituents of Essential oils.* (Library of Mass Finder 2.1 ed.). Institute of Organic Chemistry, Hamburg.

Kovacevic, N. N., Lakusic, B. S., & Ristic, M. (2001). Composition of essential oils of seven *Teucrium* species from Serbia and Montenegro. *Journal of Essential Oil Research*, *13*, 163-165.

Maccioni, S., Baldini, R., Tebano, M., & Cioni, P. L. (2007). Essential oil of *Teucrium scorodonia* L. ssp. *scorodonia* from Italy. *Food Chemistry*, *104*, 1393-1395.

Mallea, M., Chevalier, J., Bornet, C., Eyraud, A., Davin-Regli, A., Bollet, C., & Pagès, J. M. (1998). Porin alteration and active efflux: two in vivo drugs strategies used by *Enterobacter aerogenes*. *Antimicrobial Agents and Chemotherapy*, *46*, 2640-2643.

Martonfi, P., & Cernaj, P. (1989). Variability of *Teucrium chamaedrys* essential oil. *Biologia Bratislava*, *44*, 245-251.

Mass Finder (2006), In: Hochmunth, D., Version 3.0, Scientific Consulting, Germany.

Massart, D. L. (1998). *Chemometrics: A Textbook* (Elsevier Science Ltd.). New-York: Elsevier.

McLafferty, F. W., & Stauffer, D. B. (1988). *The Wiley/NBS Registry of Mass Spectra Data* (Wiley-Interscience., Vol. fourth ed.). New-York.

Menichini, F., Conforti, F., Rigano, D., Formisano, C., Piozzi, F., & Senatore, F. (2009). Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. *Food Chemistry*, *115*(2), 679-686.

Morteza-Semnani, K., Akbarzadeh, M., & Rostami, B. (2005). The essential oil composition of *Teucrium chamaedrys* L. from Iran. *Flavour and Fragrance Journal*, *20*, 544-546.

Muselli, A., Costa, J., Desjobert, J. M., Dessi, M. A., Paolini, J., & Bernardini, A. F. (2009). Chemical Composition of the Essential Oils of *Teucrium chamaedrys* L. from Corsica and Sardinia. *Journal of Essential Oil Research*, *21*, 138-143.

National Institute of Standards and Technology (1999), NIST/EPA/NIH Mass Spectral Library. PC Version 1.7. Perkin-Elmer Corp., Norwalk, CT, USA.

Ozkan, G., Kuleasan, H., Celik, S., Gokturk, R. S., & Unal, O. (2007). Screening of Turkish endemic *Teucrium montbretii* subs. *pamphylicum* extracts for antioxidant and antibacterial activities. *Food Control*, *18,* 509-512.

Piozzi, F., Bruno, M., Rosselli, S., & Maggio, A. (2005). Advances on the chemistry of furano-diterpenoids from *Teucrium* genus. *Heterocycles*, *65*, 1221-1234.

Presti, M. L., Crupi, M. L., Costa, R., Dugo, G., & Mondello, L. (2010). Seasonal variation of *Teucrium flavum* L. essential oil. *Journal of Essential Oil Research, 22*, 211-216.

Rajab, M. S., Cantrell, C. L., Franzblau, S. G., & Fischer, N. H. (1998). Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure-activity study. *Planta Medica, 64*, 2-4.

Ricci, D., Fraternale D., Giamperi L., Bucchini A., Epifano F., Burini G., & Curini M. (2005). Chemical composition, antimicrobial and antioxidant activity of the essential oil of *Teucrium marum* (lamiaceae). *Journal of Ethnopharmacology, 98*, 195-200.

Saracoglu, V., Arfan, M., Shabir, A., Hadjipavlou-Litina, D., & Skaltsa, H. (2007). Composition and antioxidant activity of the essential oil of *Teucrium royleanum* Wall. ex Benth growing in Pakistan. *Flavour and Fragrance Journal, 22*, 154-157.

Sarer, E., & Konuklugil, B. (1987). Investigation of the volatile oil of *Teucrium polium* L. *Doga: Turk Tip ve Eczacilik Dergisi, 11*(2), 317-325.

Silva Ferreira, A. C., & Guendes de Pinho, P. (2004). Nor-isoprenoids profile during port Wine ageing-influence of some technological parameters. *Analytical Chemistry, 513*, 169-176.

Stanic, G., Petricic, J., Blazevic, N., & Plazibat, M. (1993). Essential oil of *Teucrium flavum* L. from Croatia. *Journal of Essential Oil Research, 5*, 625-627.

Ultee, A., Bennik, M. H., & Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology, 68*, 1561-1568.

Ulubelen, A., Topu, G., & Sönmez, U. (2000). Chemical and biological evaluation of genus *Teucrium*. *Bioactive natural Products (Part D)* (Vol. 23, pp. 591-648). Elsevier.

Van Vuuren, S. F., & Viljoen, A. M. (2007). Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. *Flavour and Fragrance Journal*, *22*, 540-544.

Table(s)

Table 2: Main components (> 2g/100g) of *Teucrium* essential oils from Corsica

					Teucrium taxa[e]							
No.[a]	Compounds	IRI_a[b]	RI_a[c]	RI_p[d]	TMC	TM	TSC	TCH	TP	TFG	TFF	Identification[f]
12	*α*-thujene	932	923	1021					**8.1**	0.1	0.2	RI, MS
13	*α*-pinene	936	932	1023				0.8	**24.1**	**12.2**	**21.9**	RI, MS
20	sabinene	973	966	1121			1.1	tr	**4.1**	0.4	0.5	RI, MS
21	*β*-pinene	978	973	1112				0.5	**9.2**	**10.3**	**18.1**	RI, MS
25	myrcene	987	981	1332	tr		0.1	tr	1.2	1.9	2.0	RI, MS
27	isobutyl isovalerate	993	989	1154	**5.8**							RI, MS
34	*p*-cymene	1015	1014	1267				0.3	**4.0**	0.2	tr	RI, MS
37	limonene	1025	1020	1186	0.4		1.2	0.2	**5.2**	**27.4**	**20.0**	RI, MS
40	(*Z*)-*β*-ocimene	1029	1026	1207	2.3		0.9	0.1		**6.0**	**15.5**	RI, MS
52	6-methyl-3-heptyl acetate	1074	1074	1267	**19.1**	tr	tr	0.1	tr	0.7	0.3	RI, MS
57	linalool	1083	1088	1394	**5.2**	0.7	0.5	1.8	1.1	0.5	0.2	RI, MS
60	3-methyl butyl isovalerate	1092	1092	1257	**3.4**							RI, MS
72	3-octanyl-acetate	1113	1111	1315	**7.0**	tr	0.1	0.1		1.1	0.2	RI, MS
78	*cis*-verbenol	1127	1129	1651					2.6			RI, MS
95	terpinen-4-ol	1164	1160	1562	0.1		0.3	0.1	**6.6**	0.7	0.2	RI, MS
102	estragole	1175	1176	1645		**3.5**	0.1					RI, MS
103	hexyl butanoate	1177	1176	1403		2.3						RI, MS
113	citronellol	1213	1213	1726	**4.1**	0.2						RI, MS
116	pulegone	1215	1216	1603	**6.9**							RI, MS
163	α-cubebene	1355	1349	1452	tr		**6.2**	0.1				RI, MS
164	geranyl acetate	1362	1360	1715	0.9	**3.0**	0.1		0.5			RI, MS
172	α-copaene	1379	1377	1488	0.1		2.1	0.2				RI, MS
173	*β*-bourbonene	1386	1381	1483	1.7		0.1	**2.7**				RI, MS
177	*β*-cubebene	1390	1388	1513			2.3					RI, MS
185	*α*-gurjunene	1413	1413	1475	0.1		**3.5**					RI, MS
186	(*E*)-*β*-caryophyllene	1421	1418	1580		**5.3**	**21.1**	**33.9**		0.3	0.1	RI, MS
192	(*E*)-*α*-bergamotene	1434	1429	1547	0.2	**8.2**		0.1	0.9			RI, MS
194	*γ*-elemene	1429	1432	1616			**3.9**					RI, MS, Ref.
202	(*E*)-*β*-farnesene	1446	1449	1628	0.3	0.4	1.8	**5.1**		2.3	0.6	RI, MS
203	*α*-humulene	1455	1450	1665		1.3	**6.9**	**7.5**				RI, MS
211	germacrene-D	1479	1476	1666	**6.1**		**6.7**	**18.5**		0.7	tr	RI, MS
215	zingiberene	1489	1490	1717		0.3	**3.6**					RI, MS
221	(*E*,*E*)-*α*-farnesene	1498	1500	1693			0.4	tr		2.0	0.9	RI, MS
223	β-bisabolene	1503	1502	1720		**7.5**	**3.3**	1.8				RI, MS
231	*δ*-cadinene	1520	1514	1715	0.8		2.5	**4.6**	2.2			RI, MS
232	*β*-sesquiphellandrene	1516	1517	1765		**3.7**	0.7			0.2	0.1	RI, MS
243	germacrene B	1552	1556	1820			**8.3**					RI, MS, Ref.
249	caryophyllene oxide	1578	1570	1942	0.4	**9.8**	1.0	**3.1**	0.1			RI, MS
254	guaiol	1591	1584	2090					2.1	0.3	0.2	RI, MS
260	humulene epoxide II	1602	1599	2044	tr	**3.2**	0.9	0.1				RI, MS
296	(3*Z*,6*E*,10*E*)-*α* -springene	1941	1941	2190		**3.2**						RI, MS
303	(*E*)-phytol	2114	2097	2559	0.3	2.5	1.4	1.8		**4.5**	0.5	RI, MS
306	octadecyl acetate	2198	2194	2499		**3.3**				0.4	0.1	RI, MS
	Total identification (g/100g)				92.1	95.5	98.2	93.6	98.9	94.9	95.4	
	Hydrocarbon compounds				17.9	33.9	**85.3**	**80.0**	**63.7**	**66.5**	**83.0**	
	Monoterpene hydrocarbons				3.5	0	4.6	2.0	**59.3**	**59.9**	**80.7**	
	Sesquiterpene hydrocarbons				14.1	30.7	**80.7**	**77.9**	4.4	6.6	2.3	
	Hydrocarbon diterpene				0	3.2	0	0	0	0	0	

