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Study chemical composition, phytochemical, antioxidant and antimicrobial activity of some medicinal plants used in Yemen

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

In the Name of Allâh, the Most Beneficent, the Most Merciful.

Au nom d'Allah, le Tout Miséricordieux, le Très Miséricordieux.

وَأَوۡحَىٰ رَبُّكَ إِلَى ٱلنَّحۡلِ أَنِ ٱتَّخِذِي مِنَ ٱلۡجِبَالِ بُيُوتٗا وَمِنَ ٱلشَّجَرِ وَمِمَّا يَعۡرِشُونَ ﴿٦٨﴾ ثُمَّ كُلِي مِن كُلِّ ٱلثَّمَرَٰتِ فَٱسۡلُكِي سُبُلَ رَبِّكِ ذُلُلٗاۚ يَخۡرُجُ مِنۢ بُطُونِهَا شَرَابٞ مُّخۡتَلِفٌ أَلۡوَٰنُهُۥ فِيهِ شِفَآءٞ لِّلنَّاسِۗ إِنَّ فِي ذَٰلِكَ لَأٓيَةٗ لِّقَوۡمٖ يَتَفَكَّرُونَ ﴿٦٩﴾ النحل

68. and Your Lord inspired the bee, saying: "Take You habitations In the mountains and In the trees and In what they erect.

69. "Then, eat of All fruits, and follow the ways of Your Lord made easy (for You)." there comes forth from their bellies, a drink of varying colour wherein is healing for men. Verily, In This is indeed a sign for people who think. Alnahl

68. [Et voilà] ce que Ton Seigneur révéla aux abeilles: ‹Prenez des demeures dans les montagnes, les arbres, et les treillages que [les hommes] font.

69. puis mangez de toute espèce de fruits, et suivez les sentiers de votre Seigneur, rendus faciles pour vous. de leur ventre, sort une liqueur, aux couleurs variées, dans laquelle il y a une guérison pour les gens. il y a vraiment là une preuve pour des gens qui réfléchissent. Elnahl.

Dedication

To my parents, who overworked and suffered the pain to get me well-brought up, and whose care and love yet embrace me till the moment;

To my inspirer and life partner, Um Abdulrahman;

To my lovely, Abdulrahman Aayah and Iaad;

To my sisters, whom I love and respect;

To my brothers, whose life away from home;

To my whole family;

To my colleagues,

I dedicate this humble work.

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ملخص

يعود استخدام النباتات الطبية والعطرية في اليمن الى آلاف السنين ، حيث تشكل جزء هام من الثقافة العلاجية. بعض النباتات مازالت تلعب دور مهم في الصحة ونظام العناية بجسم الانسان. تعتبر النباتات مصدر رئيسي للكثير من الأدوية المستخدمة لعلاج مختلف الامراض . ترجع قدره العقاقير النباتية ضد الامراض بشكل رئيسي الى مكوناتها الكيميائية. والعقاقير النباتية اليوم تعتبر اكثر امانا مقارنه بالعقاقير المصنعة كيميائيا.

تهدف دراستنا الى الكشف عن التركيب الكيمائي وتقدير مركبات الايض الثانوية لثمانية من النباتات اليمنية، وتحديد قدرتها كمضادات للأكسدة ، وكذا قدرتها على تثبيط نمو الميكروبات الممرضة وتشمل الصبر السقطري ، الكرفس، المر، الترمس المر، التوت، حبه البركة ، الرجلة واخيرا الحلبة .

تم استخلاص النباتات بعدة مذيبات مختلفة وتم تقدير المركبات الفينولية الكلية والفلافونيدات والتانينات والقلويدات. اظهر مستخلصا الايثانول والميثانول لنبات الصبر السقطري اعلى محتوى من الفينولات الكلية (20184.16 ملغGAE / 100 غ وزن جاف، 10771 ملغ GAE/ 100غ وزن جاف) على التوالي.

تقييم فعالية النباتات كمضادات للأكسدة أجري مخبريا باستخدام طريقه تنظيف DPPH و فوق اكسيد الهيدروجين ،و اختزال الحديديك والقدرة الكلية المضادة للأكسدة. وبينت النتائج ان مستخلص اسيتات الايثيل لنبات الصبر السقطري يملك اعلى قدره على تنظيف جزيئات DPPH (IC50 = 5.34 مكغ / مل)، كما أن له قدرة على اختزال الحديديك (EC50 = 3.273 مكغ /مل)، بينما امتلك مستخلص الميثانول لنبات الصبر السقطري اعلى قدره كمضاد للأكسدة الكلية (478.0535 ملغ الفا توكوفيرول/غ وزن نبات جاف).

أجريت دراسة قدره المستخلصات على تثبيط الميكروبات بطريقه الانتشار بالأقراص، حيث تم استخدم تسع سلالات من الميكروبات الممرضة (سبع سلالات بكتيرية وسلاله واحدة من كلا الفطريات والخمائر). بينت النتائج ان مستخلصي اسيتات الاثيل والميثانول لنبات الصبر السقطري اظهرا اعلى تثبيط لنمو بكتيريا الكلبسيلة الرئوية ، اشريشيا المعويه، بكتيريا العنقودية الذهبية(18 ملم، 11ملم ، 18 ملم على التوالي). بينما المستخلص الميثانولي لكل من اوراق التوت والصبر السقطري كان لديهما اعلى قدره على تثبيط نمو البكتيريا العصوية الدقيقة (20ملم)، بينما مستخلصات اسيتات الاثيل والايثانول لأوراق التوت كانت هي الاعلى في تثبيط نمو بكتيريا ميكروكوكاس لاتيوس وبكتيريا الليستيرية المستوحدة (11 ملم و 28 ملم على التوالي).

جميع مستخلصات الهكسان لم تظهر أي فعالية ضد انواع البكتيريا المختلفة ماعدا نبات الكرفس حيث اظهر مستخلصه قدرته على تثبيط البكتيريا العصوية الدقيقة (11 ملم). وبينت النتائج ان كل مستخلصات الكرفس لها القدرة على تثبيط الخميرة المبيضية، بينما كان مستخلص الايثانول لأوراق التوت ومستخلص الهكسان لنبات الصبر السقطري لديهما قدره جيده في ثبيط نمو فطر اسبرجلس فلافوس.

كلمات مفتاحيه : النباتات الطبية اليمنية، مركبات الايض الثانوية، ، مضادات الأكسدة، تثبيط الميكروبات، التركيب الكيميائي، المعادن

ABSTRACT

The use of medicinal and aromatic plants species in Yemen goes back thousands of years, and form an important part of the culture. Some of the plants still play important role in the health and body care system. Plants are the major sources for various therapeutic agents in the treatment of a wide variety of diseases. The potency of herbal drugs against diseases is mainly due to their chemical compounds. The herbal products today symbolize safety in contrast to the synthetics.

Our study aimed at detection and determination the chemical and phytochemical composition, estimation antioxidant and antimicrobial activities of eight Yemeni plants "*Aloe perryi, Apium graveoleus L, Commiphora myrrha, Lupinus termis, Morus alba, Nigelle sativa L, Portulaca oleracea L, Trigonella foenum L"*.

Plants extracted by different solvents, Total phenol, Flavonoids, Tannins, and alkaloids of plants extracts were measured. The ethanol and methanol extracts of *Aloe perryi* revealed highest total phenols (20184.16 mg GAE /100g , 10771.78mg GAE /100g) respectively.

Estimation of Antioxidant activity was carried out *in vitro* by DPPH, hydrogen peroxide, reducing power and total antioxidant capacity methods. The results showed that ethyl acetate extracts of *Aloe perryi* has a strong scavenging of DPPH (IC_{50} = 5.34 µg/ml), and better reducing capacity (EC_{50} = 3.273 µg/ml), and methanol extract of *Aloe perryi* has highest total antioxidant capacity (478.0535mg Tocopherol /g.dw).

Evaluation of Antimicrobial study was carried out using disk diffusion method, it used 7 bacteria, 1 fungi, and 1 yeast pathogenic strains. Based on the results obtained, it was found that ethyl acetate and methanol extracts of *Aloe perryi* show the highest effective action against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus(*18 mm,11 mm and 18mm*)* respectively. The methanol extracts of *Morus alba* and *Aloe perryi* showed highest antibacterial activity against *Bacillus subtilis* (20 mm), while ethyl acetate and ethanol extracts of *Morus alba* showed significant activity against *Micrococcus luteus* and *Listeria monocytogenes* (11 mm and 28 mm) respectively. Hexane extracts of all plants didn't show activity against all the tested bacterial strains except *Apium graveoleus* which showed efficacy to inhibit *Bacillus subtilis* (11 mm) .

All extracts of *Apium graveoleus* displayed significant antifungal activity against *Candida albicans*, the ethanol extract of *Morus alba* and hexane extract of *Aloe perryi* exhibited significant antifungal activity against *Aspergillus flavus.*

Key words: Yemeni Medicinal plants, Phytochemicals, Antioxidant, Antimicrobial, Chemical composition, minerals.

RESUME

L'utilisation des plantes aromatiques et médicinales au Yémen remonte depuis des millénaire, et fait partie intégrantes de la culture locale. Certaines de ces plantes jouent encore un rôle important dans le système de santé locale. Les plantes médicinales sont la source principale de différents agents thérapeutiques impliqués dans le traitement d'une grande variété de maladies. Le pouvoir thérapeutique des drogues d'origine végétale contre les maladies est principalement dû à leurs composés chimiques. Les produits issus des plantes symbolisent, aujourd'hui, la sûreté contrairement aux produits synthétiques.

Notre étude a visé la détection et la détermination de la composition chimique et phytochimique, les activités antioxydantes et antimicrobiennes de huit plantes du Yémen, à savoir : "*Aloe perryi, Apium graveoleus L, Commiphora myrrha, Lupinus termis, Morus alba, Nigelle sativa L, Portulaca oleracea L, Trigonella foenum L*.

Les plantes médicinales choisies, ont été extraites par différents solvants, les phénols totaux, les flavonoïdes, les tannins et les alcaloïdes. Des extraits de plantes ont été mesurées, les extraits alcooliques (éthanolique et méthanolique), d'*Aloe perryi* ont révélé des teneurs élevées en phénols totaux (20184.16 mg GAE /100g, 10771.78mg GAE /100g, respectivement).

L'activité antioxydante a été mesurée *in vitro* par les méthodes suivantes, piégeage du radical libre DPPH, piégeage du peroxyde d'hydrogène, la réduction du fer et l'activité antioxydante totale. Les résultats obtenus montrent que les extraits d'acétate d'éthyl du *Aloe perryi*, exerce un grand effet sur le radical DPPH (IC_{50} = 5.34 µg/ml) et une grande capacité à reduire le fer (EC_{50} = 3.273 µg/ml). Les extraits alcooliques (méthanolique et éthanolique) d'*Aloe perryi,* ont montré une capacité antioxydante totale la plus élevée (478.0535 mg Tocopherol /g.ps).

L'étude de l'activité antimicrobienne, a été effectuée en utilisant la méthode de diffusion de disque. 10 bactéries, un moisissure, et un souche de levure pathogènes ont été utilisées. A partir des résultats obtenus, les extraits méthanolique et acétate d'éthyle d'*Aloe perryi,* montrent une activité antimicrobienne très élevée contre *Klebsiella pneumoniae*, *Escherichia coli* et *Staphylococcus aureus (*18 mm,11 mm and 18mm) respectivement. Les extraits éthanolique de *Morus alba* et *Aloe perryi*, ont montré une forte activité antibactérienne vis-à-vis de *Bacillus subtilis* (20 mm), alors que les extraits méthanolique et d'acétate d'éthyle ont révélé une activité relativement significative contre *Micrococcus luteus* et *Listeria monocytogenes* (11 mm and 28 mm) respectivement.

Les extraits hexaniques de toutes les plantes choisies dans cette étude, n'ont pas exercé une activité antibactérienne contre les différentes souches bactériennes testées, exception faite pour *Apium graveoleus,* qui a montré une activité contre *Bacillus subtilis* (11 mm). Tous les extraits d'*Apium graveoleus,* ont exercé une activité antifongique significative vis-à-vis de *Candida albicans*. L'extrait éthanolique de *Morus alba* et l'extrait hexanique d'*Aloe perryi,* ont montré une activité antifongique significative vis-à-vis d'*Aspergillus flavus.*

Mots clés : Plantes médicinales de Yémen, Phytocomposés, Antioxydants, Antimicrobiens, Composition Chimique, Minéraux.

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ANOVA	Analysis of Variance
BC	The Common Era
BCE	Before the Common Era
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C^{o}	Unit of temperature
CAM	Complementary or alternative medicine
CE	Catechin equivalent
CE	The Common Era
DMSO	Dimethylsulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazil
EC_{50}	effective concentration(the effective concentration at which the absorbance was 0.5 for reducing power)
FCR	The Folin–Ciocalteu's reagent
Fe	Iron
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid
HAT	Hydrogen Atom Transfer
IC_{50}	half maximal inhibitory concentration
K	Potassium
LDL	Low density lapoprotein
LSD	Low significant difference
masl	Meter above sea level
mg/ml	Milligram per Milliliter
MHA	Mueller Hinter Agar
MIC	Minimum inhibitory concentration
mm	Millimetre
NIDDM	Non-insulin dependent diabetes mellitus
ORS	Oxygenated reactive species
ROS	reactive Oxygen species
P	Phosphorus
Pb	Lead
RNS	Reactive nitrogen species
rpm	Revolution per minute
SD	Standard Deviation
SDA	Sabouraud Dextrose Agar

SET	Single Electron Transfer
TAOC	Total antioxidant capacity
TBHQ	tert-butylhydroquinon
TFC	Total phenolic compounds
TM	Traditional medicine
TPC	Total flavonoids compounds
WHO	World Health Organization
Wt	Weight
Zn	Zinc
µg	Microgram

THEORETICAL PART

INTRODUCTION

1- INTRODUCTION

1-1 Medicinal plants

There are approximately 250,000 to 500,000 species of plants on earth. Some of them are used as food by human and animal species (Marjorie, 1999; Moumita *et al,* 2013). Worldwide between 50,000 and 80,000 flowering plants are used medicinally (Kishan *et al,* 2008). World Health Organization (WHO) also reported that over 30% of the world's plant population has been used for medicinal purposes at one time or another (Peter, 2006).

Plants form the basis of traditional medicine (TM) practices that have been used for thousands of years by people in China, India, Bilad El-Sham, Arabian peninsula ,Iraq and many other countries, some of the earliest records of the usage of plants as drugs are found in the Artharvaveda, which is the basis for Ayurvedic medicine in India (dating back to 2000 BCE), the clay tablets in Mesopotamia (1700 BCE), and the Eber Papyrus in Egypt (1550 BCE) (Kishan *et al*, 2008). Other famous literature sources on medicinal plant include "De Materia Medica" written by Dioscorides between 78 CE and 60CE, and "Pen Ts'ao Ching Classic of Materia Medica" written around 200 CE (Kishan *et al*, 2008), the utilization of plants in the treatment of certain human diseases is an evidence of mankind ingenuity, the contribution of these plants to the therapeutic arsenal in combating diseases, currently, traditional medicine is widely practiced, especially in developing countries (Taylor *et al.*, 2001).

Nowadays, plants are still important sources of medicines, especially in developing countries that still use plant-based traditional medicine (TM) for their healthcare (Cohen PA *et al.,* 1996). Approximately 60-80% of the world's population still relies on traditional medicines for treatment of common illnesses (Rajalakshmy, *et al,* 2010).

In industrialized countries, plant-based traditional medicines or phytotherapeuticals are often termed complementary or alternative medicine (CAM) (Kishan *et al.,* 2008).

The World Health Organization (WHO) defines traditional medicine as the "diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness. There is a need to validate the information through an organized infrastructure to be used as an effective therapeutic means, either in conjunction with existing therapies, or as a tool in novel drug discovery. Traditional medicine utilizes biological resources and the indigenous knowledge of traditional plant groups, the latter being conveyed verbally from generation to generation. This is closely linked to the conservation of biodiversity and the related intellectual property rights of indigenous people (Timmermans, 2003).

1-2 Classical Arabic and North African traditional medicine

The oldest written information in Arabic traditions comes from the Sumerians and Akkadians of Mesopotamia, thus originating from the same areas as the archeological records of Shanidar IV (Heinrich *et al.,* 2004). The earliest documented record, which presumably relates to medicinal plants, dates from 60000 before the common era (BCE) found in the grave of the Neanderthal man from Shanidar IV, an archeological site in Iraq (Gurib-Fakim, 2006).

Similar documents have survived several millennia in Egypt. The Egyptians documented their Knowledge in wall paintings of tombs dating from the old Kingdom and on papyrus .The most important of these writings is the Ebers Papyrus, which contain ancient medicinal knowledge from before 3000 BC (Gurib-Fakim, 2006). The Arabian Gulf region is one of the birthplaces of herbal therapy. Herbal medicine occupies a significant part of this region's heritage and recently, it functions as a main health care system. The Eastern region of the Mediterranean has been distinguished throughout generations with a rich inventory of natural medicinal herbs used by local herbalists (Azaizeh et al., 2006).

Despite the wide variety of herbal species in the region with about 600 species in UAE (Jongbloed, 2003), 1204 in Oman (Ghaznafar, 1994), 2250 in Saudi Arabia (Migahid, 1990), 2088 in Egypt and 2367 in Palestine (Amr et al., 2007), most of

the species presented in the last two countries are also recorded in the Gulf region. Less than 10% of these species have been screened for their medicinal usage.

Table 1: Floral diversity in the Middle East region**.** (Amr *et al.*, 2007)

Region	Country	Total species	Medicinal	Endemic	Endemic (%)
Arabian Gulf	Bahrain	248		0	0
	Iran	8000		1400	17.5
	Iraq	3000		190	6.3
	Kuwait	282		0	0
	Oman	1100		150	15
	Qatar	306		0	0
	Saudi Arabia	2028	300	34	1.7
	UAE	600	99	0	0
North Africa	Algeria	3164			
	Egypt	2121		54	7.2
	Libya	1825	352		
	Morocco	3675			
	Sinai (Egypt)	984		30	3.1
	Tunisia	2196	151		
Mediterranean	Syria	300		395	13
	Palestine	2225	700	165	7.4
	Jordan	2100		145	7.3
	Lebanon	2600		311	12
	Turkey	8650	500	2675	30.9
	Middle East	15000			
	Mediterranean	25000		1000	
Over all	Europe	11000			
	China	32200		5000	
	India	35000		3000	
	USA	19473			

1-3 Plants in Yemen

1-3-1 Flora of Yemen

Yemen occupies an area about 527,970 square kilometers in the south and southwestern part of the Arabian Peninsula. Yemen is located in the South East of the Arabian Peninsula. It lies between 120° and 17°N Latitude and 43° and 56°E Longitude and with an altitudinal rang from sea level up to 3766 meters above sea level (masl). It is boarded by Saudi Arabia from the north, Sultanate Oman and Rub ´al Khali Desert from the east, Arabian sea and Gulf of Aden from the south and the Red Sea from the west see Figure (1) (Al- Kharbash & Al- Anbaawy, 1996) in Arabic.

Figure 1: Map of Yemen Location (Al- Seragy ,2009)

Yemen is a Middle East country and south west of Asia which is surrounded by Red Sea, Gulf of Aden and Arabian Sea. The entire land of Yemen is mostly covered with desert areas. This altitudinal range is largely responsible for the differentiation in climate and different geographic regions. Rainfall is generally bi-modal. Short season sparsely in the spring (March – May) and longer season in the summer (July – September), in winter rain is very rare, with two rainy seasons

merging together in the year of good rains in high mountain area. In Yemen rainfall is more closely correlated with altitude than other climatic factors. It varies from 50mm in the coastal plains to more than 800mm in the high mountain area, not only the amount of rain but also its distribution in time and space is of great ecological importance.

Relative humidity varies from very high at the coastal plain and decreases from the west to the eastern desert. Temperature in the mountain area is moderate in summer but in winter the temperature is moderate in valleys and lower plains. The mist may occur at peaks 1500 masl. The hottest months are June , July and August with a main temperature about 22 C◦. Whereas the coldest months are November – February with a mean temperature of 10 C◦. The higher the altitude the lower winter temperature . Plants life is possible under all temperature as well as on all kinds of soil. The high masses of southwest monsoon blowing during the summer from the Indian Ocean (Al- Khulaidi & Mahdi, 2000).

Yemen is characterized by the diversity of aspects of the surface and therefore has been divided into five major geographic regions (National Information Center) :

- **The coastal plain region**

  The Coastal Plain region is characterized by a hot climate throughout the year with little rainfalls ranging between 50-100 mm per annum

- **The mountain highlands region**

This region's mountains are the highest in the Arabian peninsula. The average Height of each reaches 2000m and its peaks reach to more than 3500m. The highest peak reaches 3666m in Alnabi Shuaib Mountain

- **The mountainous basins region**

This region has mountainous plains and basins located in the Mountainous Highlands, mostly located in the eastern section water division line which stretches from the farthest north to the farthest south

- **The plateau areas region**

It lies to the east and north of the Mountainous Highlands and it is parallel to them. But it widens more towards the Empty Quarter and begins a gradual decline. The surface slides toward the north and the east mildly. The majority

of the surface of this region is formed from rocky desert surface which is splitted by some valleys

- **Desert region**

It is a sandy region almost devoid of flora except in the areas of rainfall courses where rain runs through after descending from mountainous areas adjacent to this region. Height of the surface ranges between 500-1000m above sea level and it slopes without terrainous discontinuity towards the north east to the center of the Empty Quarter. Climate here is severe with high temperature, rare rainfall and low humidity.

- **Yemeni Islands**

Many islands spread along the Yemen territorial waters. They have their peculiar terrain, climate and environment. Most of these islands lie in the Red Sea, Some of the main islands on the Arab Sea are Soqatra Archipelago.

Soqatra Island:

Soqatra Island lies in the northern west part of the Indian Ocean, between latitudinal range $12^{-}\ 19\ ^{0} – 12\ ^{-}\ 42\ ^{0}$ and longitudinal range $53\ ^{-}\ 20^{0}$ - $54^{-}\ 30^{0}$, with an area of about 3625 km^2 . Topographically , it can be divided into three main geographical zones :

A) Coastal plain (0 – 100 masl).

B) Limestone plateau (100 – 800 masl).

C) Mountains, which reach up to 1550 masl.

1-3-2 Medicinal plants in Yemen

The use of Medicinal and Aromatic plant species in Yemen goes back thousands of years and form an important part of the culture (Al shameri, 2008).

Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas. Thus, phytotherapy is practiced by a large proportion of Yemen population for the treatment of several physical, physiological, mental and social ailments (Mothana & Ulrike, 2005).

In developing countries and particularly in Yemen, a large segment of the population still relies on folk medicine to treat serious diseases (Mothana *et al,* 2009).

It has been estimated that there are about 2 900 plant species belonging to 175 families in Yemen, more than 420 of them are endemic, which occur in the island Soqatra only. More than 800 plant species have been recorded to grow on the Island, of which 307 species are considered to be endemic (Al- Khulaidi & Mahdi, 2000; Ali *et al*, 2007).

1-4 Objectives

The main objectives of this study are to investigate the following:-

1. Testing the efficacy of the Yemen traditional medicinal plants including dried leaves of White Mulberry (*Morus alba L.*), dried seeds of celery *(Apium graveoleus L.Nees*), dried seeds of lupin (*Lupinus termis*.), dried seeds of Common purslane (*Portulaca oleracea L.*), dried gel and leaves of aloe (*Aloe perryi J.G.BAKER*), dried gum resin of myrrh (*Commiphora myrrha*), dried seeds of Black cumin (*Nigelle sativa L*) and dried seeds of Fenugreek *(Trigonella foenum graecum L).*

2- determining the chemical composition, minerals and phytochemicals of the Yemen traditional medicinal plants.

3- The effect of different extracts methods on the antioxidant activity obtained from Yemen traditional medicinal plants determined by methods DPPH, H_2O_2, FRAP and total antioxidant capacity.

4- The effect of the different extraction methods on the antimicrobial activity obtained from Yemen traditional medicinal plants determined by disc diffusion method.

REVIEW of LITERATURE

REVIEW OF LITERATURE

2.1 Experimental plants

2.1.1 *Aloe perryi J.G.BAKER*

2-1-1-1 Common names

English name: Aloe

Arabic name: Saber soqatri

2-1-1-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: liliopsida

Order : Liliidae

Family: Aloaceae

Genus: Aloe

Species: *A. perryi*

2-1-1-3 Botanical description

Aloe is a member of the Liliaceae family , It is a succulent and perennial plant , its leaves are 30 to 50 cm long and 10 cm broad at the base , it has pea- green and bright yellow tubular flowers with 25 to 35 cm in length arranged in a slender loose spike(figure 2) , stamens frequently project beyond the perianth tube (WHO, 1999).

Figure 2: whole plant of *Aloe perryi* (www.friendsofsoqotra.org).

2-1-1-4 Origin and distribution

Aloe perryi is native to Soqatra Islands only.(Mothana *et al,* 2009)

2-1-1-5 Medicinal Use

Aloes recommended for the treatment of atonic constipation and suppressed menstruation. It is employed as purgative, cathartic, biogenic stimulator and immune stimulator. It acts also as an anti-inflammation, it has a positive effect on the regeneration of body cells (Al shameri, 2008) .

Aloe extracts have antibacterial and antifungal activities, which may help in the treatment of minor skin infections, such as boils and benign skin cysts. *Aloe perryi* extracts have been shown to inhibit the growth of fungi that cause tinea (Sumbul *et al,* 2004)

In Bolivia. Fresh leaf juice is used as an analgesic topically for burns and wounds. Orally, the juice is used as a laxative.(Ross, 2003)

In Brazil. Fresh leaf juice is taken orally as an anthelmintic and febrifuge. Infusion of dried root is taken orally to treat colic (Hirschmann *et al,* 1990).

In Yemen. Leaf juice is taken orally against diabetes (Al-shameri, 2008).

In Tunisia. Hot water extract of the dried leaf is taken orally for diabetes, and to treat problems of venous circulation. Externally, the extract is used for eczema (Boukef *et al,* 1980). Aloe has been used in ointments and creams to assist the healing of wounds, burns, eczema and psoriasis (Thomas *et al,* 2000).

Leaf pulp and gel extracts have been administered orally to non-diabetic, Type I and type II diabetic rats, were ineffective on lowering the blood sugar level in non-diabetic rats. The leaf pulp extract has showed hypoglycemic activity on type I and type II diabetic rats. The effectiveness enhanced for type II diabetes in comparison with glibenclamide. On the contrary, the leaf gel extract has showed hyperglycemic activity on type II rats. It has been concluded from this study that the leaves devoid of the gel could be useful in the treatment of noninsulin dependent diabetes mellitus (Okyar, 2001).

2.1.2 Apium graveolens L.Nees

2-1-2-1 Common names

English name: celery wild

Arabic name: Karfas. Karfas alma'a. Karfas Barri

2-1-2-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Apiales

Family: Apiaceae

Genus: Apium

Species: *A. graveolens*

2-1-2-3 Botanical description

Celery is a biennial vegetable , although grown as an annual crop. It is a member of the Apiaceae family (Annie, 2001).

Celery is a herbaceous annual or biennial erect herb growing to a height of 60–90 cm, with conspicuous branches bearing well-developed leaves on long expanded petioles. Stems are branched, angular or fistular and conspicuously jointed. Leaves are radical, pinnate, deeply divided into three segments, once or twice divided and toothed at apex. The leaflets are ovate to suborbicular, 3 lobed, 2–4.5 cm long. The flowers are small and white (figure3,4 and5) . The inflorescence is a compound umbel. Calyx teeth are obsolete, five petioles ovate, acute with tip inflexed, carpels semiterete, subpentagonal, primary ridges distinct and filiform. The fruit is a schizocarp with two mericarps, suborbicular to ellipsoid, 1–2 mm in diameter, aromatic and slightly bitter. The seed (mericarp) resulting from the splitting of schizocarp also ribbed and much smaller than carrot seed. (Peter, 2006)

Figure 3 : Flowers of *Apium graveolens*

Figure 4 **:**Plant of *Apium graveolens*

Figure 5 : Fruit/ Seeds of *Apium graveolens*

(Mohammed, 2013)

2-1-2-4 Origin and distribution

Celery grows in the wild of Europe, the Mediterranean region, Asia and west of the Himalayas. The ancient Greeks and Egyptians cultivated celery. It was probably first grown as a medicinal plant. Celery has a long history in China, dating back to at least the 6th century AD. It is reported as being cultivated in several African countries, such as Eritrea and Ethiopia (Parthasarathy *et al*, 2008). According to Thompson & Kelly (1957), the origin site of celery extends from Sweden to Algeria, Egypt, Abyssinia, Asia Caucacus, Baluchistan and the mountain regions of India. It has been found growing in the wild of California and New Zealand. The first mention of its cultivation as a food plant was in 1623 in France.

2-1-2-5 Chemical composition of celery seeds

Celery seeds are nutritive .The major composition of seeds are carbohydrate, fat, protein and ash. They also contain micronutrient and vitamin A (Villupanoor A. Parthasarathy *et al*, 2008).

Table 2: Nutritional composition of celery seed .

Composition	Content (per /100 g)	Composition	Content (per /100 g)
Water (g)	6.04	Ca (mg)	1767
Protein (g)	18.07	Fe (mg)	45
Fat (g)	25.27	Mg (mg)	440
Carbohydrates (g)	41.35	P (mg)	547
Ash (g)	9.27	K (mg)	1400
Ascorbic acid (mg)	17.14	Na (mg)	160
		Zn (mg)	7

2-1-2-6 Medicinal Use

The seeds are used as an antispasmodic, it is also used for different treatments such as asthma, bronchitis, liver, spleen, kidney failure, bladder kidney calculi, edema, arthritis, dizziness, gout, weight loss, lowering blood pressure, relief of anxiety, insomnia and reducing blood sugar. In the European tradition, the seeds have been used as carminative, stomachic, emmenagogue, diuretic and laxative, it is also used for glandular stimulation, rheumatic complaints, nervous unrest, loss of appetite and exhaustion (Grieve, 1984; Chiej, 1984; Sayre, 2001; Villupanoor *et al,* 2008**;** Khan & Abourashed, 2010).

2.1.3 Commiphora myrrha

2-1-3-1 Common names

English name: Myrrh

Arabic name: Mor, Ogah

2-1-3-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Burseraceae

Genus: Commiphora

Species: *C. myrrha*

2-1-3-3 Botanical description

Myrrh (*Commiphora myrrha*) is a close relative and member of the Burseraceae family, Myrrh is an Arabic word which means bitter. The highly valued aromatic gum resin of myrrh has a bitter, pungent taste and a sweet, pleasing aroma. Myrrh grows to a height of about 9 ft (2.7 m). The light gray trunk is thick and the main branches are knotted with smaller branches protruding at a right angle and ending in sharp spines. The hairless, roughly toothed leaves are divided into one pair of small, oval leaflets with a larger, terminal leaflet. The yellow-red flowers grow on stalks in an elongated and branching cluster. The small brown fruit is oval, tapering to a point figures (6-8) (Longe, 2005 ; Awadh & Abdullah, 2006).

Figure 6 : *whole plant of Commiphora myrrh* (Maria, 2007)

Figure 7 : Oleo-gum-resin of *Commiphora myrrh.* (Maria, 2007)

Figure 8 : Characteristic features of *Commiphora*, with its papery bark, trifoliate leaves, ripe fruit and pseudo-aril (left); pseudo-aril with exposed black seeded stone(right). (Maria, 2007)

2-1-3-4 Origin and distribution

It native is the eastern Mediterranean Ethiopia, the Arabian peninsula, and Somalia (Al-shameri, 2008).

2-1-3-5 Major active constituents

This plant is composed of the following constituents :

- Carbohydrates Up to 60%: gum yielding arabinose, galactose, xylose, and 4-O-methylglucuronic acid following hydrolysis.
- Resins(average 20%): consisting of α-β-γ- commiphorinic acid,α- and β – heerabomyrrhols, heeraboresene and commiferin.

- Steroids: compestrol and β- sitosterol .

- Terpenoids: α- and β- amyrin and elemol.

- Volatile oil (1.5 – 17 %): containing dipentene, cadinene, heerabolene, limonene, pinene, eugenol, cresol, cinnamaldhyde, cuminaldhyde, comic alcohol and others (Al-shameri, 2008;Awadh & Abdullah, 2006).

2-1-3-6 Medicinal Use

In Yemen, the plant is used in folk medicine as an expectorant anti-inflammatory and antispasmodic for the treatment of orodental in infections, it is also used as an amulet, analgesic, treating emotional and psychological illness (Awadh & Abdullah, 2006). It is used as a mouth wash and gargle and emmenagogue. In West Africa the gum resin is boiled for treatment of inflammation of the eyes by holding the face over the steaming pot (Lemenih & Teketay , 2003; Thomas *et al*, 2000). The myrrh resin has antimicrobial properties and acts as a stimulator of a macrophage activity in the blood stream. The herb is being studied for its potential as an anticancer medication (Longe, 2005), anesthetic, antiemetic, antioxidant, fungicide (James, 2008) and antidiabetic (Soumyanath, 2006).

2.1.4 lupinus albus L

2-1-4-1 Common names

English name: White Lupin

Arabic name: Tarmis abid

2-1-4-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Lupinus

Species: *L. albus or L. termis*

2-1-4-3 Botanical description

White lupin (*Lupinus albus L.*) is a member of the family Fabaceae. It is an annual plant, with an erect and ramified stem (Figure 9), it is height can be more than one meter, compound leaves of 5 to 7 leaf and apical clusters of flower (Figure 10). The lupin seed is produced in pods that develop on the main stem of the lupin plant. Pods contain between 3 to 7 seeds, the seeds of white lupin are the largest, measuring 8 –14 mm in diameter and with a 1000-grain weight of 300 g , they have a circular flattened shape and are cream in color (Figure 11) (kurlouich, 2002).

Figure 9 : White lupin plant

Figure 10 : White lupin flower

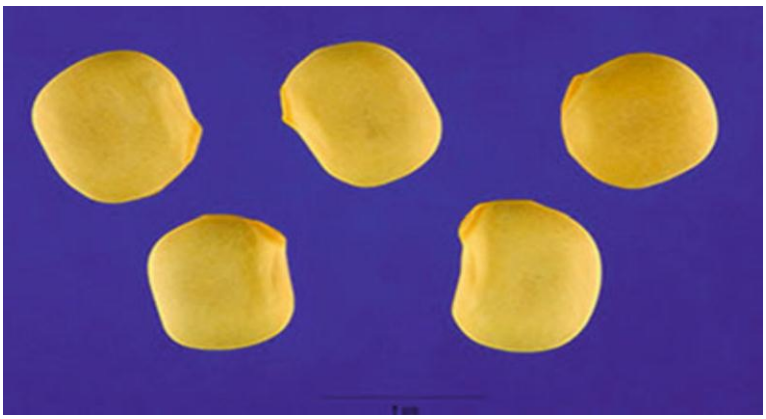

Figure 11 : White lupin seeds (Lim, 2012)

2-1-4-4 Origin and distribution

The white lupin is an old world species mainly distributed around the Mediterranean and along the Nile valley. White Lupine is widely spread as wild plants throughout the southern Balkans, the islands of Sicily, Corsica and Sardinia, and the Aegean Sea, as well as in Palestine and western Turkey. It is cultivated over all the Mediterranean region and also in Egypt, Sudan, Ethiopia, Syria, Central and Western Europe, USA and South America, Tropical , Southern Africa, Russia, and Ukraine (Gladstones, 1974).

2-1-4-5 Chemical composition of lupin seeds

Lupin seeds content high protein, fat and carbohydrates (Duke & james, 1992).

Table 3 : The major composition of lupin seeds

Content	Value (ppm)
Ash	32,800 - 36,580
fat	97,400 - 108,630
fiber	137,700 - 153,600
carbohydrates	367,000 - 450,370
protein	361,700 - 403,400
alkaloids	10,000 - 27,400
water	104,400

2-1-4-6 Medicinal Use

The seeds are used as anthelmintic, anti-inflammatory of skin and acne, headache, it increases the vitality of circulation, arthritis , extraction contraceptive and tonic for liver (AL Sayed, 2009).

The use of lupin for the treatment of diabetes is well known in North African/Middle Eastern as folk medicine. Diabetic Palestinians, Bedouins, Yemenites, and Moroccans, are among others, who add lupin seeds to their diets (Soumyanath, 2006).

2.1.5 Morus alba L

2-1-5-1 Common names

English name: white mulberry

Arabic name: Tout abid

2-1-5-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Urticales

Family: Moraceae

Genus: Morus

Species: *M. alba L*

2-1-5-3 Botanical description

Mulberries are a member of the Moraceae family. The tree grows from 6 to 12 m high. The bark is gray-brown (Figure 12). The leaves are alternate with flat grooved, somewhat hairy petioles (Figure 13). They are cordate or ovate, sessile, unevenly lobed and serrate with short rough hairs on the upper surface (Figure 14). Flower and Fruit, the plant is monoecious or dioecious. The greenish flowers are in catkin-like inflorescences (Figure 15). The male flowers are ovate to cylindrical, the female flowers ovate or globular. The flowers have a 4-bract involucre, which enlarges and becomes fleshy in the female flowers. The female flowers have 2 stigmas, the male flowers have 4 stamens. All of the fruit from the catkins develops into blackberry-like false berries, which are really a series of fleshy drupes that are edible and pleasant-tasting (Thomas , 2000).

Figure 13 : Stem of *Morus alba*

Figure 14: Leaves of *Morus alba*

Figure 15 : Whole Plant of *Morus alba*

Figure 16 : Fruit of *Morus alba*

(Bandna *et al* , 2013)

2-1-5-4 Origin and distribution

White mulberry is a Chinese tree, cultivated throughout the world wherever in Japan, Europe, North America and Africa (Yadav *et al*, 2008).

2-1-5-5 Chemical composition

Chemical composition of mulberry leaves illustrated in table (4).

Table 4: Chemical composition of mulberry leaves (Andallu & Vardacharyulu, 2001).

Content	Value (ppm)
Ash	80,000 - 204,000 ppm
Carbohydrates	400,000 - 633,000 ppm
Fat	68,000 - 74,000 ppm
Fiber	153,000 ppm;
Protein	140,000 - 288,000 ppm

In addition to 1) Flavone and Flavone Glycosides, the mulberry leaf contains compounds of rutin, quercetin, isoquercitrin and Quercetin-3-Triglucoside, etc.
2) Steroids :Including β- sitosterol, stigmasterol, campesterol, β-sitosteryl-β-D-glucoside, mesoinositol and metamorphic hormone such as inokosterone and ecdysterone.
3) Volatile Oils :Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, isocaproic acid, methyl salicylate, guaiacol, phenol, o-cresol, m-cresol, eugenol, etc., also containing oxalic acid, fumarate, tartaric acid, citric acid, succinic acid, palmitic acid, ethyl palmitate, henthriacontane and hydroxycoumarin, etc.
4) Saccharides and amino acids : Including sucrose, frucrose, fructose, glucose and 17 kinds of amino acids such as aspartic acid and glutamic acid. The content of blood level-decrease component γ – amino butanoic acid is up to 226mg / 100g. For γ-amino butanoic acid is conversed from glutamic acid, so the glutamic acid in mulberry leaf is up to 2323mg / 100g.

5) Vitamins and trace elements

Containing vitamin A, B1, B2, C, nicotinic acid, carotene and food fibres, also with zinc, copper, boron, manganese, iron, etc. In addition, mulberry leaf contains alkaloids, especially one particular component called 1-deoxynojirimycin (DNJ) (Andallu & Vardacharyulu, 2001).

2-1-5-6 Medicinal Use

Mulberry leaf shows the effect to reduce blood glucose for experimental rat with diabetes (Andallu & Vardacharyulu, 2001). Add mulberry leaf to the feed for rat with natural high blood pressure, continuously feeding several days later, you would find that the frequency of the symptom for the rat with high blood pressure decreases obviously (Ramachandran, 1986). The effect to reduce biochem cholesterol and reduce blood fat, botanic sterols in mulberry leaf can inhibit the absorption of bio- cholesterol inside intestine canal. The flavones in mulberry leaf also can inhibit the absorption of biochem cholesterol, along with improving liver function. Feeding the rabbit with high blood fat, mixed with mulberry leaf, to the result, the bio-cholesterol and neutral fat in serum decrease in different degree. Mulberry leaf and its extracts also can reduce the intensity of the protein in serum for the rabbit. Through study to pathologic section of the liver, it is proved that mulberry leaf has indeed the effect to inhibit the formation of liver fat and to reduce fat in serum (Chen *et al,* 2007). The leaves were reported to possess antipyretic, anti-inflammatory, anthelmintic and diuretic (Chatterjee *et al* , 1983; Al sayed, 2009).

2.1.6 Nigella sativa L

2-1-6-1 Common names

English name: Black cumin

Arabic and local name: Habbat al-barakah , Habbah sauda, Kammon aswad

2-1-6-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Order: Ranunculales

Family: Ranunculaceae

Genus: Nigella

Species: *N. sativa*

2-1-6-3 Botanical description

It is small prostrate annual herb which is about 45 cm high 2-3 slender leaves pinnatisect, 2-4 cm long cut into linear oblong segment. It has pale flowers, which white on solitary long peduncles, and black trigonous seeds (figure 16). The plant has stiff, erect, branching stem, bearing deeply-cut greyish-green leaves and terminal greyish blue flowers, followed by odd, toothed seed vessels, and filled with small compressed seeds, they are usually three-cornered seeds, with two flat sides and one convex, with white and oleaginous external surface. In addition, it has strong agreeable aromatic odour, like that of nutmegs, and a spicy, pungent taste. The flowers are delicate, and usually has pale blue and white color, with 5–10 petals (Fig. 1). The fruit is a large and inflated capsule composed of 3–7 united follicles, each containing numerous seeds. It has a pungent bitter taste and a faint smell of strawberries (Sharma *et al*, 2009).