Non-terpenic hydrocarbons	0.3	0.9	0	0.1	0	0	0
Oxygenated compound	**74.2**	**61.6**	12.9	13.6	**35.2**	**35.0**	12.4
Oxygenated monoterpenes	20.4	17.6	2.0	2.4	29.6	12.7	6.7
Oxygenated sesquiterpenes	7.4	18.6	6.0	6.4	4.8	1.3	0.8
Oxygenated diterpene	0	2.5	1.4	1.9	0	4.5	0.5
Non-terpenic oxygenated compounds	**46.4**	22.9	3.5	2.9	0.8	16.5	4.4
Alcohols	19.9	13.7	8.1	8.2	**22.2**	**17.8**	7.4
Aldehydes and Ketones	10.6	12.3	1.5	0.4	9.2	5.3	3.1
Esters	**42.5**	16.3	0.6	0.3	4.3	3.6	1.1
Oxides	1	**16.9**	2.5	4.2	0.9	1.4	0.8
Acids	0	1.1	0.2	0	0	0.3	0

[a] Order of elution is given on apolar column (Rtx-1).
[b] Retention indices of literature on the apolar column (IRI_A) reported from Konig et al., (2001).
[c] Retention indices on the apolar Rtx-1 column (RI_A).
[d] Retention indices on the polar Rtx-Wax column (RI_P).
[e] Corsican *Teucrium* taxa (concentrations in g/100g): *T. massiliense* (**TMC**), *T. marum* (**TM**), *T. scorodonia* subsp. *scorodonia* (**TSC**), *T. chamaedrys* (**TCH**), *T. polium* subsp. *capitatum* (TP), *T. flavum* subsp. *glaucum* (**TFG**) and *T. flavum* subsp. *flavum* (**TFF**).
[f] RI: Retention Indices; MS: Mass Spectra in electronic impact mode; Ref.: compounds identified from literature data: Koning et al., 2001 (**194**, **244**).

Table 3: Normalized percentage abundances from class compounds of Corsican *Teucrium* essential oils

Class compounds[a]		*Teucrium* taxa[b]						
		TMC	**TM**	**TSC**	**TCH**	**TP**	**TFG**	**TFF**
Hydrocarbon Compounds	Σ^{e}	**45**	**17**	**26**	**39**	**21**	**20**	**20**
	Δ^{d}	**17.5 - 22.4**	**21.6 - 56.1**	**74.1 - 87.1**	**79.1 - 85.4**	**60.1 - 67.7**	**60.5 - 74.8**	**82.3 - 83.7**
MH	Σ^{e}	4		9	12	16	10	10
	Δ^{d}	3.5 - 3.7		0.5 - 6.0	2.6 - 4.1	55.3 - 62.6	50.9 - 70.3	80.4 - 81.5
SH	Σ^{e}	28	13	27	22	5	10	10
	Δ^{d}	13.5 - 18.3	19.9 - 40.7	72.7 - 82.9	74.9 - 81.8	4.2 - 6.0	4.5 - 9.9	1.9 - 2.3
DH	Σ^{e}	1	1					
	Δ^{d}	tr - 0.1	1.1 - 17.8					
NTH	Σ^{e}	12	3		5			
	Δ^{d}	0.3 - 0.5	0.1 - 1.9		0.1 - 0.2			
Oxygenated compounds	Σ^{e}	**91**	**78**	**54**	**47**	**65**	**61**	**61**
	Δ^{d}	**67.1 - 75.8**	**33.7 - 62.1**	**9.5 - 16.0**	**11.5 - 13.6**	**31.1 - 35.0**	**19.0 - 35.2**	**11.2 - 11.4**
MO	Σ^{e}	25	22	9	8	49	27	27
	Δ^{d}	10.5 - 12.3	4.7 - 24.8	0.6 - 1.9	1.4 - 2.0	26.6 - 29.4	6.2 - 15.8	6.0 - 6.7
SO	Σ^{e}	31	9	16	14	8	3	3
	Δ^{d}	4.5 - 5.8	3.2 - 20.2	3.1 - 8.9	6.1 - 7.4	2.6 - 5.1	1.0 - 1.8	0.2 - 0.8
DO	Σ^{e}	2	2	2	3		2	2
	Δ^{d}	tr - 0.3	0.5 - 4.5	1.1 - 4.6	1.2 - 2.2		2.4 - 5.5	0.3 - 0.5
NTO	Σ^{e}	30	43	15	18	4	25	25
	Δ^{d}	50.8 - 60.7	8.8 - 27.6	2.0 - 4.7	1.9 - 2.7	0.4 - 1.0	7.3 - 14.2	3.8 - 4.7

[a] Monoterpene hydrocarbons (**MH**), Sesquiterpene hydrocarbons (**SH**), Diterpene hydrocarbons (**DH**), Non-terpenic hydrocarbons (**NTH**),Monoterpenes oxygenated (**MO**), Sesquiterpenes oxygenated (**SO**), Diterpene oxygenated (**DO**), Non-terpenic oxygenated (**NTO**).

[b] Corsican *Teucrium* taxa: *T. massiliense* (**TMC**), *T. marum* (**TM**), *T. scorodonia* subsp. *scorodonia* (**TSC**), *T. chamaedrys* (**TCH**), *T. polium* subsp. *capitatum* (TP), *T. flavum* subsp. *glaucum* (**TFG**) and *T. flavum* subsp. *flavum* (**TFF**).

[c] Σ Total number of specified components

[d] Δ Min-Max normalized % abundances

Table 4: Inhibition zones (mm) of *Teucrium* essential oils on bacterial strains

	S. aureus	*S. epidermidis*	*L. innocua*	*EAEP289*	*E. aerogenes*	*C. jejuni*
Antibiotic	46.0	29.0	24.0	22.0	31.0	42.0
TM	13.0 ± 0.6 $^{(+)}$	12.0 ± 0.0 $^{(+)}$	13.0 ± 0.6 $^{(+)}$	9.0 ± 0.6 $^{(+)}$	8.0 ± 0.3 $^{(-)}$	40.0 ± 0.6 $^{(+++)}$
TMC	13.0 ± 1.0 $^{(+)}$	28.0 ± 1.2 $^{(+++)}$	28.0 ± 1.0 $^{(+++)}$	6.0 ± 0.0 $^{(-)}$	13.0 ± 1.2 $^{(+)}$	41.0 ± 1.4 $^{(+++)}$
TP	18.0 ± 2.1 $^{(++)}$	22.0 ± 0.6 $^{(+++)}$	17.0 ±0.6 $^{(++)}$	15.0 ± 1.0 $^{(++)}$	10.0 ± 0.6 $^{(+)}$	44.0 ± 0.6 $^{(+++)}$
TFG	19.0 ± 1.2 $^{(++)}$	23.0 ± 0.6 $^{(+++)}$	22.0 ± 1.5 $^{(+++)}$	16.0 ± 0.0 $^{(++)}$	16.0 ± 0.0 $^{(++)}$	39.0 ±0.6 $^{(+++)}$
TSC	13.0 ± 0.6 $^{(+)}$	18.0 ± 0.6 $^{(++)}$	18.0 ± 0.0 $^{(++)}$	6.0 ± 0.0 $^{(-)}$	6.0 ± 0.0 $^{(-)}$	26.0 ± 1.0 $^{(+++)}$
TCH	10.0 ± 0.6 $^{(+)}$	7.0 ± 0.0 $^{(-)}$	8.0 ± 0.0 $^{(-)}$	6.0 ± 0.0 $^{(-)}$	6.0 ± 0.0 $^{(-)}$	22.0 ± 0.6 $^{(+++)}$

Values are means ± standard deviation (SD) of triplicate determinations
Essential oils are classified as $^{(-)}$ not active, $^{(+)}$ moderately active, $^{(++)}$ active and $^{(+++)}$ extremely active.
Bacteria tested: *Staphylococcus aureus* (CIP 53.156), *Staphylococcus epidermidis* (CIP 53124), *Listeria innocua* (CIP 80.11), *Enterobacter aerogenes* multi-drug resistant (MDR) strain EAEP289 and the wild type (CIP 60.86), *Campylobacter jejuni* (CIP 107370).
Corsican *Teucrium* taxa: *T. marum* (**TM**), *T. massiliense* (**TMC**), *T. polium* subsp. *capitatum* (**TP**), *T. flavum* subsp. *glaucum* (**TFG**), *T. scorodonia* subsp. *scorodonia* (**TSC**) and *T. chamaedrys* (**TCH**).

Table 5: MIC (mg/mL) of *Teucrium* essential oils on bacterial strains

Essential oils	*S. aureus*	*S. epidermidis*	*L. innocua*	*EAEP289*	*E. aerogenes*	*C. jejuni*
TM	0.4 $^{(++)}$	25.0 $^{(-)}$	12.5 $^{(+)}$	0.4 $^{(++)}$	0.4 $^{(++)}$	1.0 $^{(++)}$
TMC	0.8 $^{(++)}$	0.8 $^{(++)}$	0.8 $^{(++)}$	6.0 $^{(+)}$	6.0 $^{(+)}$	1.0 $^{(++)}$
TP	0.4 $^{(++)}$	12.5 $^{(+)}$	6.0 $^{(+)}$	0.8 $^{(++)}$	0.8 $^{(++)}$	0.2 $^{(+++)}$
TFG	0.8 $^{(++)}$	1.5 $^{(++)}$	1.5 $^{(++)}$	1.5 $^{(++)}$	1.5 $^{(++)}$	0.2 $^{(+++)}$
TSC	12.5 $^{(+)}$	> 50.0 $^{(-)}$	50.0 $^{(-)}$	0.2 $^{(+++)}$	1.5 $^{(++)}$	2.0 $^{(++)}$
TCH	3.0 $^{(+)}$	> 50.0 $^{(-)}$	3.0 $^{(+)}$	> 50.0 $^{(-)}$	> 50.0 $^{(-)}$	1.0 $^{(++)}$

Values are means of triplicate determinations
Essential oils are classified as $^{(-)}$ not active, $^{(+)}$ moderately active, $^{(++)}$ active and $^{(+++)}$ extremely active.
Bacteria tested: *Staphylococcus aureus* (CIP 53.156), *Staphylococcus epidermidis* (CIP 53124), *Listeria innocua* (CIP 80.11), *Enterobacter aerogenes* multi-drug resistant (MDR) strain EAEP289 and the wild type (CIP 60.86), *Campylobacter jejuni* (CIP 107370).
Corsican *Teucrium* taxa: *T. marum* (**TM**), *T. massiliense* (**TMC**), *T. polium* subsp. *capitatum* (**TP**), *T. flavum* subsp. *glaucum* (**TFG**), *T. scorodonia* subsp. *scorodonia* (**TSC**) and *T. chamaedrys* (**TCH**).

Figure(s)

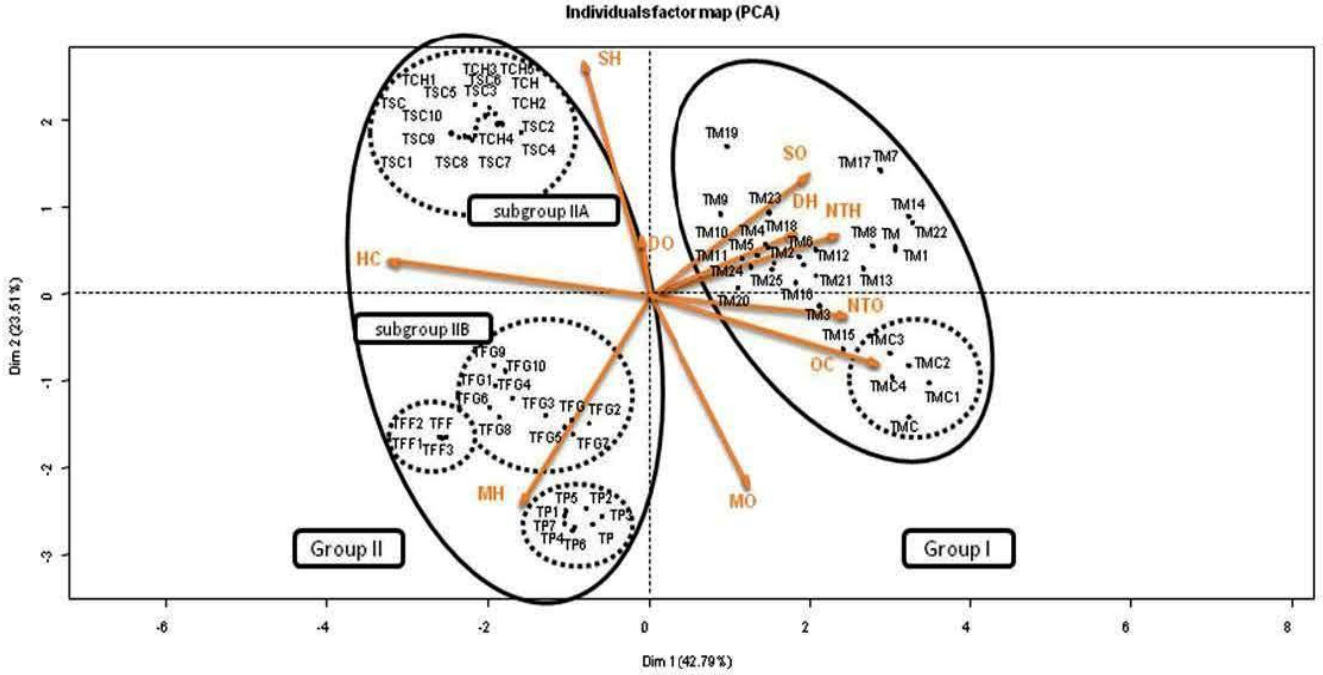

Figure 1: PCA of chemical compositions of *Teucrium* essential oils from Corsica: Distribution of variable corresponding to those of Table 3 and distribution of samples (coding numbers of locations)