Figure 16: *Nigella sativa* (whole plant, Flower and seeds) Al-shameri, 2008.

2-1-6-4 Origin and distribution

Black cumin is considered to be originally from the Mediterranean region, North Africa and West Asia (Al sayed, 2009). It is cultivated in southeastern Europe, Egypt, India, Pakistan, Iran, Iraq, Turkey and Yemen. (Hammond, 2013; Al-Dubai & Al-khulaidi, 2005).

2-1-6-5 Chemical composition of black cumin seeds

The nutritional values of the seeds have been studied by deferent researchers. Studies have shown that black cumin seeds have nutritional value: protein range from 20 to 27%, carbohydrate ranges from 23.5 to 33.2 %, moisture content range from 5.52 to 7.43% , and ash ranges from 3.77 to 4.92%. The seeds contain a fixed oil ranging from 32 to 53%. It has been reported that mineral content of the seeds ranges from 1.79 to 3.74% (calcium, phosphorous, potassium, sodium and iron) and their crude fiber content is 5.5%. The seeds contains vitamins (thiamin,

riboflavin, folic acid, niacin and biotin), Saponins and alkaloids (Guler *et al*, 2006).

2-1-6-6 Medicinal Use

Seeds have a positive effect on the immunity system and lactation, it has also a diuretic effect. In addition, it normalizes the activity of the intestines, improves appetite and normalizes menstruation (Al-shameri, 2008), it has also reported to be Analgesic and Antidiabetic (Duke *et al*, 2007). Some studies showed that it boosts antibacterial and antifungal activities (Longe, 2005). It is known for its hypotensive (El Tahir, 1993; Zaoui, 2000), hepatoprotective effects (Sharma, 2009) .

In Kuwait, a plant mixture comprising *N. sativa*, myrrh gum olibanum, gum asafetida has been commonly used against diabetes. This mixture has glucose-lowering effects in normal and diabitic STZ-treated rats without affecting plasma insulin or intestinal glucose absorption. Finally, a Moroccan group also showed that oral treatment of Wistar–Kyoto rats with a hexane extract of *N. sativa* seeds reduced serum cholesterol, triglycerides and glucose (Zaoui, 2002).

The antifertility activity of *Nigella sativa* in male rats has been established, shown by an inhibition of spermatogenesis and a significant reduction in sialic acid content of the testis, epididymis, seminal vesicles and prostate (Sharma, 2009).

2.1.7 Portulaca oleracea L

2-1-7-1 Common names

English name: common purslane

Arabic name: Raglah.Baklah.Ragnah

2-1-7-2 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Caryophyllales

Family: Portulacaceae

Genus: Portulaca

Species: *P. oleracea*

2-1-7-3 Botanical description

It is annual, prostrate or spreading, succulent and branched herb of the portulacaeae family; It is quite glabrous with 10–50 cm long. The stems are often purplish and leaves are fleshy flat (figure 17,18), obtuse, oblong-obovate and base cuneate, with 1 to 2.5 cm long. Flowers are sessile (figure 19), axillary and terminal with few-flowered heads. The heads are solitary or cymose and the buds are compressed (figure 20). Petals: five, yellow and about as long as the sepals. Stamens: 8–12 (Ross, 2005; Al-Dubai & Al-khulaidi, 2005).

Figure 17: The appearance of *P. oleracea*

Figure 18: The leaves of *P. oleracea*

Figure 19: The flower of *P. oleracea*

Figure 20: The seeds of *P. oleracea*

(Ju OK, 2011)

2-1-7-4 Origin and distribution

It is a very common weed of cultivated and undisturbed land. It is Natively to the old world tropics. Nowadays, it is found in both temperate and tropical zones, it is in South Europe where cultivated as a vegetable (Al-Dubai & Al-khulaidi, 2005).

2-1-7-5 Chemical composition

Purslane seeds are nutritive .The major composition of seeds are carbohydrate, fat, protein and ash (Shehata and Soltan ,2012;Duke& james, 1992).

Table 5: Nutritional composition of celery seed.

Composition	Content (g /100 g)
Water (g)	5.933
Protein (g)	22.34
Fat (g)	9.1
Carbohydrates (g)	33.87
Ash (g)	15.39
Crude fiber	13.37
Linoleic-Acid	67,686 ppm

2-1-7-6 Medicinal Use

In Brazil , seeds are taken orally as an emmenagogue (Roig , 1945), whereas in India, seeds are taken orally as a vermifuge (Ikram, 1981). In Peru, hot water extracts of dried seeds and of dried stems are taken orally as an anti- scorbutic, ant dysenteric, emmenagogue and vermifuge, and for jaundice (Ramirez *et al*, 1988), In China, *P. oleracea* is considered to have blood-cooling and haemostatic properties and so used internally in bleeding bacillary dysentery (Keys, 1976), haemotochezia (bloody stool), bleeding haemorrhoids (Keys, 1976), and metrorrhagia. It is also used as antiphlogistic (Ju, 2001), diarrhoea, haemorrhoids and enterorrhagia (Keys, 1976). In addition, it is also described as antidote, refrigerant and antidysenteric (Ju, 2001). The dried herb is boiled into tea/soups (Cai *et al*., 2004). *P. oleracea* also exhibits a wide range of pharmacological

effects including antibacterial (zhang *et al*., 2002), analgesic, anti-inflammatory (chan *et al.,* 2000), bronchodilatory effect (malek *et al*., 2004), and wound healing (rashed *et al.,* 2003) activities and anti-fatigue activities (yue *et al*., 2005; dong *et al.*, 1997; ling, 2004). It is reported that extracts of *P. oleracea* has antioxidant activity (peksel *et al,* 2006).

2.1.8 *Trigonella foenum graecum L*

2-1-8-1 Common names

English name: Fenugreek

Arabic name: Helbah, Holbah

2-1-8-2 Scientific classification

Kingdom: Plantae

Division : Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: Trigonella

Species: *T. foenum-graecum*

2-1-8-3 Botanical description

Fenugreek (*Trigonella foenum graecum L.*), which is one the family of Fabaceae (korand *et al*, 2013). Fenugreek is an erect annual growing plant of about 30 to 60 cm high (Figure 21). The fruit is a compressed pod containing seeds of 1 inch long, they are oblong, and rhomboidal in shape where a deep furrow dividing them into two unequal lopes (Figure 22), they have peculiar bitter taste and specific odor (Mundhe *et al*, 2012).

Figure 21: Whole plant of fenugreek (Lim, 2012)

Figure 22 : Fenugreek seeds (Lim, 2012)

2-1-8-4 Origin and distribution

Fenugreek is an annual herb which is originally from the Mediterranean region, north Africa, India and Yemen. It is widely cultivated in these areas. The material of commerce comes exclusively from cultivated plants mainly from Morocco, Turkey, India, and China (Wichtl *et al*, 1994; Bruneton, 1995; Leung *et al*, 2008).

2-1-8-5 Chemical composition of Fenugreek seeds

The general composition of Fenugreek seeds are as follows: humidity 13.7%, protein 24.8–26.2%, fiber 7.5% and ash 30%. Moreover, Fenugreek seeds contain: carotene 96 mg, thiamin 0.34 mg, rapophliphen 0.29 mg, vitamin C and payotein

(Chadha, 1976). The carbohydrates contents of fenugreek seeds are different from other plants since they contain simple sugars such as glucose, sucrose, and a few of the galactose. The most important proteins that have been found in Fenugreek seeds are albumin, globulin and prolimen. The amino acid composition of the fenugreek protein has been reported and classified by Jamalian *et al,* (1968), they include: 2.33% Lysine and 1.14% ulphur-containing amino acids (Methionine + Cysteine). The total lipid content of the seeds is 7.5% and consists of neutral lipids, glycolipids and phospholipids (Hemavathy *et al*, 1989).

The seed contains higher amount of minerals (Ca, P, Fe, Zn and Ma), it includes vitamin A, vitamin B complex, flavonoids, saponins, trigonelline and other alkaloids (Longe, 2005).

2-1-8-6 Medicinal Use

Plant possesses mucilaginous demulcent, laxattive, nutritive, expectorant orexigenic and vulnerary properties (Al-shameri, 2008). In addition to its use in flavoring foods, the antifungal and antibacterial properties of fenugreek are now being applied to food preservation, some studies also show that serum cholesterol levels in diabetics, and perhaps in others, are reduced by fenugreek (Longe, 2005).

There is some evidence that internal use of fenugreek seed can decrease some stone-forming substances in the kidney, particularly calcium oxalate. Fenugreek may encourage flagging appetite, cancer researchers are also studying fenugreek for its potential effectiveness as a cancer chemopreventive. It is thought that fenugreek may help to prevent cancer by raising the levels of vitamin C, vitamin E, and other antioxidants in the bloodstream (Longe, 2005).

For their anti-diabetic action, fenugreek seeds have been known for along time (Moissides, 1939; Mishkinsky *et al,* 1967). Moreover Sampath *et al,* (2011) stated that dietary fiber reduced plasma glucose in non-insulin dependent diabetes mellitus (NIDDM) and also diabetic women. Sharma *et al,* (1990), investigated the effect of fenugreek seeds on blood glucose in insulin-dependent type I diabetic patients and found that it reduces blood glucose level and improved glucose tolerance test.

2-2 PHENOLIC COMPOUNDS

2-2-1 Generalities

Plants synthesize a vast range of secondary metabolites with a significant portion consisting of phenolic compounds (Crozier *et al.,* 2006), structurally, they are molecules that have at least on hydroxyl group attached to an aromatic ring, generally benzene (Figure 23), most phenolic phytochemicals are present as esters or glycosides rather than as free compounds

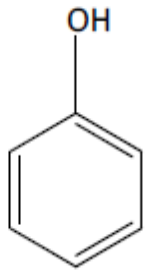

Figure 23: Phenol structure

As a large group of bioactive chemicals, they play diverse biological functions in plant growth and defense, providing an efficient protection against pathogens, predators and abiotic stresses (Popa *et al.,* 2002; Vermerris & Nicholson., 2007; Ignat *et al.,* 2011). Phenolic provide plants resistance against pathogens and herbivores by acting as phytoalexins (Bravo, 1998; Popa *et al.,* 2008) or by increasing astringency such as tannins (Jean-Blain, 1998; Iason, 2005). They also act as malabsorption, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV radiations (Naczk & Shahidi, 2006). In particular, natural phenols have been reported to have interesting properties as, food preservatives as well as playing an important role in the protection against a number of pathological disturbances, such as atherosclerosis, brain dysfunction and cancer (Gordon, 1996).

Phytochemicals comprise a wide group of structurally diverse plant compounds, which are predominantly associated with the cell wall and widely dispersed throughout the plant kingdom (Cassidy, 2005).

2-2-2 Phenolic acids

Phenolic acids have been connected with diverse plant functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis, structural components and allelopathy (Wu *et al.*, 1999).

Phenolic acids can be divided into two groups as hydroxybezoic acid and hydroxycinnamic acids both of which have single-ring structure (Manach *et al.*, 2008). Cinnamic and benzoic acid derivatives exist in virtually all plants and are physically dispersed throughout the plant in seeds, leaves, roots and stems (Robbins, 2003). Due to their ubiquitous presence in plant based foods, humans consume phenolic acids on a daily basis. According to Clifford (1999), individual intake ranges from 25mg to 1g a day depending on the diet. Phenolic acids have also been associated with sensory properties, color and nutritional quality of foods (Tomas-Barberan *et al.*, 2001).

2-2-3 Flavonoids

The term “flavonoid” is generally used to describe a broad collection of natural products that include a C6-C3-C6 carbon framework, or more specifically a phenylbenzopyran functionality (Erich, 2007), carbon structure consisting of towbenzne rings linked by an oxygen containing heterocycle as showed in (Figure 24) (Pietta, 2000) .

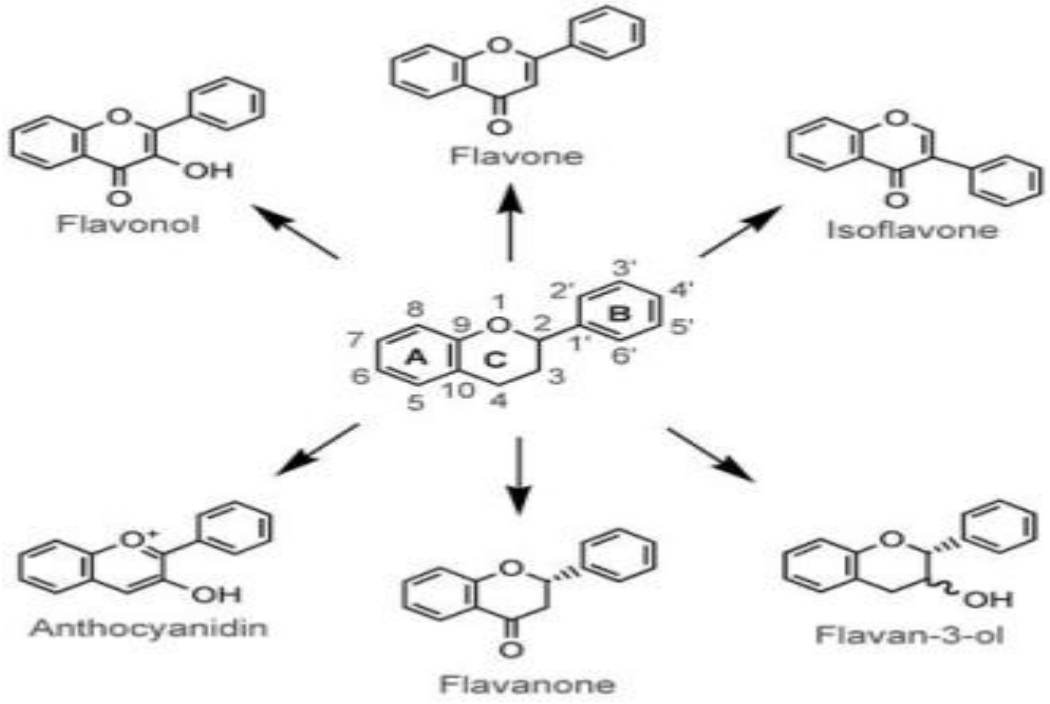

Figure 24: structures of the major flavonoids (Crozier *et al.*, 2006)

Over 5,000 different flavonoids have been identified and are classified into at least 10 chemical groups (Whiting , 2001; Le Marchand, 2002).

Among them, flavonols, flavones, flavanones, catechins, anthocyanidins and isoflavones are particularly common reviewed by Cook *et al.*, (1996).

Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. By virtue of their capacity to inhibit LDL oxidation, flavonoids have demonstrated unique cardioprotective effects (Kondo *et al.*, 1996, Mazur *et al.*, 1999).

2-2-4 Anti-oxidant of flavonoids

The most described property of flavonoids is their capacity to protect the organism against free radicals and oxygenated reactive species (ORS) produced during the metabolism of oxygen (Grace, 1994). The cellular damage by the free radicals causes a change of the net charge of cells, thus modifying their osmotic pressure and inducing their swelling and their death. The free radicals act also on the mediators of the inflammatory diseases, and accelerate the tissue damage.

Moreover, cells lesions lead to an increase in the production of the ORS which induces the consumption and the depletion of the endogenous chelating agents. To protect against oxygenated reactive species (ORS), the organism and living cells have developed several mechanisms including enzymes like the superoxyde dismutase, the catalase and the glutathion peroxidase, and also non-enzymatic homologues such as the glutathion, the ascorbic acid and α-tocopherol. The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation (Halliwell, 1995).

2-2-5 Tannins

Bate-Smith defined tannins as " water –soluble phenolic compounds having molecular weights between 500 and 3000 daltons giving the usual phenolic reaction and having special properties such as the ability to precipitate alkaloids, gelatin and other proteins" (Figure 25) (Ann,2002).

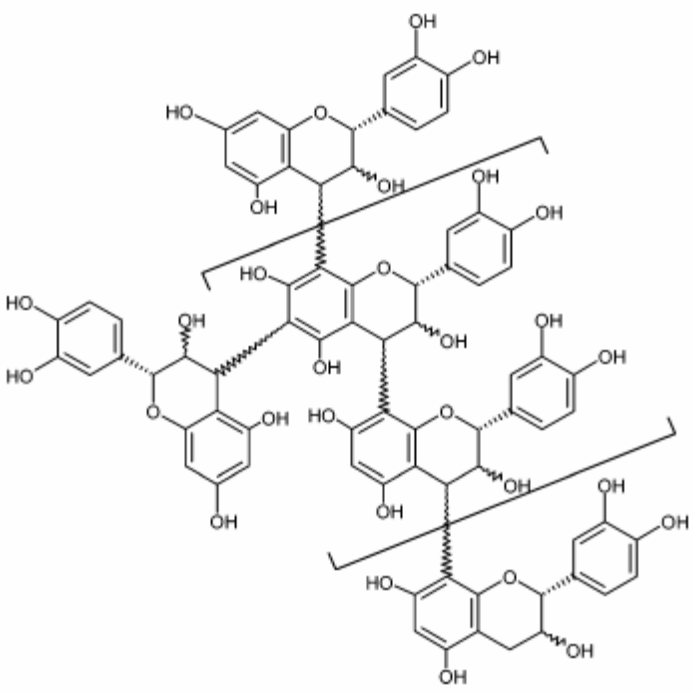

Figure 25: General structure of condensed tannins.(Lisbeth, 2006).

Tannins are classified on the basis of their structural characteristics in three major groups: the hydrolysable, the complex or partially hydrolysable and the condensed or non-hydrolysable tannins (Cos *et al.,* 2003). The first group encompasses polyesters of Gallic acid and hexahydroxydiphenic acids (gallotannins and ellagitannins, respectively) whereas the latter groups, oligomers and polymers composed of flavan-3-ol nuclei (De Bruyne *et al.*, 1999). Complex tannins consist of a flavan-3-ol unit connected to a gallo- or ellagitannin through a glycosidic linkage. They are only partially hydrolyzable due to the carbon-carbon coupling on their flavan-3-ol unit with the glycosidic portion (Figure 26). Condensed tannins, commonly referred to as proanthocyanidins or polyflavanoids, are complex mixtures of oligomers and polymers composed of phenolic flavan-3-ols. In contrast to hydrolyzable tannins, condensed tannins do not have a polyol nucleus and are therefore not readily hydrolyzed. However, upon heating in acidic alcohols, red anthocyanin pigments are obtained (Cos *et al.,* 2003). All these tannins are derived from the shikimate/chorismate pathway (De Bruyne *et al.*, 1999).

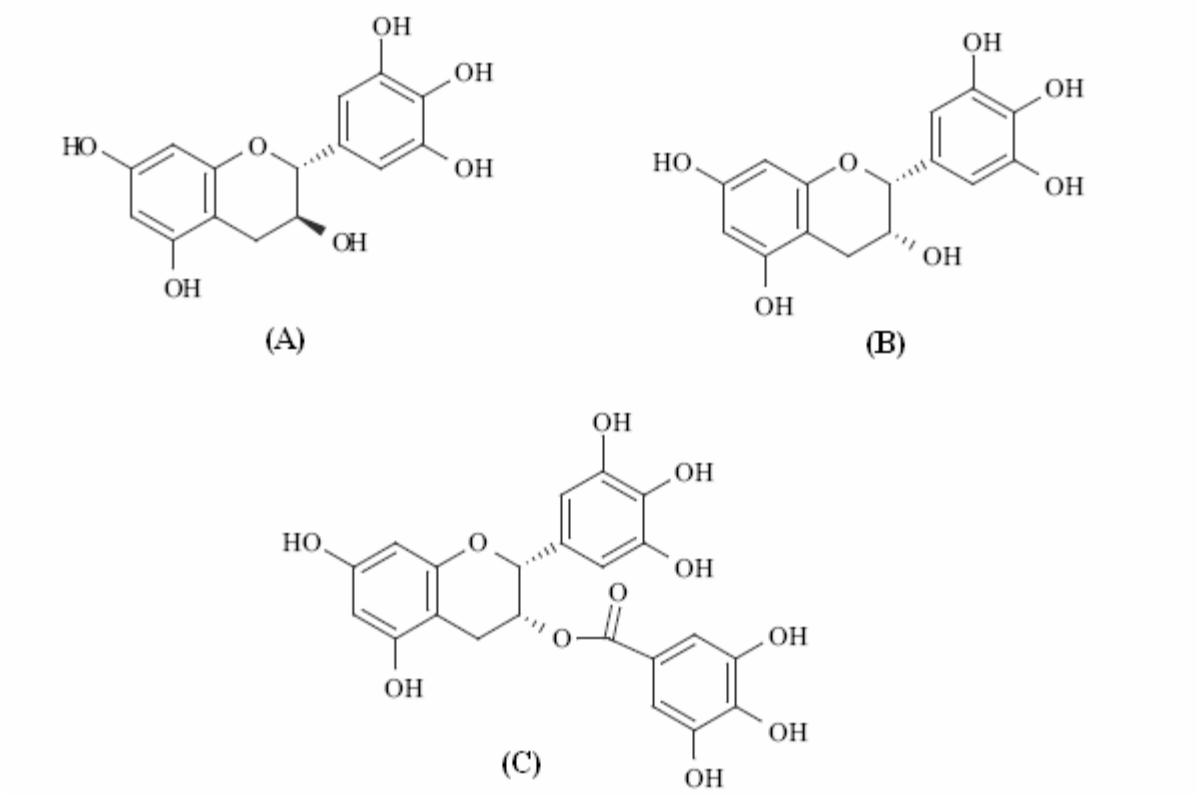

Figure 26: Chemical structures of some flavan-3-ols: (+)-Gallocatechin (A), (-)-Epigallocatechin (B) and (-)-Epigallocatechin 3-O-gallate (C).(Lisbeth, 2006).

Proanthocyanidins and flavan-3-ols derivatives are present in the fruits, leaves, bark and seeds of plants where its main function is to provide protection against microbial pathogens, insects and larger herbivores (Dixon *et al.,* 2005). Besides their biological functions, proanthocyanidins have been suggested to account for a major fraction of the polyphenols ingested in the Western diet, due to their ubiquitous existence (Santos-Buelga *et al.,* 2000). Although the bioavailability and metabolism of proanthocyanidins is still poorly understood, it seems clear that dimmers are the only proanthocyanidins that can be absorbed and found at low concentrations in plasma. Additionally, oligomeric and polymeric proanthocyanidins have been shown to be degraded by microbial flora in the gut into simple phenolic acids, which are readily absorbed (Prior *et al.,* 2005).

2-2-6 Alkaloids

A simple general definition of an alkaloid has been suggested by Pelletier "An alkaloid is a cyclic compound containing nitrogen in a negative oxidation state which is of limited distribution in living organisms" are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms, This group also includes some related compounds with neutral (IUPA,1997). Also some synthetic compounds of similar structure are attributed to alkaloids (Robert,1998).

2-2-6-1 Alkaloid classifications

2-2-6-1-1 Chemical classification

There are three main types of alkaloids:

- True alkaloids: Heterocyclic ring with hydrogen. Derived amino acids
- Proto alkaloids: Does not have Heterocyclic ring. Derived from amino acids. Colchicine is an example of a proto alkaloid (Chetan *et al.,* 2006).
- Pseudo alkaloids: Have Heterocyclic ring. Not derived from amino acids. Pseudo alkaloids can be derived from terpenoids or Purines (Chetan *et al.,* 2006).

Some alkaloids do not have the carbon skeleton characteristic of their group. So, galantamine and homoaporphines do not contain isoquinoline fragment, but are generally attributed to isoquinoline alkaloids (Chetan *et al.,* 2006).

2-2-6-1-2 Alkaloids as medicines

Many alkaloids are pharmacologically active substances, which possess various physiological activities in humans and animals. The use of alkaloid-containing plants as dyes ,spices, drugs or poisons can be traced back almost to the beginning of civilization(Roberts *et al.,* 1998). Today, several alkaloids are still in use. Caffeine and codeine are used as an antitussive, cocaine is used as a local anesthetic. Morphine is an indispensable analgesic, used for treatment of severe pain. Quinine is most known used for antimalarial and remains on the market as an antipyretic (Roberts *et al.,* 1998).

2-4 FREE RADICALS

2-4-1 Definition of free radicals

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons The presence of unpaired electrons usually confers a considerable degree of reactivity upon a free radical. Those radicals derived from oxygen represent the most important class of such species generated in living systems (Valko *et al.*, 2006).

Generation of highly reactive oxygen species (ROS) is an integral feature of normal cellular function like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation, and fertilization. Their production however, multiplies several folds during pathological conditions. The release of oxygen free radicals has also been reported during the recovery phases from many pathological noxious stimuli to the cerebral tissues (Beal, 2000).

2-4-2 Major types of free radicals

2-4-2-1 Reactive oxygen species

Reactive oxygen species is a collective term that includes all reactive forms of oxygen, including both radical and non-radical species that participate in the initiation and/or propagation of chain reaction. Free radicals represent a class of highly reactive intermediate chemical entities whose reactivity is derived from the presence of unpaired electron in their structure, which are capable of independent existence for very brief interval of time (Shiv, 2011).

2-4-2-2 Reactive nitrogen species

Reactive nitrogen species (RNS) are highly active compounds that can cause cellular damage through oxidation and nitration of biomolecules. RNS include, among others, $NO^{\cdot}$ and its derivative peroxynitrite $(ONOO)^{\cdot}$. Peroxynitrite is produced under inflammatory conditions and has been implicated in promoting the development of various pathologies (Marina &Abraham, 2006).

It are review the reaction of NO• and superoxide (O_2H_2) that forms peroxynitrite and the reactions that peroxynitrite itself undergoes.

The formation of peroxynitrite in *vivo* transforms two relatively unreactive free radicals, NO• and $O_2^{\bullet 2}$, into a much more reactive species (Giuseppe & William, 1998).

2-4-3 Production route of free radicals

Production of free radicals in the body is continuous and inescapable. The basic causes include the following (Abheri *et al;* 2010):

The immune system: Immune system cells deliberately create oxy-radicals and ROS (Reactive oxygen species) as weapons.

Energy production: During energy-producing cell generates continuously and abundantly oxy-radicals and ROS as toxic waste. The cell includes a number of metabolic processes, each of which can produce different free radicals. Thus, even a single cell can produce many different kinds of free radicals.

Stress: The pressures common in industrial societies can trigger the body's stress response to mass produce free radicals. The stress response races the body's energy-creating apparatus, increasing the number of free radicals as a toxic by-product. Moreover, the hormones that mediate the stress reaction in the body (cortisol and catecholamine) themselves degenerate into particularly destructive free radicals.

Pollution and other external substances: Air pollutants such as asbestos, benzene, carbon monoxide, chlorine, formaldehyde, ozone, tobacco smoke, and toluene ,Chemical solvents such as cleaning products, glue, paints, and paint thinners , Over-the-counter and prescribed medications, perfumes, pesticides, Water pollutants such as chloroform and other trihalomethanes caused by chlorination, cosmic radiation, electromagnetic fields, medical and dental x-rays, radon gas, solar radiation ,the food containing farm chemicals, like fertilizers and

pesticides, processed foods containing high levels of lipid peroxides, are all potent generator of free radicals.

General factors: Aging, Metabolism, Stress

Dietary factors: Additives, alcohol, coffee, foods of animal origin, foods that have been barbecued, broiled, fried, grilled, or otherwise cooked at high, temperatures, foods that have been browned or burned, herbicides, hydrogenated vegetable oils, pesticides, sugar.

Toxins: Carbon tetrachloride, Paraquat, Benzo (a) pyrene, Aniline dyes, Toluene

Drugs: Adriamycin, Bleomycin, Mitomycin C, Nitrofurantoin, Chlorpromazine

Figure 27: Production routes of free radicals

2-4-4 Mechanisms for formation of oxygen free radicals

Oxygen is required for the generation of all ROS, RNS, and reactive chlorine species. The major reactions for the production of oxygen and nitrogen free radicals in the body are illustrated in (Figure28) (Yun-Zhong *et al;* 2002).

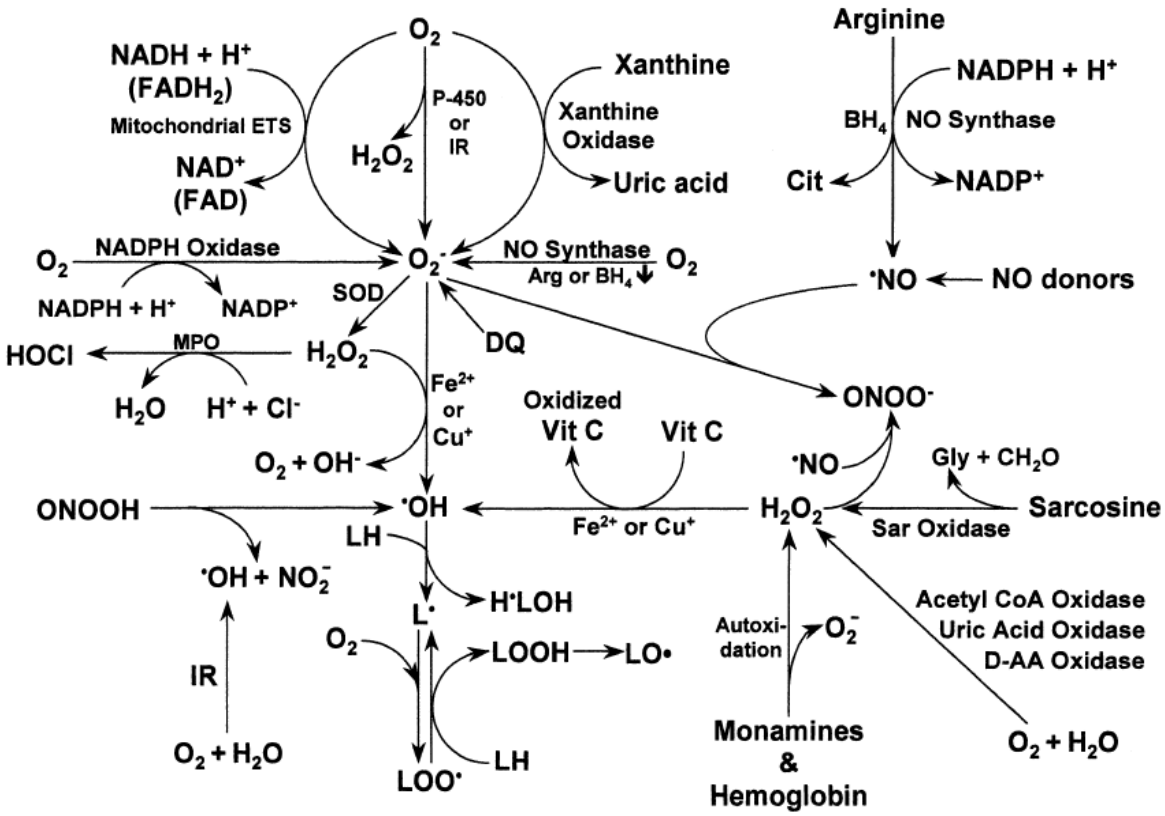

Figure 28: production of oxygen and nitrogen free radical and other reactive species in mammalian cells .

All radical groups formed during oxidation processes, are primarily responsible for certain diseases involving many organs. For example and unpredictable effect of oxidation in DNA can lead to cancers. The following disorders which are related to free radical chain reactions are demonstrated in (Figure 29).

The negative effects caused by free radicals can be observed not only in the body but also in food materials and drugs. Antioxidants are protective substances against these negative effects and can be found in many natural and synthetic sources (Simone, 1992).

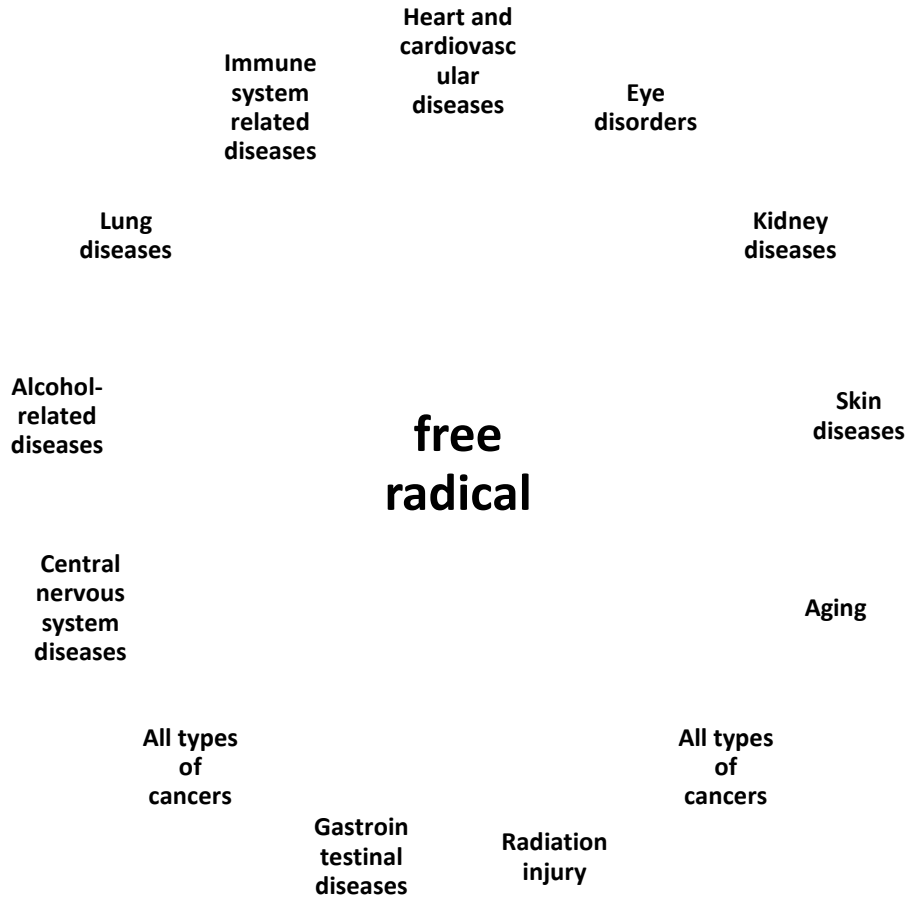

Figure 29: Free radicals disorders

2-4-6 Oxidative stress

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.(John *et al;* 1999)
Oxidative stress is involved in the process of aging (Kregel and Zhang 2007), and various chronic diseases such as atherosclerosis (Fearon & Faux, 2009), diabetes (Ceriello *et al,* 2004), and eye disease (Li *et al,* 2009).

2-4-7 Antioxidant

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. The most common antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances (Hamid, 2010). Antioxidants, traditionally, are divided into two groups, primary antioxidant (chain breaking) and secondary antioxidant (preventing) (Antolovich *et al;* 2002). In a more detailed way, antioxidants can be grouped as follows;

• Inhibitors of free-radical oxidation reactions (inhibits the formation of lipid radicals).

• Inhibitors interrupting the propagation step of autoxidation.

• Singlet oxygen quenchers.

• Synergist antioxidants (those show no antioxidative effect when used alone but increasing the activity of primary antioxidants when used together).

• Reducing agents (transforms hydroperoxides into stable forms).

• Metal chalators (transforms metal ions into stable forms) (Pokorny, 2007).

Antioxidants can deactivate radicals by two major mechanisms, Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). Antioxidants that work with HAT mechanism quench free radicals simply by donating hydrogen atom while those of which work with SET mechanism transfer one electron to reduce any compound such as metal ions, radicals and carbonyls as shown in equations below (Antolovich et al.,2002).

$$L\bullet + AH \rightarrow LH + A\bullet$$

$$LOO\bullet + AH \rightarrow A\bullet + LOOH$$

$$LO\bullet + AH \rightarrow A\bullet\ LOH$$

HAT antioxidants, when present in trace amounts, may delay or prevent the initiation step by reacting with lipid radical or directly inhibit the propagation step by reacting with peroxyl or akoxyl radicals while donating their hydrogen atom

$$.LOO\bullet + A\bullet \rightarrow LOOA$$

$$LO\bullet + A\bullet \rightarrow LOA$$

The antioxidant free radicals can interfere with chain-propagation reactions and form peroxyantioxidant groups due to their hydroxyl groups (Figure 2.2.).

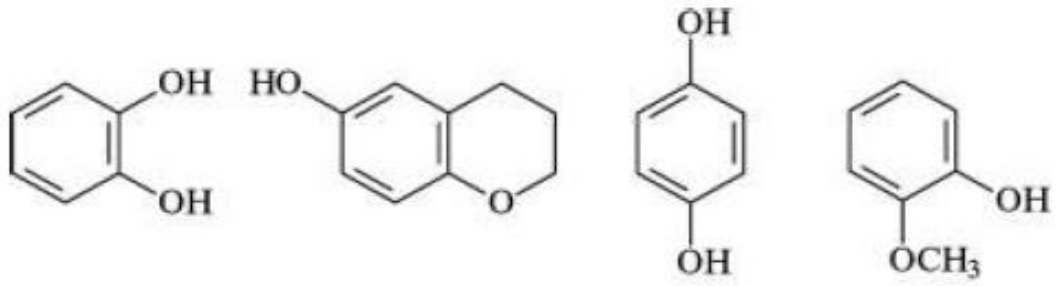

Figure 30: Basic chemical structure of antioxidants (Pokorny, 2007).

There have been some discussions about undesired effects of use of synthetic antioxidants. Antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) tert-butylhydroquinon (TBHQ) and propyl gallate are widely used in many foods. These compounds, for example, are added to oils and fats as additives at low concentrations ranged from 50 to 200 ppm to prevent formation of peroxides during storage (Fki *et al;* 2005).

As an example, use of BHT in rat feed caused them to develop fatal haemorrhages in the pleural and peritoneal cavities and in organs such as epididymis testes and pancreas (Fki et al., 2005). This effect is attributed to the ability of BHT to reduce vitamin K dependent blood clotting factor (Hakkim *et al;* 2007). On the other hand BHA, has become under attack due to its potential action as carcinogenesis

promotion and causing lesion formation in rat forestomach (He & Shahidi 1997; McCarthy *et al;* 2001).

Although synthetic additives have been widely used in food industry, to inhibit the process of lipid oxidation. The trend is to decrease their use because of the growing concern among customers about such chemical additives (Fernandez-Lopez *et al;* 2005). Accordingly, there is a strong argument for the effective isolation of organic antioxidants from natural sources such as phytochemicals especially polyphenols and essential oils obtained from plant extracts as alternatives of synthetic antioxidants (McCarthy *et al;* 2001).

2-5 Antimicrobial activity of plants

2-5-1 Historic use of plants as antimicrobials

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold in the development of new drugs, they may become the base for the development of medicine, a natural blueprint for the development of new drugs, or, a phytomedicine to be used for the treatment of disease. There are numerous illustrations of plant derived drugs. Some selected examples, including those classified as anti-infective, are presented below (Janick , 1999). Since antiquity, humans have used plants to treat common infectious diseases. we need, some traditional medicines are still part of customary treatment of these diseases in some societies.

In the past decade, a worldwide increase in the incidence of infections and a rise in the resistance of some species of microorganisms to different antibiotics used in medicinal practice have been observed. Hence, there is a great demand for novel antimicrobials, across a wide range of structural classes that can act on new targets with fewer side effects (Mohd *et al* , 2013). Plants are rich in a wide variety of secondary metabolites, which have been found to have diverse biological properties. Some plants have also shown the ability to overcome resistance to some microorganisms, which has prompted researchers to investigate their mechanisms of action and isolate their active compounds (Mohd *et al* , 2013).

2-5-2Major groups of antimicrobial compounds in plants

Infectious diseases are the world's leading cause of premature death, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The abusive and indiscriminate use of antimicrobial compounds over many years is the main factor responsible for the appearance of the phenomenon of bacterial resistance to such compounds (Venketeshwer, 2012).

Recently, the growing occurrences of multi-drug resistant strains of bacteria and the appearance of strains with decreased susceptibility to antibiotics have led to a resurgence of research interests in the discovery of novel antimicrobial agents from natural sources for therapeutic and preventive purposes against microbial diseases, food preservatives and feed additives in the animal industry. The ethnopharmacologists, botanists, microbiologists and natural-product chemists are constantly in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are comparatively meager compared to the vast number of plant population. Plants produce a great diversity of compounds. The structures of close to 50,000 compounds have already been elucidated and there are perhaps hundreds of thousands of such compounds in plant (Amlan & Patra, 2012).

Microbial resistance occurs mainly in three general mechanisms: prevention of interaction of the drug with target, direct destruction or modification of the drug, and efflux of the drug from the cell. These mechanisms were used by different microorganisms and led to the emergence of many pathogenic bacterial strains (Alanis, 2005). With pathogenic fungi, the situation is not so bright also, where Amphotericin B was for many years the only treatment available for fungal infections (Iraj , 2011).

Since antiquity, humans have used plants to treat common infectious diseases. we need, some traditional medicines are still part of customary treatment of these diseases in some societies. Plants are rich in a wide variety of secondary metabolites, which have been found to have diverse biological properties. Some plants have also shown the ability to overcome resistance to some microorganisms, which has prompted researchers to investigate their mechanisms of action and isolate their active compounds (Mohd *et al* , 2013).