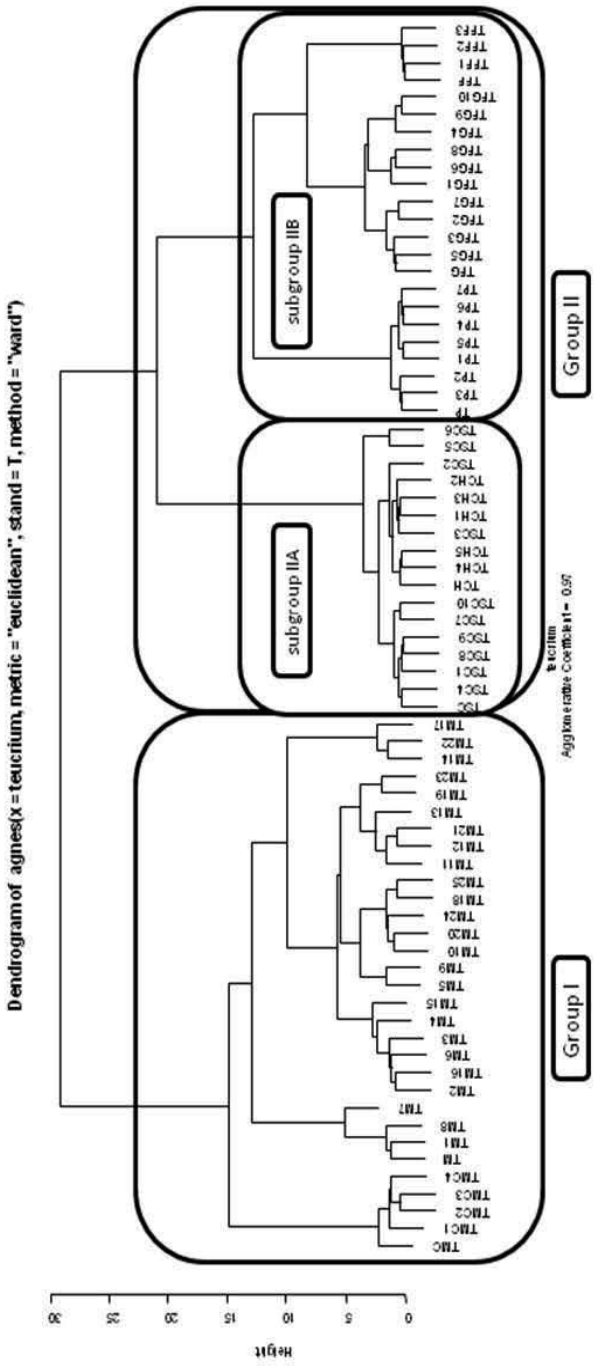

Figure 2: Cluster Analysis of chemical compositions of *Teucrium* essential oils from Corsica

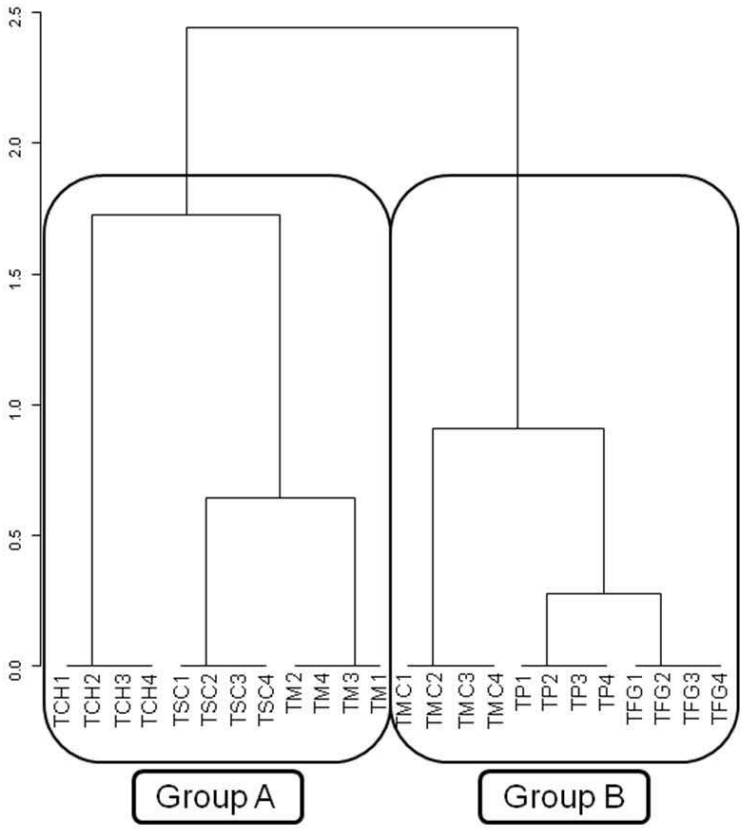

Figure 3: Cluster analysis of antibacterial activity of *Teucrium* essential oils from Corsica

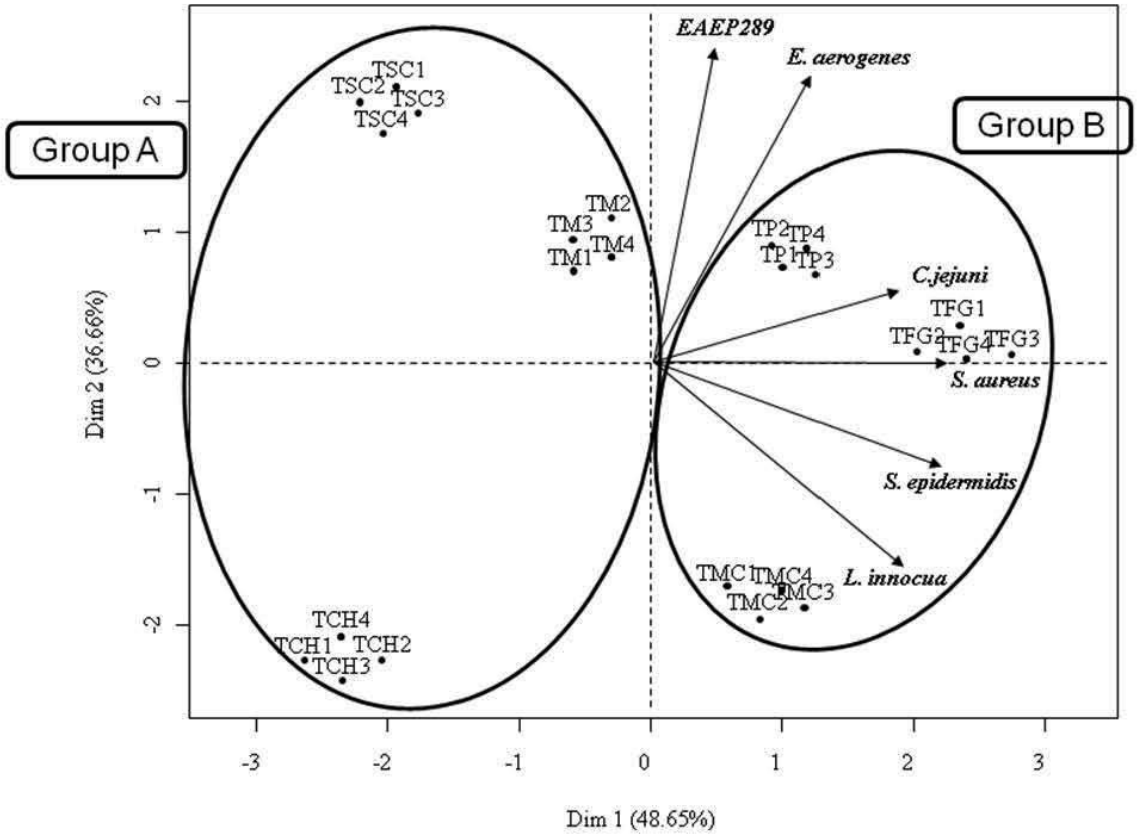

Figure 4: PCA of antibacterial activity of *Teucrium* essential oils from Corsica

Distribution of variables corresponding for each experiment to the mean value (Table 5) and to the three repetitions (data not shown).

Table 1(Supplementary material): Chemical compositions of *Teucrium* essential oils from Corsica

					Teucrium taxa[e]							
No.[a]	Compounds	*I*RI$_A$[b]	RI$_A$[c]	RI$_P$[d]	TMC	TM	TSC	TCH	TP	TFG	TFF	Identification[f]
1	hexanal	780	772	1100		0.1						RI, MS
2	(*E*)-hex-3-en-1-ol	812	823	1330						0.1	0.2	RI, MS
3	*trans*-2-hexenal	832	834	1216		0.6	0.1			0.1	0.2	RI, MS, Ref.
4	ethyl-2-methyl butyrate	843	829	1011	0.2							RI, MS
5	(*Z*)-hex-3-en-1-ol	851	832	1368	0.2		0.1	tr		0.1	0.2	RI, MS
6	1-hexanol	837	841	1331		0.2		tr				RI, MS, Ref.
7	(*E*)-hex-2-en-1-ol	851	854	1389						0.2	0.1	RI, MS
8	pentyl acetate	881	881	1137		tr						RI, MS
9	2-heptanol	880	884	1307						0.1	0.2	RI, MS, Ref.
10	isobutyl isobutyrate	899	899	1065	0.7							RI, MS
11	2-hexyl acetate	920	917	1124	0.4							RI, MS, Ref.
12	*α*-thujene	932	923	1021					8.1	0.1	0.2	RI, MS
13	*α*-pinene	936	932	1023				0.8	24.1	12.2	21.9	RI, MS
14	benzaldehyde	941	933	1512		tr	0.3	0.1				RI, MS
15	camphene	950	944	1067				tr	0.5	0.2	0.3	RI, MS
16	6-methyl-3-heptanol	945	945	1320	1.6							RI, MS
17	oct-1-en-3-ol	962	960	1429	tr	0.7	0.9	2.1		0.2	tr	RI, MS
18	octan-3-one	969	963	1207	tr					0.4	0.2	RI, MS
19	6-methylhept-5-en-2-one	978	963	1340					0.1			RI, MS
20	sabinene	973	966	1121			1.1	tr	4.1	0.4	0.5	RI, MS
21	*β*-pinene	978	973	1112				0.5	9.2	10.3	18.1	RI, MS
22	1,8-dehydro-cineole	980	979	1195					0.1			RI, MS
23	3-octanol	981	980	1366	0.4		0.3	0.1		0.5	0.7	RI, MS
24	octanal	980	981	1310		0.1						RI, MS
25	myrcene	987	981	1332	tr		0.1	tr	1.2	1.9	2.0	RI, MS
26	decene	988	982	1423	tr							RI, MS, Ref.
27	isobutyl isovalerate	993	989	1154	5.8							RI, MS
28	yomogi alcohol	991	996	1376						0.1	0.1	RI, MS, Ref.
29	3-methyl butyl isobutyrate	994	997	1167	0.2			0.1				RI, MS
30	α-phellandrene	1002	997	1162					0.1			RI, MS
31	2-methyl butyl isobutyrate	1002	1001	1167	0.8							RI, MS
32	phenyl acetaldehyde	1012	1007	1608		1.0		tr		0.1	tr	RI, MS
33	*α*-terpinene	1013	1010	1170			0.1		0.7			RI, MS
34	*p*-cymene	1015	1014	1267				0.3	4.0	0.2	tr	RI, MS
35	2-heptyl acetate	1024	1014	1356	0.4							RI, MS, Ref.
36	4,6-dimethylhept-5-en-2-one	1016	1015	1362					0.5			RI, MS
37	limonene	1025	1020	1186	0.4		1.2	0.2	5.2	27.4	20.0	RI, MS
38	*β*-phellandrene	1023	1025	1209				0.1	0.1			RI, MS
39	1,8-cineole	1024	1025	1203					0.1			RI, MS
40	(*Z*)-*β*-ocimene	1029	1026	1207	2.3		0.9	0.1		6.0	15.5	RI, MS
41	2,6-dimethyl-5-heptanal	1039	1034	1348	tr	0.2						RI, MS
42	(*E*)-*β*-ocimene	1041	1036	1221	0.5		0.3	tr	0.2	1.2	2.2	RI, MS
43	2-methylbutyl butyrate	1041	1042	1258		1.0		tr				RI, MS
44	*γ*-terpinene	1051	1049	1230			0.1	tr	1.5			RI, MS
45	non-1-en-3-ol	1058	1050	1514						0.1	0.1	RI, MS, Ref.
46	1-octanol	1063	1053	1524						0.2	0.1	RI, MS
47	*trans*-linalool oxide THP	1058	1054	1435	tr	0.3						RI, MS
48	camphenilone	1059	1058	1452					0.1			RI, MS
49	*trans*-linalool oxide THF	1072	1061	1440	tr							RI, MS
50	fenchone	1069	1068	1412		0.2			0.3			RI, MS
51	*p*-cymenene	1075	1074	1415					0.2			RI, MS
52	6-methyl-3-heptyl acetate	1074	1074	1267	19.1	tr	tr	0.1	tr	0.7	0.3	RI, MS
53	terpinolene	1082	1080	1263			0.8	tr	0.1			RI, MS
54	rose furane	1091	1080	1563						0.3	0.4	RI, MS, Ref.
55	folifolone	1090	1082	1423					0.1			RI, MS, Ref.

56	nonanal	1086	1087	1514	0.2	0.6	0.4			0.3	0.3	RI, MS
57	linalool	1083	1088	1394	5.2	0.7	0.5	1.8	1.1	0.5	0.2	RI, MS
58	*cis*-sabinene hydrate	1087	1090	1528			0.1	tr	0.1			RI, MS
59	*α*-thujone	1089	1091	1397					0.1			RI, MS
60	3-methyl butyl isovalerate	1092	1092	1257	3.4							RI, MS
61	isochrysanthenone	1088	1092	1430					0.1			RI, MS, Ref.
62	1-oct-3-enyl acetate	1093	1094	1363		1.0	0.1	tr				RI, MS
63	2-methyl butyl isovalerate	1094	1094	1260	0.9	0.5		tr				RI, MS
64	isophorone	1100	1095	1537					0.2			RI, MS
65	(*Z*)-rose oxide	1100	1098	1362		0.1						RI, MS
66	fenchol	1099	1099	1561					0.1			RI, MS
67	*β*-thujone	1103	1101	1427					0.3			RI, MS
68	chrysanthenone	1110	1103	1482					0.1			RI, MS
69	*trans*-*p*-mentha-2,8-dien-1-ol	1113	1104	1614						0.4	0.7	RI, MS
70	*α*-campholenal	1109	1105	1480					0.9			RI, MS
71	nopinone	1111	1109	1583					0.6			RI, MS
72	3-octanyl-acetate	1113	1111	1315	7.0	tr	0.1	0.1		1.1	0.2	RI, MS
73	*cis*-*p*-mentha-2,8-dien-1-ol	1125	1117	1651						0.7	0.7	RI, MS
74	1,2-epoxy-limonene	1117	1120	1148		tr						RI, MS
75	camphor	1123	1122	1513					0.1			RI, MS
76	*trans*-pinocarveol	1126	1125	1643				tr	2.3			RI, MS
77	neo-allo-ocimene	1126	1126	1363	0.3							RI, MS
78	*cis*-verbenol	1127	1129	1651					2.6			RI, MS
79	citronellal	1129	1131	1456	0.5	1.6						RI, MS
80	menthone	1136	1133	1446	0.1							RI, MS
81	*trans*-verbenol	1139	1135	1659					0.1			RI, MS
82	sabina ketone	1132	1137	1601					0.1			RI, MS, Ref.
83	*trans*-pinocamphone	1139	1139	1503					0.1			RI, MS, Ref.
84	pinocarvone	1140	1139	1557					0.5	1.5	1.3	RI, MS
85	*p*-mentha-1,5-dien-8-ol	1148	1140	1689					0.1	0.6	0.6	RI, MS
86	isomenthone	1146	1141	1452	0.1							RI, MS
87	2-methyl benzofurane	1149	1146	1573			0.1			0.3	0.2	RI, MS, Ref.
88	umbellulone	1151	1150	1610					0.2			RI, MS
89	borneol	1150	1150	1705					1.5			RI, MS
90	*cis*-pinocamphone	1151	1152	1533					0.1			RI, MS, Ref.
91	isopulegone	1150	1154	1621	0.2							RI, MS, Ref.
92	methyl-4-acetophenone	1157	1156	1747	0.2				0.2			RI, MS
93	cryptone	1160	1156	1655						0.1	0.2	RI, MS
94	*p*-cymen-8-ol	1169	1159	1812					0.4			RI, MS
95	terpinen-4-ol	1164	1160	1562	0.1		0.3	0.1	6.6	0.7	0.2	RI, MS
96	(*Z*)-3-hexenyl butyrate	1170	1168	1450				0.2				RI, MS, Ref.
97	methyl salicylate	1171	1170	1720			0.2	0.1				RI, MS
98	myrtenal	1172	1171	1614					1.2			RI, MS
99	*α*-terpineol	1176	1172	1700	0.1		0.2	tr	1.2	1.0	0.2	RI, MS
100	myrtenol	1178	1173	1780		0.1	0.1	tr	1.6	1.2	0.3	RI, MS
101	2,6-dimethyl-3-octyl acetate	1170	1174	1356	0.5							RI, MS, Ref.
102	estragole	1175	1176	1645		3.5	0.1					RI, MS
103	hexyl butanoate	1177	1176	1403		2.3						RI, MS
104	*trans*-isoperitenol	1182	1179	1720						1.1	0.4	RI, MS
105	verbenone	1184	1185	1680					1.9			RI, MS
106	decanal	1188	1188	1498	tr	0.3	0.1			0.6	0.4	RI, MS
107	decanol	1197	1194	1732		tr						RI, MS
108	*trans*-carveol	1200	1199	1821					0.3	0.4	0.2	RI, MS
109	dodecane	1200	1201	1500	tr							RI, MS
110	*cis*-*p*-mentha-1(7),8-dien-2-ol	1206	1206	1871						0.2	0.2	RI, MS
111	fenchyl acetate	1205	1207	1456					0.1			RI, MS
112	nerol	1210	1210	1675		0.8	0.1	tr	0.1	0.2	0.1	RI, MS
113	citronellol	1213	1213	1726	4.1	0.2						RI, MS
114	cuminaldehyde	1217	1214	1745					0.2			RI, MS
115	carvone	1214	1216	1726					0.8	0.4	0.1	RI, MS
116	pulegone	1215	1216	1603	6.9							RI, MS
117	(*Z*)-3-hexenyl-2-methyl butyrate	1220	1219	1457	tr	0.2		tr				RI, MS