The idea that certain plants have healing potential and they contain what we would currently call antimicrobial compounds was well accepted before humans actually , discovered the existence of microbes, Since then, mankind have used plants to

treat common infectious diseases and some of these traditional medicines are still being performed as antimicrobial treatments (Haq, 2012), for example table (6)

Table 6: Selected plants containing antimicrobial activity (Donald *et al* , 2006)

Common name [a]	Scientific name	Compound	Class	Activity against [b]
Aloe	*Aloe barbadensis, Aloe vera*	Latex	Complex mixture	Corynebacterium , Salmonella, Streptococcus, S. aureus
Bay	*Laurus nobilis*	Essential oils	Terpenoids	Bacteria, fungi
Black pepper	*Piper nigrum*	Piperine	Alkaloid	Fungi, Lactobacillus, Micrococcus, E. coli, E. faecalis
Blueberry	*Vaccinium spp.*	Fructose	Monosaccharide	E. coli
Chamomile	*Matricaria chamomilla*	Anthemic acid	Phenolic acid, coumarins	M. tuberculosis, S. typhimurium, S. aureus, helminths, viruses
Chappara	*Larrea tridentata*	Nordihydroguaiaretic acid	Lignan	Skin bacteria
Echinacea	*Echinaceae angustifolia*	-		General
Eucalyptus	*Eucalyptus globulus*	Tannin	Polyphenol, Terpenoid	Bacteria, viruses
Garlic	*Allium sativum*	Allicin, ajoene	Sulfoxide, sulfated Terpenoids	General
Ginseng	*Panax notoginseng*		Saponins	E. coli, S. schenchii, Staphylococcus spp., Trichophyton
Golden seal	*Hydrastis canadensis*	Berberine, hydrastine	Alkaloids	Bacteria, Giardia duodenale, trypanosomes, plasmodia
Green tea	*Camellia*	Catechin	Flavonoid	General,

	sinensis			Shigella, Vibrio, mutans, viruses
Licorice	*Glycyrrhiza glabra*	Glabrol	Phenolic alcohol	S. aureus, M. tuberculosis
Olive oil	*Olea europaea*	Hexanal	Aldehyde	General
Papaya	*Carica papaya*	Latex	Mix of terpenoids, organic acids, alkaloids	
Rosemary	*Rosmarinus officinalis*	Essential oil	Terpenoid	General
Willow	*Salix alba*	Salicin, tannins, essential oil	Phenolic glucoside, polyphenols, terpenoid	
Wintergreen	*Gaultheria procumbens*	Tannins	Polyphenols	General

aBold indicates plants are available as commercial preparations.
b‘‘General’’ denotes activity against multiple types of microorganisms, i.e., bacteria, fungi, and protozoa; ‘‘bacteria’’ denotes and gram-negative bacteria and activity against gram-positive

Plants produce a great diversity of compounds. The structures of close to 50,000 compounds have already been elucidated and there are perhaps hundreds of thousands of such compounds in plants (Amlan & Patra, 2012). Most of these compounds are secondary metabolites, Many of these aromatic compounds have been found to be plant-defense mechanisms against predation by microorganisms, insects, and herbivores. Terpenoids give plants their odors, quinones and tannins are responsible for the plant pigments, other compounds are responsible for plant flavors. Antimicrobial phytochemicals can be divided into several categories, as described below and summarized in Table (7)

Table 7: Selected classes of antimicrobial compounds from plants and their mechanism of action (Donald *et al* , 2006).

Class	Type	Antimicrobial mechanism
Phenolics	Simple phenols	Substrate deprivation Membrane disruption
	Quinones	Bind to adhesions Complex with cell wall
	Flavonoids, Flavones	Bind to adhesions Complex with cell wall Inactivate enzymes Inhibit HIV reverse transcriptase
	Tannins	Bind to proteins: Bind to adhesions Enzyme inhibition Substrate deprivation Complex with cell walls Membrane disruption Metal ion complexation
Terpenoids, essential oils		Membrane disruption
Alkaloids	Berberine, piperine	Intercalate into cell wall and/or DNA
Coumarins	Warfarin	Interaction with eukaryotic DNA (antiviral activity)
Lectins, polypeptides	Mannosespecific Agglutinin	Block viral fusion or adsorption
	Fabatin	Form disulfide bridges

2-5-4 Antimicrobial activity of *Aloe perryi*

There are many studies showing that resistance to infection is enhanced by Aloe either in humans or in animals, whether even if the infective agent is a bacterium, virus or fungus (Khaing, 2011).

The efficacy of Aloe liquid as an antibacterial agent is shown to have a wide range against Gram positive and Gram negative bacteria. The antimicrobial agents of *Aloe vera* gel was reported to effectively kill or greatly reduce or eliminate the growth of *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus*

pyogenes, Pseudomonas aeruginosa, Escherichia coli, Propionibacterium, Helicobacter pylori and Salmonella typhi. Whole leaf components are proposed to have direct antibacterial properties include anthraquinones and saponin, while polysaccharides have been attributed within direct bacterial activity through the stimulation of phagocytic leucocytes to destroy bacteria (Rubina *et al* , 2009).While roots *of aloe perryi* do not have any activity against *Staphylococcus aureus. Bacillus subtilis , Micrococuss flavus, , Escherichia coli, Pseudomonas aeruginosa* and one fungal strain *Candida maltosa* (Mothana *et al,* 2009).

2-5-5 Antimicrobial activity of *Apium graveolens L*

Sesquiterpene lactones from *Apium graveolens L* showed activity against *Bacillus subtilis* and *Proteus vulgaris* and also tested fungi (Villupanoor , 2008). Essential oil of celery also inhibited the growth of *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica, Pseudomonas aeruginosa, Lactobacillus plantarum, Aspergillus niger, Geotrichum* and *Rhodotorula*. The root extracts possess high activity against *B. cereus* and *Enterococcus faecalis* only (Parthasarathy *et al ,* 2008).

The essential oil of celery showed marked antibacterial activity against, *Bacillus subtilis, Bacillus pumilus, Vibro cholera, Staphylcoccus aureus, Streptococcus Pyrogens, Salbus, Shigella dysentrica, Coryne bacterium diphtheria, Salmonella typhii, Salmonella faecalis, Pseudomonos solanacearum*. The essential oil of celery also showed bacteriostatic effect against all the above microbes. The essential oil of celery has further been reported to be effective against *S typhii, S. albus* and *V. cholera*. In some other studies the essential oil of celery has been found effective against *C. albicans, S. aureus, B. subtilis and P. Maltocida* and against *Staphylococcus aureus* and *Sarcina lutea*. (Mohammed *et al* , 2013).
Celery seeds in methanolic extract have showed antimicrobial activity against *Shigella dysenteriae* , while it do not showed antimicrobial activity against, *Bacillus cereus Staphylococcus aureus* and *Salmonella typhy* ,while in petroleum ether extracts showed activity against *Staphylococcus aureus and Salmonella*

typhy ,while not activity against *Bacillus cereus and Shigella dysenteriae* (Manisha & Rita, 2010). The antifungal activity of steam distilled oil by comparing the inhibition activity of the oil with respect to eight standard antifungal drugs namely Griseofulvin 1%, Hamycin 0.5%, Zinc undecnoate 1%, cetrimide 1%, benzoic acid 1%, Salicylic acid1%, hexachlorophene 1% and Chlorobenzoic acid 1%. The steam distilled oil of celery showed marked activity in comparison to standard drugs répet! against *Trichophyton-terrestre, Histoplasma-capsulatum, A niger, A nidulans, Epidermophylon floccosum, Fusarium-oxysporum* (Mohammed *et al* , 2013).

2-5-6 Antimicrobial activity of *Morus alba*

Heat Stable Proteins of *Morus alba* tested for the antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis* and compared with antibiotic chloramphenicol. Area of zone of inhibition increased with the increase in the concentration of the Heat Stable Proteins for all the microbes tested by the mulberry varieties. For *Escherichia coli* Minimum inhibitory concentration (MIC) was at 25 µl by *Morus alba* was more effective against *E. coli* at 100µl (Bandna *et al* , 2013). In an another study petroleum ether, chloroform and methanol sequential leaf extracts of *Morus alba* at different concentrations of the extracts were tested for antimicrobial activity against various bacterial strains and fungal strains (*Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Shigella flexneri, Aspergillus niger, Candida albicans)*. The effects of these extracts were compared to standard drugs, results of the antimicrobial activity revealed that all the extracts showed noticeable antimicrobial activity in a dose dependant manner against the organisms studied (Aditya *et al* , 2012).

2-5-7 Antimicrobial activity of *Portulaca oleracea*

Antimicrobial activity in aerial parts of chloroform and ethanolic extracts of *Portulaca oleracea* by agar diffusion method against five bacteria and three fungi (bacteria like *Staphylococcus aureus, Bacillus cereus, Klebisilla pneumonia and fungi* like *Aspergillus fumigates* and *Nerospora crassa*), ethanolic crude extract

showed maximum effect on organisms like *Staphylococcus aureus, Klebisilla pneumonia* and *Nerospora crassa*, whereas chloroform extract showed moderate effect on *Klebisilla pneumonia, Aspergillus niger* and *Nerospora crassa* (Chowdhary *et al* 2013). Dhole *et al* (2011) had reported the antmicrobial activity in root and leaves parts of aqueous extract and ethanolic extract have showed antimicrobial activity against *Bacillus subtilis , Staphylococcus aureus, P. aeruginosa, Aspergillus niger*. Whereas chloroform and ethanolic extract of *Portulaca oleracea* showed antimicrobial activity *against Escherichia coli, Pseudomonas aurnginosa, Klebsilla peumoniae, Aspergillus niger, Aspergillus fumigate and Neurospora Crassa* (Ramesh & Hanumantappa , 2011).

EXPERIMENTAL PART

MATERIALS

&

METHODS

3. MATERIALS AND METHODS

3.1. The sources and collection of study plants

The choice of plants is based on a survey of ethnopharmacological population with knowledge of their use in traditional medicine.

A total of eight plants were collected from August to September 2011, from different regions of Yemen. Scientific name, English common name, Region of collection, Part use and the date of collection are described in Table (8)The collection of plans was carried out with the assistance and under the supervision of Yasin House of medicinal plants (Alzubairy Street - Sana'a- Yemen).

Plant species Identification accomplished in the Laboratory of botany (Department of Biology, Faculty of Science, Sana'a University- Yemen). After collection, fresh plant samples was air dried at ambient temperature (25°C) in the laboratory for approximately 15 days. The leaves of plants were separated from the bark and any fruit present was removed and stored separately. Then the plant samples preserved in sterilized polyethylene sacks for further experimental use. The plant material was then milled to a fine powder using a miller before each analysis.

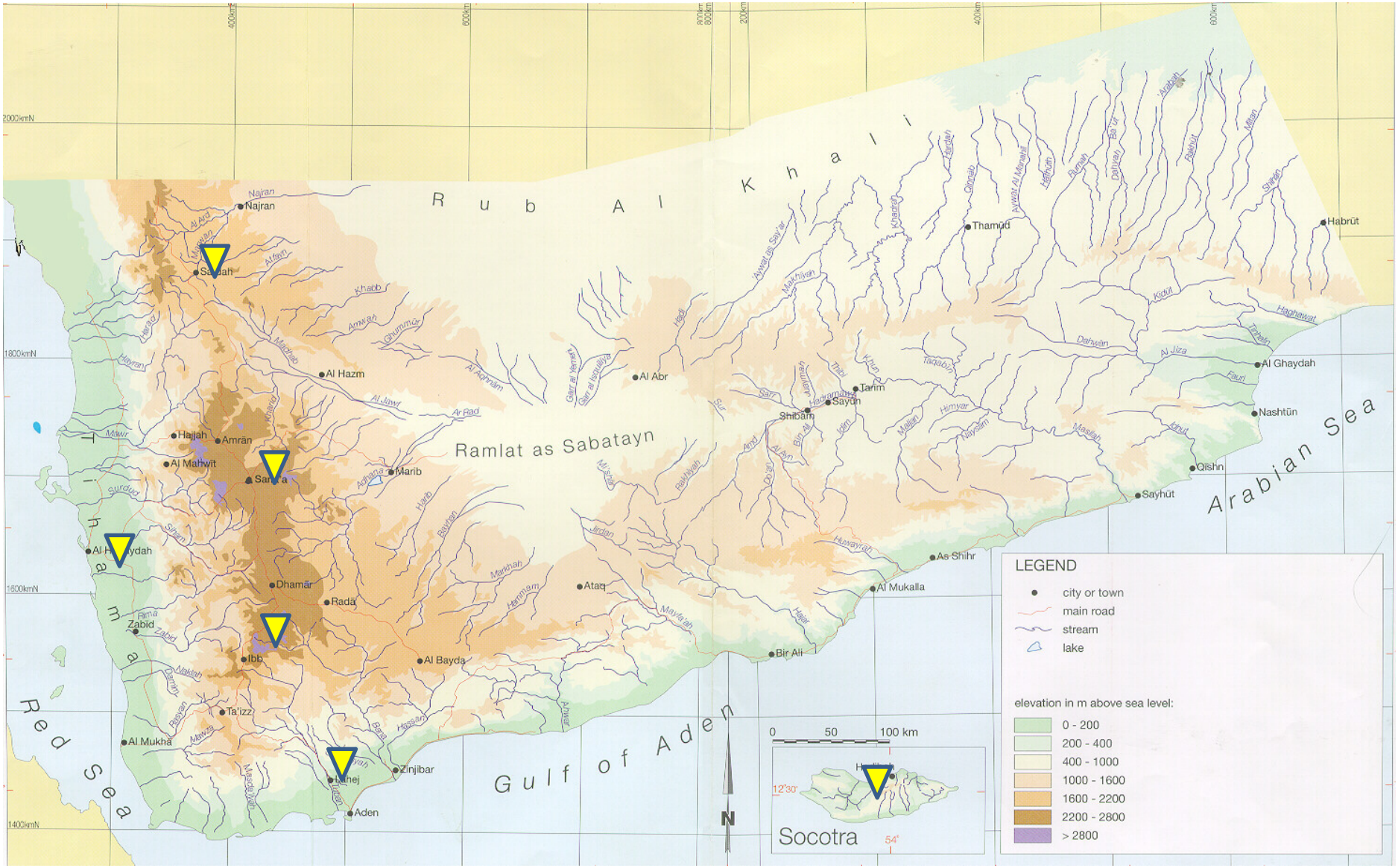

Figure 31: Map of Yemen (indicated the source of experimental plants

Table 8: Description of the plants of study

Latin name	English common name	Region of collect	Part used	Date of collection
Aloe perryi J.G.BAKER	Aloe	Soqatra	Leaves	September
Apium graveoleus L.Nees	Celery	Lahj	Seeds	September
Commiphora myrrha	Myrrh	Soqatra	Resin	September
Lupinus termis	Lupin	Sana'a	Seeds	September
Morus alba L	White Mulberry	Sana'a	Leave	September
Nigelle sativa L	Black Cumin	Sadh	Seeds	September
Portulaca oleracea L	Common Purslane	Alhodidh	Seeds	September
Trigonella foenum L	Fenugreek	Ibb	Seeds	August

3.2. Chemical analysis

3.2.1. Determination of crude fibre content

Defatted sample (1g) was placed in a glass crucible and attached to the extraction unit(In Kjel, D-40599, behr Labor-Technik GmbH, Dusseldorf, Germany). 150 ml boiling1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace (MF-1-02, PCSIR Labs., Lahore, Pakistan) for 2 h, cooled in a desiccator and reweighed(W2). Extracted fiber was expressed as percentage of the original undefatted sample was and calculated according to the formula:

$$\text{Crude fibre (\%)} = \frac{\text{Digested sample (W1)} - \text{Ashed sample (W2)}}{\text{Weight of sample}} \times 100$$

(A.O.A.C, 2000)

3.2.2. Determination of crude protein:

The crude protein was determined using micro Kjeldahl method (A.A.C.C. 2000). Two grams of oven-dried material was taken in a Kjeldahl flask and 30 ml conc. H_2SO_4 was added followed by the addition of 10 g copper sulphate. The mixture was heated first gently and potassium sulphate and 1 g then strongly once the frothing had ceased. When the solution became colorless or clear, it was heated for another hour, allowed to cool, diluted with distilled water and transferred to a 800 ml Kjeldahl flask, washing the digestion flask. Three or four pieces of granulated zinc, and100 ml of 40% caustic soda were added and the flask was connected with

the splash heads of the distillation apparatus. Next 25 ml of 0.1 N receiving flask and distilled. When two-thirds of the liquid sulphuric acid was taken in the had been distilled, it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content.

3.2.3. Determination of moisture

Approximately 2 g of the material under test is accurately weighed (to 0.001 g) into a small dish. This is then placed in the oven for 1 hour, removed from the oven and put in the desiccator to cool. It is then weighed. The dish is replaced in the oven for 30 minutes and the process repeated to constant weight (Marwaha, 2010). The moisture content is found using the following

formula

$$\% \text{ moisture} = \frac{\text{inital weight} - \text{ final weight}}{\text{inital weigh}} \times 100$$

3.2.4. Determination of total ash

For determination of ash content, 10 g of each sample was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about3–5 h at 600°C. It was cooled in a desiccator and weighed to ensure completion of ashing. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or greyish white). Weight of ash gave the ash content (A.A.C.C., 2000).

3.2.5. Determination of minerals

Weigh 2g of the dried and milled (to 1 mm) sample into a silica crucible and place in a cold muffle furnace with the chimney vent open, and allow to heat up to 450°C. Close the vent and maintain at this temperature overnight. Remove from the furnace and allow to cool, then add15 drops HCl from a polythene Pasteur pipette, being careful to moisten all the sample. Using a fume cupboard, gently evaporate off all the HCl on a hotplate at moderate heat, then remove and cool. Dissolve the residue in 0.1 M HCl, and transfer quantitatively to a 10-ml volumetric flask. Make up trace element standards in 0.1 M HCl covering the expected ranges in the sample solutions and analyse by Atomic absorption spectrophotometer. AI 1200. Aurora. Canada) according to the instrument manufacturer's instructions Calculation. The sample solution is of 2 g in 10 ml, therefore the concentrations in μg ml–1 of the trace element should be multiplied by 5 to give the concentration in μg g–1 of the trace element in the dried sample (Faithfull , 2002).

3.2.6. Determination of the lipid content

Lipid content determined as described by (Chaturvedi, 2006), where 3-5 g of sample Weighted into a completely dried thimble and Place thimble in the Soxhlet's apparatus and fill the flask ¾ with ether. Start the water in condenser and heat the flask and set on 5-6 drops per second for four hours, Take out the thimble. Keep it at room temperature for evaporation of ether and then keep overnight in the oven at 105C°, Remove the thimble from oven, cool it in a desiccator and weigh.

Calculation :-

Wt. of sample = (Wt. of thimble + sample) – wt. of thimble

Wt. of fat = (Wt. of thimble + sample) - (Wt. of thimble + sample after extraction)

$$\text{Ether extract (\%)} = \frac{\text{wt .of fat}}{\text{wt of sample}} * 100 .$$

3.2.7. Determination of total carbohydrate:

Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein) (Shumaila & Mahpara, 2009).

3.3. Phytochemical analysis

3.3.1. Determination of alkaloids:

alkaloids contents of the plants were determined using the method that described by Harborne (1998), using soxhlet, ten grammes of the powdered sample was extracted with 250 mL of ethanol period five hours, extracted of ethanol was evaporated to dryness with a rotary evaporator, under reduced pressure at 40 C◦. dry residue repeat by 150 mL of chloroform and acidify by HCl 5% pH 3 ,it let pillow during 30 minutes in the room temperature, the phase acid aqueous were extracted by 150 ml of chloroform , basify by the NaHCO3 5% pH 9 and lit it during 15 minutes in the room temperature . the chloroform phase was evaporated to dryness with a rotary evaporator under reduced pressure. The dry residue are the total alkaloids.

3.3.2. Determination of Tannins

Five grams of each part were milled into powder. The powder was extracted with 100 ml acetone–water (70/30, V/V), and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40 C^0 to remove acetone. The washed with 30 ml dichloromethane to remove lipid soluble remaining solution was substances. After that, the solution was further extracted with ethyl acetate at a ratio of 30/30 (V/V). The water layer was separated and extracted twice more similarly. Then the resulting water layer was evaporated to dryness, and the resulting substance was weighed (Zhang *et al*, 2008).

3.3.3. Determination of total phenols:

The powdered plant material (2g) was extracted with methanol ,ethanol, Ethyl acetate, water and hexane at room temperature overnight. The solvent extracts were combined and concentrated under reduced pressure on a rotary evaporator. Total phenolic content of each plants extract was determined with the Folin–Ciocalteu's reagent (FCR) according to the published method (Slinkard & Singleton, 1977). Each sample (0.5 ml) was mixed with2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na2CO3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at30C^{o} for 90 min. Results were expressed as Gallic acid equivalent (mg Gallic acid/100g dried extract).

3.3.4. Determination of Total flavonoids

The total flavonoid content of extract of plants was determined by a colorimetric method as described in the literature (Zhishen *et al,* 1999). Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15%). After 6 min, 0.15 ml of aluminum chloride ($AlCl_3$) solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at510 nm versus prepared water blank. Results were expressed as Catechin equivalent (mg Catechin/100g dried extract).

3.4. determination of antioxidant activity of plants

3.4.1 Preparation of plant extracts

The dried parts of the plant (5 g each) were powdered and macerated with 125ml from methanol, ethanol, water ,ethyl acetate and hexane for 48 hour at room temperature and filtering through Whitman No. 4 filter paper, After evaporation of the solvent under reduced pressure , All the extracts were kept in tightly stoppered bottles under refrigeration (4 °C) until used for the biological testing .

3.4.2 DPPH scavenging assay

The hydrogen atom donation ability of chemical compounds in plant samples was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical (Concepción *et al*, 1998). Fifty microliter of various concentrations of the extracts in methanol were added to 1950 ml of a 0.025 g/l methanol solution DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a control at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity}(\%) = \frac{\text{control} \_ \text{Asample}}{\text{control}} \times 100$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound.

3.4.3 Determination of ferric reducing power (FRAP) of the extracts

The reducing power of extract of plants was evaluated according to the method described by (Aiyegoro & Okoh, 2010). The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of the extracts and standards prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10%

w/v), which was then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

3.4.4 Measurement of hydrogen peroxide scavenging activity

1 ml of sample was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4), and then 0.6 ml of 43 mM solution of H_2O_2 in the same buffer were added. The mixture was incubated at room temperature for 40 min and the absorbance values of the reaction mixtures at 230 nm were recorded against a blank solution containing phosphate buffer without H_2O_2 for each sample (kesenkaş *et al,* 2011). The hydrogen peroxide scavenging activity was measured according to the following

$$H_2O_2 \text{ scavenging activity}(\%) = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

3.4.5 Determination of total antioxidant capacity

The total antioxidant capacity (TAOC) of hexane, ethyl acetate, methanol, ethanol and water extract were evaluated by the method of (Prieto *et al,* 1999). An aliquot of 0.1 ml of sample solution (1 mg/ml) was combined with 1 ml of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions. The antioxidant capacity was expressed as the number of equivalents of α-tocopherol (mg/g of dry weight).

3.5 Antimicrobial assay

3.5.1. Microbial strains sources

The samples were evaluated against a various microorganisms, including the bacterial strains:

Staphylococcus aureus (ATCC 8739),+

Listeria monocytogenes ATCC19115+
Bacillus subtilis 6633 +

Micrococcus luteus (ATCC 9341),+

Pseudomonas aeruginosa (ATCC 27853),-

Klebsiella pneumoniae (ATCC 700603),-

Escherichia coli (ATCC 8739), -

The yeasts:

Candida albicans (ATCC 10231).

The fungi
Aspergillus flavus MNHN 994294
All microorganisms obtained from Pastor institute – Algeria

2.5.4. Preparation of plant extracts

The dried parts of the plant (5 g each) were powdered and macerated with 125ml from methanol, ethanol, water ,ethyl acetate, hexane for 48 hour at room temperature and filtering through Whitman No. 4 filter paper, After evaporation of the solvent under reduced pressure , All the extracts were kept in tightly stoppered bottles under refrigeration (4 °C) until used for the biological testing .

3.5.5. Preparation of Inoculum

The gram positive (*Bacillus subtilis* , *Listeria monocytogenes, Micrococcus luteus* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were pre-cultured in nutrient agar overnight in incubation at 37°C, , pellet was suspended in double

distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculum (*A. flavus*) was prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 105 spores/ml. (Mahesh & Satish , 2008)

3.5.6. Agar disc diffusion technique

PRINCIPLE

This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth.(Ruangpan& Tendencia, 2004)

3.5.7. Antibacterial testing

The dried plant methanol, ethanol, ethyl acetate extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 100 mg/ml. while water extract was dissolved in sterilizer water and hexane extracts was dissolved in hexane. Antibacterial tests were then carried out by the disc diffusion method (Ali-Shtayeh *et al*, 1998), using an inoculum containing 106 bacterial cells/ml spread on Muller–Hinton agar plates (1 ml inoculum/plate). The discs. (diameter= 6 mm) were impregnated with extract (2,1,0.5 mg/disc) at a concentration and placed on the inoculated agar and incubated at 37°C for 24 h.

3.5.8. Antifungal testing:

The antifungal activity was tested by disc diffusion method (Mahesh & Satish , 2008).The potato dextrose agar plates were inoculated with each fungal culture by point inoculation. The filter paper discs (diameter= 6 mm) were impregnated with extract (2,1,0.5 mg/disc) at a concentration, Blank disc impregnated with solvent hexane followed by drying off was used as negative control and Nystatin (30 µg /disc) used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

3.6 Statistical Analysis

The experimental data were expressed as mean ± SD. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA. The multiple comparison carried out by tukey test. The level of significance was set at $p < 0.05$.

RESULTS

RESULTS

4- 1- Experiment 1

4-1-1Chemical analysis

4-1-1-1 Nutrient compositions of plants

Chemical compositions of experiment plants are shown in Table (9)

Results show that the moisture contents in plant samples were ranged between 3.75% and 10.09%, *Commiphora myrrha* resin contains a highest percentages of moisture (10.09%), *Apium graveoleus L* comes in the second order with a moisture rate of 7.6%, after that *Trigonella foenum L* and *Lupinus termis* samples were in the third order where it gain 6.57 % and 6.26% respectively, followed by *Morus alba*, *Portulaca oleracea L*, and *Aloe perryi* which were contained 5.76% ,4.47% and 4.57% respectively, finally *Nigelle sativa* were the lowest value 3.75%, of moisture contents. the highest content of total ash *Portulaca oleracea L*, *Morus alba*, and *Apium graveoleus L* with 15.04%, 14.22%, and 13.2% respectively, followed by *Commiphora myrrha*, *Nigelle sativa* and *Trigonella foenum L* that were 6.20%, 4.11% and 4.03% respectively while the minimum values of ash were in *Lupinus termis* and *Aloe perryi* with 2.70% and 1.87% respectively.

The results in table (9), the third in column showed Proteins results of plant samples, *Lupinus termis* samples gave the great values of protein which was 37.17%, followed by *Trigonella foenum L* (26.78%), *Morus alba* came in third grade 20.92 %, the fourth and fifth plant in protein were *Apium graveoleus L* and *Portulaca oleracea L* which gain 16.37% and 16.31%, followed by *Commiphora myrrha* 10.45% and *Aloe perryi* was poor in proteins which has only 2.05%. in fourth column in the table (9), we can see lipid contents of experimental plants, the

major rate showed for *Nigelle sativa* 44.67%, followed by *Portulaca oleracea L*, *Apium graveoleus L*, *Lupinus termis* and *Trigonella foenum L* with rates of 15.49%, 9.61% 8.52%, and 6.35% respectively, and the *Morus alba* 3.02%, *Commiphora myrrha* and *Aloe perryi* were lack in lipids matters 0.54% and 0.19%. Crude fiber appear the highest rate in *Portulaca oleracea L* (33.37%), in the next *Apium graveoleus L Lupinus termis* and *Morus alba* 14.77%,10.39% and 9.53%, then *Nigelle sativa* and *Commiphora myrrha* with percentage of 2.42% and 1.51% , and in the end of list *Aloe perryi* come to show the lowest rates of crude fibers 0.03%.

Table 9: Chemical compositions of experiment plants

Plant Sample	Moisture %	Total Ash %	Protein % N×6.25	Lipid (%)	Crude Fiber %	Carbohydrate %
Aloe perryi	4.57	1.87	2.05	0.19	0.03	91.29
Apium graveoleus L	7.60	13.2	16.37	9.61	14.77	38.63
Commiphora myrrha	10.09	6.20	10.45	0.54	1.51	71.21
Lupinus termis	6.26	2.70	37.17	8.52	10.39	34.97
Morus alba	5.76	14.22	20.92	3.02	9.53	46.54
Nigelle sativa L	3.75	4.11	13.28	44.67	2.42	31.77
Portulaca oleracea L	4.47	15.04	16.31	15.49	33.37	15.32
Trigonella foenum L	6.57	4.03	26.78	6.35	6.75	49.52

In contradistinction with it content of total carbohydrates that was very large quantity in comparing with the other plants with 91.29%, *Commiphora myrrha* comes the next order after *Aloe perryi* with large difference 71.21%, followed by *Trigonella foenum L* and *Morus alba* 49.52% and 46.54%, and then *Apium graveoleus L* ,*Lupinus termis* and *Nigelle sativa* 38.63%, 34.97% and 31.77% respectively, while *Portulaca oleracea L* was the poorest in carbohydrates 15.32%. In general carbohydrates quantities in the experimental plants was the first class, and proteins represent the second class in plant chemical composition, followed by lipids that ranked in third grade, and the crude fibers which stand in the fourth mark and finally ash which always came in least of chemical materials in all of plants.

4-1-1-2 Mineral compositions of experiment plants

Mineral compositions of experiment plants are shown in Table (10).

Table 10: The mineral compositions of plants

Plant	Ca (ppm)	Na (ppm)	Zn (ppm)	Ag (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)	ASH %
Aloe perryi	3225	1500	25.5	0.00	15	101.25	3.50	1.87
Apium graveoleus L	14000	900	6.5	0.00	16	432.5	5.25	8
Commiphora myrrha	7115.6	1918	7.72	0.11	9.9	68.22	25.05	1.45
Lupinus termis	6525	1053	32.67	0.27	9.18	47.52	4.86	3
Morus alba	3600	800	10	0	11,75	65,5	6,5	2.20
Nigelle sativa L	22000	1225	36.5	0.025	20.75	239.25	5.00	12
Portulaca oleracea L	4000	1450	11.75	0.175	8.75	210.5	4.25	7.1
Trigonella foenum L	16250	1175	19.5	0.125	8.25	85.75	7.00	15.05

A total ash content was 15.05 % as maximum level in *Trigonella foenum L*, while the minimum level was 1.45 % in *Commiphora myrrha*., the limited concentration of Ca was 22000 *ppm* in *Nigelle sativa L. and* 3225 ppm in *Aloe perryi* , and Na was high 1500 *ppm* in *Commiphora myrrha*. and low 800 ppm in *Morus alba*, and Zn concentration ranged from 36.5ppm in *Nigelle sativa L* to 6,5 ppm in *Apium graveoleus L*., Ag was 0,175 *ppm* in *Portulaca oleracea L.* and 0,025 *ppm* in

Nigelle sativa L. And it was disappeared in *Apium graveoleus L., Morus alba, and Aloe perryi* , The major value of Cu 20.75 ppm found in *Nigelle sativa L.* and the minor value 8.25 *ppm* in *Trigonella foenum L.* And Fe was raised in *Apium graveoleus L. 43*2.5 ppm and increased in *Lupinus termis* 47,52 *ppm.* While Pb had the higher concentration 7 *ppm* in *Trigonella foenum L.* and the lower concentration 4.25 *ppm* in *Portulaca oleracea L.*

The concentration of elements in the plants which are analyzed in the study decreases in the following order: Ca, Pb, Na, Cu, Zn, and Ag. The order of plant according their minerals contents is as follows *Nigelle sativa L, Trigonella foenum L, Apium graveoleus L, Commiphora myrrha, Lupinus termis, Portulaca oleracea L, Aloe perryi* and *Morus alba*

4-1-2 phytochemical Study

4-1-2-1 The yield of extraction

The yield of extraction of plant parts in methanol and water were showed in table(11) .After extraction and recovering the solvents from the extracts , the dry matters were weighed to determine the yield of each plants in both of solvent. *Aloe perryi*, gave (82.225 %) of yielded when extracted using methanol, and (53.955%) as water extract. *Commiphora myrrha* gave value of (57.93 %) from the weight of row plants as water extract yielded, while the methanol extract produced was (14.72 %) . The yield of *Lupinus termis* in water produced (33.53 %) from raw material of plant part, and methanol extract yielded was (7.26 %) . The lowest yield was gained in extract of *Portulaca oleracea L* as water extract which was (2.29 %) and the methanol extract was (6.48 %). Therefore we can make order of plants according its yields in both of methanol and water as follows, *Aloe perryi* , *Commiphora myrrha , Lupinus termis,* and *Portulaca oleracea L.* The yield of methanol extracts was higher in *Aloe perryi Portulaca oleracea L., Trigonella foenum L. and Morus alba.* In the other side The yield of methanol extracts was higher *Commiphora myrrha, Nigelle sativa L.* and *Lupinus Termis.*

Table 11: yield of extraction by water and methanol

Extracts / plants	Methanol g/100g	Water g/100g
Aloe perryi	82.225±5.025	53.955±0.06363961
Apium graveoleus L	13.67±1.44	13.6±1.18
Commiphora myrrha	14.726±4.212529921	57.938±7.578678777
Lupinus Termis	7.26±2.06	33.53± 0.694766148
Morus alba	9.3376±1.5443	7.354±3.611
Nigelle sativa L	9.696±0.6853	10.8483±1.84896142
Portulaca oleracea L	6.48±2.38	2.293±1.537
Trigonella foenum L	7.69±0.599437236	5.3983±1.167072548

4-1-2-2 The yield of alkaloid

The results obtained for the total alkaloids in deferent plants were presented in Table (12) in first column. The highest content of total alkaloid appear in *Lupinus termis* (6200.32 mg/100g dw), *Nigelle sativa L* (758.65 mg/100g dw). *Trigonella foenum L* was (755.9 mg/100g dw) and (547.3 mg/100g dw) for *Portulaca oleracea L* , the total alkaloid content in raw *Morus alba* , *Aloe perryi* were (2304.3 mg/100g dw) ,(523.26 mg/100g dw) respectively. The alkaloid is the lowest in *Commiphora myrrha* (1.626 mg/100 g dw).

Table 12: Alkaloids and Tannins Yields of plant samples

Sample of Plants	Alkaloid mg/100g (dry Wight)	Tannin mg/100g (dry Wight)
Aloe perryi	523.26±3.153	1215±15
Apium graveoleus L	164.59±43.99	5216.1±205.35
Commiphora myrrha	1.626±0.122	840.34±109.65
Lupinus termis	6200.32±200.05	810.6±20.50
Morus alba	2304.3±106.61	2422.31±332.45
Nigelle sativa L	758.65±68.8	1340.66±80.03
Portulaca oleracea L	547.3±37.871	260.07±29.88
Trigonella foenum L	755.9±50.501	2032.53±131.95

4-1-2-2 The yield of tannin

The table (12) is showed rustle tannin in column tow. *Apium graveoleus L , Morus alba* and *Trigonella foenum L* have tannin contents of (5216.1mg/100g), (2422.31 mg/100g) and (2032.53 mg/100g) respectively, while the lowest contents was in *Portulaca oleracea, Commiphora myrrha , Aloe perryi* and *Nigelle sativa L* (260.07 mg/100g dw), (840.34 mg/100g dw) , (1215 mg/100g dw) and (1340.66mg/100g dw) respectively

4-1-2-3 Total phenolic and flavonoids

Total phenol compounds, as determined by Folin Ciocalteu method, are reported as Gallic acid equivalents by reference to standard curve ($y = 0.0235x - 0.0906$, $R^2 = 0.9964$) .

Total phenols in methanol extract varied from 129.35 mg/100g dry weight to 10771.7 mg/100g dry weight table (13), plant samples ordered in correspondence their phenolic contents as follows , *Aloe perryi Lupinus Termis, Commiphora myrrha, Nigelle sativa L., Morus alba, Trigonella foenum L., Apium graveoleus L* and *Portulaca oleracea L. Aloe perryi* showed high phenolic value in comparing with the other plants samples. Phenolic composition of water extracts of plant samples were totally lower than that of methanol extracts of these plants with the exception of water extract of *Apium graveoleus L* which was 2549.99±234.16 mg/100g dry weight. Phenolic composition of water extracts were ranged between 17.50 mg/100g dry weight and 7752.3 mg/100g dry weight, the highest value was showed in *Aloe perryi* and the lowest value in *Portulaca oleracea L* in parallel with than in methanol extracts with the exception of water extract of *Apium graveoleus L,* as we mentioned above.

Table 13: Total phenolic and total flavonoids in plant samples

Plants	TPC mg GAE/100g dw		TFC mg CE/100g dw	
	extraction		extraction	
	methanol	water	methanol	water
Aloe perryi	10771.7±776.9	7752.3±309.9	1993.79±13.8	1997.14±239.6
Apium graveoleus L	437.64±41.96	2549.99±234.16	209.96±32.84	317.6±24.72
Commiphora myrrha	1420.55±28.5	211.96±54.4	529.17±8.55	93.91±27.93
Lupinus termis	1729.2±26.6	480.59±14.34	310.43±7.07	78.77±2.69
Morus alba	518.2±54.7	134.33±62.5	199.8±41.2	91.49±69.61
Nigelle sativa L	1028.47±6.57	394.55±12.89	177.64±46.68	83.15±10.7
Portulaca oleracea L	129.35±2.283	17.50±13.1	99.34±51.59	4.92±3.5
Trigonella foenum L	505.53±21.48	115.37±12.01	128.25±3.8	54.94±7.79

TPC : Total phenolic content ,mg equivalents of Gallic acid/100g dry matter. TFC: and total flavonoids content ,mg catechin equivalent (CE) /100g dry matter

The total flavonoid contents are reported as mg catechain equivalent/100g of extract powder, by reference to standard curve ($y = 0.0062x - 0.0026$, $R^2 = 0.9991$) .

Total flavonoids of plant samples are illustrated in table (13) in the second column, total flavonoids in methanol extract were placed between 99.34±51.59 mg/100g dry weight and 1993.79±13.8 mg/100g dry weight, while it ranges between 4.92±3.5 and 1997.14±239.6 mg/100g dry weight in water extracts of plant samples, the highest value were found in *Aloe perryi* and the lowest was in *Portulaca oleracea L.* in both of methanol and water extract for all plants, these results are in accordance with the results of total phenols. The relation between total phenol and total flavonoids in plants is parallel relationship as well as between methanol and water extracts.

4-1-2 determination of antioxidant activity of plants

4-1-2-1 DPPH scavenging assay

The results of DPPH scavenging activities of plant materials of the experiments are illustrated in table(14). The DPPH assay carried out for tow extracts of plant samples, plant samples are ordered according their activity on DPPH in the following order, *Morus alba, Apium graveoleus L., Portulaca oleracea L , Aloe perryi, Commiphora myrrha, Nigelle sativa L., Trigonella foenum L.,. Lupinus termis*.

Table 14: DPPH free radical scavenging activity of medicinal plants(IC_{50} µg/ml)

Plants	Methanol	water
Aloe perryi	133.0195±2.824	9550±1117.22
Apium graveoleus L	83.825±1.039	191.855±3.231
Commiphora myrrha	208.8±17.833	ND
Lupinus termis	2848.975±31.614	1262±67.87
Morus alba	35.355±3.783	81.895±1.534
Nigelle sativa L	406.71±4.610	1064.025±23.369
Portulaca oleracea L	132.445±3.740	168.9165±0.966
Trigonella foenum L	620.955±20.216	296.08±15.202
BHA	71.61±5.39 µg/ml	

In methanol extract, and their grade in DPPH scavenging activity when they extracted by water was, *Morus alba, Portulaca oleracea L., Apium graveoleus L., Trigonella foenum L., Nigelle sativa L,. Lupinus termis, Aloe perryi.* The results posted as IC_{50} so the low values indicate to the high activity, IC_{50} of methanol extract gained the low value 35.355±3.783 µg/ml with *Morus alba* extract and the elevated value was 2848.975±31.614 µg/ml in *Lupinus termis*. And the lowest weight that can scavenging 50% of DPPH in water extract was 81.895±1.534 µg/ml in *Morus alba* too, while the highest weight was 9550±1117.22 µg/ml in *Aloe perryi*. Moreover the activity of *Morus alba* was high not only on comparing

with other plants, but also it was elevated vis-à-vis the synthesized antioxidant BHA where the activity of *Morus alba* was twofold that of BHA. When the activity against DPPH radical for the both of water and methanol extract where compared, it's found that methanol extract was more effective than that of water extract for all plants samples except that of *Trigonella foenum L. and Lupinus termis* where the antioxidant activity of water extract were high than that of methanol extracts of these plants.

4-1-2-2 Determination of ferric reducing power of the extracts

The reducing power activities of plant matters are demonstrated in table (15), as EC_{50}. Methanol extract of *Aloe perryi* revealed high capacity to reduce ferric ions (8.915±0.190 µg/ml) in comparing with the other plants, and also with the standard antioxidant ascorbic acid. Next *Commiphora myrrha* 47.245±1.03 µg/ml came in the second order , after that *Morus alba* take the third order with 49.096±4.375 µg/ml, and then *Portulaca oleracea L* 57.6125±0.150 µg/ml, and in the following class *Apium graveoleus L, Lupinus termis, Nigelle sativa L*, and *Trigonella foenum L.*, with reducing power activities of 109.555±8.704 µg/ml, 200.5±2.078 µg/ml, 218.23±12.699 µg/ml, and 280.68±34.549 µg/ml respectively.

Water extracts of plant samples also showed in table (15), where we can see that *Portulaca oleracea L* have the lowest weight of plant that reduced 50% of ferric ions which was 28.005±0.077 µg/ml, followed by, *Apium graveoleus L* 65.3±0.113 µg/ml, and *Nigelle sativa L* 178.393 ±0.363 µg/ml, *Trigonella foenum L* 231.41±8.711 µg/ml , *Morus alba* 258.855 ±19.339 µg/ml in third to 6th orders, while *Lupinus termis* came in the 7th with 366.65 ±3.747 µg/ml, and finally *Aloe perryi* and *Commiphora myrrha* 653.6875 ±4.507 µg/ml and 1220.25 ±0.707 µg/ml placed in the tow last degrees in contrast that their methanol extract which showed high reducing power activities.

Table 15: values of concentration EC_{50} of extracts of plant (µg/ml)

Plants	Methanol extracts	Water extract
Aloe perryi	8.915±0.190	653.6875±4.507
Apium graveoleus L	109.555±8.704	65.3±0.113
Commiphora myrrha	47.245±1.039	1220.25±0.707
Lupinus termis	200.5±2.078	366.65±3.747
Morus alba	49.096±4.375	258.855±19.339
Nigelle sativa L	218.23±12.699	178.393±0.363
Portulaca oleracea L	57.6125±0.150	28.005±0.077
Trigonella foenum L	280.68±34.549	231.41±8.711
Ascorbic acid	3.5±0.654	

4-2 Experiment 2

4-2-1 Result of phytochemical structure

4-2-1-1 The yield of extraction

Table (16) the results of yields of the plants of study with using the five solvents water, methanol, ethanol, ethyl acetate, and hexane, the yields are expressed as g/100 g of dray weight of plant materials, the results reveal the solubility of plant contents in the various solvent to provide idea about the characteristics of these contents, and its quantities in each solvent for each plant in order to therefore photochemical analysis and the determination of their antioxidant activities, as in the table (16), ethanol, showed the highest capability to solve the composites of *Aloe perryi* where the yield was 88.045±0.700 g/100 g, and methanol come in next with yield 82.225±7.106 g/100 g of dry weight, and water also exhibited good yield in the 3rd order. And then ethyl acetate with little degree yielded 17.085±8.464 g/100 g, while hexane was poor in yield of *Aloe perryi.* For *Portulaca oleracea L* also ethanol extract gave high quantity of yield 15.0275±0.880 g/100 g of dry weight, while ethyl acetate, methanol, hexane

surpassed water in their yields that gain in this plant, where the lowest yield of water extract was only 2.293±2.173 g/100 g of dry weight, similar with that of hexane with *Aloe perryi*. But water outmatched all the other solvent with *Morus alba* where its yield was 13.0775±0.449 g/100 g of dry weight. Followed by methanol, ethanol, ethyl acetate, and hexane which take the last degree 9.337±2.1840 g/100 g, 4.41±0.205 g/100 g, 3.435±1.294 g/100 g, 0.6191±0.107 g/100 g of dry weight respectively. In *Apium graveoleus L.*, ethyl acetate yielded the high percent 17.3025±8.925 g/100 g, and ethanol show up good yield 6.8165±6.813 g/100 g, whereas both of water and methanol give similar rates13.6±5.33193 g/100 g of dry weight and 13.67±5.111 g/100 g of dry weight respectively, the yield of Hexane arise with these plant to near from the other solvents 12.95±4.585 g/100 g of dry weight.

Table 16: yield of sample plants in different solvent

plants	Extracts	Yield %
Aloe perryi	Water	53.955±0.063
	Methanol	82.225±7.106
	Ethanol	88.045±0.700
	Ethyl acetate	17.085±8.464
	Hexane	2.805±0.431
Apium graveoleus L	Water	13.6±5.33193
	Methanol	13.67±5.111
	Ethanol	16.8165±6.813
	Ethyl acetate	17.3025±8.925
	Hexane	12.95±4.585
Morus alba	Water	13.0775±0.449
	Methanol	9.337±2.1840
	Ethanol	4.41±0.205
	Ethyl acetate	3.435±1.294
	Hexane	0.6191±0.107
Portulaca oleracea L	Water	2.293±2.173
	Methanol	6.48±3.365
	Ethanol	15.0275±0.880
	Ethyl acetate	13.5375±4.818
	Hexane	4.0325±0.286

The results of phytochemical analysis of plant samples (total phenolic compounds TPC and total flavonoids compounds TFC) are given in the table (17), for various solvents, ethanol extract of *Aloe perryi* yielded high quantity of total phenolic compounds arrived to 20184.16±1120.082 mg GAE /100 g of dry weight, whereas

methanol extract was approximately the half quantity, water take the third class with 7752.38±309.946 mg GAE /100 g of dry weight, followed by ethyl acetate with 1905.90±217.655 mg GAE /100 g of dry weight, and hexane was the lowermost of phenolic compounds with 40.07±11.284 mg GAE /100 g of dry weight. But total flavonoids in water was the uppermost quantity followed by ethanol and then methanol 1997.14±239.691 mg CE/100g, 1984.12±663.97 mg CE /100g, and 1993.79±13.838 mg CE /100 g of dry weight respectively. *Aloe perryi* showed a high amounts of both of total phenolic compounds TPC and total flavonoids compounds TFC in all of used solvents comparing with the other plants of experiment. The classification of the extracts of *Portulaca oleracea L,* according their contains of both of total phenolic compounds (TPC) and total flavonoids compounds (TFC) is ethanol, hexane, ethyl acetate, water and methanol, all extracts of *Portulaca oleracea L* were showed low amounts of phenol and flavonoids in compare *Aloe perryi.* And it they can ordered for *Morus alba* as follows, methanol, water, ethanol, ethyl acetate and then hexane, the maximum phenolic compounds was 518.26 mg GAE/100g and the minimum was 88.107 mg GAE /100g, the order is the same for flavonoids with 199.8 mg CE/100g as a maximum with methanol extract and 23.18 mg CE/ 100g as a minimum values in hexane extract. Water extract gave high quantity if phenols and flavonoids for *Apium graveoleus L,* followed by methanol, ethyl acetate, ethanol, and finally hexane. Generally the contents of phenols and flavonoids in *Apium graveoleus L* were high in all of the used solvents in comparing with the other plants in this study, and it came in second class after *Aloe perryi*.

Table 17: Total phenolic content(TPC) and total flavonoids content(TFC) of plant samples in different solvent

Plants	Extracts	TPC mg GAE/100g (dry matter)	TFC mg CE/100g (dry matter)
Aloe perryi	Water	7752.38±309.946	1997.14±239.691
	Methanol	10771.78±776.955	1993.79±13.838
	Ethanol	20184.16±1120.082	1984.12±663.971
	Ethyl acetate	1905.90±217.655	239.148±35.802
	Hexane	40.07±11.284	43.442623±66.210
Apium graveoleus L	Water	2549.9±234.16	590.3±54.34
	Methanol	437.6±41.967	209.9±32.8
	Ethanol	245.7±1.162	120.08±11.55
	Ethyl acetate	388.16±32.201	312.8±25.427
	Hexane	166.1±44.27	46.34±36
Morus alba	Water	134.33±62.598	91.49±69.61
	Methanol	518.26±54.724	199.8±41.208
	Ethanol	183.1±43.003	155.7±17.354
	Ethyl acetate	102.5±86.899	65.18±30.209
	Hexane	88.107±4.767	23.183±2.194
Portulaca oleracea L	Water	17.507±13.127	4.920 ±1.517
	Methanol	129.3581±2.283764	99.34±51.59
	Ethanol	932.8±172.309	78.29815±4.928
	Ethyl acetate	107.51±27.844	55.37±11.633
	Hexane	183.8±29.347	73.54±3.435

TPC : Total phenolic content ,mg equivalents of Gallic acid/100g dry matter. TFC: and total flavonoids content ,mg catechin equivalent (CE) /100g dry matter

4-2-2 determination of antioxidant activity of plants

4-2-2-1 DPPH scavenging assay

The results of antioxidant activity of plant samples of study are given in table (18), the results are expressed as the weight of plant samples that extracted by the five solvent which scavenging amount of 50% of DPPH radical (IC_{50} µg/ml), as in the table, the ethyl acetate extract of *aloe perryi* exhibited more aptitude to scavenging DPPH radicals with little bulk of extracted compounds 5.34 µg/ml, ethanol and methanol extracts of *Aloe perryi* also scavenging DPPH radicals with relatively little masses 66.57 and 133.0195 µg/ml respectively, while the weight of the

extracts of hexane and water of *Aloe perryi* that were necessary to reduce 50% of DPPH molecules in the reaction medias were getting up attained to 5460.54 µg/ml and 9550 µg/ml correspondingly.

According to the extracts of *Portulaca oleracea L.*, ethyl acetate extract in contrasts to its affective in the state of *Aloe perryi*, its activity against DPPH here was weak where it was needed to grand quantity (3660.43 µg/ml) to reduce 50% of DPPH radicals, the same results were happened with ethanol which also needed 2655.6 µg/ml to reduce 50% of DPPH radicals. And similar behavior was displayed by hexane extract where the quantity of this extract which consumed to reduce the 50% of DPPH were increased to reach 9553.54 µg/ml. And also oppositions were occurred with water extract of *portulica oleracea L* from *Aloe perryi* but in this time for the positive side where water extract here exposed great effectiveness in DPPH radical scavenging with IC_{50} 168.9165 µg/ml. While methanol extract were maintained its activity with both of *Aloe perryi* and *portulica oleracea L*.

Very good results obtained from both of *Morus alba* and *Apium graveoleus L* in all of solvents that used in their extracting, with the exception of hexane extracts of these two plants, wherever the reduction of DPPH radicals were achieved by little masses of the extracts of these plant ranged between 35.355 and 257.567 µg/ml, in particularly *Morus alba* which were sometimes but often preferable more from that of the synthesized standard antioxidant BHA. generally, according the present results *Morus alba* considered the best antioxidant against DPPH radicals in all solvents comparing with the other plants in the study.

Table 18: DPPH free radical scavenging activity of medicinal plants in different solvent (IC_{50} µg/ml)

plants	solvent	IC_{50} µg/ml± SD
Aloe perryi	Water	9550±1117.22
	Methanol	133.0195±2.824
	Ethanol	66.57±4.242640687
	Ethyl acetate	5.34±0.657
	Hexane	5460.54±223.54
Apium graveoleus L	Water	191.855±3.231
	Methanol	83.825±1.039
	Ethanol	257.567±4.84
	Ethyl acetate	101.415±9.53
	Hexane	5500±28
Morus alba	Water	81.895±1.534
	Methanol	35.355±3.783
	Ethanol	64.79±3.43
	Ethyl acetate	168.8±4.9205
	Hexane	4869.18±1131.82
Portulaca oleracea L	Water	168.9165±0.966
	Methanol	132.445±3.740
	Ethanol	2655.6±315,87
	Ethyl acetate	3660.43±453.21
	Hexane	9553.54±507.54
BHA	71.61±5.39 µg/ml	

The results are the mean ± SD

Concentrations in water and four different organic solvents, ethanol, methanol, ethyl acetate, and hexane. The results reveals that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

The results of the Scavenging % against DPPH radicals of *Aloe perryi* at several concentrations in water and four different organic solvents, ethanol, methanol, Ethyl acetate, and hexane are exposed in Figure (33) The results reveals that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

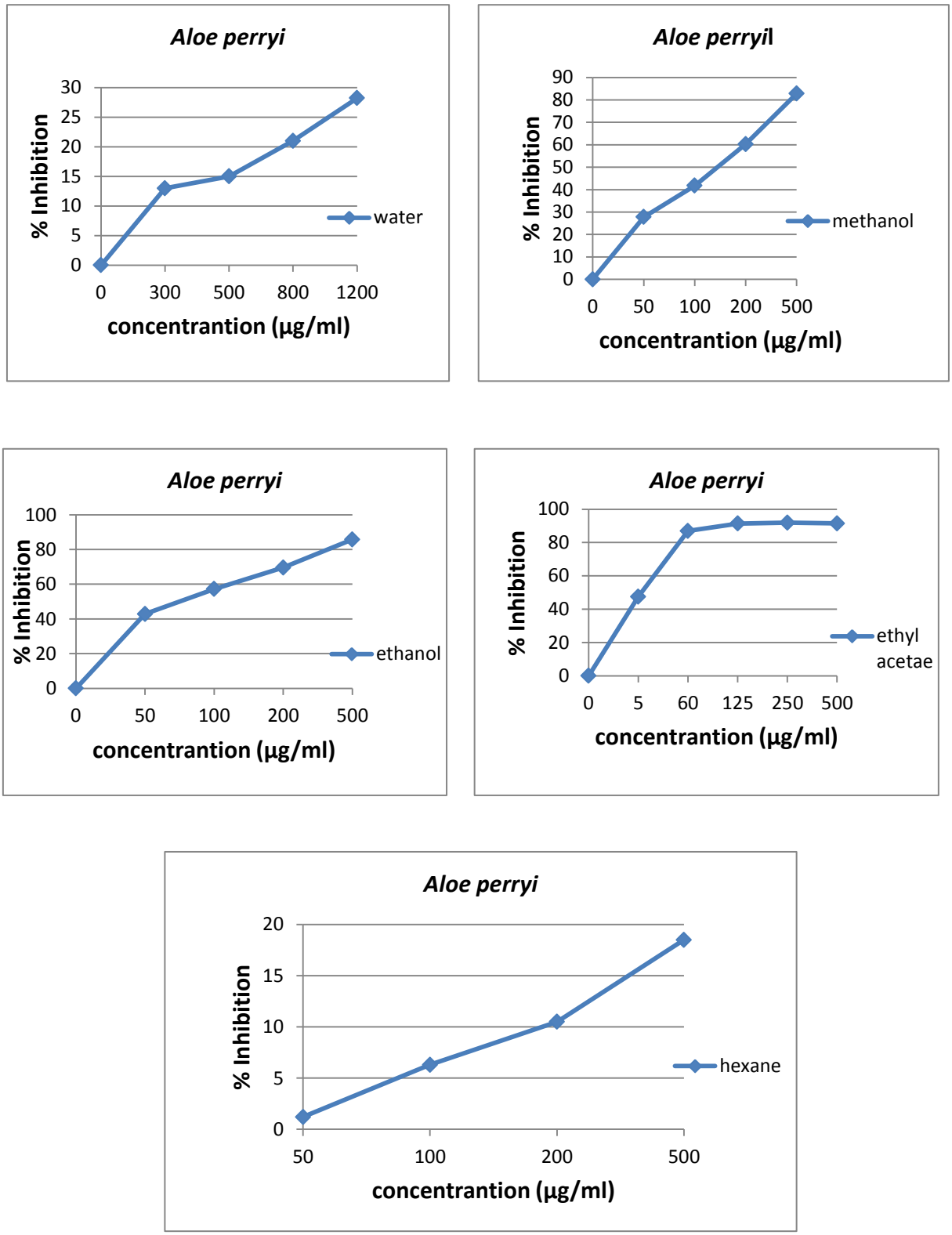

Figure 32: DPPH free radical scavenging activities of extracts of *Aloe perryi*

The results of the Scavenging % against DPPH radicals of *Portulaca oleracea L* at several concentrations in water and four different organic solvents, ethanol, methanol, Ethyl acetate, and hexane are exposed in Figure (34) The results reveals that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

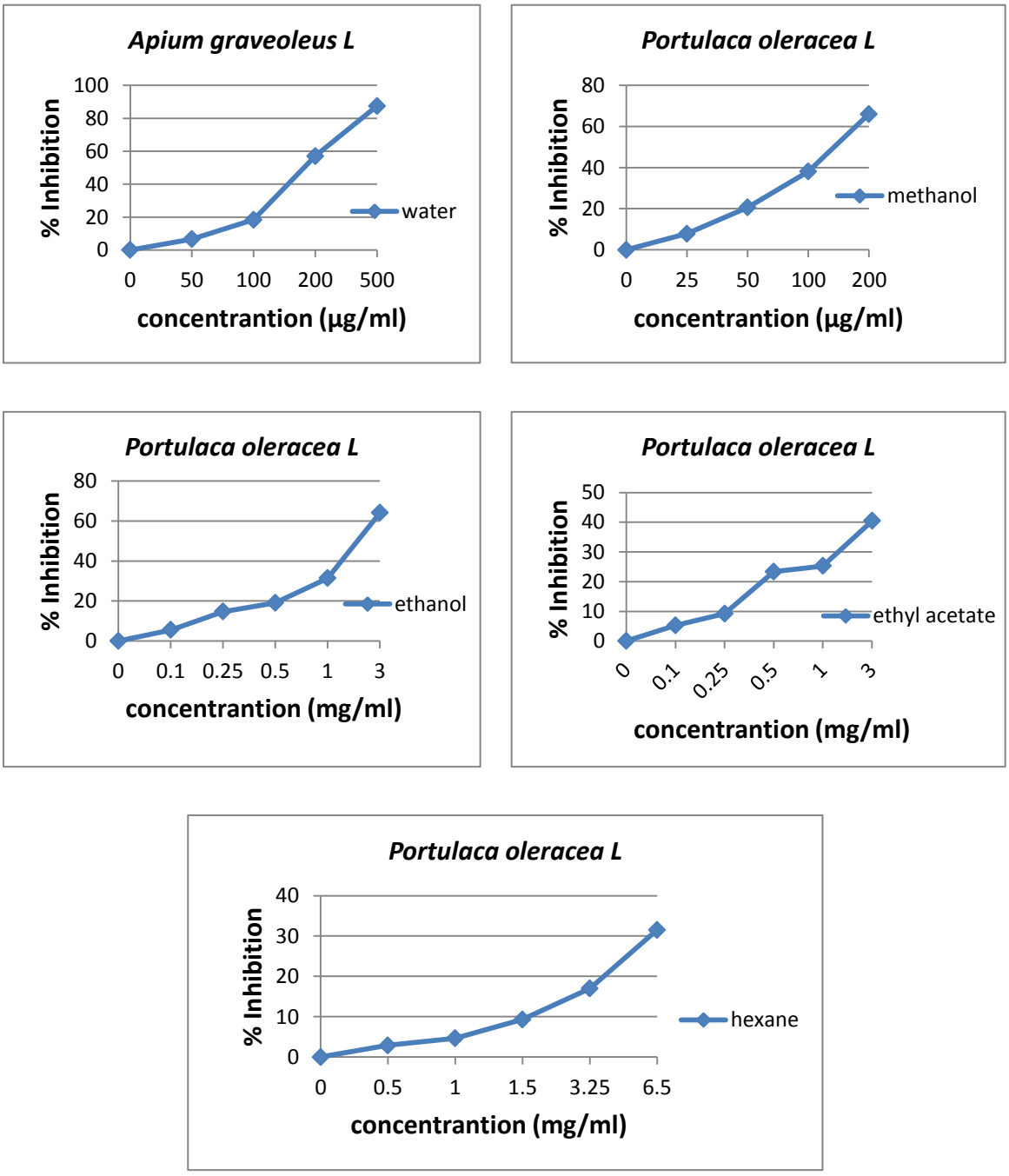

Figure 33: DPPH free radical scavenging activities of extracts of *Portulaca oleracea L*

The Scavenging activity % of DPPH radicals by the water and the four different organic solvents, ethanol, methanol, ethyl acetate, and hexane extracts, of *Morus alba*. at the different concentrations are explained in Figure (35). These results reveal that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's

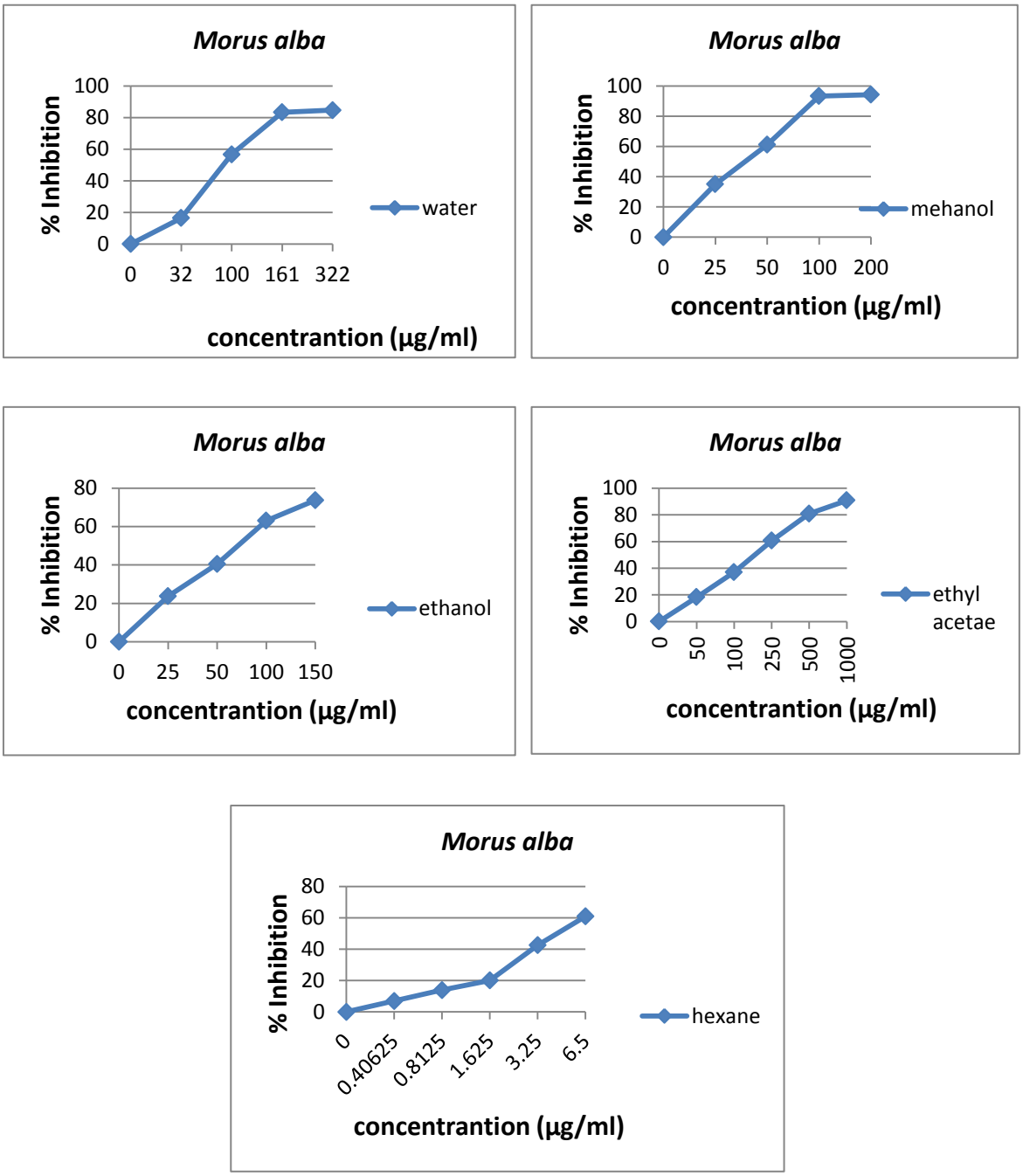

Figure 34: DPPH free radical scavenging activities of extracts of *Morus alba*

. The Scavenging activity % of DPPH radicals by the water and the four different organic solvents, ethanol, methanol, ethyl acetate, and hexane extracts, of *Apium graveoleus* at the different concentrations are explained in Figure (36). These results reveal that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

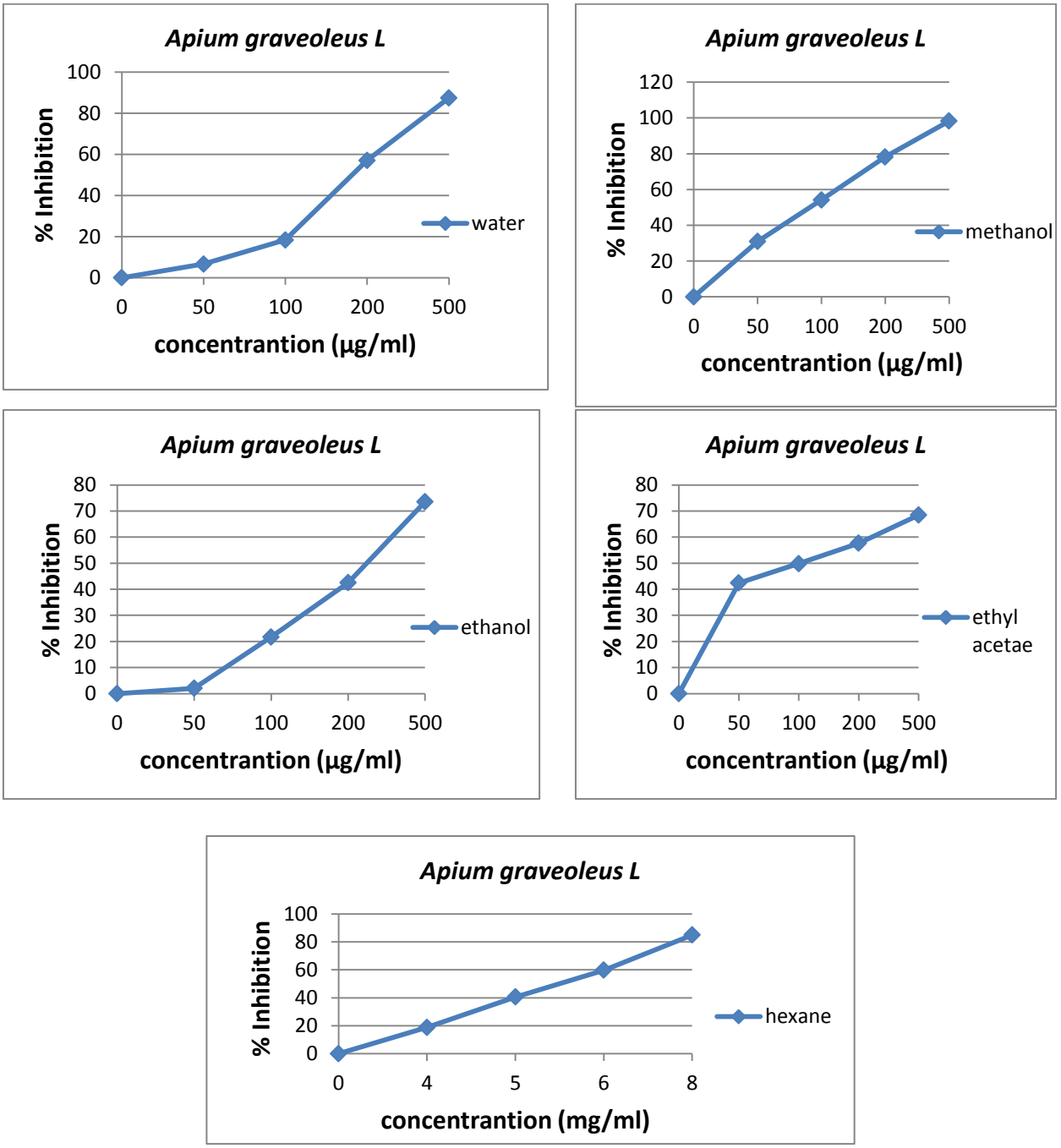

Figure 35: DPPH free radical scavenging activities of extracts of *Apium graveoleus*

4-2-2-2 Reducing power activity of plant samples

In the table (19) the results of reducing power of plant extracts of the different solvents, which give their abilities to reduce iron ions in alkaline media, reducing power are expressed as the weight of plants samples that can reduce 50% of ferric ions to ferrous ions (EC_{50}), as we can see in the table the results of *Aloe perryi* showed that ethyl acetate exhibited high capability in ferric ion reducing where a very small quantity (3.273 µg/ml) was capable to reduce ferric ions, in the second methanol was placed with weight of (8.915 µg/ml), while ethanol was the third with (26.3095 µg/ml), and for first time hexane extract has the antecedence from water extract while the weight of water extract was three fold that of hexane extract 653.6875 and 292.174 µg/ml respectively. Water extract with a opposes of its result with *Aloe perryi* its reducing power with *Portuleca oleracea L* was very high, where the weight that reduce 50% of iron ions was the smallest 28.005 µg/ml, followed by methanol, ethanol, ethyl acetate and finally hexane with progressive order, but in general the reducing power of the extracts of *Portuleca oleracea L.* were high in all of solvent more than that of *Aloe perryi. Morus alba* extracts were in the meddle in their reducing power activity between *Portuleca oleracea L,* and *Aloe perryi.* Hexane extract take the second order after ethanol and methanol came the third and ethyl acetate the fourth while water extract was the last. For the extracts of *Apium graveoleus L.* ethyl acetate was take the first class this once with weigh of 34.85 µg/ml, and the second extract was water extract with 56.3 µg/ml, and then methanol, ethanol and hexane respectively.

Table 19: values of concentration EC_{50} of extracts of plants (µg/ml)

Samples	Solvent	EC_{50}± SD
Aloe perryi	Water	653.6875±4.507
	Methanol	8.915±0.1909
	Ethanol	26.3095±0.928
	Ethyl acetate	3.273±0.4935
	Hexane	292.174±14.405
Apium graveoleus L	Water	65.3±0.1131
	Methanol	109.555±8.704
	Ethanol	111.835±3.174
	Ethyl acetate	34.85±1.626
	Hexane	191.13±4.532
Morus alba	Water	258.855±19.339
	Methanol	49.096±4.375
	Ethanol	22.713±0.0042
	Ethyl acetate	87.36±3.125
	Hexane	25.88±3.436
Portulaca oleracea L	Water	28.005±0.077
	Methanol	57.6125±0.150
	Ethanol	76.110±94.935
	Ethyl acetate	120.85±2.559
	Hexane	143.24±8.980
Ascorbic acid	3.5±0.654µg/ml	

The reducing power at 700_{nm} of the water and the four different organic solvents, ethanol, methanol, ethyl acetate, and hexane extracts, of *Aloe perryi* at the different concentrations are explained in Figure (36). These results reveal that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

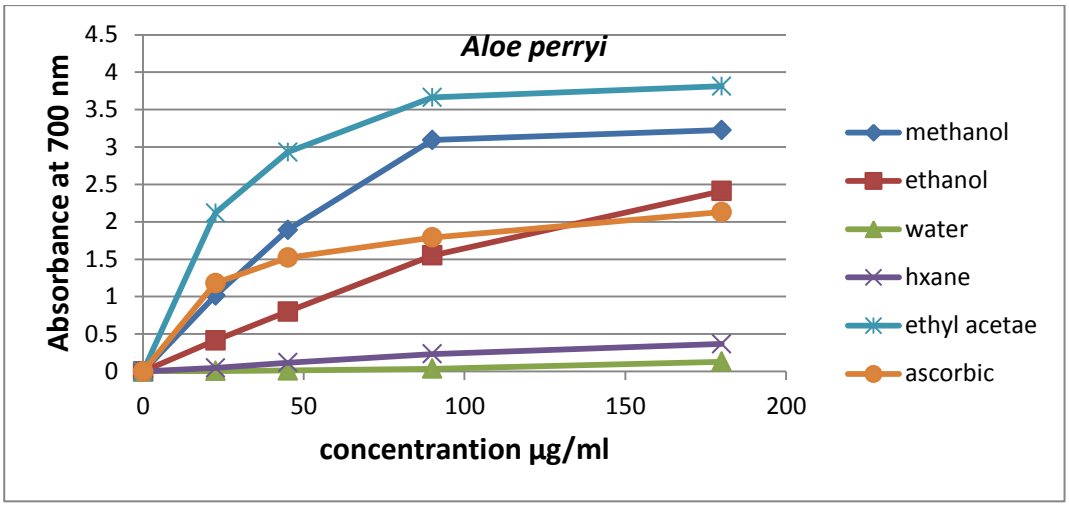

Figure 36. Reducing powers of methanol, ethanol , water ,hexane and ethyl acetate extracts of *Aloe perryi*.

Figure (37) illustrate the antioxidant activity as reducing power expressed by the absorbance at 700 $_{nm}$ of *Portulaca oleracea L*, at several concentrations in water and four different organic solvents, Ethanol, methanol, Ethyl acetate, and hexane. The results reveals that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

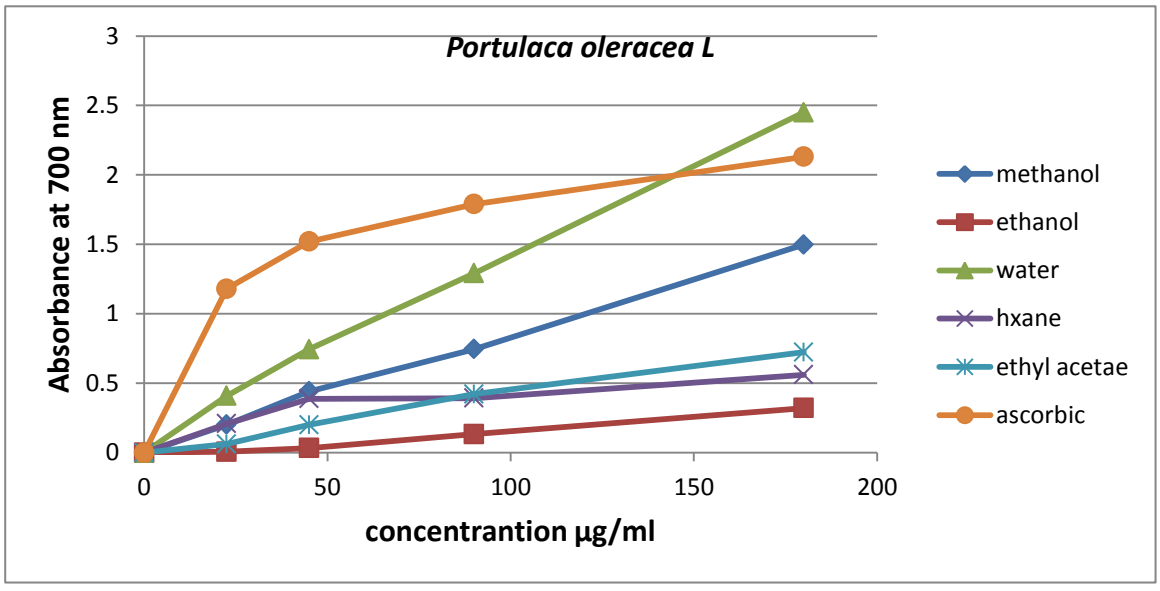

Figure 37: Reducing powers of methanol, ethanol , water ,hexane and ethyl acetate extracts of *Portulaca oleracea L*

The reducing power activity of the water and the four different organic solvents, ethanol, methanol, ethyl acetate, and hexane extracts, of *Morus alba* at the different concentrations of ferric ions are explained in Figure (38). These results reveal that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

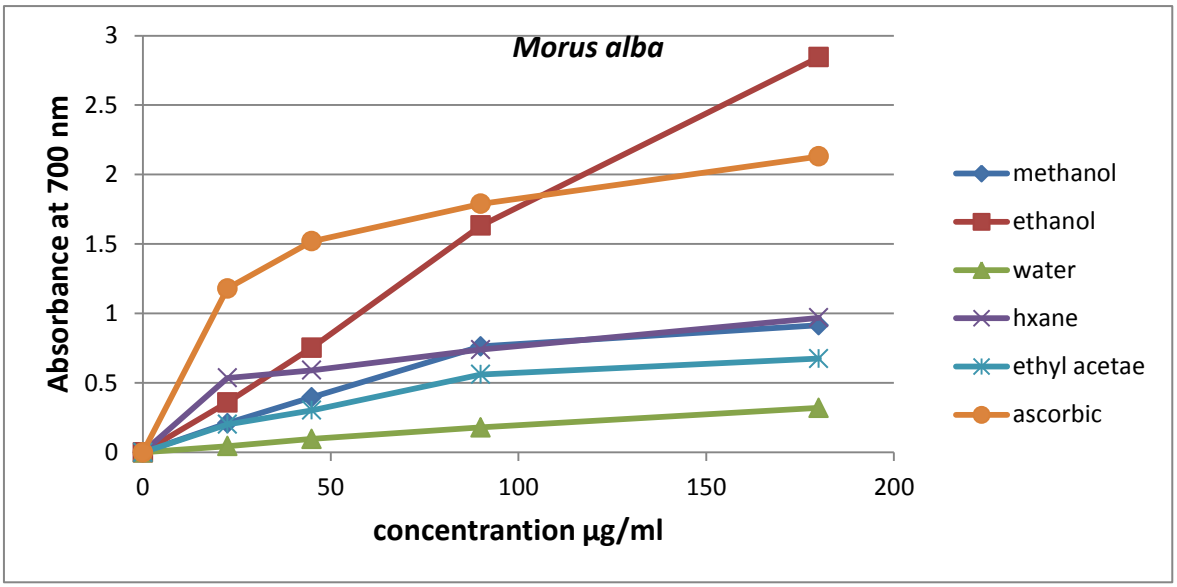

Figure 38: Reducing powers of methanol, ethanol , water ,hexane and ethyl acetate extracts of *Morus alba*

Figure (39) illustrate the antioxidant activity as reducing power expressed by the absorbance at 700 nm of *Apium graveoleus L*, at several concentrations in water and four different organic solvents, ethanol, methanol, ethyl acetate, and hexane. The results reveals that the antioxidant activity were increased as the concentration of the extracts of plant samples were increasing in all of solvent used in this study, with variant of concentrations activity between the extract each other's.

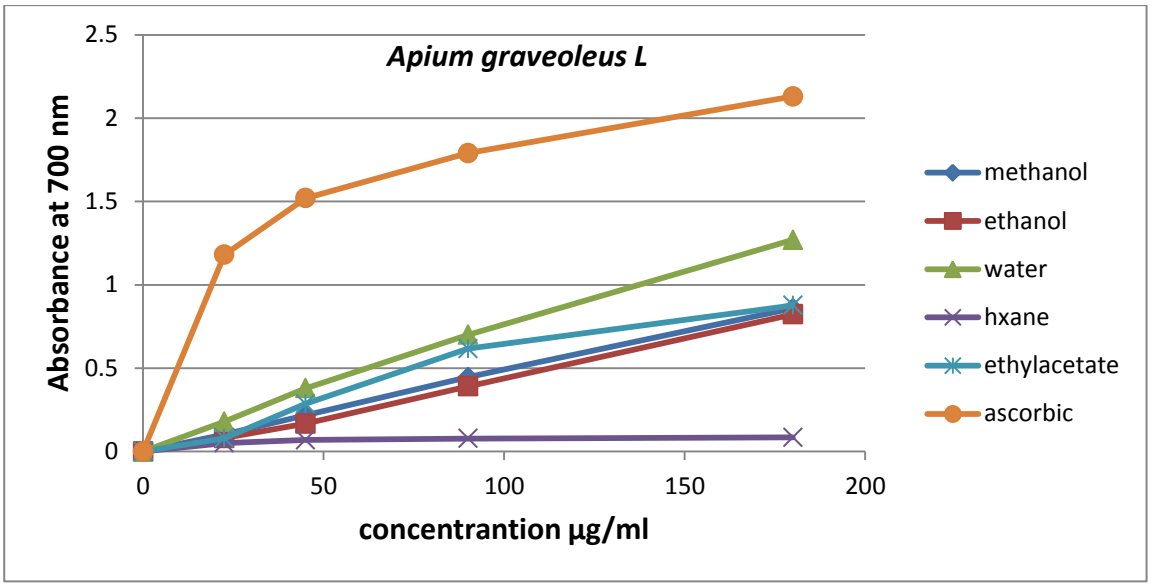

Figure 39: Reducing powers of methanol, ethanol , water ,hexane and ethyl acetate extracts of *Apium graveoleus L*

4-2-2-3 Scavenging of hydrogen peroxide

Table (20) gives the results hydrogen peroxide scavenging activities of plant extracts, hydrogen peroxide scavenging expressed as the mass of sample extract µg/ml that scavenge 50% of H_2O_2, ethyl acetate extract of *Aloe perryi* displayed excellent activity against hydrogen peroxide, while the mass of this extract that react with 50% of H_2O_2 molecular was only 57.475 µg/ml, in the next methanol were came, and after that ethanol and water and the end hexane. In *Portulaca oleracea L*, ethanol has the priority even of on BHA, where the weight that scavenging H_2O_2 were less than that of BHA with 14.06 µg/ml and ethyl acetate came in the second order with 43.28 µg/ml, and the third was methanol with 46.46 µg/ml, and then water extract with 100 µg/ml, whereas hexane didn't exhibit any activity against H_2O_2. The mass that degraded H_2O_2 by the extracts of *Morus alba* were ranged between 19.06 µg/ml of ethanol extract and 459.28 µg/ml of hexane, the activity of *Apium graveoleus L* against H_2O_2 were very high with of solvent extracts wile it were ranged between 38µg/ml for ethanol and 74.9 µg/ml for hexane extract which are small comparing with the other plants and near from the synthesized standard antioxidant BHA.

Table 20: H_2O_2 Scavenging by different solvent of extract plants, IC_{50} (µg/ml)

plants	solvent	IC_{50}± SD
Aloe perryi	Water	134.9075±41.08643952
	Methanol	72.455±14.95530842
	Ethanol	80.105±3.726452737
	Ethyl acetate	57.475±4.702260095
	Hexane	372.155±32.46
Apium graveoleus L	Water	65.035±4.744
	Methanol	60.49±1.725
	Ethanol	38.38±11.15
	Ethyl acetate	64.055±1.887975106
	Hexane	74.09±1.265
Morus alba	Water	92.575±9.270169901
	Methanol	82.865±2.202637623
	Ethanol	19.06±0.862670273
	Ethyl acetate	41.06±5.982123369
	Hexane	459.28±12.54
Portulaca oleracea L	Water	100.985±13.38553137
	Methanol	46.4675±2.839033726
	Ethanol	10.92±3.592102448
	Ethyl acetate	43.285±3.754737008
	Hexane	Ne
BHA	14.06	

Figure (40) illustrate the H_2O_2 scavenging activity of *Aloe perryi*, the results recorded high activity for ethyl acetate extract followed be methanol, ethanol, and then hexane extract, while water extract showed the lowest activity, the statistical analysis showed that the mean difference is significant at the $P \leq 0.05$ level for all of the extracts, between groups and also within groups, the high significant was showed for ethyl acetate extract while the lowest significant was recorded for water extract according to the post hoc multiple comparisons LSD tests.