118	(*Z*)-piperitenone oxide	1232	1230	1722	0.3							RI, MS
119	2-methylbutyl hexanoate	1235	1235	1475		tr						RI, MS
120	geraniol	1235	1235	1801	1.0	1.5	0.1	0.2	0.2	0.2	tr	RI, MS
121	(*E*)-2-decenal	1240	1238	1644		tr		tr	tr	0.3	0.1	RI, MS
122	(*Z*)-myrtanol	1242	1240	1869		tr						RI, MS
123	linalyl acetate	1239	1241	1557	0.1				0.8			RI, MS
124	geranial	1244	1243	1707	0.7	1.0						RI, MS
125	perilla aldehyde	1260	1248	1774					0.1	0.1	tr	RI, MS
126	*p*-menth-1-en-7-al	1250	1249	1699					0.2			RI, MS
127	citronnellyl formate	1259	1256	1583	0.4	0.4						RI, MS
128	*p*-mentha-1,3-dien-7-al	1260	1258	1765					0.1			RI, MS, Ref.
129	neryl formate	1265	1262	1649	tr							RI, MS
130	nonanoic acid	1263	1266	2098		0.4				0.1	tr	RI, MS
131	cuminol	1265	1266	2089					0.3			RI, MS
132	thymol	1267	1267	2168					0.2			RI, MS
133	decanol	1268	1267	1749			0.1					RI, MS, Ref.
134	lavandulyl acetate	1275	1268	1593	tr							RI, MS
135	bornyl acetate	1270	1269	1572					0.9			RI, MS
136	*p*-mentha-1-en-9-ol	1283	1269	1929						0.5	0.1	RI, MS
137	benzyl isobutanoate	1269	1271	1817	tr							RI, MS
138	undecan-2-one	1273	1273	1592		1.0						RI, MS
139	vitispirane	1276	1273	1510				0.1				RI, MS, Ref.
140	perillyl alcohol	1280	1273	1980						0.1	0.1	RI, MS
141	menthyl acetate	1278	1274	1553		0.4						RI, MS
142	carvacrol	1278	1276	2171					0.1			RI, MS
143	isobornyl acetate	1276	1280	1580						0.1	tr	RI, MS
144	geranyl formate	1284	1280	1666	0.3	tr						RI, MS
145	dihydroedulan II	1284	1281	1504				tr				RI, MS, Ref.
146	undecan-2-ol	1284	1285	1719		tr						RI, MS
147	dihydroedulan I	1288	1289	1474				tr				RI, MS, Ref.
148	(*E*,*E*)-2,4-decadienal	1290	1291	1820	tr	0.4						RI, MS
149	theaspirane I	1299	1291	1480	tr		0.1			0.5	0.1	RI, MS
150	tridecane	1300	1302	1308				tr				RI, MS
151	theaspirane II	1313	1306	1517	0.1		0.1			0.3	0.1	RI, MS
152	piperitenone	1318	1309	1870	0.7							RI, MS
153	hexyl tiglate	1333	1314	1578		0.3						RI, MS
154	(*Z*)-carvyl acetate	1318	1316	1720						0.2	0.1	RI, MS
155	myrcenyl acetate	1328	1329	1725		0.3						RI, MS
156	α-terpinyl acetate	1335	1332	1693					1.1	0.4	0.2	RI, MS
157	eugenol	1331	1334	2157				0.1		0.7	0.2	RI, MS
158	citronellyl acetate	1337	1334	1626	1.3	1.0				0.1	tr	RI, MS
159	neryl acetate	1342	1335	1694	tr		0.1		0.1			RI, MS
160	δ-elemene	1340	1336	1535	tr							RI, MS, Ref.
161	bicycloelemene	1338	1339	1477				0.1				RI, MS, Ref.
162	*trans*-2-undecenal	1346	1342	1726	tr					0.1	tr	RI, MS
163	α-cubebene	1355	1349	1452	tr		6.2	0.1				RI, MS
164	geranyl acetate	1362	1360	1715	0.9	3.0	0.1		0.5			RI, MS
165	(*E*)-β-damascenone	1363	1361	1770	0.3	0.3	0.1	tr		0.9	0.2	RI, MS
166	benzyl isovalerate	1374	1362	1851		0.3						RI, MS, Ref.
167	Isoledene	1382	1366	1462	tr							RI, MS
168	methyl-eugenol	1369	1367	2113		0.4						RI, MS
169	dodecanoic acid	1369	1369	2469						0.2	tr	RI, MS
170	α-ylangene	1376	1371	1476	tr		0.5					RI, MS
171	hexyl hexanoate	1363	1372	1599		0.3						RI, MS
172	α-copaene	1379	1377	1488	0.1		2.1	0.2				RI, MS
173	β-bourbonene	1386	1381	1483	1.7		0.1	2.7				RI, MS
174	α-bourbonene	1389	1383	1521						0.3	0.3	RI, MS, Ref.
175	1,5-diepi-β-bourbonene	1389	1384	1483	0.1			0.3				RI, MS, Ref.
176	dolicholactone	1385	1385	2134		0.7						RI, MS
177	β-cubebene	1390	1388	1513			2.3					RI, MS
178	β-elemene	1389	1388	1571			1.9					RI, MS
179	dodecanal	1389	1389	1402		0.3						RI, MS

180	dodecan-2-one	1381	1391	1711		tr						RI, MS
181	iridomyrmecine	1397	1397	2112		0.5						RI, MS
182	tetradecane	1392	1402	1402	tr							RI, MS
183	neryl acetone	1412	1409	1851		0.4						RI, MS
184	cinnamyl acetate	1408	1411	2152	0.1							RI, MS
185	α-gurjunene	1413	1413	1475	0.1		3.5					RI, MS
186	(*E*)-β-caryophyllene	1421	1418	1580		5.3	21.1	33.9		0.3	0.1	RI, MS
187	β-ylangene	1420	1419	1534	0.6			0.3				RI, MS
188	2-undecanyl acetate	1425	1421	1632		0.5				0.1	0.1	RI, MS
189	α-barbatene	1414	1422	1563		1.2						RI, MS, Ref.
190	β-copaene	1430	1423	1581	0.3			0.2				RI, MS
191	citronnellyl propionate	1427	1426	1549	tr							RI, MS
192	(*E*)-α-bergamotene	1434	1429	1547	0.2	8.2		0.1	0.9			RI, MS
193	geranyl acetone	1430	1431	1834		tr				0.1	tr	RI, MS
194	γ-elemene	1429	1432	1616			3.9					RI, MS, Ref.
195	α-sesquisabinene	1435	1433	1626	0.1							RI, MS
196	veratraldehyde	1432	1435	2370						0.1	tr	RI, MS
197	α-guaiene	1440	1437	1538			0.4					RI, MS
198	aromadendrene	1443	1437	1605	0.1							RI, MS
199	α-sesquisabinene	1435	1439	1626						0.1	tr	RI, MS
200	β-barbatene	1445	1441	1593		1.1						RI, MS, Ref.
201	isogermacrene D	1445	1446	1681			0.1	0.2				RI, MS, Ref.
202	(*E*)-β-farnesene	1446	1449	1628	0.3	0.4	1.8	5.1		2.3	0.6	RI, MS
203	α-humulene	1455	1450	1665		1.3	6.9	7.5				RI, MS
204	allo-aromadendrene	1462	1455	1605	1.4		0.3	0.6				RI, MS
205	neryl propionate	1451	1456	1782		0.2						RI, MS
206	βhumulene	1460	1456	1605	tr							RI, MS
207	β-ionone	1468	1465	1918		0.3				0.1	0.1	RI, MS
208	α-curcumene	1473	1470	1731	0.3	0.4	0.9					RI, MS
209	γ-muurolene	1474	1471	1642	tr							RI, MS
210	*trans*-β-bergamotene	1480	1474	1608		0.4				0.1	tr	RI, MS
211	germacrene-D	1479	1476	1666	6.1		6.7	18.5		0.7	tr	RI, MS
212	γ-humulene	1483	1477	1685	tr							RI, MS
213	zingiberene	1489	1483	1687	0.2							RI, MS
214	4-epi-cubebol	1490	1487	1870	0.1			0.4				RI, MS
215	zingiberene	1489	1490	1717		0.3	3.6					RI, MS
216	bicyclogermacrene	1494	1492	1685	0.5		0.4	1.6				RI, MS
217	β-bisabolene	1503	1493	1720						0.4	0.2	RI, MS
218	β-dihydroagarofuran	1500	1495	1705					0.1			RI, MS, Ref.
219	α-muurolene	1496	1496	1719			0.3	0.2				RI, MS
220	α-cuparene	1498	1497	1840		0.5						RI, MS
221	(*E,E*)-α-farnesene	1498	1500	1693			0.4	tr		2.0	0.9	RI, MS
222	β-curcumene	1503	1500	1687	0.3							RI, MS
223	βbisabolene	1503	1502	1720		7.5	3.3	1.8				RI, MS
224	(*Z*)-γ-bisabolene	1505	1505	1709	0.4							RI, MS
225	γ-cadinene	1507	1507	1749			0.5	0.1				RI, MS
226	sesquicineole	1507	1508	1737				0.2				RI, MS
227	cubebol	1514	1510	1924	tr			tr				RI, MS
228	apofarnesal	1522	1511	1760		0.4						RI, MS, Ref.
229	(*E*)-calamenene	1517	1512	1816			0.2					RI, MS
230	7-epi-α-selinene	1519	1513	1742				0.1				RI, MS, Ref.
231	δ-cadinene	1520	1514	1715	0.8		2.5	4.6	2.2			RI, MS
232	β-sesquiphellandrene	1516	1517	1765		3.7	0.7			0.2	0.1	RI, MS
233	(*E*)-α-bisabolene	1530	1524	1733		0.4	1.3			0.2	0.1	RI, MS
234	cadina-1,4-diene	1523	1526	1757				tr				RI, MS
235	(*E*)-γ-bisabolene	1531	1527	1733	0.1							RI, MS, Ref.
236	α-calacorene	1527	1530	1875					0.8			RI, MS
237	α-agarofuran	1537	1531	1860					0.5			RI, MS, Ref.
238	α-cadinene	1534	1531	1743			0.3					RI, MS
239	β-caryophyllene oxide	1546	1540	1964			0.2					RI, MS, Ref.
240	β-calacorene	1541	1545	1935					0.1			RI, MS
241	dodecanoic acid	1554	1546	1874		0.5	0.2					RI, MS, Ref.

242	(*Z*)-3-hexenyl benzoate	1553	1550	2131				0.1				RI, MS, Ref.
243	germacrene B	1552	1556	1820			8.3					RI, MS, Ref.
244	(*E*)-nerolidol	1553	1556	2037		0.8	0.1	0.1				RI, MS
245	1,5-epoxy salvial-4(14)-ene	1554	1558	1910	0.2							RI, MS
246	spathulenol	1572	1560	2065	1.2		0.3					RI, MS
247	palustrol	1562	1562	1920	0.1		0.1					RI, MS
248	germacrene D-4-ol	1571	1565	2025	0.1							RI, MS
249	caryophyllene oxide	1578	1570	1942	0.4	9.8	1.0	3.1	0.1			RI, MS
250	globulol	1589	1575	2074	0.3		0.1					RI, MS
251	germacradiene-11-ol	1579	1580	2065	0.1							RI, MS
252	6,7-bisepoxy-sec-calamenene	1585	1580	2107					0.1			RI, MS, Ref.
253	fokienol	1582	1582	2180		0.7						RI, MS
254	guaiol	1591	1584	2090					2.1	0.3	0.2	RI, MS
255	dodecyl acetate	1585	1585	1960		0.7						RI, MS, Ref.
256	viridiflorol	1592	1586	2071	0.1		0.1	0.1				RI, MS
257	copaborneol	1595	1592	2159				0.1				RI, MS
258	ledol	1600	1589	1996	0.1		0.2					RI, MS
259	*β*-oplopenone	1594	1591	2023	0.6							RI, MS
260	humulene epoxide II	1602	1599	2044	tr	3.2	0.9	0.1				RI, MS
261	hexadecane	1600	1601	1602	tr							RI, MS
262	7-epi-*γ*-eudesmol	1610	1609	2075					0.1			RI, MS
263	ar-curcumen-7-ol	1610	1611	2240	0.5							RI, MS, Ref.
264	1,10-diepicubenol	1615	1612	2031			0.2					RI, MS
265	*α*-acorenol	1623	1617	2130		0.7						RI, MS
266	isospathulenol	1625	1618	2175	0.4							RI, MS, Ref.
267	epi-cubenol	1623	1623	2059	tr		0.3					RI, MS
268	cubenol	1630	1633	2013	tr		0.2					RI, MS
269	*τ*-muurolol	1633	1634	2109	0.7		0.6	0.3				RI, MS
270	*τ*-cadinol	1633	1635	2125	0.5		0.7	0.7				RI, MS
271	4α-hydroxydihydroagarofuran	1636	1635	2217					0.1			RI, MS, Ref.
272	valerianol	1647	1635	2208					0.1			RI, MS
273	hinesol	1632	1636	2193					0.1			RI, MS
274	caryophylla-4(14)-8(15)-dien-5*α*-ol	1641	1636	2292	0.1	0.7	0.8	0.4				RI, MS, Ref.
275	*β*-eudesmol	1641	1636	2210					0.4			RI, MS
276	*α*-cadinol	1643	1641	2184	0.3		0.2	0.2				RI, MS
277	bulnesol	1665	1651	2194					0.2	0.1	0.1	RI, MS
278	cadalene	1659	1653	2200					0.9			RI, MS, Ref.
279	(*Z*,*Z*)-farnesol	1635	1654	2224	0.8							RI, MS
280	eudesma-4(15),7-dien-1*β*-ol	1671	1671	2348	0.8			0.4				RI, MS
281	*α*-bisabolol	1673	1679	2201		1.5				0.9	0.5	RI, MS
282	3*β*,4*β*-oxydoagarofuran	1685	1685	2282					0.1			RI, MS
283	(*Z*,*E*)-farnesol	1685	1688	2311		0.5						RI, MS
284	1-pentadecanal	1702	1693	2085		1.2						RI, MS, Ref.
285	heptadecane	1700	1701	1703	tr							RI, MS
286	mint sulfide	1734	1733	2152				0.3				RI, MS, Ref.
287	lepidozenal	1744	1737	2232	0.1							RI, MS, Ref.
288	benzyl benzoate	1741	1744	2575				tr				RI, MS, Ref.
289	tetradecanoic acid	1748	1755	2164		0.2						RI, MS, Ref.
290	bulnesyl acetate	1782	1784	2156					0.8			RI, MS
291	1-hexadecanal	1782	1796	2320		0.6						RI, MS, Ref.
292	octacosane	1800	1803	1798				0.1				RI, MS
293	diisobutyl phthalate	1826	1829	2522						0.5	0.1	RI, MS, Ref.
294	hexahydrofarnesyl acetone	1845	1842	2125	tr	0.6	0.5	tr		0.1	tr	RI, MS, Ref.
295	2-heptadecanone	1870	1888	2427		0.2						RI, MS, Ref.
296	(3*Z*,6*E*,10*E*)-*α* -springene	1941	1941	2190		3.2						RI, MS
297	isophytol	1949	1946	2296				0.1				RI, MS
298	hexadecyl acetate	1970	1951	2407	tr	0.6						RI, MS, Ref.
299	hexadecanoic acid	1962	1969	2826	tr							RI, MS, Ref.
300	abietatriene	2046	2036	2218	0.1							RI, MS, Ref.
301	methyl heptadecanol	2030	2037	2482	0.4							RI, MS, Ref.
302	octadecanol	2070	2073	2529	0.3	1.7				1.4	0.1	RI, MS
303	(*E*)-phytol	2114	2097	2559	0.3	2.5	1.4	1.8		4.5	0.5	RI, MS