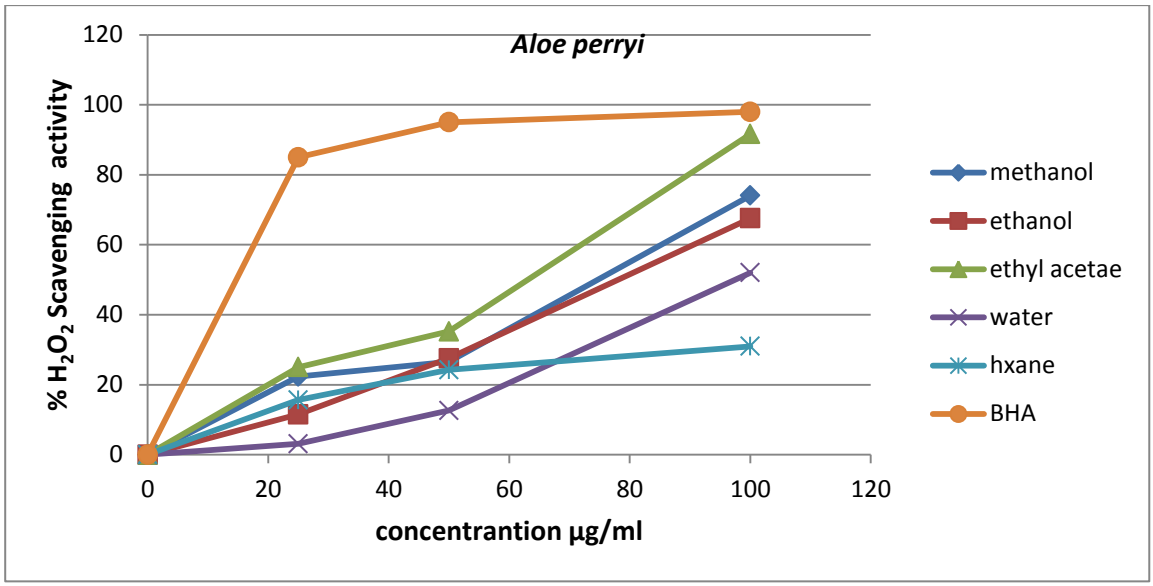

Figure 40: Hydrogen peroxide scavenging activity of different extracts of *Aloe perryi*

In figure (41) the results of the activity of *Apium graveoleus*, to scavenging H_2O_2 were exemplified, as it noted for both of *Portulaca oleracea* and *Morus alba*, ethanol extract showed the highest activity, but the different between ethanol extract of *Apium graveoleus* and the previous plants, was appeared at 100 µg/ml concentration where ethanol her was lowest comparing with the other extracts. Other different appeared in the activity of *Apium graveoleus* from the previous plants which are the activity of hexane activity of this plant which were higher than that of methanol, water and ethyl acetate extracts at the first tow concentrations. The highest activity at the highest concentration were recorded for methanol extract followed by ethyl acetate and then ethanol extract. A significant differences were obtained showed for all solvent used to extraction at $P \leq 0.05$ by ANOVA test, and the post hoc multiple comparisons LSD test showed significant difference between all concentrations for all extracts.

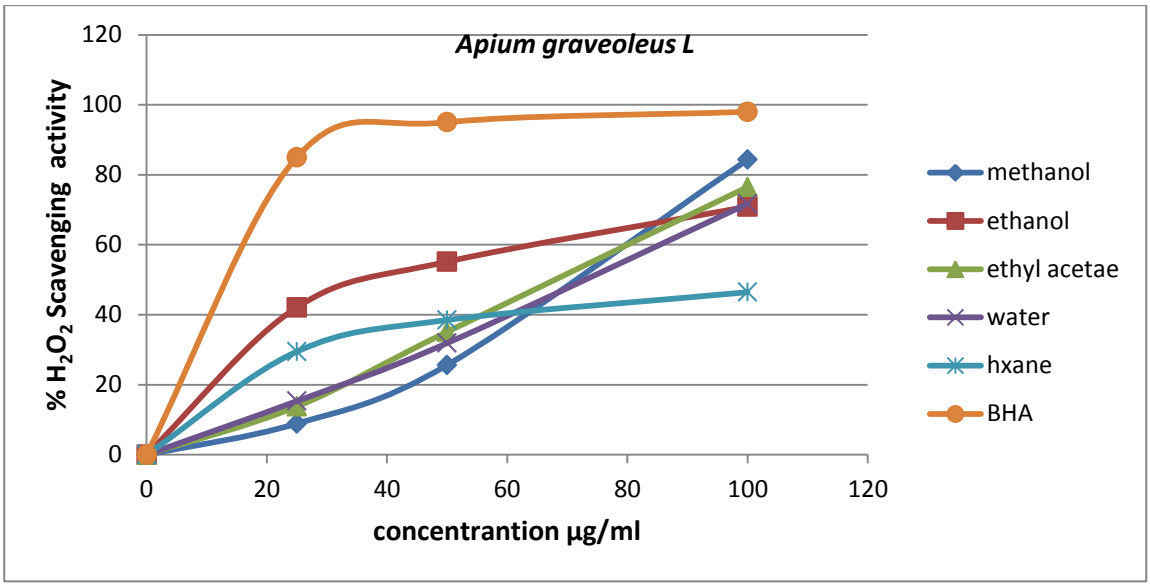

Figure 41: Hydrogen peroxide scavenging activity of different extracts of *Apium graveoleus*

By seeing the results in the figure (42), that exposing the activities of several extracts that applied to study *Morus alba* activity against H_2O_2 radical, it is clear that ethanol extract had appeared to be the best scavenging agent for H_2O_2, comparing with the other extracts of the same plant and even when compared with BHA and the other plants in the experiment. Ethyl acetate extract of *Morus alba* was the next in its activity against H_2O_2 radical, and both of methanol and water extracts were similar in their activity, while hexane extract of *Morus alba* present the lowest activity. The statistical analysis of *Morus alba* showed that the mean difference is significant at the $P \leq 0.05$ level for all of the extracts, between groups and also within groups, the high significant was showed for ethanol extract while the lowest significant was recorded for hexane extract according to the post hoc multiple comparisons LSD tests.

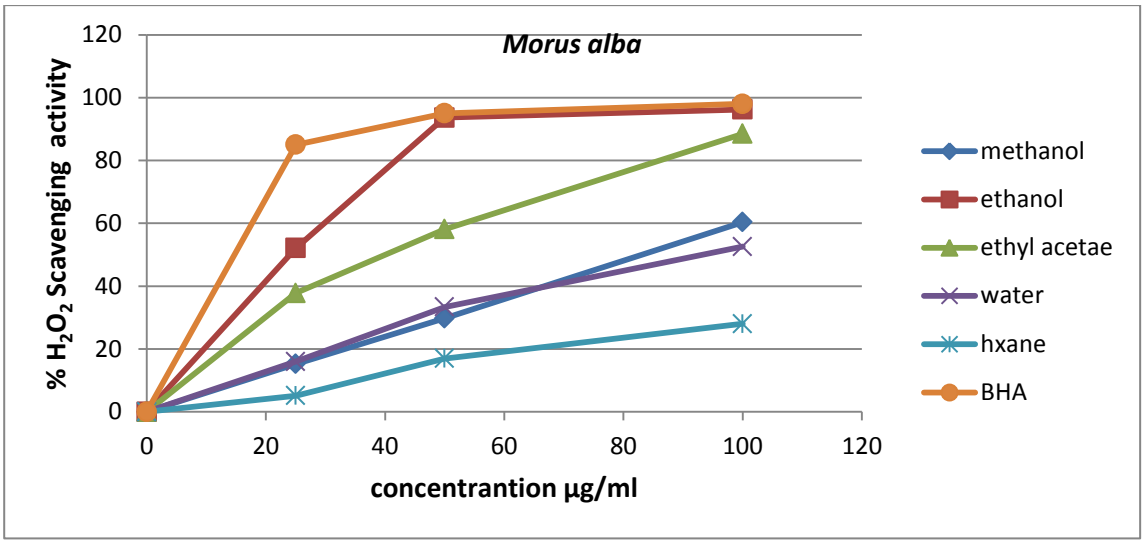

Figure 42: Hydrogen peroxide scavenging activity of different extracts of *Morus alba*

The results of H_2O_2 scavenging activities of the different extracts of *Portulaca oleracea* are demonstrated in figure (43), these results showed that the ethanol extract gave the major activity where its activity were similar to that of BHA methanol and ethyl acetate extracts were showed similar activity were they placed in the second class after ethanol extract, while water extract gave similar result of this extract of *Aloe perryi* in the last order, and no effects were shown for hexane extract at all concentrations. ANOVA test showed significant differences for all solvent used to extraction at $P \leq 0.05$, the post hoc multiple comparisons LSD test showed significant difference between all concentrations for all extracts.

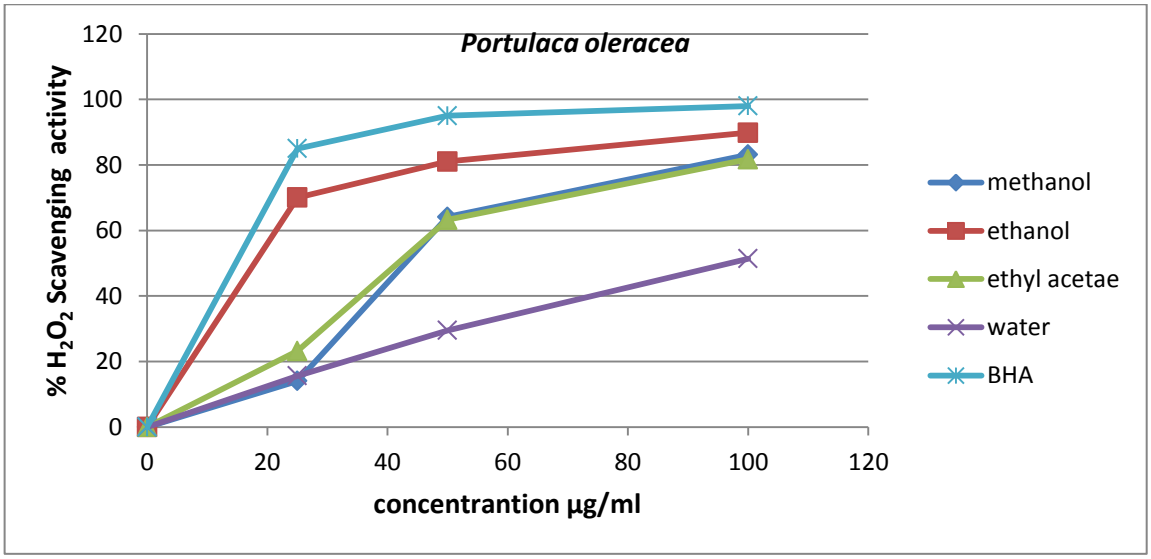

Figure 43: Hydrogen peroxide scavenging activity of different extracts of *Portulaca oleracea*

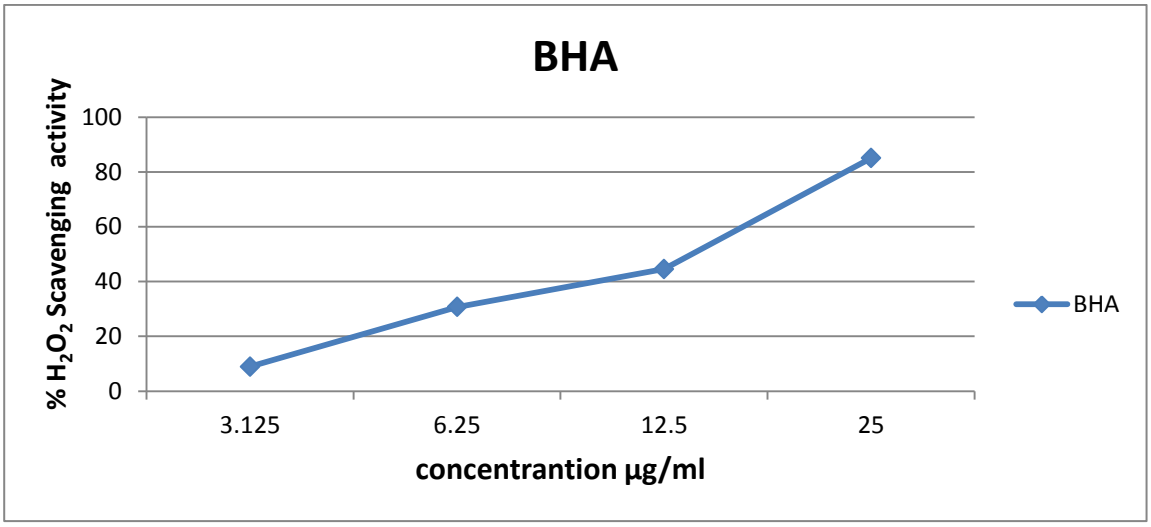

Figure 44: Hydrogen peroxide scavenging activity of different concentration of BHA

4-2-2-4 Determination of total antioxidant capacity

The total antioxidant capacity results of *Aloe perryi* are illustrated in figure (45),total antioxidant capacity are exposed as mg tocopherol/g.dw. the largest quantity was gained by ethanol extract, followed by methanol, water, ethyl acetate and hexane extract as the lowest quantity. *Aloe perryi* extracts of each of ethanol methanol, water, ethyl acetate and hexane was showed significant difference from each of *Portulaca oleracea L* , *Morus alba* and *Apium graveoleus L*, with the exceptions of the extract of *Aloe peryyi* of ethyl acetate with *Apium graveoleus L* and the extract of *Aloe peryyi* with *Morus alba*.

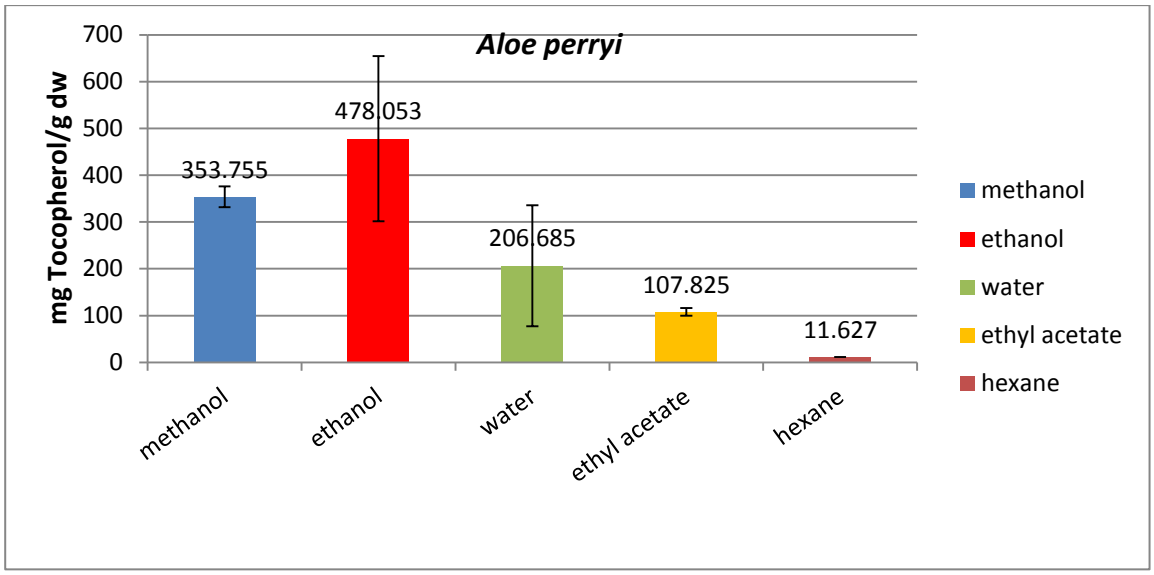

Figure 45: Total antioxidant capacity of different extracts of *Aloe perryi*

Figure (46) demonstrated the total antioxidant capacity of *Apium graveoleus*, the graph showed increasing of total antioxidant capacity of ethyl acetate as the larger value, in the second order, water extract, and the third extract was methanol extract, while hexane came in fourth order and at least ethanol extract. The statistical analysis of *Apium graveoleus*, showed significant differences between the methanol extract of *Apium graveoleus* and all of other plants, and between methanol extract *Apium graveoleus* and *Aloe perryi*, and also between its extract of ethyl acetate with both of *Portulaca oleracea L* and *Morus alba*. While it differed significantly only from water extract of *Aloe perryi* and hexane extract of *Portulaca oleracea L* at level $P \leq 0.05$.

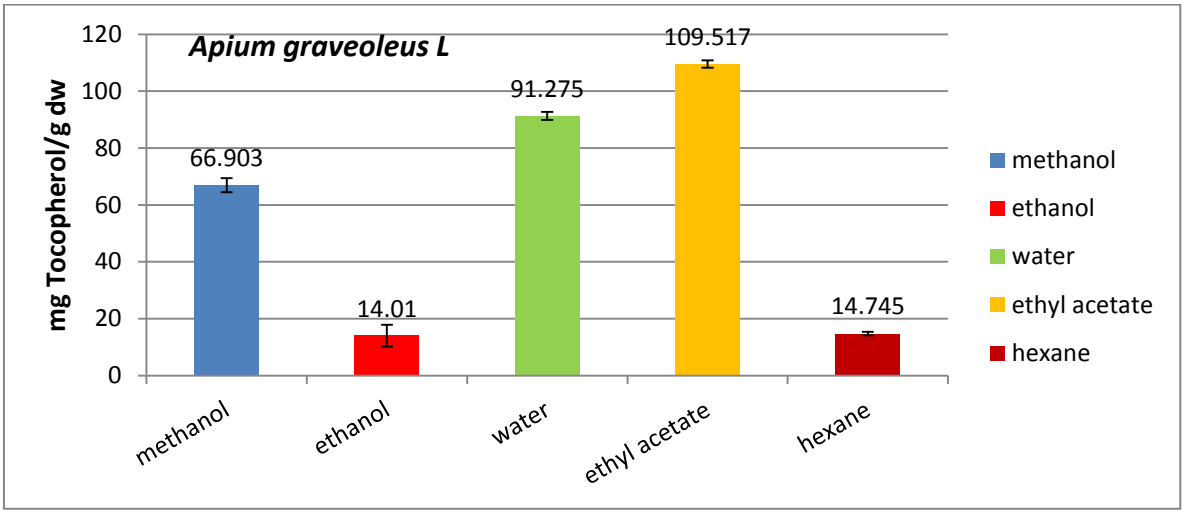

Figure 46: Total antioxidant capacity of different extracts of *Apium graveoleus*

Total antioxidant capacity of *Morus alba* were exposed in the figure (47), the results revealed increasing of water extract capacity of this plant with the contrast of the other plants where water extract was low in total antioxidant capacity, methanol extract was placed in next class, and ethyl acetate in the third class and the fourth and fifth were ethanol and hexane respectively. When we analyzed this data statistically, it were found that *Morus alba* methanol extract was varied significantly from methanol extract of *Aloe perryi* and celery, and ethanol extract was differed from ethanol extract only of *Aloe perryi*, while ethyl acetate of *Morus alba* showed significant different in comparing with ethyl acetate of *Aloe perryi* and *Apium graveoleus*, and with water extract of *Aloe perryi* and hexane extract of *Portulaca oleracea L* at level $P \leq 0.05$.

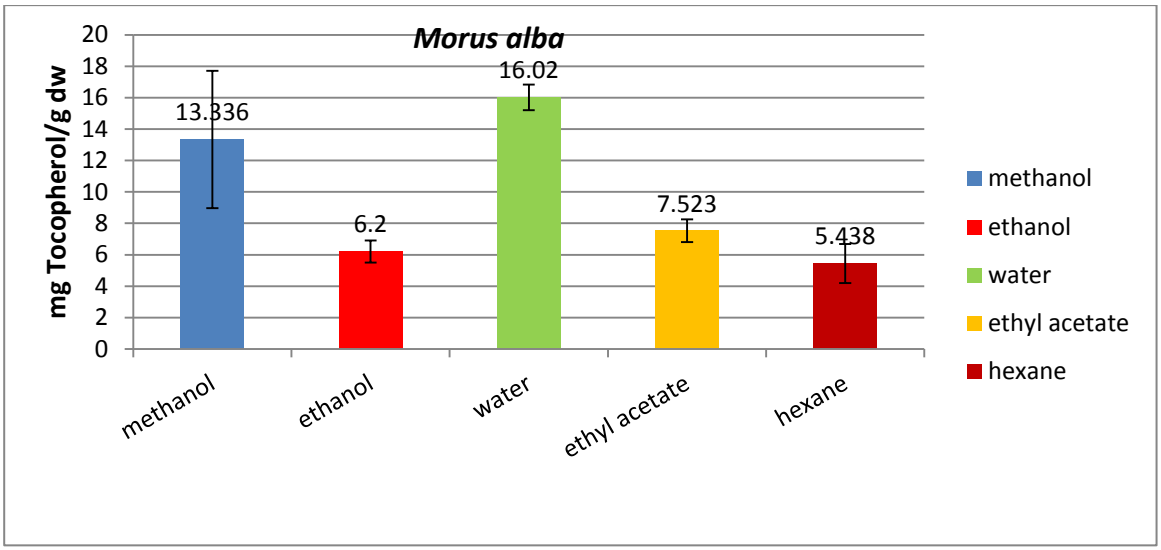

Figure 47: Total antioxidant capacity of different extracts of *Morus alba*

The results of Total antioxidant capacity of *Portulaca oleracea* are shown in figure (48), in this results as a contrast with all of experimental plants, hexane extract of *Portulaca oleracea* came to light and recorded the high total antioxidant capacity, with a large differences from the other extracts with more than 170 mg eq. tocopherol/g. dw. Methanol and ethyl acetate came in the second and third class while ethanol and water extracts recorded negligible values. The statistical results showed high significant differences between hexane extracts and other extracts of *Portulaca oleracea*, and between the other extracts of *Portulaca oleracea* and the

extracts of other plants, while no significant differences between the other extracts of *Portulaca oleracea* each other's at level P ≤ 0.05.

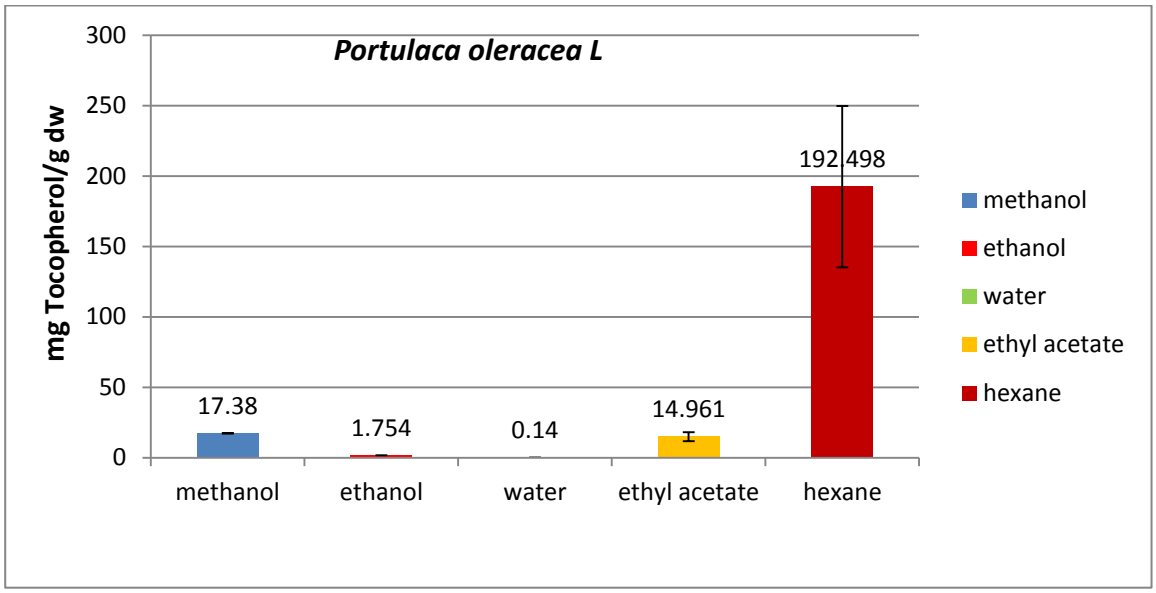

Figure 48: Total antioxidant capacity of different extracts of *Portulaca oleracea*

The overall statistical Tests of Between-Subjects Effects showed significant differences between plants an also between solvents at level P≤ 0.05. Pairwise Comparisons tests between plants in general reported significant differences between *Aloe perryi* and each of *Portulaca oleracea*, *Morus alba*, and *Apium graveoleus* at level P ≤ 0.05. while *Portulaca oleracea* was varied significantly from *Aloe perryi* but not varied from both of *Morus alba* and *Apium graveoleus*. A significant differences were showed between white and both of *Aloe perryi* and *Apium graveoleus*, but not with *Portulaca oleracea*. The same results showed for *Apium graveoleus* at level P ≤ 0.05. the Pairwise Comparisons tests for total antioxidant capacity according the solvent that used in plant extraction, methanol extract revealed significant difference with both of ethyl acetate and hexane extract, in the other side there are no significant differences between methanol and both of ethanol and water extracts at level P ≤ 0.05. ethanol extract was differed significantly from all of the other extracts with the exception of methanol extract. And ethyl acetate was in significant different with both of ethanol and ethanol extract, but not with water and hexane extracts. While water extract didn't showed any significant different with all of extracts except that of ethanol. And finally,

hexane extract present significant different from both of alcohols extracts but not from water and ethyl acetate at level $P \leq 0.05$.

4-2-3 Antimicrobial assay

Table (21) demonstrate antimicrobial activity of *Aloe perryi* against several bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus subtilis*), and one strain of yeast (*Candida albicans*) and also one fungi (*A. flavus*). Each of ethanol, methanol, ethyl acetate, water and hexane extract were tested using three concentrations (0.5, 1.0, and 2.0 mg/disk). The inhibition of bacterial growth by all extracts increased by the increasing of plant concentration, except that of hexane extract as it appeared with all of extract didn't show any activity on all types of microorganisms.

The effect of methanol and ethyl acetate on *Klebsiella pneumoniae* were high in all of concentrations followed by both of ethanol and water extract, while hexane extract didn't show any inhibition for bacteria, similar inhibitions were occurred for *Escherichia coli* with different in zone of inhibition where it were larger in the case of *Klebsiella pneumoniae* than that of *Escherichia coli*. The effects of *Aloe perryi* water extract on *Micrococcus luteus* was high comparing with the other extracts, followed by ethanol and ethyl acetate in the same order and then ethanol. The extracts of *Aloe perryi* were ordered according their activity on *Pseudomonas aeruginosa* as follows, ethanol in the 1st order and water in the second order and both of methanol and ethyl acetate were in the last class. Water extract of *Aloe perryi* the highest inhibition activity against *Listeria monocytogenes* with 20 mm of inhibition zone comparing the other extracts and even with the other bacterial strains, followed by ethanol extract an then ethyl acetate and finally methanol extract. Methanol extract was the first inhibitor for *Bacillus subtilis* and ethyl acetate came in the next and then water and ethanol with the same inhibitor zone size. No inhibition were showed for all of *Aloe perryi* extracts neither with *Candida albicans* nor with *A. flavus*.

Table 21: Zone of Inhibition of various extracts of Aloe perryi for antimicrobial activity(mm)

sample	solvent	Concentration (mg/disk)	Zone of inhibition (mm)								
			Bacteria							Yeast	Fungi
Aloe perryi			*Kp*	*Ec*	*Ml*	*Pa*	*Sa*	*Lm*	*Bs*	*C. albicans*	*A. flavus*
	water	2	11	9	11	10	15	20	12	0	0
		1	0	7	10	10	10	11	12	0	0
		0.5	8	7	7	0	9	8	10	0	0
	Methanol	2	18	11	10	9	18	10	20	0	0
		1	10	8	9	8	17	8	15	0	0
		0.5	9	0	7	0	10	7	14	0	0
	Ethanol	2	11	9	9	11	12	16	12	0	0
		1	9	8	8	9	11	12	9	0	0
		0.5	7	0	7	7	8	10	7	0	0
	Ethyl acetate	2	18	11	10	9	10	12	19	11	0
		1	11	8	9	8	9	8	18	0	0
		0.5	10	0	7	0	7	0	17	0	0
	Hexane	2	0	0	0	0	0	0	0	0	12
		1	0	0	0	0	0	0	0	0	0
		0.5	0	0	0	0	0	0	0	0	0

Kp= Klebsiella pneumonia; Ec= Escherichia coli ; Ml= Micrococcus luteus ; Pa= Pseudomonas aeruginosa, Sa= Staphylococcus aureus ; Lm=, Listeria monocytogenes ; Bs= Bacillus subtilis

Table 22: Zone of Inhibition of Antibiotic(mm)

Antibiotic	*Kp*	*Ec*	*Ml*	*Pa*	*Sa*	*Lm*	*Bs*	*C. albicans*	*A. flavus*
VA30	0	7	9	0	18	16	10	-	-
OX1	0	21	0	0	31	12	0	-	-
AM10	0	0	22	10	42	10	11	-	-
Nystain	-	-	-	-	-	-	-	16	21

VA30 :Vancomycin 50µg/disk, OX1 :Oxacillin 50µg/disk, Ampicillin50µg/disk , Nystain30µg/disk
Kp= Klebsiella pneumonia; Ec= Escherichia coli ; Ml= Micrococcus luteus ; Pa= Pseudomonas aeruginosa, Sa= Staphylococcus aureus ; Lm=, Listeria monocytogenes ; Bs= Bacillus subtilis

In table (23) the inhibition activity of the extracts of *Portulica oleracea L* as in this table we can note that there are no activity where shown for hexane extract with all of microorganisms, and all extracts didn't appeared any effects on *A. flavus*, but with candida there were activity of methanol and ethyl acetate extracts but the other extracts didn't effect in *Candida albicans* growth. Water extract of *Portulica oleracea L* wasn't caused any inhibitor for each of *Staphylococcus*

aureus , Listeria monocytogenes , and *Bacillus subtilis* strains, while it inhibited the growth of other bacteria with various values ranged between 7 and 15 mm., methanol didn't effects on *Klebsiella pneumonia*, and the zone of its inhibition against the other strains varied from 8 to 15 mm in the high concentration and 7 to 10mm in the low concentration. Ethanol extract of *Portulica oleracea* L was more effective against all of bacterial strains, the highest activity of this extract was with *Listeria monocytogenes* in all concentrations used, and ethyl acetate extract was take the second class after ethanol extract.

Table 23: Zone of Inhibition of various extracts of *Portulaca oleracea L* for antimicrobial activity(mm).

sample	Concentration (mg/disk)	Zone of inhibition (mm)								
		Bacteria							Yeast	Fungi
Portulaca oleracea L		*Kp*	*Ec*	*Ml*	*Pa*	*Sa*	*Lm*	*Bs*	*C. albicans*	*A. flavus*
Water	2	15	8	10	10	0	0	0	0	0
	1	9	7	8	7.5	0	0	0	0	0
	0.5	0	0	7	0	0	0	0	0	0
Methanol	2	0	9	10	12	8	23	15	11	0
	1	0	8	8	9	7	13	13	8	0
	0.5	0	7	7	0	7	10	10	0	0
Ethanol	2	10	10	12	11	14	23	13	0	0
	1	8	8	10	11	11	21	10	0	0
	0.5	7	7	8	8	9	16	9	0	0
Ethyl acetate	2	10	10	8	0	12	20	15	10	0
	1	9	8	7	0	9	15	12	8	0
	0.5	0	7	0	0	7	12	11	7	0
Hexane	2	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0	0	0

Kp= Klebsiella pneumonia; Ec= Escherichia coli ; Ml= Micrococcus luteus ; Pa= Pseudomonas aeruginosa, Sa= Staphylococcus aureus ; Lm=, Listeria monocytogenes ; Bs= Bacillus subtilis

The results of antimicrobial activity of *Morus alba* extracts of ethanol, water, methanol, ethyl acetate and hexane, at 0.5, 1, 2 mg /disk concentrations in table (24) , showed that there no activity of each of water, methanol and hexane extracts on *A.flavuse*, and there were inhibition occurred only with the high dose if both of ethanol and ethyl acetate extracts. And also no activity were found of water extract of *Morus alba* on *Candida albicans* and the high concentration of methanol and the high, and medium dose of ethanol and ethyl acetate showed inhibitor activities

on *Candida albicans*. Water extract didn't effect on *Staphylococcus aureus* and its low concentration didn't showed activities on each of *Escherichia coli* , *Micrococcus luteus* , and *Bacillus subtilis* , and its high activity was occurred with *Pseudomonas aeruginosa*, followed by *Listeria monocytogenes* , *Klebsiella pneumonia, Micrococcus luteus* respectively. The low concentration of methanol extract didn't influence on the growth of each of *Klebsiella pneumonia, Escherichia coli* , *Micrococcus luteus* , and *Staphylococcus aureus* , and the high activity of this extract where occurred with *Bacillus subtilis* , and the size of inhibition zone in each *of Escherichia coli* , *Micrococcus luteus* , *Pseudomonas aeruginosa*, and *Staphylococcus aureus* , similar results were showed with ethanol extract, with few different in the inhibition zone of *Listeria monocytogenes* , ethyl acetate showed good activity against al bacterial strains with all of concentrations varied from 7 to 19mm.

Table 24: Zone of Inhibition of various extracts of *Morus alba* for antimicrobial activity(mm)

sample	Concentration mg/disk	Zone of inhibition (mm)								
		Bacteria							Yeast	Fungi
Morus alba		*Kp*	*Ec*	*Ml*	*Pa*	*Sa*	*Lm*	*Bs*	*C. albicans*	*A. flavus*
Water	2	9	7	8	11	0	11	8	0	0
	1	8	7	7	10	0	9	0	0	0
	0.5	7	0	0	9	0	8	0	0	0
Methanol	2	7,5	10	10	10	10	10	20	10	0
	1	7	7	0	9	9	8	15	0	0
	0.5	0	0	0	7	0	7	14	0	0
Ethanol	2	7.5	10	10	10	10	28	16	17	12
	1	7	7	0	9	9	17	9	10	0
	0.5	0	0	0	7	7	12	8	0	0
Ethyl acetate	2	12	10	11	19	12	18	18	14	10
	1	9	9	10	9	8	12	14	8	0
	0.5	8	8	7	8	8	9	9	0	0
Hexane	2	0	0	0	0	0	00	0	0	0
	1	0	0	0	0	0	0	0	0	0
	0.5	0	0	0	0	0	0	0	0	0

Kp= Klebsiella pneumonia; Ec= Escherichia coli ; Ml= Micrococcus luteus ; Pa= Pseudomonas aeruginosa, Sa= Staphylococcus aureus ; Lm=, Listeria monocytogenes ; Bs= Bacillus subtilis

In table (25) as in the results of the other plant extracts there no effects of *Apium graveoleus* on *A. flavus*. But in contrast of all the experimental plants *Apium graveoleus* in all solvent appeared good activity against *Candida albicans* by all concentrations, in the same state happened with hexane extract of *Apium graveoleus* where there were good inhibitor occurred on Bas strain, and hexane extract of *Apium graveoleus* appeared to be good antifungal where the size of inhibition zone were 20, 14, and 11mm with *Candida albicans* and 10, 8, and 0 mm with *A. flavus*. Antibacterial activity of the extracts of *Apium graveoleus* showed high activity on *Bacillus subtilis* , and there were inhibition occurred for all bacteria stains with all of *Apium graveoleus* extracts with various different, the inhibitor zone were ranged between 6.5 and 20mm, with the exception of water and methanol extract on *Klebsiella pneumonia* where there are no effects found on this strain.

Table 25: Zone of Inhibition of various extracts of *Apium graveoleus* for antimicrobial activity(mm)

sample	Concentrtion mg/disk	Zone of inhibition (mm)								
		Bacteria							Yeast	Fungi
Apium graveoleus		*Kp*	*Ec*	*Ml*	*Pa*	*Sa*	*Lm*	*Bs*	*C. albicans*	*A. flavus*
Water	2	0	10	7,5	8	0	19	18	11	0
	1	0	8	7	7	0	14	10	8	0
	0.5	0	7	0	6.5	0	11	8	0	0
Methanol										
	2	0	10	12	0	12	10	8	10	0
	1	0	9	9	0	9	8	0	8	0
	0.5	0	7	8	0	0	0	0	0	0
Ethanol	2	13	10	15	12	13	11	15	17	0
	1	11	8.5	10	10	9	10	12	11	0
	0.5	8.5	7	8	8	8	8	8	8	0
Ethyl acetate	2	10	9	9	0	10	20	13	15	10
	1	8	8	8	0	8	18	9	11	8
	0.5	0	8	8	0	7.5	15	8	0	0
Hexane	2	0	0	0	0	0	0	11	20	10
	1	0	0	0	0	0	0	10	14	8
	0.5	0	0	0	0	0	0	8	11	0

Kp= Klebsiella pneumonia; Ec= Escherichia coli ; Ml= Micrococcus luteus ; Pa= Pseudomonas aeruginosa, Sa= Staphylococcus aureus ; Lm=, Listeria monocytogenes ; Bs= Bacillus subtilis

The statistical analysis of antimicrobial activity of experimental plants showed that there are significant differences at level $P \leq 0.05$ for each of *Aloe perryi, Morus alba, Portulaca oleracea, Apium graveoleus L.* between groups and within groups when microorganisms were used as the factor for comparison, the post hoc Tukey HSD test didn't showed any significant different between the activity of *Aloe perryi* and *Morus alba* against all microorganisms, while there were high significant different between the activity of *Portulaca oleracea* against both of *Escherichia coli* and *Listeria monocytogenes* and it were also showed high significant at level $P \leq 0.05$ between the activity of *Portulaca oleracea* against both of *Micrococcus luteus* and *Listeria monocytogenes* and also between it activity against *Pseudomonas aeruginosa* and *Listeria monocytogenes* at the same levels. And it appeared that there are high significant different at level $P \leq 0.05$ between the activity of *Apium graveoleus L,* except that between *Escherichia coli and Bacillus subtilis* and between the activity against *Klebsiella pneumonia* and *Bacillus subtilis*, and against *Pseudomonas aeruginosa* and *Bacillus subtilis* and between *Staphylococcus aureus* and *Bacillus subtilis.*

ANOVA test of antimicrobial activity of plant's extracts when using the solvents as factor exhibit significant difference between groups and within groups for all of plant's extracts. The Post Hoc multiple comparisons Tukey HSD Tests between the different extracts didn't showed significant differences for *Aloe perryi* between the four methanol, ethanol, water and ethyl acetate extracts, while hexane extract exhibit high significant differences from all the other solvents at level $P \leq 0.05$. and the ethanol extract of *Morus alba* was differed significantly from each of methanol, water and hexane extracts, and not from ethyl acetate extract. And methanol extract of *Morus alba* had high significant difference comparing with all of other solvent's extracts with the except of water extract, ethyl acetate extract of *Morus alba* also showed significant difference from the other solvents except that of ethanol extract, while water extract of this plant demonstrated significant difference in its antimicrobial activity from all of solvent except that of methanol extract. And hexane extract of *Morus alba* was differed significantly from all of the other solvent at level $p \leq 0.05$.

All of extracts of *Portulaca oleracea* didn't showed any significant different between each other, only hexane extract of this plant which differed significantly from all the other solvent's extracts at $P \leq 0.05$. The same results were noted for the antimicrobial activity of the extracts of *Apium graveoleus L.*

DISCUSSION

5- Discussion

World health organization (WHO) has estimated that about 36,000 plant species are used worldwide for medical purposes out of the estimated 250,000 to 350,000 species identified so far, covering the health needs of more than 75 percent of the population. In developing countries the usage of herbal medicines is quite prevalent. Meanwhile in developed countries people are seeking alternate medical facilities as they are disillusioned with current health care. Harmful effects of most modern drugs and effectiveness of newly developed plant medicines due to improved science and technology has lead to resurgence of plant based remedies (Geetha , 2011).

Plant products have been used medicinally for various ailments for years. Medicinal plants constitute the main source of new pharmaceuticals and health care products (Ivanova *et al*., 2005). Extraction and characterization of several active phytocompounds from these green factories have given birth to some of the high activity profile drugs (Mandal *et al*., 2007). It is believed that crude extracts from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana & Shekhawat, 2010). Phytochemical screening of plants has revealed the presence of numerous chemicals including phenols, flavonoids, alkaloids, steroids, saponins and glycosides. These secondary metabolites of plants serve as medicine in the treatment of various infectious diseases (Cowan, 1999). Herbal medicines have become more popular in the treatment of many diseases due to the belief that green medicine is safe, easily available and with fewer side effects. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents which are natural, stable, non toxic and multifunctional (Ramya.2010).

Chemical analysis were carried out in this study to determine the major nutrients compositions of eight species of Yemeni medicinal plants(*Aloe perryi., Apium graveoleus L, Commiphora Myrrha., Lupinus termis., Morus alba., Nigelle sativa L., Portulaca oleracea L., Trigonella foenum L.*), that used traditionally in the

medication of diabetes mellitus and such diseases. Determination of moisture contents is one of the most fundamental and important analytical procedure. The dry matter that remains after moisture removal is commonly referred to as total solids. This analytical value is of great economic importance to a food manufacturer because water is inexpensive filler, and moisture assays can be one of the most important analyses performed on a food product and yet one of the most difficult from which to obtain accurate and precise data (Parks and Row 1941; Nielsen S. Suzanne.1998). Moisture is a quality factor in the preservation of some products and affects stability in spices and herbs (Nielsen , 1998).

Average moisture content was (11.71%) in *Aloe barbadensis* (Adesuyi *et al*., 2012). The main feature of all the *Aloe vera* leaf was their high water content. Approximately 97.42 ± 0.13% moisture contents were observed (Ahmed & Hussain., 2013). Earlier investigations indicated 90-98% moisture in *A. vera* leaf (Muaz & Hussain, 2013; Parks & Row, 1941). The moisture content of *Aloe perryi* that tested in this study was 4.57% of the dry matter of the leaf of *Aloe perryi.* This low content of *Aloe perryi* indicates its content of solid matter, and it is useful in the preservation of plant during the storage, and diminish its contamination by microorganisms.

Total ash refers to the inorganic residue remaining after total incineration of organic matter present in food. Because of its non-variable nature, the ash content can be used for assessing the quality of food product with respect to the presence of inorganic substance in it. Ash content represents the total mineral content in foods. Determining the ash content may be important for several reasons. It is a part of the proximate analysis for nutritional evaluation. Ashing is the first step in the preparation of a food sample for specific elemental analysis. Because certain foods are high in particular minerals, ash content becomes important. We can usually expect a constant elemental content from the ash of animal products, but that from plant sources is variable (Harbers, 1998).

Ash contents of *Aloe vera* leaves in the study of Ahmed *et al* (2013) Was (16.88 ± 0.04%) and it was in previously estimated by Femenia et *et al*., 1999,

which was (7.12-7.30%), and it found 2.36% in *Aloe barbadensis* that studied by Adesuyi *et al*., (2012). Our results reveal high content of ash contents 1.87% in *Aloe perryi* in comparing that of *Aloe vera* and *Aloe barbadensis* in the previous studies with the consideration of water contents.

After considerable deliberation, the American Association of Cereal Chemists (AACC) proposed the following new definition (Prosky, 2003), the crude fiber represents the cell wall material left after boiling with dilute acid and alkali. It contains a mixture of cellulose, lignin and pentosans, together with silica and other mineral matter locked in the tissues and a little nitrogenous matter. No digestible carbohydrates could all be categorized as dietary fiber. Average crude fibers in *Aloe barbadensis* was previously reported by Adesuyi *et al*., (2012) to be (7.84%), *Aloe perryi* leaves are rich source of fibers, as these represented greater than 70% proportion (73.35 ± 0.30%) in the study of Ahmed *et al*., (2013). *Aloe perryi* in this study seems to be poor in crude fiber where it was 0.03% from the dry weight of leaves.

Carbohydrates in plant foods can be classified as digestible (monosaccharides, β-linked di- and oligosaccharides, and starch) or nondigestible (dietary fiber, nonstarch polysaccharides, β-linked di- and oligosaccharides, and resistant starch). The major distinction is that the former are hydrolyzed by human digestive enzymes and absorbed in the upper gastrointestinal tract, whereas the latter are not. Digestible carbohydrate is the most important biological fuel provider in human nutrition. Nondigestible carbohydrates can, however, provide some energy (approximately 2 kcal/g in the form of short-chain fatty acids) if they are fermented by bacteria in the colon (Livesey, 1990).Adequate consumption of nondigestible carbohydrates is considered to confer many health benefits, and epidemiological studies suggest an inverse relationship between a higher intake of dietary fiber and the risk of diseases such as colorectal cancer (Bingham *et al*., 2000), and coronary heart disease (Liu *et al*., 2002).

The results in the present study showed that *Aloe perryi* contains high quantity of carbohydrates compounds with a percent of 91.29%, this results conceder high

in the comparison that of *Aloe barbadensis* as recorded in previously the study of Adesuyi *et al*., (2012). The high carbohydrates contents of *Aloe perryi* make very good medical plant promising, which can used in the treatment of money disease because its high contains of oligosaccharides which serve as good prebiotics and it can entered in some food manufacturing and also in drug industry as a carrier substance and or as affective substances. Carbohydrates of *Aloe perryi* are characterized by their special physiological properties in particularly thickness and viscosity, which can be useful in improvement of drugs products such as creams, syrups, gels, and other pharmaceutical forms.

Proteins perform many functions in the body. They are antibodies, structural proteins, transporters, many hormones, receptors, chemokines, and enzymes. Some proteins have more than one function. Protein analysis is a subject of enormous economic and social interest. The market value of the major agricultural commodities (cereal grains, legumes, flour, oilseeds, milk, livestock feeds) is determined partly by their protein content. Protein quantitative analysis is necessary for quality control and is have varying aesthetic appeal to the consumer. Compliance with religious dietary restriction means excluding certain protein (sources) from the diet. The variety of protein consumed is also extremely important in relation is a growing problem. Proteins show differencing nutritional quality or ability to support dietary needs. In summary, protein analysis has legal, nutritional. health ,safety, and economic implication for the food industry (Tallent, 1979).

Protein analysis is important for the biological activity determination, Some proteins, including enzymes or enzyme inhibitors, are relevant to food science and nutrition: for instance. The proteolytic enzymes in the tenderization of meats, pectinases in the ripening of fruits, and trypsin inhibitors in legume seeds are proteins. In addition to Functional property investigation. Proteins in various types of food have unique food functional properties: for example, gliadin and glutenins in wheat flour for bread making, casein in milk for coagulation into cheese products, and egg albumen for foaming. And for Nutrition labeling (Chang, 1998).

Average crude protein in *Aloe barbadensis* was (4.73%), (Adesuyi *et al*., 2012). A previous studies on compositional features of *Aloe vera* tissues reported 7.56-15.4% crude proteins (Femenia *et al*, 1999). And a recent study on the protein composition of *Aloe vera* reported (6.86 ± 0.06%) (Ahmed & Hussain, 2013). The protein content of a lyophilized product indicated a level that corresponds to about 0.013% in commercial aloe gel products (McKeown, 1983). The results of our study showed that *Aloe perryi* contains 2.05 % of proteins from the dry weight, that indicates *Aloe perryi* would serve as enzymatic catalyst, mediate cell responses, control growth and cell differentiation (Whitney & Rolfes , 2005). And it can serve as emulsion agent in money drug products. And also it can provide human body with some essential amino acids.

Lipids are, by definition, organic compounds that are poorly soluble in solutions such as water and soluble in organic solutions such as ether. As a group of molecules that is defined functionally by solubility, lipids consist of diverse chemical structures. Chemically, lipids are either compounds that yield fatty acids when hydrolyzed or complex alcohols that can combine with fatty acids to form esters. A number of lipids are clinically important. This group includes fatty acids, triacylglycerols (or triglycerides), cholesterol, and phospholipids. Lipid are considered important carrier of many important bioactive molecules including, carotenoids, steroids, fat soluble vitamins,… Lipids and their derivatives can be used in food and drugs industrials (Arneson & Brickell, 2007).

On dry matter basis, lipids represented a minor fraction in present analysis (2.91 ± 0.09%) in *Aloe vera* (Ahmed & Hussain , 2013), it was showed a low quantity of lipids (0.27) in *Aloe barbadensis* (Adesuyi *et al*., 2012), this data was similar to our results 0.19% , but this quantity of lipids are adequate to carry the bioactive compounds and also help in improve some technological properties of foods and drugs, and it can also contribute in nutrition.

The results of the present study of *Nigelle sativa L.* showed that the moisture was (3.75%). These results were partially in accordance with the findings of AI-Jassir (1992), which was 4.6 % ± 0.45 and it was less than the results of Sultan *et*

al., (2009), that reported 6.46% of moisture in *Nigelle sativa L.*, and also the results that recorded by Babayan *et al*., (1978) were it was 5.52 %. The low amount of moisture in *Nigelle sativa L.* that cultured in Yemen to advantages of the dry materials in this plants, and also it can contribute in the preservation of this seeds, and prolong its storage periods, and diminish the occurrences of rancidity of the oil in these seeds, and prevent the seeds from adulteration by microorganisms.

The results of ash parentage of *Nigelle sativa L.* in this investigation was 4.11%, these results is similar with the pervious findings of AI-Jassir (1992), which was 4.4 % and also the results that recorded by Sultan *et al*., (2009), that reported 4.20 ± 0.11% of ash in *Nigelle sativa L.*, and, and it was highr than the results of Babayan *et al*., (1978) that record 3.77%.

The protein contents of *Nigelle sativa L.* that growing in Saudi Arabia was 20.9%, and Sultan *et al*., (2009) record 22.80 ± 0.60%, and also protein in *Nigelle sativa L.* was 21.26% (Babayan *et al*., 1978). While the protein composition in *Nigelle sativa L.* in this study was low13.28% in comparing with the findings from the previous studies, These differences may be due to variations in the environmental factors in the areas where the black cumin seeds were grown. And also it may be referred to difference in source and types of this plant.

The crude fiber content of 7.94% in *Nigelle sativa* seeds makes it a source of dietary fiber (AI-JassirI, 1992). Other studies carried out in *Nigelle sativa L*, found high quantity of fibers varied from 5.50 to 6.03 (Babayan *et al*., 1978; Sultan *et al*., 2009). As it was in protein results of *Nigelle sativa L.* the rates of fibers in our study was only 2.42% which considered low.

Total lipid quantity of *Nigelle sativa L*, in the present study showed to be large quantity where it was 44.67%, this results make *Nigelle sativa L*. good source of food energy and also it provide human body with the essential and unsaturated fatty acids, in addition, it can be good source of tocopherols, carotenoids, vitamins A,E,K, and their precursors. In other hands *Nigelle sativa L* can be utilized in food pharmaceutical industries because its high and specific contains of lipids. Our

results were very high in comparing with the results from the other studies of each of Al-Jassir (1992), Sultan *et al*., 2009, Babayan *et al*., (1978), and Al-Naqeep *et al*., (2009), which found that the percentage of lipids in *Nigelle sativa L*. were 38-2 + 2.20%, 31.16 ± 0.82%, 35.49%, and 30–48% respectively.

According the results of this study, the carbohydrates contents of *Nigelle sativa L* was 31.77%. This results showed that carbohydrates contents of *Nigelle sativa L* that analyzed in this study lower than that reported in the other studies where carbohydrates rates ranged between 29% and 34% (AI-Jassir I 992; Sultan *et al*., 2009; Babayan *et al*., 1978; Al-Naqeep *et al*., 2009).

The results of chemical composition of *Trigonella foenum L* showed that this plant's seeds contains 6.57% of moisture. These results were lower than that of Mullaicharam *et al,* (2013), where the moisture in *Trigonella foenum L*. was 9%, and that which reported by Muhammad *et al*., (2013) which ranged between 7.5 and 8.1 %, our results were higher than the result of Balgees, (2013) which record 5.89%.

Ash composition of *Trigonella foenum-graecum* in this investigation was 4.03%, our findings was higher than that of whole seeds of *Trigonella foenum-graecum* 3% (Mullaicharam *et al,* 2013), and it is in agreement with 4.65% of *Trigonella foenum-graecum* in sudan (Balgees, 2013), but our results were higher than 3.1% and 3.9% that reported by (Muhammad *et al,* 2013).

Seeds used in this study had a high protein content (26.78) as compared to 24% observed in seeds from Egypt (Gerhartz, 1987), and agreed with 26% in seeds from India (Sharma *et al,* 1990), and it is also agreed with that of Indian line 26.0%, while its lower than Crude protein content in seed of lines growing Southern Alberta (Lethbridge) (Acharya *et al,* 2006). Differential crude protein content in seed in this experiment may be attributed directly to the genotypic potential.

Lipid amounts in *Trigonella foenum L* was 6.35%. these results seems to be less than that reported for the whole seeds of fenugreek where it was 8%

(Mullaicharam *et al,* 2013), and our results were in partially in accordance with that of Balgees, (2013), which was 6.16%, and that of Muhammad, (2013) of Fenugreek (*Trigonella foenum-graecum*) from two regions of Pakistan which was 6.47%. While the results of the present study were higher in comparing with 5.4 % (Megha *et al,* 2012).

Fiber composition of *Trigonella foenum-graecum* from this study showed to be high percentage 6.75%, these amount make *Trigonella foenum-graecum* good source of dietary fibers, which would be useful in moderate of blood lipids, in partially cholesterol, and it can reduce blood glucose concentration, in addition to amelioration of digestion process. In comparing our results with 48% that reported for whole seeds of *Trigonella foenum-graecum* (Mullaicharam *et al,* 2013), it is clear that the last result are very large from our results, and also the results of Balgees (2013), which were14.04% which present tow fold of our results, but in regarding to their results carbohydrates contents were very low, so they may calculated some carbohydrates (polysaccharides) as fibers, and the findings of Muhammad *et al*., (2013), indicate the fiber content of *Trigonella foenum-graecum* ranged between 7.6% and 8.5%, which support our results.

the results of Carbohydrates are higher comparing with 6% of (Mullaicharam *et al,* 2013), and that (40.66% nitrogen free extract) of *Trigonella foenum-graecum* in sudan (Balgees, 2013), and 48.1% (Muhammad, 2013), but it was lower than 52.83% that of results of (Muhammad *et al,* 2013) and also lower when compare with 58% of Megha *et al,* (2012).

Results of chemical composition of *Lupinus albus* in our study showed the moisture contents was 6.26%, these results are lower than 10.81% that reported by Mostafa *et al*., (2013), and these results which previously described by Nwokolo *et al*., (1996) which were 9%. These is may be due to over drying of *Lupinus albus,* that used in this study, and also to the environmental conditions of their culturing.

Ash concentration of *Lupinus albus* in the present work was 2.70%, this results can be described as low in the comparing with results from the previous studies where ash concentration were 3%, 4%, and 3.41% that reported by each of Mohamed *et al*., (1995), and Mostafa *et al*., (2013), and Nwokolo *et al*.,,(1996), respectively. That can be referred to differential genotype of the plant used in this study, and also to the structure of the soil where the plant were grew and other environmental factors.

Protein concentration of *Lupinus albus* in this investigation was 37.17%, these results were in harmony with the findings of the percent of 38% , 38.60%, and 40% which reported by each of Mohamed *et al*., (1995), and Mostafa *et al*., (2013), and Nwokolo *et al*., (1996), respectively. These results shows that *Lupinus albus* are a rich source of protein and amino acids in particularly essential amino acids, and it increase the possibility of the presence of antioxidant enzymes such as catalase, and others, the presence of proteins in high quantities also provide the body with the elements that considered a part of amino acid structure such as sulfur in cysteine and methionine and else, *Lupinus albus,* because its contains of proteins can used in the supplementation of some food products to increase their nutrition values, and contribute in the solving of the protein deficiency in world wide. And the fermentation of *Lupinus albus* can produce polypeptides with can be healthy useful.

The results of Mohamed *et al*., (1995), showed that the lipids composition of *Lupinus albus* were 10%, and Mostafa *et al*., (2013), indicates that the percent of lipids in *Lupinus albus* were 9.94%, while in other study the rates of lipid compounds in this plant seeds were found to be 13% (Nwokolo *et al*., 1996). In contrasts , in the lipid concentration of *Lupinus albus* was diminished to be 8.52%, this is may be due to the differences in the genetic origin and the culturing factors.

In regarding to the findings of present work *Lupinus albus* seems to be rich in dietary fibers where the concentration of fiber in the dry matter of this seeds were 10.39%, this results are considered high in comparing with the results from foregoing study 9% (Nwokolo *et al*., 1996).

Lupinus albus L contained 3% starch a significantly lower content when compared with the values reported for other legumes. Lupin cotyledons have been reported to store reserve galactose-rich polysaccharides in the cell wall that are depleted during germination (Parker 1984, Trugo & Almeida , 1988).

A comparison of stachyose + verbascose content in the *lupin variety , lupin albus* L.2043N with other lupin species and soybeans suggested that a lower flatulence factor would be expected in the former. This may be an advantage of *L. albus* that could be exploited to enhance its food use (Macrae & Zand-Moghaddam, 1978). The amount of oligosaccahrides in lupin is influenced by the climate and the soil. *Lupinus albus* (cv. Hamburg) grown in Germany and *L. albus* (cv. Ultra) grown in South America contained a higher oligosaccarides content than the *L. albus* L.2043N. Germination and lactic acid bacteria fermentation are among the proposed methods to lower oligosaccharides content in lupin (Trugo & Almeida , 1988). Sucrose was found (5%) in *L. albus* grown in Argentina (Trugo & Almeida , 1988). Only a trace of fructose was detected in the lupin sample reported here. Horbowicz & Obendorf, (1994) reviewed and surveyed 19 species of seeds, including lupin, at mature dry state and found only a trace of glucose or fructose.

The total carbohydrates content of lupin seeds used in this study was 34.97% . This amount is lower comparable to the 48% in *Lupinus albus*(Mohamed *et al*., 1995). And it were higher than 265 that reported in the previous study (Nwokolo *et al*., 1996).

Syed *et al*., (2012) reported that the moisture content of purslane powder was 5.14% and celery (5.1-11%). Water content almost the same in all 5.62 of celery at dray weight basis. These results are in agreement with those obtained by Michael *et al*., (1999), Lee *et al*., (2011). Other research found that the moisture concentration of celery were 5.62% and Celery seeds 6.39 % in Egypt (Shehata & Soltan., 2012).

The results of chemical composition of *Apium graveoleus L* in Yemen showed that the percent of water and volatile compounds were 7.60%.

Ash contents of *Apium graveoleus L* in the present study was 13.2% , while the results of Shehata & Soltan , (2012) reported that the concentration of ash were in celery seeds 20.83% and in it were celery 21.91 %, Shehata & Soltan , (2012) also recorded the high value of ash for celery (21.96%), which are larger than our results, that is may be mentioned to the soil of growing and the water of irrigation.

The results of Shehata & Soltan., (2012) reported the protein contents of celery and celery seed (19.27 and 18.19%) respectively. Syed *et al*., (2012) reported the crude protein levels in celery seed was 18.1%.

Our research revealed that the proteins contents of celery (*Apium graveoleus L*), were 16.37 %, These results were partially in accordance with the findings of the other research above.

Lipid concentration of celery in our results was 9.61%, these results were higher than findings of Reem *et al*., (2009) who reported that the lipid content in celery 1.36%. and that of Shehata & Soltan., (2012) which showed that total lipids of celery were 1.5% and celery seed (3.25%).

our results also showed that the quantity of total fiber and total carbohydrates in celery seeds were 14.77% and 38.63% respectively. Which are larger than 21% of celery in Egypt (Shehata & Soltan., 2012).

This result is in agreement with previous studies reported by Lee *et al,* (2011), Kabulov & Tashbekov, (1979), Ezekwe *et al*., (1994), Obied *et al*., (2003) and Besong *et al*., (2011), they found that the protein of purslane was ranged from 17.9% to 26.7%.

The role of inorganic elements like Zn, Cr, V, Fe, Cu, and Ni in the improvement of impaired glucose tolerance and their indirect role in management of diabetes mellitus are being increasingly recognized (Narendhirakannan *et al*., 2005).

Levine *et al*., (1983) has observed that there is tissue zinc deficiency in genetically obese, insulin-resistant diabetic mice. Copper is widely distributed in biological tissues, where it occurs largely in the form of organic complexes, many of which are metalloproteinase and function as enzymes. Copper enzymes are involved in a variety of metabolic reactions, such as the utilization of oxygen during cell respiration and energy utilization (Aras & Ataman, 2006). Calcium is the major component of bone and assists in teeth development (Rahmatollah & Rabani , 2010). Iron is also required for the activity of certain enzymes involved in energy production and about 10 % of the body pool of iron is used in this way (Aras & Ataman, 2006).

Some literatures suggested that abnormal zinc metabolism play a role in the pathogenesis diabetes and/or its complications (Faille *et al*., 1983). The complexes of zinc and insulin in varying ratios are stored in pancreatic β-cells and released into the circulation via the portal vein (Scott & Fisher, 1938). Enzymes that do not contain a trace element as an integral part but are activated by metals such as Cu, Fe, and Ni respond to in vitro addition of several transition elements with a dose-dependent activation (Speck , 1949).

Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting, and the regulation of cell permeability. It also plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system(Indrayan *et al*, 2005).

Mean dietary intakes of children have been reported to be in the range 9-278 µg of lead per day and for adults 20-282 µg day .A typically high dietary intake (e.g .500 µg of lead per day) was found in one Indian investigation (Aras & Ataman, 2006).

According to our results the lead contains in the plants are adequate and in the save range.

Our results demonstrate that the concentration of Major elements and Micronutrients in all of experimented plants are in Sufficient or normal ranges according to reference and standards values (kalra, 1998). The importance of these elements cannot be overemphasized because many enzymes require them as cofactors (Akpanabiatu *et al*., 1998).

Adesuyi *et al*., (2012), reported that *Aloe vera* that grown in local garden in Lagos in Nigeria, contains following mineral concentrations, Mg, 325.7, Zn 71.5, Fe 28.8, Mn 128,Cu 15.3, Pb 1.7, P 6650.1, Na 5170, K 10615 ppm.

Aloe perryi in the present study was contained the same concentration of Cu, in and the concentration of Fe and Pb in *Aloe perryi* were more than that of *Aloe vera*, while *Aloe vera* were surpassed *Aloe perryi* in it contents of both of Na, Zn.

Nigelle sativa L., that grown in Saada- Yemen which analyzed in this study, appeared to bettered in comparing with black cumin (*Nigelle sativa L*) that purchased from Riyadh Saudi Arabia, which contained, Potassium 7.6, Phosphorus 1-8 , Sodium 0.75, Iron 0.15, Zinc 0.06, Calcium 0.04, Magnesium 0.03, Manganese 0.02, Copper 0-02 (mg/100 g) (AI-Jassir, 1992).

In the present study was very large in comparing with the results of Abdel-Nabey & Damir, (1999), while their results showed Zn and Fe were more than that of our study. The results of minerals in our study for fenugreek were approximately similar with that of (Choudhury, 2008) with exception of Ca and Fe where it was the double in our results for Ca and the contrast for Fe. And Ca content in fenugreek in turkey was higher in comparing with the same plant in Yemen, while the other minerals contents were similar (Ozcan, 2004). Awadh & Abdullah (2006) , found lower concentration of Zn and Cu in Myrrh.

In the presnet study, *Lupinus termis* grains found to be better in its contents of each of calcium, sodium, copper, and zinc in comparing with that of lupin grain (at 87% dry matter) that reported by Nwokolo & Smartt (1996), while their results found grand quantity of Iron in comparing with our results.

Celery (*Apium graveoleus L.*), had been reported to contained, Calcium 1.767-1.800 (g) , Phosphorus 547-550(mg), Sodium 160-170 (mg), Potassium 1400 (mg), and Iron 44.9 - 44.9(mg) (Parthasarathy *et al*., 2008). These results indicated that high concentration of calcium in compare with our results, while sodium concentration in Yemeni celery was more than that of these study, and the quantities of iron were similar in the two studies.

According to study of Masood *et al* (2008), the concentrations of minerals in *Morus alba* were Iron 19.00-50.00, Zinc 0.72-3.65, Calcium 786.66-2726.66, Phosphorus 970 Magnesium 720 (mg/100 g). And Omidiran *et al*., (2000) found that in the mineral composition of two types of *Morus alba*, calcium was 1.550 and 1.510 and zinc 2.400 and 3.600(mg/100 g), while iron concentration in the study of Masood *et al* (2008), was more than this value in the present study.

The results of this investigation, reported that calcium concentration in *Morus alba* that grown in Yemen, were less than calcium in first study and more than that of the other study, while zinc concentration in our study was surpassed the results of these studies.

The difference between mineral concentrations in the plants from different regions can be referred to the difference of soil and water structures, the climate and weather conditions.

The concentration of elements in the plants which are analyzed in the study decreases in the following order, Ca, Pb, Na, Cu, Zn, and Ag.

The order of plant according their minerals contents is as follows *Nigelle sativa L*, *Trigonella foenum L*, *Apium graveoleus L*, *Commiphora myrrha*, , *Lupinus termis*, , *Portulaca oleracea L*, *Aloe perryi* and *Morus alb.*

From the present study, it is concluded that the presence of various inorganic trace elements such as, zinc, copper, iron, potassium, and sodium in the plants could account for the hypoglycemic nature of these plants. Further, the data obtained on individual element concentration in each plant will be useful in

deciding the dosage of herbal drugs prepared from these plant materials for the management of diabetes related metabolic disorders.

And the present of these minerals in this plant in good balance to improve and maintenance of human health and to help meet daily mineral needs. In addition to these findings the results from the traditional use of these plants in Yemen gives affirmative assurances to the benefits of these plants in the treatment of diabetes.

Plant extracts and herbal formulations are getting more considerations being safe antioxidant and free radical protectors. Phenolic composition of medicinal plants is mainly responsible for antioxidant activity and contributes to their biofunctionalities such as reduction of chronic and degenerative diseases including cancer, cardiac, and infectious diseases etc. (Gulcin *et al.*, 2007).

Extraction is a very critical step for the recovery of bioactive compounds. Extraction solvents and the conditions have great impact on yield and bioactivities of extracts (Me *et al*., 2007). Prior to determine biological activities, proper extraction of bioactive compounds from medicinal plants were performed by sequential extraction with solvents of different polarities .

There is a general principle which is " like dissolves like". This means non polar solvent will extract non-polar substances while polar materials will be extracted by polar solvents (Peter & Amala, 1998). As extraction solvents have differing polarities and hence affinities for the compounds of plant materials other than essential oils, it is important to know that the yield as well as the solvents. The yield composition of extracts varies with the solvent polarity (Moyler *et al.*, 1992). According to study that have done by Duffy & Power, (2001), different extracts of several solvent give different antioxidant potentials

. Tow solvents were used in the extraction process of *Commiphora myrrha, Lupinus termis, Nigelle sativa L and Trigonella foenum,* and five solvents were used in extraction process of *Aloe perryi*, *Apium graveoleus L*, *Morus alba* and *Portulaca oleracea L.*

The solvents were selected based on the different polarity levels. Hexane was selected because it is a very non polar solution, extremely volatile and evaporates very quickly and cleanly, hexane can extract the compounds with low polarity such as volatile oils, fats, waxes, Flavonols, phenolic acids, simple phenolics, anthocyanins Polyphenols from olive leaves, oleuropein and rutin (Ioana *et al*., 2011). Meanwhile, Methanol is a polar solution and was selected because it can extract the compounds with high polarity such as amino acids, glycosides, sugar and other polyphenols (Peter & Amala, 1998). However, methanol is not normally used for extraction from aqueous solutions because they are too soluble in water.

Ethanol have been extensively used to extract antioxidant compounds from various plants and plant-based foods (fruits, vegetables etc.) such as plum, strawberry, pomegranate, broccoli, rosemary, sage, sumac, rice bran, wheat grain and bran, mango seed kernel, citrus peel, and many other fruit peels (Bushra & Muhammad, 2009). Ethanol and different aqueous forms (10–90%, v/v) can extract the compounds such as Anthocyanins, flavonols, free phenolic acids (Balas & Popa, 2007; Wang *et al*., 2009; Bleve *et al*., 2008, Bucic´-Kojic´ *et al*., 2000).

Water is a polar molecule, the polarity of water molecule arises due to its greater electronegativity or electron loving nature, than hydrogen. Even though the pairs of electrons are shared with the hydrogen atoms, they are not shared equally as oxygen pulls the electrons more towards itself. This gives rise to a greater negative charge on the side of oxygen atom and a positive charge on the hydrogen atoms. The polarity of water molecule makes it a universal solvent. It can dissolve salts, acids, sugars, as well as alkalis and gases. Most cell components including proteins, polysaccharides and DNA dissolve in water making it the basis of life. Thus water was selected as one of the solvents, while ethyl acetate can extract the compounds of Phenolic acids, flavonols, antocyanins (Pinelo *et al*., (2005); Russell *et al*., (2008)

Alkaloids a secondary metabolite compound which may be responsible for the, antidiabetics, antiaging and antiviral activities of this herbal plant (Evans & Trease , 2002). Alkaloids has the biological property of toxicity against cells of foreign

organisms. It activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications.(Adesuyi *et al*., 2012)

Recently, Mostafa *et al*., (2013) found that *Lupinus termis* contained 1.47g/100g of alkaloids, and the results of. Nwokolo *et al*., (1996) showed that the alkaloids were between 0.0001 to 5 g/100g in *Lupinus termis.* Both of these results were overtaken by the results of this study, where, the alkaloids concentration of *Lupinus termis* were reached to 6.2 g /100g.

The findings of this work demonstrated that, *Morus alba* had a high alkaloids concentrations which were, 2.304 g/100g , this amount of alkaloids are considered greater as compared to 0.830 g/100 g that observed recently by the study of Omidiran *et al*., (2012). Our results also confirmed by the results of other study in *Morus alba* which was reported that *Morus alba* contains alkaloids compounds (Lim, 2012).

Alkaloids concentrations of *Portulaca oleracea L* in this investigation was 547.3±37.871 mg/100 g, this results are established by the recent findings of Ju OK(2011), which reported that *Portulaca. oleracea* contains alkaloids. There no information about the quantity of alkaloids in *Portulaca oleracea L* were reported in the literatures, so it assured that this study is the unique or the first study that determined the concentration of alkaloids in *Portulaca oleracea L* this study didn't only determined concentration of alkaloids in *Portulaca oleracea L.*

Leaves of *Aloe perryi* that originated in Socotra island in the republic of Yemen in this study had appeared to include 523.26 mg/100g, of alkaloids, this quantity considered elevated as compared to 247.1 mg/100g that observed in *Aloe barbadensis* by (Adesuyi *et al*., 2012).

The results of the present study are inconsistent with the results of the recent study that made by Ibegbulem & Chikezie, (2012), who reported that *Aloe perryi*

were not contained alkaloids compounds. The inconsistency may attributed to the differences at the level of genotype and the environmental factors, and the methods by which the plant samples were subjected to.

Commiphora myrrha in this study had lower amount of alkaloids 1.626±0.122 mg /100g , in comparing with the other plants in this study in each of solvents, but this results were agreed with that of Al-Daihan *et al*., (2013), who reported that *C.myrrha* contains alkaloids, while there are no findings were provide the structure of *Commiphora myrrha* from alkaloids compounds, our investigation is the forerunner work in this field.

Tannins are found in leaf, bud, seed, root, and stem tissues (Rabiah , 2012).The presence of tannins may be responsible for ability to cure diseases such as diabetes (Cushine & Lamb, 2005), Tannins have shown potential antiviral, (Lü L *et al*, 2004), antibacterial (Akiyama *et al.*, 2001), and antiparasitic effects(Kolodziej & Kiderlen, 2005). It have been reported to inhibit digestive enzymes , affect the utilization of vitamins and minerals and are capable of binding and precipitating protein causing a reduction in nutritional value (Hahn *et al*, 1984, Chavan *et al*, 2001). Tannins are known to be effective in the treatment of sore throat, diarrhoea and haemorrhage. Tannins are known for their abilities to precipitate with iron and other metals, thereby reducing their absorption (Adesuyi *et al*., 2012).

In this study, *Apium graveoleus L* had the highest concentration of tannins, and there are no evidence of the concentration of tannins in *Apium graveoleus L*, except that of Mohammed *et al*., (2013) who reported that seeds of *A. graveoleus* contains of tannins, but didn't mentioned its quantity in *Apium graveoleus L.*

The results of the present study, revealed that tannins amounts were found in high quantity reached in *Trigonella foenum L*, this results are supported by the results of Sumayya *et al*, (2012) who reported that *Trigonella foenum L.* contains tannins, but they didn't declared its quantity in *Trigonella foenum L.* it seem that this study is the first study that determine the quantity of tannins in seeds.

The average of tannins components of *Morus alba* in our study were very high when compared with (130mg/100g) that observed in *Morus alba* in a previous study that have been done by Wang *et al*., (2012).

Recently Padmaa (2010), demonstrated that Black cumin appeared to be containing tannin, his results are corroborate the results of this study .

Ibegbulem & Chikezie (2012) showed that *Aloe perryi* appeared to be contained tannins in its leaf's structure, and Adesuyi *et al*., (2012) showed that *Aloe barbadensis* comprised 0.155 g/100g tannins in its structure, this amount considered very low comparing with the results of this study.

Commiphora myrrha in the present study had of tannins, in contrast to that of Al-Daihan *et al*., (2013) who reported that *C. myrrha* doesn't contains tannins.

Lupinus termis content tannins in this study, which are very large as compared to (74.28 mg/100 g) that showed by Norma *et al*., (2012).

In this study, the lowest amount tannins was appeared in *Portulaca oleracea L,* that which confirmed recently by Ju OK (2011) showed that *Portulaca. oleracea* contains tannins.

In recent years interest in plant Polyphenols has increased due to their nutraceutical importance. Phenolic compounds are secondary metabolites which synthesize in plants, they are important by their contribution to human health. They possess biological properties such as: antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Xiuzhen Han *et al*., 2007)

Spectrophotometeric methods are most commonly used for the determination of phenolic contents. Folin-Ciocalteu is very sensitive reagent contains phosphomolybdate and phosphotungstate, which formed blue complex in alkaline solution by the reduction of phenols. Absorbance is measured at 765 nm and

phenolic compounds are quantified with the help of standard curve prepared from pure phenolic standard. This method was developed by Singleton and Rossi 1965, and further improved by many scientists (Slinkard *et al*., 1977; Porter *et al*., 1986).

Flavonoids are naturally occurring substances in plants that are thought to have positive effects on human health. Several members of the flavonoids family have been reported to possess antiviral and antiinflammatory properties, vacuole-protector and anti-thrombotic action, spasmolytic activity, estrogenic actions, antioxidant and liver protecting effects ,report from researches indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". *In vitro* studies have also showed that flavonoids also have anti-allergic, anti-inflammatory , anti-microbial , anti-cancer and anti-diarrheal activities . Flavonoids (both flavonols and flavanols) are most commonly known for their antioxidant(Rabiah, 2012).

Flavonoids may play a role in the prevention of several chronic diseases such as cancer, cardiovascular disease, inflammation, neurodegenerative disorders, and other pathologies associated with oxidative stress. Epidemiological studies have shown an inverse relationship between the consumption of plant foods rich in flavonoids and the incidence of certain diseases. Thus, the intake of foods like soy, rich in isoflavones, green tea as a source of flavanols, fruits, etc. may protect against different types of cancers (Aggarwal, *et al*., 2006; Block, *et al*., 1992; Duthie, *et al*., 2000; Kris-Etherton, *et al*., 2002).

These findings from epidemiological, human and animal intervention studies, as well as from in vitro mechanistic experiments have encouraged the study of these food bioactive components and their use as functional food ingredients and nutraceuticals. A nutraceutical can be defined as any food or part of a food with health beneficial effects, including a reduction on the risk of suffering from certain diseases, as well as with potential use in the treatment against different pathologies (Hurst, 2008).

Different spectrophotometric methods for the quantification of phenolic compounds in foods have been developed. Spectrophotometric methods are based

on the formation of a compound or colored complex that is measured at a certain wavelength. To avoid interference, an effective extraction of flavonoids is necessary before spectrophotometric measurement (Hurst, 2008).

In this study, *Commiphora myrrha* had total phenol in both methanolic and water extract, this results are in agreement with that results reported previously by Lumir Hanuša *et al* (2005), where *C. myrrha* contains compound phenolic in similar quantity as in our study in both of used solvents.

The results of the present study revealed that, the average total phenol content *Nigelle sativa L* (1028.47±6.57 mg/100g , 394.55±12.89 mg/100g) in methanolic extract and water extract respectively, and its content of total flavonoids were (177.64±46.68mg/100g, 83.15±10.7mg/100g) in both of methanolic and water extract respectively, total phenols in the methanolic extracts were significantly differed from total phenols in water extract. The amounts of total phenols in this study were lower in comparing with (2780 mg/100g, 3210 mg/100 g) the found in methanol and water extract respectively of *Nigelle sativa L* that grew Iran and stored in Malaysia in the study of Mariod *et al*., (2009) , the increasing of total phenol in that study in comparing with our results can be referred to the additional daring which applied on *Nigelle sativa L* in their study, where it subjected to drying overnight at 40 °C, resulting the concentration of total phenols in the plant samples, in addition, before the determination of total phenols, they extract the oil of *Nigelle sativa L.* by hexane, that explain the increasing of total phenols in water extract an it's decreasing in methanolic extract, where, a part of lipid soluble phenols were extracted with the oils part. Our results revealed a great increasing in total phenols in comparing with the result of Souri *et al*., (2008), who reported that, *Nigelle sativa L* seeds contains (122.6 mg/100g) total phenol in methanolic extract. The variances of total phenols that found in our study and the other studies may be attributed to the differences in the environmental and genotypes of *Nigelle sativa L* that used in these studies, in addition to the storage and handling of plants and also to the pretreatment and treatment of the tested samples.

From the results of this study, Total phenol in *T.foeum L.* reached (505.53±21.48 mg/100g , 115.37±12.01mg/100g) in methanol and water extract respectively, this results are in full agreements with the previous studied observed by Syeda Birjees Syeda *et al*., (2008), which reported that the methanol extract of *T.foeum L.* had (575mg/100g), while total phenol contains of *T.foeum L.* in the present study was better than total phenol contains of *T.foeum L.* that observed early by Souri E *et al*., (2008), which was only 194.63mg/100g.

According to our investigations, we suggest that methanol are more efficient solvent in phenols extractions comparing with water, that is because the tendency of phenol compounds in these plants to the solving in methanol more than that in water.

The study of Cristina *et al*., (2009) showed that *Lupinus albus* contains 182 mg /100g of total phenols as ferrulic acid, and they were 253182mg ferrulic acid /100g. Ragab (2012) showed that *Lupinus termis* had 13.817 GAE mg/100g of total phenol in water extract , while they contains 0.893 mg QE/100g DW of flavonoids in same extract. In other study, Tsaliki E., and al (1999) reported that *Lupinus termis* contains polyphenol(13.6 g/100g- 20.7g/100g) .

The amount of total phenols in *Lupinus termis* in the present study was (1729.2±26.6 mg/100g,) in methanolic and it was (480.59±14.34 mg/100g) in water extract. Extraction by methanolic extract better than water extract, This is in agreement with the reports of Hertog *et al*., (1993), and Yen *et al*., (1996), so methanol is a widely used and effective solvent for extraction of antioxidants.

Ethanol extraction the best for extraction total phenol (20184.16±1120.082mg/100g), followed methanol extraction (10771.78±776.955 mg/100g), it is in agreement with the reports of Bushra *et al*., (2009). Average amount content of total phenol in ethyl acetate was (1905.90±217.655mg/100g), in water extract was (7752.38±309.946mg/100g), Kakkar *et al*,(2012), showed that *Aloe vera* contains total phenol (10223 mg/100g ,12163 mg/100g and 13918 mg/100g) in ethyl Acetate extract, ethanol extract and water extract respectively , while Bushra Sultana *et al*., (2009) showed TPC of *Aloe barbadensis* leaves were

8250 mg GAC/100g and 6530mg 100g in methanol and ethanol extracts respectively. Hexane extraction the lowest extraction of total phenol(40.07±11.284mg/100g) , this is agreement that *Aloe perryi* had not fat or lipids.

Average amount of total flavonoids in *Aloe perryi* were (1997.14±239.691 mg GAC /100g, 1993.79±13.838 mg GAC /100g, 1984.12±663.971 mg GAC /100g, 239.148±35.802 mg GAC /100g, 43.442623±66.210 mg GAC /100g) in water, methanol, ethanol, ethyl acetate and hexane extract respectively , as compered (10639 mg/100g ,12163mg/100g and 15238 mg/100g) observed in *Aloe vera* in ethyl Acetate extract, ethanol extract and water extract respectively (Kakkar *et al*., 2012). While Bushra *et al*., (2009) showed total phenol content of *Aloe barbadensis* leaves were (2910mg CE/100g and 1680 mg CE/100g) in methanol and ethanol extracts.

Bushra *et al*., (2009) reported that ethanol, methanol and ethyl acetate solvents use for extraction compound antioxidant but in this study we observe extraction of water better than ethyl acetate for reason yield of water solvent was (53.955±0.063 g/100g) while was (17.085±8.464g/100g) in ethyl acetate, for reason *Aloe perryi* have polysaccharides (Ammar *et al*., 2010). Sheng-Quan *et al* (2010) reported that hot water extraction is the most widely used technology for polysaccharides extraction.

Apium graveoleus L contains (2549.9±234.16 mg/100g , 437.6±41.967 mg/100, 388.16±32.201mg/100, 245.7±1.162 mg/100g , 166.1±44.27 166.1±44.27mg/100g) in water, methanol, ethyl acetate, ethanol and water extract respectively , as compered (132.4101mg GAC/100g) in methanol extract (Manisha. *et al*., 2010), while average total phenol amount in leaves and fruit were (680 GAC/100g, 436.05 mg GAC/100g) respectively . (Gui-Fang *et al*., 2013, Souri *et al*., 2008)

The highest phenol content in *Morus alba* was in methanolic extract (518.26±54.724 mg GAC/100g DW), while the lowest content was found in

hexane extract (88.107±4.767 mg GAC/100g DW), whereas total phenol were (183.1±43.003 mg GAC/100g , 134.33±62.598 mg GAC/100g and 102.5±86.899 mg GAC/100g) in ethanol extract, water extract and ethyl acetate extract. Arabshahi-Delouee *et al*., (2007) found that extract methanolic better than water extract ,that is agreement our study , He found that leaves of *Morus indica* contains 932mg/100g TPC in methanolic whereas total phenol content in water extract was 710mg/100g .

Portulaca oleracea L contains total phenol (932.8±172.309 mg GAC/100g, 183.8±29.347 mg GAC/100g, 129.3581±2.283764 mg GAC/100g, 107.51±27.844 mg GAC/100g and 17.507±13.127 mg GAC/100g) in ethanol extract , hexane extract, methanol extract , ethyl acetate extract and water extract, while Souri *et al* (2008), showed that methanolic extract of seeds of *Portulaca oleracea L* had 33.66 mg/100g, whereas Chowdhary *et al*., (2013), found seeds of *Portulaca oleracea L* contains 174.5 to 348.5 mg GAE/100g in methanolic extract, Ali (2012), who reported leaves and stem of *Portulaca oleracea L* contains 586 /100g , in aqueous extracts for Aerial parts was total phenol content 600mg/100g, while in methanolic extract 440mg/100g (Yizhong *et al*., 2003). According to the results obtained by Zilha *et al* (2013) , they showed there are deferent content between use tow solvents water and ethanol extract. Nonetheless, extraction and accurate quantification of phenolic compounds in food matrix could be problematic due to complex structure and physiochemical properties of the natural source of phenolics. Phenolic compounds can exist in diversified forms, ranging from simple free acids to esterified, glycosylated or polymeric compounds. Besides, phenolic acids may also coexist as complexes with proteins, carbohydrates, lipids or other plant components (Luthria & Mukhopadhyay, 2005).

The phenolic compounds in plant extract are more often associated with other molecules like proteins, polysaccharides, terpenes, chlorophyll and inorganic compounds.(Brahmi *et al*, 2012; Milan *el al*, 2011).