304	n-heneicosane	2100	2101	2102	tr							RI, MS
305	nonadecanol	2150	2144	2605						0.5	0.1	RI, MS, Ref.
306	octadecyl acetate	2198	2194	2499		3.3				0.4	0.1	RI, MS
307	n-docosane	2200	2201	2204	tr							RI, MS
308	2-eicosanone	2200	2213	2730		0.3						RI, MS, Ref.
309	2-heneicosanone	2300	2280	2836		0.6						RI, MS, Ref.
310	n-tricosane	2300	2301	2302	0.1			tr				RI, MS
311	tetracosane	2400	2402	2403	tr			tr				RI, MS
312	pentacosane	2498	2502	2498	tr			tr				RI, MS
313	hexacosane	2598	2584	2598	tr							RI, MS
314	heptacosane	2700	2703	2700	tr							RI, MS
	Total identification (g/100g)				92.1	95.5	98.2	93.6	98.9	94.9	95.4	
	Hydrocarbon compounds				17.9	33.9	**85.3**	**80.0**	**63.7**	**66.5**	**83.0**	
	Monoterpene hydrocarbons				3.5	0	4.6	2.0	**59.3**	**59.9**	**80.7**	
	Sesquiterpene hydrocarbons				14.1	30.7	**80.7**	**77.9**	4.4	6.6	2.3	
	Hydrocarbon diterpene				0	3.2	0	0	0	0	0	
	Non-terpenic hydrocarbons				0.3	0.9	0	0.1	0	0	0	
	Oxygenated compound				**74.2**	**61.6**	12.9	13.6	**35.2**	**35.0**	12.4	
	Oxygenated monoterpenes				20.4	17.6	2.0	2.4	29.6	12.7	6.7	
	Oxygenated sesquiterpenes				7.4	18.6	6.0	6.4	4.8	1.3	0.8	
	Oxygenated diterpene				0	2.5	1.4	1.9	0	4.5	0.5	
	Non-terpenic oxygenated compounds				**46.4**	22.9	3.5	2.9	0.8	16.5	4.4	
	Alcohols				19.9	13.7	8.1	8.2	22.2	17.8	7.4	
	Aldehydes and Ketones				10.6	12.3	1.5	0.4	9.2	5.3	3.1	
	Esters				**42.5**	16.3	0.6	0.3	4.3	3.6	1.1	
	Oxides				1.0	**16.9**	2.5	4.2	0.9	1.4	0.8	
	Acids				0	1.1	0.2	0	0	0.3	0	

[a] Order of elution is given on apolar column (Rtx-1).
[b] Retention indices of literature on the apolar column (IRI$_A$) reported from Konig et al., (2001).
[c] Retention indices on the apolar Rtx-1 column (RI$_A$).
[d] Retention indices on the polar Rtx-Wax column (RI$_P$).
[e] Corsican *Teucrium* taxa (concentrations in g/100g): *T. massiliense* (**TMC**), *T. marum* (**TM**), *T. scorodonia* subsp. *scorodonia* (**TSC**), *T. chamaedrys* (**TCH**), *T. polium* subsp. *capitatum* (TP), *T. flavum* subsp. *glaucum* (**TFG**) and *T. flavum* subsp. *flavum* (**TFF**).
[f] RI: Retention Indices; MS: Mass Spectra in electronic impact mode; Ref.: compounds identified from literature data: Koning et al., 2001 (**3**, **6**, **9**, **11**, **28**, **35**, **45**, **54**, **55**, **61**, **82**, **83**, **87**, **90**, **91**, **133**, **174**, **175**, **189**, **194**, **200**, **218**, **228**, **235**, **237**, **239**, **241**, **243**, **252**, **255**, **263**, **266**, **271**, **278**, **284**, **289**, **291**, **294**, **300**); Jennings and Shibamoto, 1980 (**96**, **166**, **242**, **288**), Mass Finder, 2006 (**161**, **201**, **230**, **287**); National Institute of Standards and Technology, 1999 (**101**, **275**, **294**, **296**, **301**, **305**, **308**, **309**) and McLafferty and Stauffer 1994 (**26**, **128**, **139**, **145**, **147**, **160**, **287**, **298**, **299**).

ملخـــص

إن ثـــراء المحـيط النـبـاتي بـجـزيـــرة كـورسـيـكـا و كـذلك منـطـقـة الـغرب الـجـزائـــري غـنـــي عن الـــتـعـريـف.

إن حـوض بـحـر الأبيض المتـوسط يعتبر من الأمـاكـن الـسـاخـنـة الذي يـتـواجـد فيـه مـختلف النـبـاتـات ذات ثـــراء خـاص ودائـم، غـيـر أنـــه مـهـدد بالإنـقـداد النـاتج عن عـدة عـوامـل منـهـا الأخـطار الـخـارجيـة عن المحيط و الذي هـو في تـزايـد مستمـر.

إن الـزيـــوت الـهـامـة منـتـوج ذات جـودة عـاليـة مـستعملة في إطـار إنتـاج العطور، وأدوات التـجميل و الزينـة و كـذلك في التـغديـة الزراعـية و ذلك في مجـال الصيدلـة.

هـي مشـكـلـة مثل خليط معقد و مكـون مـن العشـرات بل المئـات التـراكيب عـامـة تـربـينيـك مـوجـودة بـكـميـة غير مـستقـرة.

إن إنـتـاج الـزيـــوت الـهـامـة، رقـابـة الـجـودة تـبـرهـن عـلى مـدى إستـعـمـال الأدوات العـامة في تـحـضـيـر بـطرق حـضـريـة ة تـحـليليـة.

هـذا العـمل يدخـل في إطـار دراســة الـزيـــوت الـهـامـة و أجـنـاس من نـوع الـمـوجـــودة في teucrium) جـزيـــرة(كـورسـيـكـا و كـذلك غـرب الـجـزائـــري.

إن هـــدفـنـــا هـو الـمســاهـمـة في تـطـويـر المـعـلـومـات الـخـاصـة يالثـروة النـبـاتيـة الـموجـودة في الغـطـاء الـنـبـــاتي بـطريـقـــة عـلـمـيـة حـديـثـــة و فـعـــالـــة

الكلمات المفتاحية : توكرييوم – الزيوت الهامة – الشفرة الجينية – النشاطات البيولوجية.

Résumé

La richesse de la flore de Corse et de l'ouest algérien n'est plus à démontrer. Le bassin méditerranéen est l'un des « points chauds » ou hotspot dans lesquels se trouve concentrée la biodiversité. A l'instar des autres zones rouges régionales, ces deux régions sont caractérisées par une richesse spécifique, un taux d'endémisme élevé mais aussi par des menaces anthropiques fortes et en augmentation rapide.

Les huiles essentielles sont des produits à forte valeur ajoutée utilisées dans des domaines aussi divers que la parfumerie, les cosmétiques, l'agro-alimentaire ou encore l'aromathérapie et la pharmacie. Elles se présentent sous forme de mélanges complexes de plusieurs dizaines voire de plusieurs centaines de composés en général terpéniques présents dans des proportions variables. La production et la caractérisation des huiles essentielles, le contrôle de leur qualité tout autant que la mise en évidence d'une éventuelle spécificité nécessite la mise en œuvre des méthodes de préparation et d'analyses les plus modernes.

Ce travail de thèse repose sur l'étude des huiles essentielles et des fractions volatiles de 9 espèces et sous-espèces du genre *Teucrium*, poussant en Corse et à l'ouest algérien.Notre objectif est de contribuer à l'amélioration des connaissances des ressources naturelles issues de la biomasse végétale en fournissant des informations scientifiques objectives. Ce travail de thèse a été l'occasion de développer un travail méthodologique et appliqué totalement complémentaire. Ainsi trois grands volets ont été développés : Le principal volet est chimique, il concerne la caractérisation des compositions chimiques des huiles essentielles, les deux autres volets sont totalement complémentaires : (i) un volet génétique dont le but est d'établir les séquences génétiques des plantes étudiées et examiner l'impact du patrimoine génétique sur la production de métabolites secondaires (ii) un volet microbiologique basé sur la mise en évidence d'activités antimicrobiennes des huiles essentielles étudiées.
Mots clefs : *Teucrium*, Huiles essentielles, Diversité génétique, Activités biologiques

Abstract

Corsican and western Algerian flora is rich and well known. The Mediterranean basin is one of the Hotspot where biodiversity is concentrated. Like other regional red areas, these two regions are characterized by high richness endemism but also by strong anthropogenic threats growing rapidly.

Essential oils are high value added products used in fields as diverse as perfumes, cosmetics, food processing or aromatherapy and pharmaceuticals. It is present generally in the form of complex mixture of several tens or even hundreds of terpene compounds in variable proportions. Production and characterization of essential oils, the quality control as well as the identification of a possible specificity requires the implementation of modern methods of preparation and analyzes.

This work is based on the study of essential oils and volatile part of nine species and subspecies of *Teucrium* genius, growing in Corsica and western Algeria. The aim of this work is to contribute to improve knowledge of natural resources from plant biomass by providing objective scientific information. Three major axes have been developed: the main stand concerns the characterization of chemical compositions of essential oils; the other two stands are fully complementary: (i) a genetic axe that aims to establish the genetic sequence of *Teucrium* plants and examine the impact of the genetic information to production of secondary metabolites (ii) a microbiological demonstration based to antibacterial activity of essential oils studied.
Keywords: *Teucrium*, Essential oils, Genetic diversity, biological activities.