For extraction, the solvent is chosen as a function of the type of required flavonoid. Polarity is an important consideration here. Less polar flavonoids (e.g.,

isoflavones, flavanones, methylated flavones, and flavonols) are extracted with ethyl acetate, while flavonoid glycosides and more polar aglycones are extracted with alcohols and water. Glycosides have increased water solubility and aqueous alcoholic solutions are suitable (Andersen & Kenneth, 2006).

In comparing between the extraction capabilities of the solvents that used in this study to according their contains of total phenols and total flavonoids for all experimental plants, it have been found that, *Aloe perryi* showed a high amounts of both of total phenolic compounds TPC and total flavonoids compounds TFC in all of used solvents comparing with the other plants of experiment. Similarly the contents of phenols and flavonoids in *Apium graveoleus L.* were high in all of the used solvents in comparing with the other plants in this study, and it came in second class after *Aloe perryi*.

Ethanol appeared to had the highest ability to extract phenols and flavonoids from *Aloe perryi* followed by methanol, water, and ethyl acetate, while hexane was very poor in its contains of phenols and total phenols, this results can be clearly referred to the high polarity of each of ethanol, methanol and water, while hexane is the lowest polarity comparing with other solvents.

The classification of the extracts of *Portulaca oleracea L,* according their contains of both of total phenolic compounds, total phenol content and total flavonoids compounds total, flavonoids content is ethanol, hexane, ethyl acetate, water and methanol, all extracts of *Portulaca oleracea L,* were showed low amounts of phenol and flavonoids in compare *Aloe perryi.* The high quantity of phenols and flavonoids in *Portulaca oleracea L,* this results can be explained by the elevated concentration of lipid compounds in *Portulaca oleracea L.* those high phenol compounds may be combined with lipids and then extracted excellently in hexane.

For extraction of phenols from *Morus alba,* methanol was more efficient than other solvent, and then water, ethanol, ethyl acetate and hexane in the last. The same order had found for flavonoids with 199.8 as a maximum with methanol extract and 23.18 as a minimum values in hexane extract.

With *Apium graveoleus L*, water extract gave great quantity if phenols and flavonoids followed by methanol, ethyl acetate, ethanol, and finally hexane.

Generally, ethanol appeared to be the efficient solvent of phenols and flavonoids from both of *Aloe perryi* and *Portulaca oleracea L*, while methanol was the effective solvent for phenols and flavonoids from *Morus alba*, and water disclosed great ability to extract phenols and flavonoids compounds from *Apium graveoleus.*

According the results of the present study, we conclude that the polar solvents varied in their abilities to extraction of phenols and flavonoids from plants, these suggested that majority of phenols and flavonoids compounds in these plants are from the categories of compounds whose soluble in the polar solvents.

In vitro antioxidant analysis of phenolic compounds depends upon their free radical scavenging potential. *In vitro* analysis often *uses* chemicals and reagents to generate free radicals so that the radical scavenging ability of the test antioxidant can be determined. Several methods have been developed in which the antioxidant activity is assessed by the scavenging of synthetic radicals in polar organic solvents. Most commonly used synthetic free radicals are 1-1-diphenyl 2-picrylhydrazyl (DPPH) and 2,2‘-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) ABTS.

A significant number of studies are reported regarding the phenolic contents and antioxidant activity of various medicinal plants. Antioxidant with remarkable antioxidant activity has been found in many medicinal plants.

A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. *In vitro* methods can be divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capacity(ORAC) (Chanda *et al*, 2009), Total radical trapping antioxidant potential (TRAP)(Cristina Popovici *et al*,2009) and β carotene bleaching. 2) Electron transfer reactions like trolox equivalent antioxidant capacity(TEAC), Ferric reducing antioxidant power (FRAP), α, α diphenyl-β-picryl-hydrazyl radical scavenging assay(DPPH), Superoxide anion

radical scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Total phenol assay (Huang *et al,*2005). These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals .(Chanda *et al,*2009). Taking this into account, the in vitro antioxidant activity of crude methanolic, water extract of *Commiphora myrrha, Lupinus termis, Nigelle sativa L , Trigonella foenum L* and crude methanolic extract, water extract, ethanol extract, ethyl acetate extract and hexane extract compared to that ascorbic acid, BHA as positive references standard, were evaluated using five different assays, namely DPPH radical scavenging assay, reducing power assay, metal chelating assay, haemolysate catalytic assay and lipid hydroperoxide assay (Liu *et al,*2007). Each of these methods differs in the principles, characteristics and applications.

Radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biology systems. Excessive formation of free radicals accelerates the oxidation of lipids in food and decreases food quality and consumer acceptance (Buyukokuroglu & Gulcin, 2009). DPPH assay has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances in food systems (Cotelle *et al,*1996). This is a decolorization assay that measures the capacity of antioxidants (AH) to directly react with DPPH radicals by monitoring the decrease in absorbance at 517 nm due to the reduction by antioxidants or reaction with a radical species (R •), DPPH is a stable free radical showing a maximum absorbance at 517 nm. However, when it encounters a proton-donor substrate like an antioxidant, the radicals are scavenged and absorbance is reduced (Charles, 2013).

The DPPH radical is a purple-colored stable organic nitrogen centered free radical which becomes colorless when reduced to its non-radical form by AH. (Foti *et al,* 2004 ; Musialik & Litwinienko, 2005)

The DPPH radical scavenging capacity estimation is simple and as such has been used in screening the antioxidant properties of pure compounds and botanical

extracts. The major advantage of this method over other assays is its broad solvent compatibility with aqueous and polar and nonpolar organic solvents (Cheng *et al,*2006).

The amount of sample required to decrease the initial DPPH concentration (IC_{50}) by 50% is a parameter widely used to measure the antioxidant activity. The lower the IC_{50} value, the higher the antioxidant activity . From the result obtained , it was found that methanol extracts of *Commiphora myrrha, Lupinus termis, Nigelle sativa L* and *Trigonella foenum L* had antioxidant activity better than water extract , This is in agreement with the reports of Hertog *et al,* (1993), and Yen *et al,* (1996) that methanol is a widely used and effective solvent for extraction of antioxidants (Arabshahi-Delouee *et al,*2007).

Mothana *et al,* (2009) were found that DPPH scavenging of Bark of *Commiphora ornifolia* was 20.8, 85.9, 95.0, 95.4 and 95.2 % at concentration of methanolic extract 10(µg/ml) ,50(µg/ml) , 100(µg/ml) , 500(µg/ml) and 1000(µg/ml) while in extraction of water were 10.6, 25.2,49.1, 66.2and 72.9 % at concentration 10(µg/ml) ,50(µg/ml) , 100(µg /ml) , 500(µg/ml) and 1000(µg/ml). Paraskevaa *et al,* (2008) studied methanolic extraction of stem and leaves of different of species of *Commiphora* was found DPPH were between 98.61µg/ml - 7.31µg/ml , while Daniele Fraternale *et al,* (2011) found that scavenging DPPH activity of oil resin of *Commiphora erythraea* is low 9.077 mg/ml. The difference in the IC_{50} value can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs (Lisiewska *et al*., 2006).

Recent study showed that methanol extracts of lupin flour exhibited a marked antioxidant activity, higher than that of soya flour extracts (Lim, 2012).

A study carried out by Mariod *et al,* (2009), has shown the DPPH radical scavenging activity of *Nigelle sativa L* seeds with an IC_{50} value of 2.26 mg/ml and 32. mg/ml in methanol and water extract respectively, the present study has shown the inhibition at a lower concentration. This may be due to the difference between

antioxidant capacity of different varieties and cultivars of the same plant grown in different regions(Kedage *et al*., 2007)

Earlier studies on DPPH scavenging ability of *Trigonella foenum L* seeds by Khalaf *et al,* (2008) had shown IC_{50} of DPPH radicals at a concentration of 444.1µg/ml of methanol extract, while Souri., *et al,* (2008) had reported IC_{50} of DPPH radicals was at a concentration of 91.66 µg/ml.

It had been reported that, due to the presence of the antioxidant polyphenols, indoles, and alkaloids, the *A. vera* leaf gel shows antioxidant capacity as confirmed by ORAC and FRAP analyses (Nejatzadeh-Barandozi. 2013).

From the results obtained in the present study, it was found that the ethyl acetate, ethanol and methanol extracts of *A. perryi* had the highest antioxidant activity which may be due to the increased concentration of polyphenols. The antioxidant activity was proportional to the polyphenol content of the solvent extracts. Earlier study on DPPH scavenging ability of *A. vera* leaves parts by Saritha *et al,* (2010) had shown methanol extracts (93.14%) , (57.21%) and (23.59%) scavenging of DPPH of methanol extract , ethanol extract and hexane extract , their results were in agreement with our results, where alcohols solvents were better than hexane in scavenging activity. Khaing (2011) showed DPPH radical scavenging activity of *Aloe vera* with an IC50 value of 58.36 µg/ml in ethanol extract , it was also agreed with the result of our study.

Crude methanol extract of *Apium graveoleus L* had very strong antioxidant activity, while crude hexane extract had weak antioxidant activity, this is in according to that of Nurhanani *et al,* (2008) who showed that methanol extract better than hexane extract for antioxidant activity in *Anacardium occidentale*. Souri., *et al,* (2008), was reported DPPH radical scavenging activity of *Apium graveoleus L.* with an IC_{50} value of 34.75 µg/ml in methanol extract.

One study on DPPH scavenging ability of *Morus alba* leaves by Khalaf *et al,* (2008) had shown IC_{50} of DPPH radicals at a concentration of 79.53µg/ml of methanol extract but water extract no antioxidant activity. But, the present study

shown inhibition at lower concentration. This may be due to the difference between antioxidant capacity of different varieties and cultivars of the same plant grown in different regions (Henriquez *et al*., 2009).

A study carried out by Sanja *et al*. (2009) shown the DPPH radical scavenging activity of *Portulaca oleracea L* aerial parts with an IC_{50} value of 12.67µg/ml. while the recent study of Chowdhary *et al,* (2013) reported the DPPH radical scavenging activity of *Portulaca oleracea L* aerial parts with an IC_{50} value between (1.3 to 1.71 mg/ml). This may be due to the difference between antioxidant capacity of different varieties and cultivars of the same plant grown in different regions (Henriquez *et al*., 2009).

The FRAP assay also takes advantage of electron-transfer reactions. Herein a ferric salt, Fe(III)(TPTZ)2Cl3 (TPTZ) 2,4,6-tripyridyls- triazine), is used as an oxidant

Fe (III) reduction is often used as an indicator of electrondonating activity, which is an important mechanism of phenolic antioxidant action , in the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability (Dejian *et al,*2005; Ebrahimzadeh *et al,*2010).

In the present study, crude ethyl acetate extract, methanol extract and ethanol extract of *Aloe perryi* exhibited stronger reducing activity than other extracts. They showed a positive correlation with the polyphenol content. A study carried out by Saritha *et al,* (2010) had shown the reducing power of *Aloe vera* gel in concentration 2mg/ml , 4mg/ml , 6 mg/ml and 10 mg /ml of methanol extracts was, 0.015 , 0.025 , 0.05 respectively. While Bushra *et al,* (2009) showed reducing power in 10mg/ml was 2.01and 1.56 in methanol and ethanol extract .in our study, result of reducing power was the best . the difference in reducing power can be

attributed to the distribution of secondary metabolites that may fluctuate between different plant Species.

All of the individual *A. vera* leaf gel antioxidant polyphenols identified may contribute to the prevention of the above-mentioned diseases to a greater or lesser extent. The individual contributions of these to disease prevention would, however, depend on their concentrations, antioxidant capacities, bioavailabilities, and specific mechanisms of action. Although the individual phenolic acids/polyphenols occurring in the highest concentrations were benzoic acid, p-toluic acid, p-coumaric acid, psalicylic acid, protocatechuic acid, hydroxyphenylacetic acid, ferulic acid, aloe emodin, and vanillic acid, it is wellknown that the protective health benefits of polyphenols are mainly through a combination of additive and/or synergistic effects between the individual compounds (McAnuff *et al*., 2005).

Ethanol extract of *Morus alba* have stronger reducing power as compared to other solvents. Due to The ethanol extract of *M. alba* leaves contains oxyresveratrol and 5,7-dihydroxycoumarin 7-methyl ether which have antioxidant potential ,The leaves of mulberry contains higher amount of quercetin which is responsible for reduction of oxidation process in vivo and in vitro (Zafar *et al*,2013). It was observed that Hexane extract showed strong reducing activity, may be due to *Morus alba* have high tannins that Mudasir Sultana *et al*, (2012) reported extract hexane content high tannins as compared to acetone extract.

Crude water extract of *Apium graveoleus L* showed stronger reducing activity than other extract. This is due to water extract have the highest of total phenol. The antioxidant activity may be directly correlated to the phenolic content in different solvent extracts (Chu *et al*., 2000; Singh *et al*., 2002; Cai *et al*., 2004). The differences between the results obtained from DPPH assay and reducing power in both extracts water and methanol in seeds of *Apium graveoleus L* and *Morus alba* in hexane extract can be explained in terms of the difference in types, relative amounts and reaction of the antioxidant constituents in the extracts to the colorimetric assays, DPPH assay may not give a true picture of total antioxidant capacity as compared to FRAP due to its less sensitiveness towards hydrophilic

antioxidant and the interaction of antioxidant compound with DPPH depends on its structural conformation.(Fariza *et al,*2011) .

The results of this investigation, displayed that reducing power of seeds of *Apium graveoleus L* better than the that which found in the leaves of *Apium graveoleus L* that reported in pervious study (ju OK, 2011).

For the reducing power activity of crude extracts of *Portulaca oleracea L* in the different types of solvents , all extracts have antioxidant activity, but water extract showed higher reducing capacity compared to other extracts. may contain the compound antioxidant such as vitamin C, saponins, polysaccharides and glutathione.(Kamal Uddin. *et al,*2012, Shehata & Soltan, *2012*)

H_2O_2 is converted to oxygen and water by catalase. A common assay that claims to measure H_2O_2 scavenging capacity of dietary antioxidants uses horseradish peroxidase to oxidize scopoletin to a no fluorescent product. In the presence of antioxidants the oxidation is inhibited. The nature of the inhibition is ambiguous because there are several potential inhibition pathways. The antioxidants can inhibit the reaction by (a) reacting directly with H_2O_2, (b) reacting with intermediates formed from enzyme and H_2O_2, or (c) inhibiting the horseradish peroxidase from binding H_2O_2. Therefore, it is difficult to explain the actual chemical meaning of the data (Dejian *et al,*2005)

Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (Patel D.S *et al,*2012) From the present study, it was found that the hydrogen peroxide scavenging activity of *Aloe perryi* and *Morus alba* extract was low with hexane extract. While hexane extract of *Portulaca oleracea L* scavenging no activity. But all solvents had ability to scavenging hydrogen peroxide. Scavenging of H_2O_2 by the extracts may be attributed to their phenolics, which donate electron to H_2O_2, and thus reduce it to water.(Olubunmi & Anthony , 2011) .

Patel *et al,* (2012) demonstrated that water extract of *Aloe vera* scavenges 60% H_2O_2, this result is in agreement to our study. Earlier reports on extracts of *Aloe*

ferox Mill have shown ability acetone , ethanol , methanol and aqueous extract on scavenging H_2O_2 (Olubunmi& Anthony ,2011). Peksel *et al,* (2006), had shown that aerial parts of water extract *Portulaca oleracea L* at concentration 100µg/ml scavenges 13.75% H_2O_2 only , The difference in our study and early study can be attributed to the distribution of secondary metabolites that may fluctuate between different plant organs (Lisiewska *et al*., 2006).

The results obtained for H_2O_2 scavenging ability of *Apium graveolens L* seeds in this work, were closed to the study that determined H_2O_2 scavenging ability of *Apium graveolens L* seeds by Tülin & Becerik, (2011), and found the IC_{50} of H_2O_2 scavenging was obtained at a concentration of *52.3* µg/ml of methanol extract.

Total antioxidant capacity by phospho-molybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analytic and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphor-molybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of α-tocopherol (Raghu *et al,*2011,. Raghavendra *et al,*2013). The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts (Rohan & Anup,2014). Strong Total Antioxidant Capacity was found in the ethanol, methanol and water crude extract of *Aloe perryi* , due to that extract contain the highest of total phenol as compared to other plants and extract. Early studies have reported there are correlation between antioxidant activity and total phenolic content (Adriana *et al,*2012, Alali *et al,* 2007 ; Velioglu *et al,*1998).

A study carried out by Sonia Miladi & Damak, (2008) has shown the hexane fraction and ethyl acetate fraction in total antioxidant capacity (TAOC) in *Aloe vera* was 471.300 ± 0.013 and 420.700 ± 0.010 (µg α-tocopherol/g of extract) fraction of ethanol. The different between our study and that result may be due to the difference between antioxidant capacity of different varieties and cultivars of the same plant grown in different regions . And a study carried out by Khan *et al,* (2013) revealed the different parts of *Morus alba* have total antioxidant capacity in

methanol extract, it found that root bark had total antioxidant capacity higher than leaves and stem bark. Infectious diseases are the world's leading cause of premature deaths, killing almost 50000 people every day, in recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world, with the continuous use of antibiotics, microorganisms have become resistant.(Parmar Namita & el 2012)

The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover, the use of herbal remedies has risen in the developed countries in the last decade. Plants have provided a source of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold namely; they provide key chemical structure for the development of new antimicrobial drugs and also as a phytomedicine to be used for the treatment of disease(Abukakar *et al*., 2008).A large number of anti-microbial agents derived from traditional medicinal plants are available for treating various diseases caused by micro-organisms(Neeraj K, 2010). It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin *et al*., 1985). The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al*., 1998). Diffusion method is extensively used to investigate the antimicrobial activity of natural substances and plant extracts. These assays are based on the use of discs or holes as reservoirs containing the solutions of substances to be examined (Rauha *et al*., 2000).

The methanol, ethanol, hexane, ethyl acetate and water extracts of the leaves of *Aloe perryi*, *Morus alba* , seeds of *Apium graveoleus* and *Morus alba* were subjected to a preliminary screening for antimicrobial activity against seven human common pathogenic bacteria, one yeast and one pathogenic fungi .

Methanolic extract and ethyl acetate of *Aloe perriy* leaves show high inhibition against *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Bacillus subtilis.* these results are in accordance with the results of Meenatshi *et al*., 2013.

The bioactive compounds from the leaves of *Aloe vera* were screen the antimicrobial activity selected human clinical pathogens by agar diffusion method. The maximum antibacterial activities were observed in petroleum extracts (24mm) other chloroform extract. Antifungal activity of *Aloe vera* was analyzed strains *Aspergillus Niger*, *Aspergillus fumigates* and *Neurospora crassa*. The maximum antifungal activity was observed in petroleum ether and ethanol extracts (22mm and 22mm) when compared to chloroform extracts (Kedarnath *et al*, 2012).

Earlier studies have also reported the greater activity of ethanol extracts compared to other solvent extracts in *Aloe vera* leaves (Praveen Dahiya & Sharmishtha Purkayastha,2012), Aditi Grover1 *et al* (2011) studied the antimicrobial activity of methanol extract of *Aloe vera* against *B. Subtilis, S. aureus, E. coli, P.aeruginosa, C. albicans* and showed extract of plant not inhibit the growth of microorganisms. While Saritha *et al* (2010) has also shown the methanol extract of *Aloe vera* has activity against *Bacillus subtilis no effict, Listeria monocytogenes Staphylococcus aureus , Escherichia coli , Psudomonas aeroginosa.* Arunkumar & Muthuselvam (2009) reported that ethanol extract has activity against *Pseudomonas aeruginosa Staphylococcus aureus* , it is according our study , while they has showed water extract has not effect against *E.coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* and they showed that ethanol and water extract no inhibtion *Aspergillus flavus, it* is in agreement with our study. In this study methanol, ethanol, water and hexane extract inactive against *C. albicans*, it is according to Aditi *et al* (2011).

Aloe vera extracts have been shown to inhibit the growth of fungi that cause tinea, however evidence for control beneath human skin remains to be established. For bacteria, leaf gel from *Aloe vera* was shown to inhibit growth of *Streptococcus* and *Shigella* species in vitro. In contrast, *Aloe vera* extracts failed to show antibiotic properties against *Xanthomonas* species (ferro *et al*, 2003).

Extracts from *Aloe perryi*, *Capparis cartiligenea*, *Indigofera oblongifolia*, *Lawsonia inermis*, *Meriandra benghalensis*, *Oxalis corniculata* and *Ziziphus spina-christi* exhibited the most promising results against Gram-positive strains,

whereas extracts from *Anisotes trisulcus, Calotropis procera, Melia azedarach, Nigella sativa* and *Withania somnifera* showed only moderate activity (Awadh *et al*, 2001).

The water extract of the leaves of *Aloe perriy* exhibited pronounced activity against *Listeria monocytogenes*, it also showed activity against all bacteria. In our study ethyl acetate and methanol extract of leaves *Aloe perryi* showed a better antimicrobial activity, this is in agreement with Meenatshi NP *et al* (2013).

Early study showed that water extract of *Apium graveolens* seeds has antibacterial against *Escherichia coli* (Nadheera Falih Naema *et al* 2010), this result is agreement to our study. Other study by Manisha *et al* (2010) has showed that methanol extract of *Apium graveolens* no antimicrobial against *Bacillus cereus, Staphylococcus aureus* and *Salmonella typhy* but petroleum ether extracts has activity against *Staphylococcus aureus* and *Salmonella typhy* . Fethi *et al* (2013) was found that essential oils of leaves and stem *Apium graveolens* inhibition *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *candida albicans* , but it is not activity against *klebsiella pneumonia* and *Pseudomonas aeruginosa* . While Patil *et al* (2011) has reported that essential oils of *Apium graveolens* exhibited activity against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and inactivity against *Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi.* Osama (2011) have analysed the ethanolic extract of the seeds of *Apium graveolens* for their antimicrobial action against *Staphylococcus aureus, Pseudomonas Aeruginosa and Escherishia coli* . The results the effectiveness of ethanloic extracts of *Apium graveolens*. This result is agreement to this study.

The finding from the present study also indicate significant antibacterial activity in a polar solvent like methanol, ethanol, ethyl acetate and aqueous extract of *Morus alba*. They has shown the strong antibacterial activity special in ethyl acetate. A study by Aditya *et al* (2012) , who explored the antimicrobial activity of leaf extracts of three *morus* species . *Morus alba, Morus serrata* and *Morus laevigata.* and three solvents. They has found all species had activity against

microorganism and the comparison of results in all three species revealed antimicrobial activity in the order of *Morus alba*>*Morus serrata* > *Morus laevigata,* and methanolic extract more acivity than petroleum ether extract and chloroform extract against *Bacillus subtilis , Pseudomonas aeruginosa , Proteus vulgaris , Salmonella typhi , Shigella flexneri , Aspergillus niger , Candida albicans.* Shipra *et al* (2013) has showen that the ethanol extract of *Morus Alba* seed and leaf demonstrated a strong activity against gram positive, gram negative activity.

In the present study, ethanol, methanol, ethyl acetate and water seeds extract of *Portulaca oleracea L* exhibited broad spectrum activity against both Gram positive and Gram negative bacteria. While methanol and ethyl acetate extracts has showed antifungal activity against *C.albicans* .

The methanolic extract of leaves of *Portulaca oleracea L* a broad spectrum of antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus*, and *Candida albicans,* while aqueous extract has inactivity and both methanol and aqueous extract no activity against *Aspergillus Niger* (Bakkiyaraj *et al*, 2011). Ramesh *et al* (2011) had reported the antimicrobial activity in aerial parts of chloroform and ethanolic extracts of *Portulaca oleracea L* by agar diffusion method against five bacteria and three fungi (Bactria like *Staphylococcus aureus, Bacillus cereus, Kleisilla pneumonia* and fungi like *Aspergillus fumigates* and *Nerospora crassa*). Ethanol crude extract showed maximum effect on organisms like *Staphylococcus aureus, Kleisilla pneumonia.* Whereas chloroform extract showed moderate effect on *Kleisilla pneumonia, Aspergillus niger* and *Nerospora crassa.*

A study by Dhole *et al* (2011) had also shown the broad spectrum activity of *Portulaca oleracea L* leaves and root against *Bacillus subtilis, Staphylococcus aureus , Pseudomonas aeruginosa and Aspergillus niger* in the ethanol and aqueous extract. Both extract showed effect antimicrobial .

In this study, extract hexane of all plants has showed inactivity to inhibition bacteria, whereas it is has activity against *Bacillus subtilis, Candida albicans,*

Aspergillus flavus of *Apium graveoleus* . While polar solvent observed high antibacterial activity ,the reason to due that The activities could be attributed to the presence of flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins which have multiple biological effects, including antioxidant, wound healing etc. which are toxic to the microorganisms. Flavonoids, phenolic compounds in particular are important for the plant growth and defense against infection and injury (Aditya *et al,* 2012, Manisha *et al,* 2010). Many reports are available in the literature, wherein an alcoholic extract (ethanolic or methanolic) of various plant parts has shown better antimicrobial activity than other solvent extracts (Kavitha, 2010).

It is observed in extracts of *Apium graveoleus* has showed antibacterial activity against Gram- positive more than Gram-negative bacteria. The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances(Ramya , 2012) .

The result of this investigation was different when compared to findings from other studies that had used the same plant for its antibacterial property screening. There are several factors which could have influenced the result namely, different extraction methods used, the different strains of tested bacteria (Pesewu *et al,* 2008), and plant materials sources and the part of the plant used for extraction. In this study, the whole plants were used. According to Yu *et al* (2007), the different parts of a plant may contain different chemical components that contribute to the strength of its antimicrobial activity.

CONCLUSION

6- Conclusions

Due to the increasing demand and interest in the consumption of medicinal plants as an alternative therapy, it is thus important to carry on some investigation to support the therapeutic claims. Besides that, investigations of plants also ensure that the plants are safe for human consumption. Traditionally *Aloe perryi*, *Apium graveoleus L*, *Commiphora myrrha*, *Lupinus termis*, *Lupinus termis*, *Morus alba*, *Nigelle sativa L*, *Portulaca oleracea L* and *Trigonella foenum L* have been used for the treatment of diabetic diseases and other diseases by locals. The objectives of the study to evaluate the nutritional value, the antioxidant potential and antimicrobial activities of different extracts of plants. The results from the current studies thus provide scientific validation on the use of these plants.

In the result of nutritional value , it showed high concentration of protein in *Lupinus termis*, *Trigonella foenum L* and *Morus alba*, while high total fiber were in *Portulaca oleracea*, *Apium graveoleus L* and *Lupinus termis.*

According to our results the lead contains in the plants are adequate and in the save range. Our results demonstrate that the concentration of Major elements and Micronutrients in all of experimented plants are in Sufficient or normal ranges according to reference and standards values.

From the present study, it is concluded that the presence of various inorganic trace elements such as, zinc, copper, iron, potassium, and sodium in the plants could account for the hypoglycemic nature of these plants. Further, the data obtained on individual element concentration in each plant will be useful in deciding the dosage of herbal drugs prepared from these plant materials for the management of diabetes related metabolic disorders.

And the present of these minerals in this plant in good balance to improve and maintenance of human health and to help meet daily mineral needs. In addition to

These findings the results from the traditional use of these plants in Yemen gives affirmative assurances to the benefits of these plants in the treatment of diabetes.

Leaves of *Aloe perryi* that originated in Socotra island in the republic of Yemen in this study had appeared to include 523.26 mg/100g, of alkaloids, this quantity considered elevated as compared.

The inconsistency may attributed to the differences at the level of genotype and the environmental factors, and the methods by which the plant samples were subjected to.

While there are no findings were provide the structure of *Commiphora myrrha* from alkaloids compounds, our investigation is the forerunner work in this field.

The variances of total phenols that found in our study and the other studies may be attributed to the differences in the environmental and genotypes of *Nigelle sativa L* that used in these studies, in addition to the storage and handling of plants and also to the pretreatment and treatment of the tested samples.

According to our investigations, we suggest that methanol are more efficient solvent in phenols extractions comparing with water, that is because the tendency of phenol compounds in these plants to the solving in methanol more than that in water.

Aloe perryi showed a high amounts of both of total phenolic compounds (TPC) and total flavonoids compounds (TFC) in all of used solvents comparing with the other plants of experiment. Similarly the contents of total phenols in *Apium graveoleus L* were high in water extract compared with all of the used solvents and in comparing with the other plants in this study, and it came in second class after *Aloe perryi*. Ethanol appeared to had the highest ability to extract phenols and flavonoids from *Aloe perryi* followed by Methanol, Water and Ethyl acetate, while Hexane was very poor in its contains of phenols and total phenols, this results can be clearly referred to the high polarity of each of ethanol, methanol and water, while hexane is the lowest polarity comparing with other solvents. The classification of the extracts of *Portulaca oleracea L,* according their contains of both of total phenolic compounds (TPC) and total flavonoids compounds (TFC) is ethanol, hexane, ethyl acetate water and

methanol, all extracts of *Portulaca oleracea L.* were showed low amounts of phenol and flavonoids in compare *Aloe perryi.* The high quantity of phenols and flavonoids in *Portulaca oleracea L.* This results can be explained by the elevated concentration of lipid compounds in *Portulaca oleracea L.* Those high phenol compounds may be combined with lipids and then extracted excellently in hexane. For extraction of phenols from *Morus alba*, Methanol, was more efficient than other solvent, and then water, ethanol, ethyl acetate and hexane in the last. The same order had found for flavonoids with 199.8 as a maximum with methanol extract and 23.18 as a minimum values in hexane extract. With *Apium graveoleus L.*, water extract gave great quantity of phenols and flavonoids followed by methanol, ethyl acetate, ethanol, and finally hexane. Generally, ethanol appeared to be the efficient solvent of phenols and flavonoids from both of *Aloe perryi* and *Portulaca oleracea L.* While methanol was the effective solvent for phenols and flavonoids from *Morus alba*, and water disclosed great ability to extract phenols and flavonoids compounds from *Apium graveoleus L.* According the results of the present study, we conclude that the polar solvents varied in their abilities to extraction of phenols and flavonoids from plants, these suggested that majority of phenols and flavonoids compounds in these plants are from the categories of compounds whose soluble in the polar solvents.

From this work we conclude that extract methanol in all plants more exhibit significant scavenging activity than water extract towards 1, 1-di phenyl picryl hydrazyl, but *Lupinus termis* was contrary. In all the plants , ethyl acetate extract of *Aloe perryi* was found to have high scavenging activity than other extracts and other plants. Scavenging activity of ethyl acetate may be due to presence of the flavonoids, phenolics. Ethyl acetate of *Aloe perryi* and methanol extract of *Morus alba* were more scavenging activity than BHA. In the reducing power assay, ethyl acetate extract of *Aloe perryi* showed strong reducing powers (EC_{50} =3.273±0.4935 µg/ml) when compared with standard Ascorbic acid (EC_{50} = 3.5±0.654 µg/ml). In scavenging H_2O_2, ethanol extract of *Portulaca oleracea L* showed strong H_2O_2 reducing activity in comparison to butylated hydroxyanisole (BHA). In total antioxidant capacity, ethanol, methanol and water extracts of *Aloe perryi* exhibited high potent antioxidant activity. Hexane extracts of all plant showed weak antioxidant except in *Portulaca oleracea L.*

Our study showed that *Aloe perryi* have high antioxidant in all method, the result support the common belief that ethnopharmalogical selection of *Aloe* is a useful criterion in drug discovery, and the result from the present study showed that *Aloe perryi* have the highest antioxidant as compared other species of Aloe.

The result showed that polarity solvent in all plants were found to possess high antibacterial, while hexane extract was showed no antibacterial. *Aloe perryi* were in general more antibacterial than the other plants in this study. We found that the methanol extract of *Morus alba* gave stronger antifungal effects against the species of yeast, also the result showed that ethyl acetate extract in all plant the best as compared to other solvents. Hexane extract of *Aloe perryi* and ethanol extract of *Morus alba* were more effective than other solvents.

Suggestions and recommendations : The outcome of the present study has opened the way for addressing several other research problems in the current scenario. Some of the suggestions for the future research include the following:

The phytochemical fractions may be purified further to isolate the single active component from the fractions and its structure can be elucidated special in *Aloe perryi* and *Morus alba*.

Aloe perryi showed that it have stronger antioxidant than BHA, Further studies are required to investigate its efficacy in food products preservation and delaying their deterioration by rancidity.

In additions another studies are desired to confirm antioxidant activity of these plant in vivo. Anticancer studies also are wanted for all of these plants.

According the rich contents of the plants of this work it recommended to be used as food supplements for enhancement nutrition values of some foods and improve consumer's health.

According to our results, all of properties of the plants of the work indicates that these plants have health benefits against money disease, in particular diabetes, so antidiabete activity studies are in need for these plants.

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Annex 1: Standard curve of Gallic acid, Catechin and tocopherol

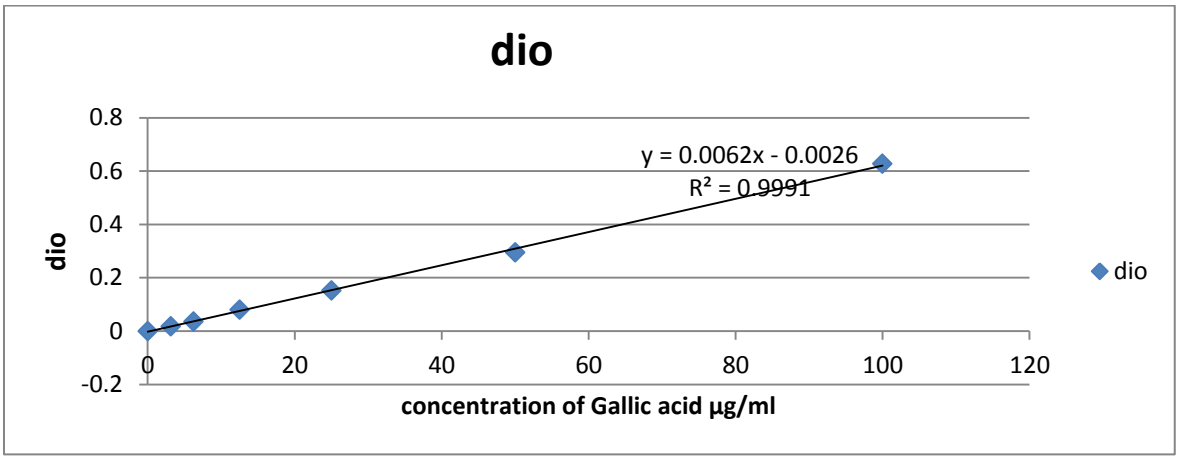


Figure 1 : Standard curve of Gallic acid

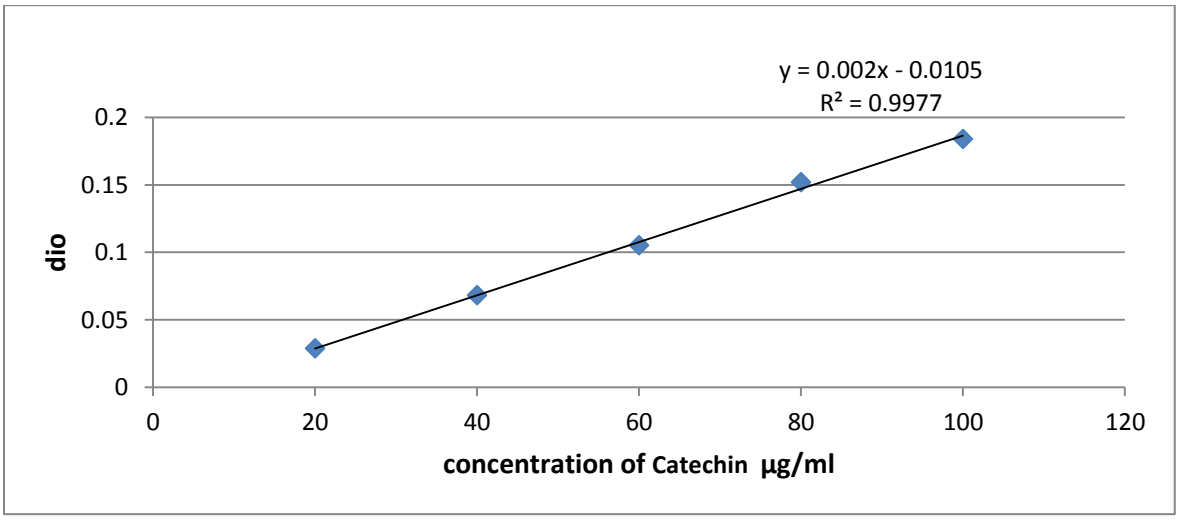


Figure 2. Standard Curve of Catechin

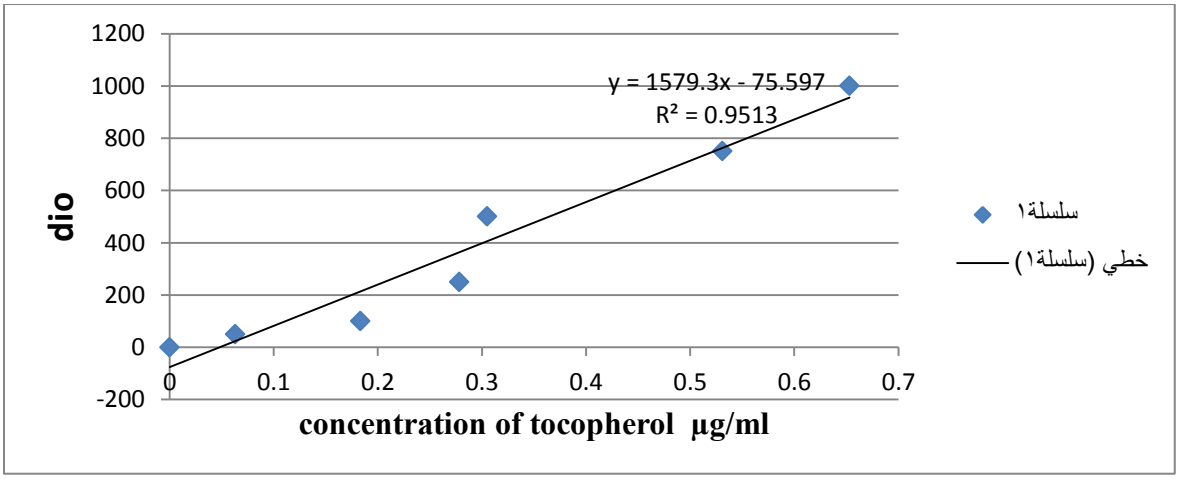
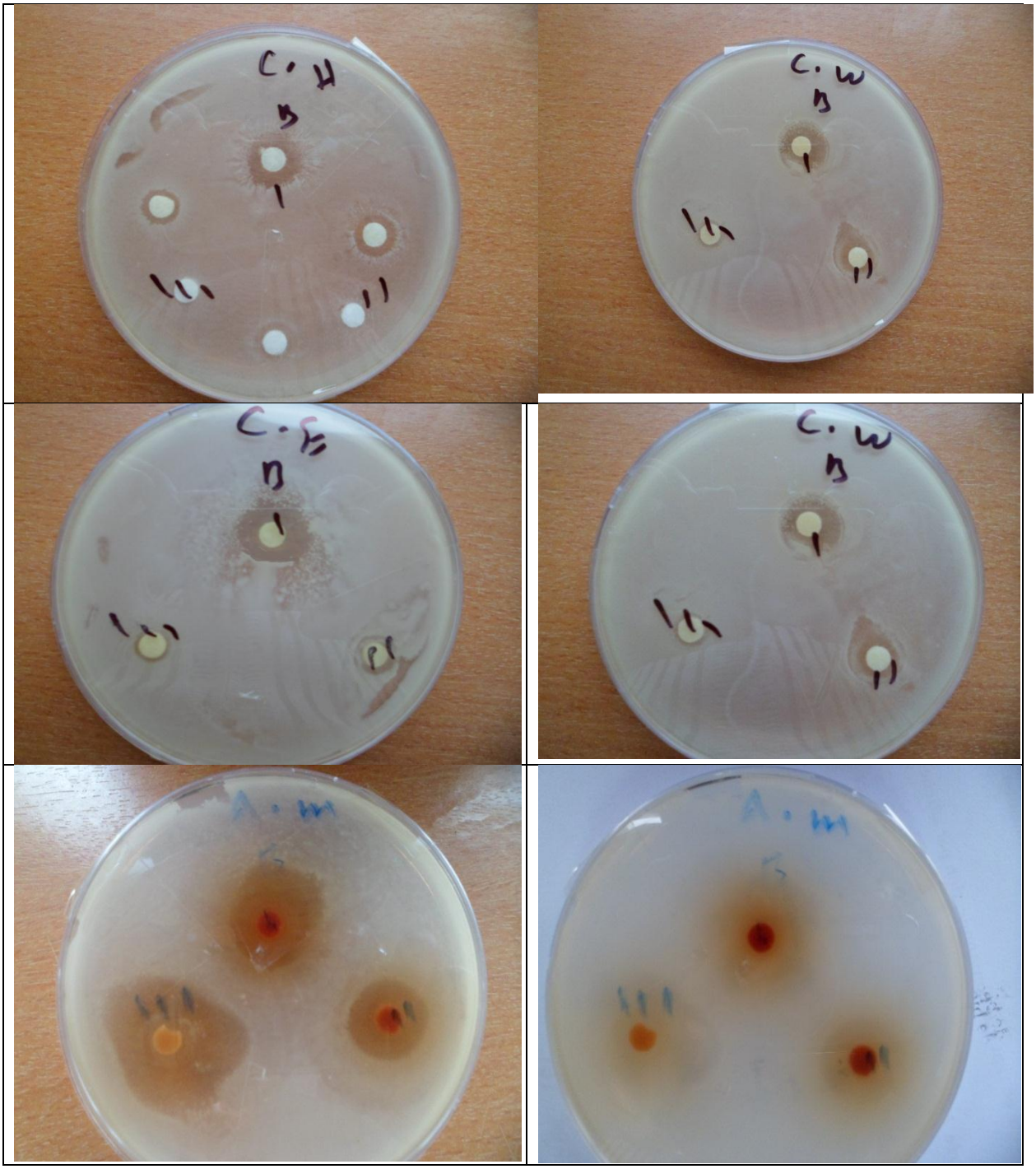
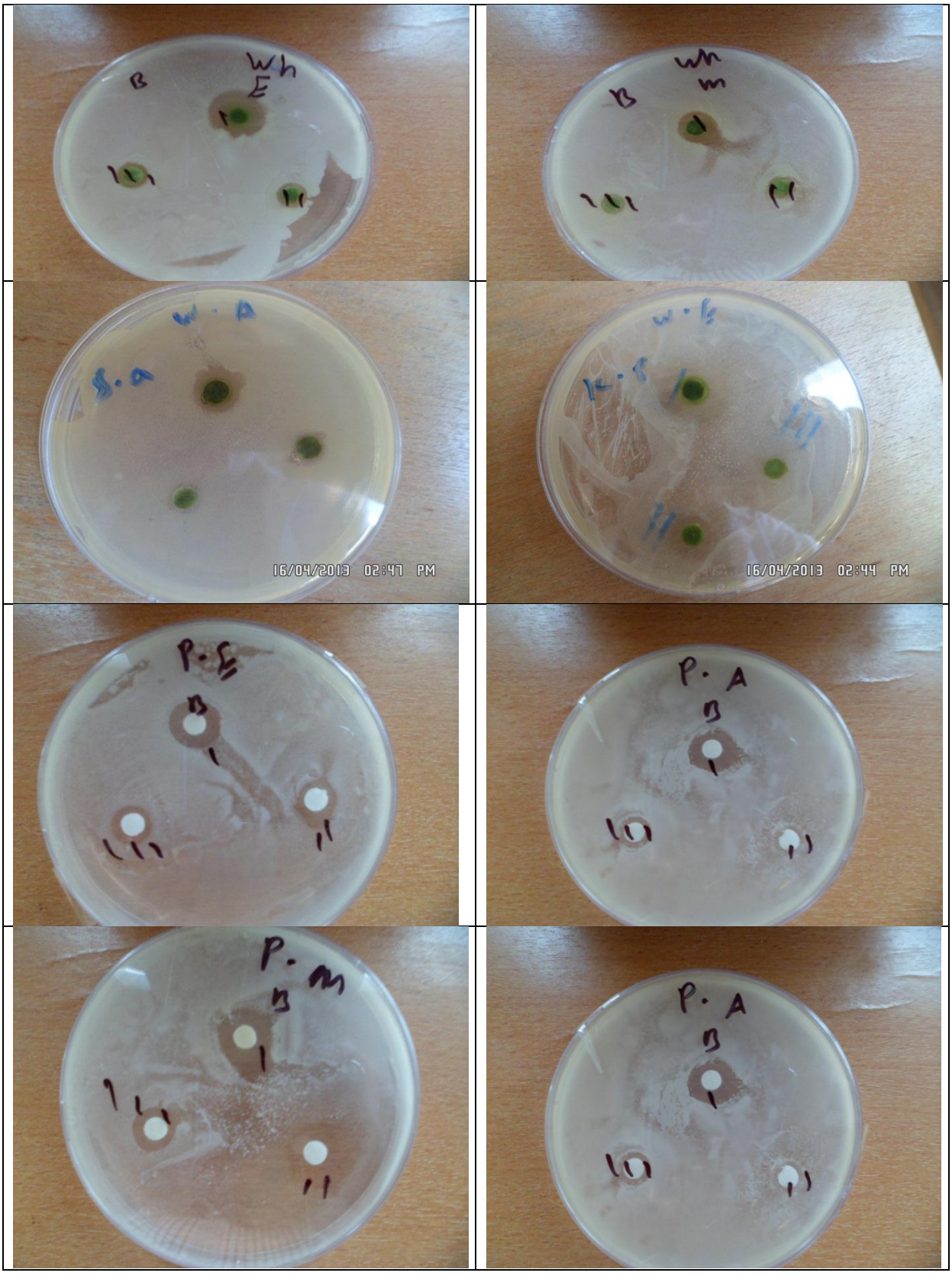


Figure 3. Standard Curve of tocopherol

Annex2 : Antimicrobial activity of plants extracts

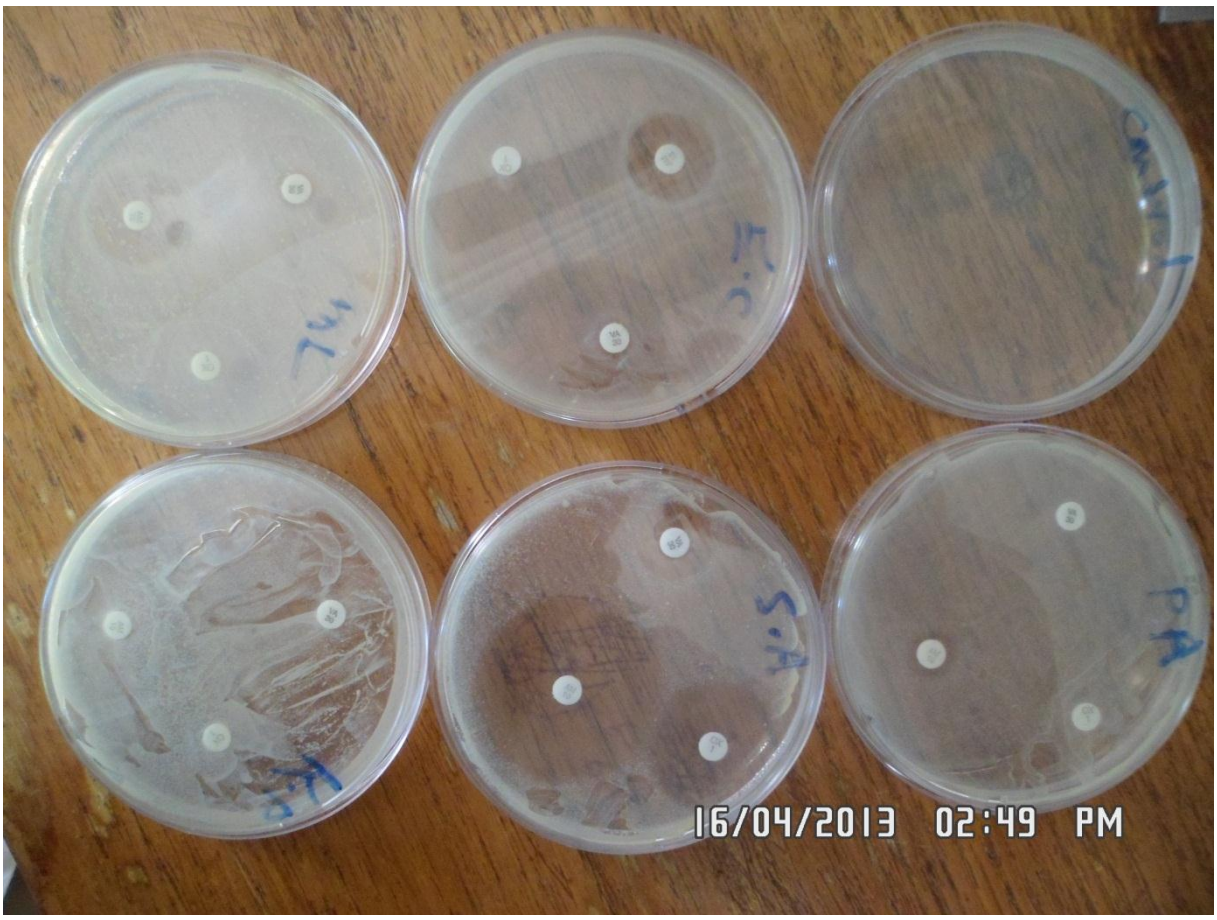


B



C

Annex 3: antimicrobial of antibiotic



Annex 4: statistical results of antioxidant activity of plants

A. ANOVA OF FRAB

		Sum of Squares	df	Mean Square	F	Sig.
purslfrab	Between Groups	10.182	4	2.546	14.287	.000
	Within Groups	7.127	40	.178		
	Total	17.309	44			
celeryfrab	Between Groups	3.063	4	.766	8.254	.000
	Within Groups	5.103	55	.093		
	Total	8.165	59			
whitfrab	Between Groups	9.620	4	2.405	8.743	.000
	Within Groups	15.128	55	.275		
	Total	24.748	59			
aloefrab	Between Groups	98.123	4	24.531	76.644	.000
	Within Groups	17.603	55	.320		
	Total	115.726	59			

B. Descriptives OF FRAB

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
purslfrab	hex	9	.1627	.12697	.04232	.0651	.2603	.03	.32
	Etha	9	.3770	.16729	.05576	.2484	.5056	.17	.56
	Acet	9	.4311	.23662	.07887	.2492	.6130	.19	.76
	water	9	1.4951	.75414	.25138	.9154	2.0748	.74	2.49
	meth	9	.8945	.47122	.15707	.5323	1.2567	.42	1.50
	Total	45	.6721	.62721	.09350	.4836	.8605	.03	2.49
celeryfrab	hex	12	.7141	.31040	.08960	.5169	.9114	.28	1.12
	Etha	12	.6317	.43101	.12442	.3579	.9056	.17	1.31
	Acet	12	.0705	.01462	.00422	.0612	.0797	.04	.09
	water	12	.3652	.30026	.08668	.1744	.5559	.08	.84
	meth	12	.4055	.30229	.08726	.2134	.5975	.10	.88
	Total	60	.4374	.37202	.04803	.3413	.5335	.04	1.31
whitfrab	hex	12	.6141	.50056	.14450	.2960	.9321	.17	1.91
	Etha	12	.1601	.10872	.03139	.0910	.2291	.04	.33
	Acet	12	1.3974	.99711	.28784	.7639	2.0309	.36	2.87
	water	12	.7090	.17765	.05128	.5961	.8219	.52	.97
	meth	12	.5706	.29519	.08521	.3831	.7582	.21	.97
	Total	60	.6902	.64766	.08361	.5229	.8575	.04	2.87

aloefrab	hex	12	.3278	.18544	.05353	.2100	.4456	.12	.61
	Etha	12	1.8316	.74143	.21403	1.3605	2.3026	.79	2.61
	Acet	12	3.5426	.37396	.10795	3.3049	3.7802	2.91	3.85
	water	12	.1120	.10606	.03062	.0446	.1793	.01	.28
	meth	12	2.3215	.93011	.26850	1.7306	2.9125	1.01	3.33
	Total	60	1.6271	1.40052	.18081	1.2653	1.9889	.01	3.85

C. Descriptives results of H_2O_2

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
celeryh2o2	Erh	9	56.0428	13.26045	4.42015	45.8499	66.2357	39.22	76.43
	Meth	9	39.6033	34.31152	11.43717	13.2292	65.9775	7.26	85.43
	Wat	9	39.6433	25.27508	8.42503	20.2152	59.0715	13.22	77.17
	Acet	9	41.7967	27.65347	9.21782	20.5403	63.0530	11.55	77.30
	Hex	9	38.1367	7.39372	2.46457	32.4534	43.8200	28.90	47.20
	Total	45	43.0446	23.57422	3.51424	35.9621	50.1270	7.26	85.43
Aloeh2o2	Erh	9	34.0517	24.36245	8.12082	15.3250	52.7783	9.76	67.56
	Meth	9	40.9600	25.62117	8.54039	21.2658	60.6542	19.35	85.32
	Wat	5	11.4120	9.24353	4.13383	-.0654	22.8894	3.11	26.37
	Acet	9	50.6233	31.20059	10.40020	26.6404	74.6062	20.27	92.70
	Hex	9	23.6167	6.90292	2.30097	18.3106	28.9227	12.13	31.80
	Total	41	34.1543	24.97700	3.90075	26.2706	42.0380	3.11	92.70
puslanh2o2	Erh	9	80.3138	9.00253	3.00084	73.3938	87.2338	65.21	91.79
	Meth	9	53.8017	31.06265	10.35422	29.9248	77.6785	13.06	85.56
	Wat	9	32.1957	15.80887	5.26962	20.0439	44.3475	13.54	54.85
	Acet	9	56.0841	25.99461	8.66487	36.1029	76.0653	20.13	83.15
	Hex	9	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	44.4791	33.19620	4.94860	34.5058	54.4523	.00	91.79
whiteh2o2	Erh	9	81.0778	21.90784	7.30261	64.2379	97.9176	50.54	100.00
	Meth	9	35.1156	20.09413	6.69804	19.6699	50.5613	12.56	61.65
	Wat	9	33.9750	15.99252	5.33084	21.6821	46.2679	15.32	55.65
	Acet	9	61.4557	22.21410	7.40470	44.3805	78.5310	34.56	90.54
	Hex	9	7.8350	7.05133	2.35044	2.4149	13.2551	1.20	17.46
	Total	45	43.8918	30.88178	4.60358	34.6139	53.1697	1.20	100.00

D. ANOVA of H2O2

		Sum of Squares	df	Mean Square	F	Sig.
celeryh2o2	Between Groups	1962.078	4	490.520	.872	.489
	Within Groups	22490.648	40	562.266		

	Total	24452.726	44			
Aloeh2o2	Between Groups	6443.451	4	1610.863	3.133	.026
	Within Groups	18510.575	36	514.183		
	Total	24954.026	40			
puslanh2o2	Between Groups	32714.862	4	8178.716	20.742	.000
	Within Groups	15772.591	40	394.315		
	Total	48487.453	44			
whiteh2o2	Between Groups	28500.715	4	7125.179	21.172	.000
	Within Groups	13461.406	40	336.535		
	Total	41962.120	44			

E. Descriptives RESULS OF DPPH

						95% Confidence Interval for Mean			
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
dpphaloe	60	15	34.1000	32.51025	8.39411	16.0964	52.1036	2.22	87.90
	125	15	43.0403	34.17268	8.82335	24.1161	61.9645	4.69	92.77
	250	15	50.3748	34.88025	9.00604	31.0587	69.6908	7.52	93.80
	500	15	58.7737	35.53418	9.17489	39.0955	78.4518	13.50	92.00
	Total	60	46.5722	34.64749	4.47297	37.6218	55.5226	2.22	93.80
dpphpurslan	60	12	14.8929	12.33480	3.56075	7.0557	22.7300	1.41	29.09
	125	12	25.9906	21.11578	6.09560	12.5742	39.4069	4.19	51.08
	250	12	38.1694	27.78334	8.02036	20.5167	55.8221	5.67	71.86
	500	12	51.9903	32.56724	9.40135	31.2981	72.6825	16.39	92.62
	Total	48	32.7608	27.63966	3.98944	24.7351	40.7865	1.41	92.62
dpphwhite	60	12	43.4248	15.12370	4.36584	33.8156	53.0339	22.68	64.40
	125	12	67.2069	18.96365	5.47433	55.1579	79.2558	42.79	94.44
	250	12	83.0036	14.03847	4.05256	74.0840	91.9232	59.61	95.34
	500	12	91.8376	6.77094	1.95460	87.5356	96.1397	80.51	97.44
	Total	48	71.3682	23.23533	3.35373	64.6214	78.1151	22.68	97.44
dpphcelery	60	12	26.5584	16.55542	4.77914	16.0396	37.0772	9.76	45.40
	125	12	42.1123	16.40114	4.73460	31.6915	52.5330	24.53	62.62
	250	12	62.3425	14.47005	4.17714	53.1487	71.5363	44.11	84.03
	500	12	84.3650	14.07416	4.06286	75.4227	93.3073	67.97	98.70
	Total	48	53.8445	26.53248	3.82963	46.1403	61.5488	9.76	98.70

F. ANOVA OF DPPH

		Sum of Squares	df	Mean Square	F	Sig.
dpphaloe	Between Groups	4970.479	3	1656.826	1.409	.250
	Within Groups	65855.976	56	1176.000		
	Total	70826.455	59			
dpphpurslan	Between Groups	9169.497	3	3056.499	5.030	.004
	Within Groups	26736.190	44	607.641		
	Total	35905.688	47			
dpphwhite	Between Groups	16230.402	3	5410.134	26.033	.000
	Within Groups	9143.974	44	207.818		
	Total	25374.375	47			
dpphcelery	Between Groups	22630.743	3	7543.581	31.744	.000
	Within Groups	10455.977	44	237.636		
	Total	33086.719	47			

G. Descriptive results of TAC

plant	solvents	Mean	Std. Deviation	N
Aloe perryi	metha	347.3500	22.18757	3
	Etha	528.9257	176.22635	3
	Acet	105.4200	8.33116	3
	Water	243.9727	129.18643	3
	Hex	11.6483	.07217	3
	Total	247.4633	205.56541	15
purslane	metha	17.3797	.15350	3
	Etha	1.7540	.06930	3
	Acet	14.9610	3.22071	3
	Water	.1403	.00635	3
	Hex	192.4957	58.20140	3
	Total	45.3461	79.59823	15
white	metha	13.3367	4.37054	3
	Etha	6.2000	.70711	3
	Acet	7.5233	.72210	3
	Water	16.0200	.82024	3
	Hex	5.4383	1.23849	3
	Total	9.7037	4.70267	15
celery	metha	66.9035	1.75650	3
	Etha	14.0100	2.72800	3
	Acet	109.5175	.91250	3

	Water	91.2750	.97500	3
	Hex	14.7450	.41500	3
	Total	59.2902	40.47927	15
Total	metha	111.2425	144.39669	12
	Etha	137.7224	247.62843	12
	Acet	59.3554	50.49673	12
	Water	87.8520	114.85057	12
	Hex	56.0818	85.99576	12
	Total	90.4508	143.57592	60

H. Pairwise Comparisons

Dependent Variable:TAC

(I) solvents	(J) solvents	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
metha	Etha	-26.480	20.764	.210	-68.446	15.486
	Acet	51.887*	20.764	.017	9.921	93.853
	Water	23.390	20.764	.267	-18.576	65.357
	Hex	55.161*	20.764	.011	13.194	97.127
Etha	metha	26.480	20.764	.210	-15.486	68.446
	Acet	78.367*	20.764	.001	36.401	120.333
	Water	49.870*	20.764	.021	7.904	91.837
	Hex	81.641*	20.764	.000	39.674	123.607
Acet	metha	-51.887-*	20.764	.017	-93.853	-9.921
	Etha	-78.367-*	20.764	.001	-120.333	-36.401
	Water	-28.497	20.764	.178	-70.463	13.470
	Hex	3.274	20.764	.876	-38.693	45.240
Water	metha	-23.390	20.764	.267	-65.357	18.576
	Etha	-49.870-*	20.764	.021	-91.837	-7.904
	Acet	28.497	20.764	.178	-13.470	70.463
	Hex	31.770	20.764	.134	-10.196	73.737
Hex	metha	-55.161-*	20.764	.011	-97.127	-13.194
	Etha	-81.641-*	20.764	.000	-123.607	-39.674
	Acet	-3.274	20.764	.876	-45.240	38.693
	Water	-31.770	20.764	.134	-73.737	10.196

Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

*. The mean difference is significant at the .05 level.

I. Univariate Tests

Dependent Variable:TAC

	Sum of Squares	df	Mean Square	F	Sig.
Contrast	57861.580	4	14465.395	5.592	.001
Error	103478.359	40	2586.959		

The F tests the effect of solvents. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

J. ANOVA of phytochemical structure

		Sum of Squares	df	Mean Square	F	Sig.
TPC mg/100g dw	Between Groups	4062955.790	4	1015738.947	.	.
	Within Groups	.000	0	.		
	Total	4062955.790	4			
yield %	Between Groups	16.408	4	4.102	.	.
	Within Groups	.000	0	.		
	Total	16.408	4			
TFC mg/100 g dw	Between Groups	179586.257	4	44896.564	.	.
	Within Groups	.000	0	.		
	Total	179586.257	4			
IC50 DPPH	Between Groups	22843645.163	4	5710911.291	.	.
	Within Groups	.000	0	.		
	Total	22843645.163	4			

K. Correlations between phytochemical stricture

Control Variables			yield %	TPC mg/100g dw	TFC mg/100 g dw	IC50 DPPH	extrats
-none-[a]	yield %	Correlation	1.000	-.324	-.054	-.523	-.026
		Significance (2-tailed)	.	.594	.931	.365	.967
		df	0	3	3	3	3
	TPC mg/100g dw	Correlation	-.324	1.000	.921	-.322	.329
		Significance (2-tailed)	.594	.	.026	.597	.588
		df	3	0	3	3	3
	TFC mg/100 g dw	Correlation	-.054	.921	1.000	-.554	.118
		Significance (2-tailed)	.931	.026	.	.333	.850

		df	3	3	0	3	3
	IC50 DPPH	Correlation	-.523	-.322	-.554	1.000	-.331
		Significance (2-tailed)	.365	.597	.333	.	.587
		df	3	3	3	0	3
	extrats	Correlation	-.026	.329	.118	-.331	1.000
		Significance (2-tailed)	.967	.588	.850	.587	.
		df	3	3	3	3	0
extrats	yield %	Correlation	1.000	-.335	-.051	-.564	
		Significance (2-tailed)	.	.665	.949	.436	
		df	0	2	2	2	
	TPC mg/100g dw	Correlation	-.335	1.000	.941	-.239	
		Significance (2-tailed)	.665	.	.059	.761	
		df	2	0	2	2	
	TFC mg/100 g dw	Correlation	-.051	.941	1.000	-.549	
		Significance (2-tailed)	.949	.059	.	.451	
		df	2	2	0	2	
	IC50 DPPH	Correlation	-.564	-.239	-.549	1.000	
		Significance (2-tailed)	.436	.761	.451	.	
		df	2	2	2	0	

a. Cells contain zero-order (Pearson) correlations.

L. Descriptives of antimicrobial activity results

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALOEPERRYI	Kp	15	11.5333	6.88546	1.77782	7.7203	15.3464	.00	19.00
	Ec	15	8.0000	4.30946	1.11270	5.6135	10.3865	.00	12.00
	Ml	15	8.0000	4.25944	1.09978	5.6412	10.3588	.00	12.00
	Pa	15	8.2000	4.34906	1.12292	5.7916	10.6084	.00	12.00
	Sa	15	11.0000	6.36979	1.64467	7.4725	14.5275	.00	18.80
	Lm	15	11.6000	6.99775	1.80681	7.7248	15.4752	.00	21.00
	Bs	15	12.6000	7.41716	1.91510	8.4925	16.7075	.00	20.80
	C. albicans	15	2.2000	4.55443	1.17595	-.3222	4.7222	.00	11.00
	A. flavus	15	2.4000	4.96847	1.28285	-.3514	5.1514	.00	12.00
	Total	135	8.3926	6.63054	.57067	7.2639	9.5213	.00	21.00
Morus alba	Kp	15	7.2000	4.11652	1.06288	4.9203	9.4797	.00	12.90
	Ec	15	7.4000	4.03007	1.04056	5.1682	9.6318	.00	10.70
	Ml	15	7.8000	4.17236	1.07730	5.4894	10.1106	.00	11.70

	Pa	15	10.0000	6.25974	1.61626	6.5335	13.4665	.00	20.00
	Sa	13	7.3846	5.21837	1.44732	4.2312	10.5380	.00	13.00
	Lm	15	13.4000	9.63261	2.48713	8.0656	18.7344	.00	29.10
	Bs	15	12.4000	7.73286	1.99662	8.1177	16.6823	.00	22.00
	C. albicans	15	8.2000	7.30166	1.88528	4.1565	12.2435	.00	17.00
	A. flavus	15	4.4000	5.61630	1.45012	1.2898	7.5102	.00	12.00
	Total	133	8.7068	6.65421	.57699	7.5654	9.8481	.00	29.10
Portulaca oleracea	Kp	15	9.0000	5.10000	1.31681	6.1757	11.8243	.00	16.00
	Ec	15	7.6000	4.03024	1.04060	5.3681	9.8319	.00	10.90
	Ml	15	7.6000	4.27718	1.10436	5.2314	9.9686	.00	13.00
	Pa	11	5.7273	5.52034	1.66445	2.0187	9.4359	.00	11.80
	Sa	13	8.7692	6.17999	1.71402	5.0347	12.5038	.00	15.00
	Lm	13	14.5385	10.23568	2.83887	8.3531	20.7238	.00	24.00
	Bs	13	9.9231	6.96971	1.93305	5.7113	14.1348	.00	16.00
	C. albicans	15	4.0000	5.07093	1.30931	1.1918	6.8082	.00	10.00
	A. flavus	15	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	125	7.3440	6.74730	.60350	6.1495	8.5385	.00	24.00
Apium graveoleus L	Kp	15	7.2000	6.22438	1.60713	3.7531	10.6469	.00	14.00
	Ec	15	7.8000	4.12657	1.06548	5.5148	10.0852	.00	11.00
	Ml	13	9.5769	6.21103	1.72263	5.8236	13.3302	.00	16.00
	Pa	15	6.4000	5.65433	1.45994	3.2687	9.5313	.00	13.00
	Sa	15	7.2000	6.22438	1.60713	3.7531	10.6469	.00	14.00
	Lm	15	12.6000	7.43351	1.91933	8.4835	16.7165	.00	21.00
	Bs	15	14.4000	2.55790	.66045	12.9835	15.8165	10.00	19.00
	C. albicans	15	16.0000	3.07060	.79282	14.2996	17.7004	11.00	20.00
	A. flavus	15	4.6667	5.16398	1.33333	1.8070	7.5264	.00	10.00
	Total	133	9.5376	6.41897	.55659	8.4366	10.6386	.00	21.00

M. ANOVA of antimicrobial activity results

		Sum of Squares	df	Mean Square	F	Sig.
ALOEPERRYI	Between Groups	1788.859	8	223.607	6.868	.000
	Within Groups	4102.333	126	32.558		
	Total	5891.193	134			
Morus alba	Between Groups	936.887	8	117.111	2.959	.005
	Within Groups	4907.878	124	39.580		
	Total	5844.765	132			
Portulaca oleracea	Between Groups	1834.365	8	229.296	6.980	.000
	Within Groups	3810.863	116	32.852		
	Total	5645.228	124			
Apium graveoleus L	Between Groups	1834.556	8	229.319	7.889	.000
	Within Groups	3604.256	124	29.067		
	Total	5438.812	132			

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Chemical Composition, Phytochemical Constituents and Antioxidant Activities of the Seeds Extract of *Apiumgraveoleus L* from Yemen

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ABSTRACT

The traditional medicine still plays an important role in the primary health care in Yemen. Celery seeds are known to have carminative, stimulant, stomachic, emmenagogue, diuretic, antirheumatic, anti-inflammatory, and laxative properties. The antioxidant activities of ethanol, methanol, water, hexane and ethyl acetate of *Apiumgraveoleus L* were investigated spectrophotometrically against 1,1- diphenyl-2-picrlhdrazyl, hydrogen peroxide, and by ferric reducing power , total antioxidant capacity methods .Total phenols ,flavonoids, tannins, alkaloids were also determined using the standard methods. The five extracts showed the beset of total phenol in water extracts (2549.9 mg/100g dw) but the lowest was in hexane extract (166.19 mg/100g). Methanol extracts exhibited the strongest activity against DPPH radicals,but the lowest was in hexane extract.The ferric reducing activities of the extracts were significantly lower than the standard drugs used in this order : vitamin C > water > ethyl acetate > methanol > ethanol > hexane extract

Keywords: celery. antioxidant. total phenol, total flavonoids.

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INTRODUCTION

The use of Medicinal and Aromatic plant species in Yemen goes back thousands of years and form an important part of the culture, Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas.[1] Free radicals are defined as molecules having an unpaired electron in the outer orbit.[2] They are generally unstable and very reactive. Examples of oxygen free radicals are superoxide, hydroxyl, peroxyl ($RO^{\bullet}_2$), alkoxyl ($RO^{\bullet}$), and hydroperoxyl ($HO^{\bullet}_2$) radicals. Nitric oxide and nitrogen dioxide ($NO^{\bullet}2$) are two nitrogen free radicals .[3] They occur continuously in the cells as a result of enzymatic and non-enzymatic reactions to different molecules in the body .[4] Free radicals represent a class of highly reactive intermediate chemical entities whose reactivity is derived from the presence of unpaired electron in their structure, which are capable of independent existence for very brief interval of time .[5] Free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to x-rays, ozone, cigarette,smoking, air pollutants, industrial chemicals .[6] Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function, Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction .[7] Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases[8]. Antioxidants are beneficial components that neutralize free radicals before they can attack cell proteins, lipids and carbohydrates[9].Synthetic drugs such as butylatedhydroxytoluene (BHT), rutin, and butylated hydroxyl anisole (BHA) are commonly used. However, they have been reported to cause adverse side effects such as toxicity, cell damage, inflammations, and atherosclerosis in animals and humans[10].

Medicinal plants are in important source of antioxidants. The secondary metabolites like phenolic and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark[11]

Celery seeds are known to have carminative, stimulant, stomachic, emmenagogue, diuretic, antirheumatic, anti-inflammatory, and laxative properties, it is prescribed for epilepsy or psychiatric problems due to its tranquilizing effect. The oil is used to treat asthma, flatulence, and bronchitis. Leaves and petioles are used for skin problems in addition to the above-mentioned uses[12].

MATERIALS AND METHODS

Plant sources and preparation

Celery seeds collected also in 2010 from (Lahj –Yemen). Plant samples were collected and transferred in separated sterilized plastic sacks. The seeds were ground into fine powder using a hammer mill and stored at 4◦C until analysis.

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Chemical analysis

Determination of the lipid content:

Weigh 3-5 g of sample into a completely dried thimble and Place thimble in the Soxhlet's apparatus and fill the flask ¾ with ether. Start the water in condenser and heat the flask and set on 5-6 drops per second for four hours, Take out the thimble. Keep it at room temperature for evaporation of ether and then keep overnight in the oven at 105C°, Remove the thimble from oven, cool it in a desiccator and weigh.[10]

Calculation Wt. of sample = (Wt. of thimble + sample) – wt. of thimble

Wt. of fat = (Wt. of thimble + sample) - (Wt. of thimble + sample after extraction)

Ether extract (%) = $\frac{\mathbf{wt.of fat}}{\mathbf{wtofsample}} * \mathbf{100}$.[13]

Determination of crude fibre content:

Defatted sample (1g) was placed in a glass crucible and attached to the extraction unit (InKjel, D-40599, behr Labor-Technik GmbH, Dusseldorf, Germany). 150 ml boiling 1.25% sulphuric acid solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25% sodium hydroxide solution (150 ml) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace (MF-1- 02, PCSIR Labs., Lahore, Pakistan) for 2 h, cooled in a desiccator and reweighed (W2). Extracted fiber was expressed as percentage of the original undefatted sample and calculated according to the formula:

$$\text{Crude fibre (\%)} = \frac{\text{Digested sample (W1)} - \text{Ashed sample(W2)}}{\text{Weight of sample}} \times 100 .^{[14]}$$

Determination of crude protein

The crude protein was determined using micro Kjeldahl method [15]. Two grams of oven-dried material was taken in a Kjeldahl flask and 30 ml conc. H2SO4 was added followed by the addition of 10 g copper sulphate. The mixture was heated first gently and potassium sulphate and 1 g then strongly once the frothing had ceased. When the solution became colorless or clear, it was heated for another hour, allowed to cool, diluted with distilled water and transferred to a 800 ml Kjeldahl flask, washing the digestion flask. Three or four pieces of granulated zinc, and100 ml of 40% caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N receiving flask and distilled. When two-thirds of the liquid sulphuric acid was taken in the had been distilled, it was tested for completion of reaction. The flask was removed and

titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content.

Determination of moisture

Approximately 2 g of the material under test is accurately weighed (to 0.001 g) into a small dish. This is then placed in the oven for 1 hour, removed from the oven and put in the desiccator to cool. It is then weighed. The dish is replaced in the oven for 30 minutes and the process repeated to constant weight[16].The moisture content is found using the following formula

$$\% \text{ moisture} = \frac{\text{inital weight} - \text{ final weight}}{\text{inital weigh}} \times 100$$

Determination of total ash

For determination of ash content, 10 g of each sample was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about3–5 h at 600°C. It was cooled in a desiccator and weighed to ensure completion of ashing. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or greyish white). Weight of ash gave the ash content[15].

Determination of minerals

Weigh 2.0000 g of the dried and milled (to 1 mm) sample into a silica crucible and place in a cold muffle furnace with the chimney vent open, and allow to heat up to 450°C. Close the vent and maintain at this temperature overnight. Remove from the furnace and allow to cool, then add15 drops HCl from a polythene Pasteur pipette, being careful to moisten all the sample. Using a fume cupboard, gently evaporate off all the HCl on a hotplate at moderate heat, then remove and cool. Dissolve the residue in 0.1 M HCl, and transfer quantitatively to a 10-ml volumetric flask. Make up trace element standards in 0.1 M HCl covering the expected ranges in the sample solutions and analyse by (spectrophotomètre d' absorptim atomique.AI 1200.Aurora. Canada) according to the instrument manufacturer's instructions Calculation. The sample solution is of 2 g in 10 ml, therefore the concentrations in µg ml–1 of the trace element should be multiplied by 5 to give the concentration in µg g–1 of the trace element in the dried sample [17].

Determination of total carbohydrate:

Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein)[18].

Determination of alkaloids:

Alkaloids contents of the plants were determined using the method that described byHarborne[19].using soxhlet,ten grammes of the powdered sample was extracted with 250 mL of ethanol period five hours, extracted of ethanol was evaporated to dryness with a rotary evaporator,under reduced pressure at 40 ◦C. dry residue repeat by 150 mL of chloroform and acidify by HCl 5% pH 3 ,it let pillow during 30 minutes in the room temperature, the phase acid aqueous were extracted by 150 ml of chloroform , basify by the NaHCO3 5% pH 9 and lit it during 15 minutes in the room temperature . the chloroform phase was evaporated to dryness with a rotary evaporator under reduced pressure. The dry residue are the total alkaloids.

Determination of Tannin

Five grams of each part (leaves, stems) was milled into powder. The powder was extracted with 100 ml acetone–water (70/30, V/V), and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40 8C to remove acetone. The washed with 30 ml dichloromethane to remove lipid soluble remaining solution was substances. After that, the solution was further extracted with ethyl acetate at a ratio of 30/30 (V/V). The water layer was separated and extracted twice more similarly. Then the resulting water layer was evaporated to dryness, and the resulting substance was weighed [20].

Determination of total phenols:

The powdered plant material (2g) was extracted with methanol, at room temperature overnight. The methanol extracts were combined and concentrated under reduced pressure on a rotary evaporator.Total phenolic content of each plants extract was determined with the Folin–Ciocalteu's reagent (FCR) according to the published method [21]. Each sample (0.5 ml) was mixed with2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na2CO3 (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at30C^{o} for 90 min. Results were expressed as Gallic acid equivalent (mg Gallic acid/100g dried extract).

Determination of Total flavonoids

The total flavonoid content of celery extract was determined by a colorimetric method as described in the literature [22]. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO2 solution (15%). After 6 min, 0.15 ml of aluminum chloride (AlCl3) solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at510 nm versus prepared water blank. Results were expressed as Catechin equivalent (mg Catechin/100g dried extract).

Determination of ferric reducing power of the extracts

The reducing power of the seeds extract of celery was evaluated according to the method described by [23]. The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of the extracts and standards prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), which was then centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

DPPH scavenging assay

The hydrogen atom donation ability of chemical compounds in leaves and stems was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical [24]. Fifty microliter of various concentrations of the extracts in methanol were added to 1950 ml of a 0.025 g/l methanol solution DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Ablanc} \_ \text{Asample}}{\text{Ablanc}} \times 100$$

Where A blank is the absorbance of the control reaction(containing all reagents except the test compound), A sample is the absorbance of the test compound.

Measurement of hydrogen peroxide scavenging activity

1 ml of sample was mixed with 2.4 ml of 0.1 M phosphate buffer (pH 7.4), and then 0.6 ml of 43 mM solution of H_2O_2 in the same buffer were added. The mixture was incubated at room temperature for 40 min and the absorbance values of the reaction mixtures at 230 nm were recorded against a blank solution containing phosphate buffer without H2O2 for each sample[25]. The hydrogen peroxide scavenging activity was measured according to the following

$$H_2O_2 \text{ scavenging activity(\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Determination of total antioxidant capacity

The total antioxidant capacity (TAOC) of hexane, ethyl acetate, methanol,ethanol and water extract of celery seeds was evaluated by the method of Prieto[26]. An aliquot of 0.1 ml of sample solution (1 mg/ml) was combined with 1 ml of reagent solution (600 mMsulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was

measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions. The antioxidant capacity was expressed as the number of equivalents of α-tocopherol (mg/g of extract).

RESULTS

Results show that celery seeds contains a highest percentages of Carbohydrate and protein and lowest percentage of moisture and lipid. table (1).

Table1 : Nutritive values of experiment's plants parts

Plant Sample	Moisture %	Total Ash%	Protein% N×6.25	Lipid (%)	CrudeFiber%	Carbohydrate %	Nutritive value (Cal/100 g)
Celery	7.60	13.2	16.37	9.61	14.77	38.63	306.49

The concentration of Ca, Na, Zn, Ag, Cu, Fe and Pb.in Celery samples is given in Table (2).The highest concentrations of Ca, Na,. And the lowest concentrations of Ag, Pb, and Zn

Table 2:Mineral composition of plants samples

Plant Sample	Ca(ppm)	Na(ppm)	Zn(ppm)	Ag(ppm)	Cu(ppm)	Fe(ppm)	Pb(ppm)
Celery	6425	1425	45.5	0.025	18.75	162,25	6,25

The yields of the extracts were calaulated as percent by weight of the celery seeds. According to the chemical composition and polar or no polar nature of phenolic compounds, celery contains a relatively high percentage yield in Ethyl acetate ,ethanol,methanol and water while lower in hexane .yeild ,total phenolic and total flavoinods shown in table 3

Table 3:Yeild , Total phenolic content (TPC), flavonoid content (FC) and chelating activity of organic solvent extracts of fenugreek expressed as gallicacid,andcachain and t, respectively.

sample	yeild	TPC mg GAC/g dw	TFC mg cach/g dw
methanol Extraction	13.67±1.44	437.64±41.96	209.9±32.8
ethanol Extraction	16.81±2.23	245.75±11.6	120.08±11.5
Ethyl acetate Extraction	17.30±3	388.16±32.2	312.8±25.4
water Extraction	13.6±1.18	2549.9±234.16	590.37±54.34
hexane Extraction	12.95±0.68	166.19±44.27	46.34±3.6

The total phenolic content was determined by following a modified Folin-Ciocalteu reagent method. Using a standard curve of Gallic acid (R2 = 0.9964).In table 3 the results

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were expressed as Gallic acid equivalent.TPC was in the range of 2549.9 - 166.19 mg/100g of celery seeds extract. The amounts of total phenolic compounds were higher in water extract 2549.9mg/100g while lowest for hexane 166.19mg/100g.

Using the ALCl3 reagent and Catechin as standard (R2=0.9991), the total flavonoids are in the range from 590.37- 46.34 mg/100g of Catechin equivalent (Table 3).The highest value for the water was 590.37 mg/100g of celery with the following decreasing order of the extract water> Ethyl acetate > methanol > ethanol > hexane.

The potential of the plant extracts Fe3+ to Fe2+ by electron transfer is an in dictation of their antioxidant ability. The reducing power of the extracts in comparison with the standards (vitamin C) is presented in Figure 1. The observed change of yellow color of test solution to various shades of blue and green, depending on the concentration of the extract, is an indication of its antioxidant activity. The ferric reducing activities of the extracts were significantly lower than the standard drugs used in this order : vitamin C > water > ethyl acetate > methanol > ethanol > hexane extract.

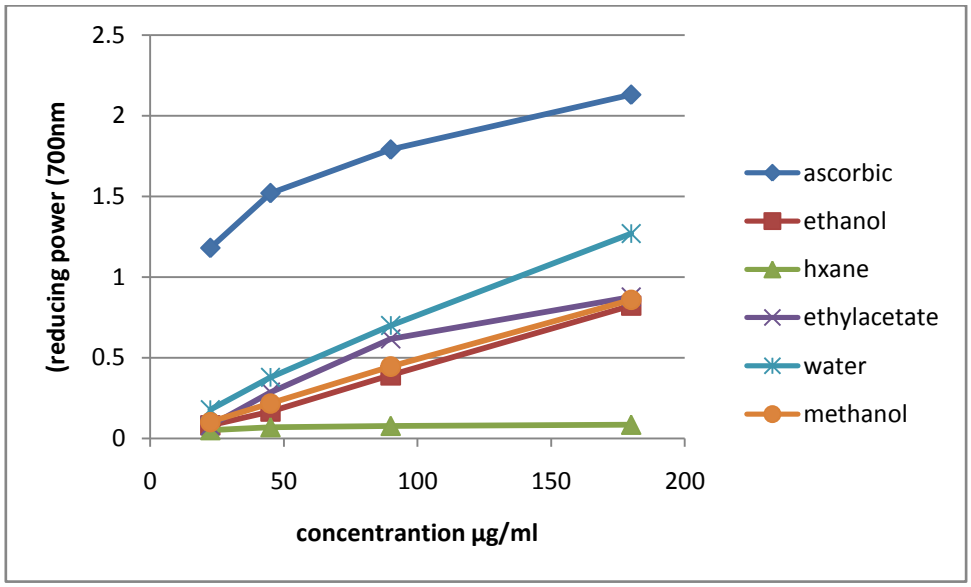

Figure 1: Reducing power of different solvent extracts of celery

The DPPH scavenging potential of the seeds extracts of celery concentration dependent as shown in Figure 2 .The IC50 of DPPH by the various solvent extracts and the standard drugs was recorded in decreasing order :BHA> methanol> vitamin C > ethyl acetate > water > ethanol > hexane, it was 71.61 ± 5.39 µg/ml, 83.825 ±1.03 µg/ml, 90.495 ± 22.8µg/ml, 101.415 ± 9.53µg/ml, 191.855 ± 3.23 µg/ml, 191.855 ± 3.23 µg/ml, 257.567±4.84µg/ml, 5.5±0.028 mg/ml respectively.

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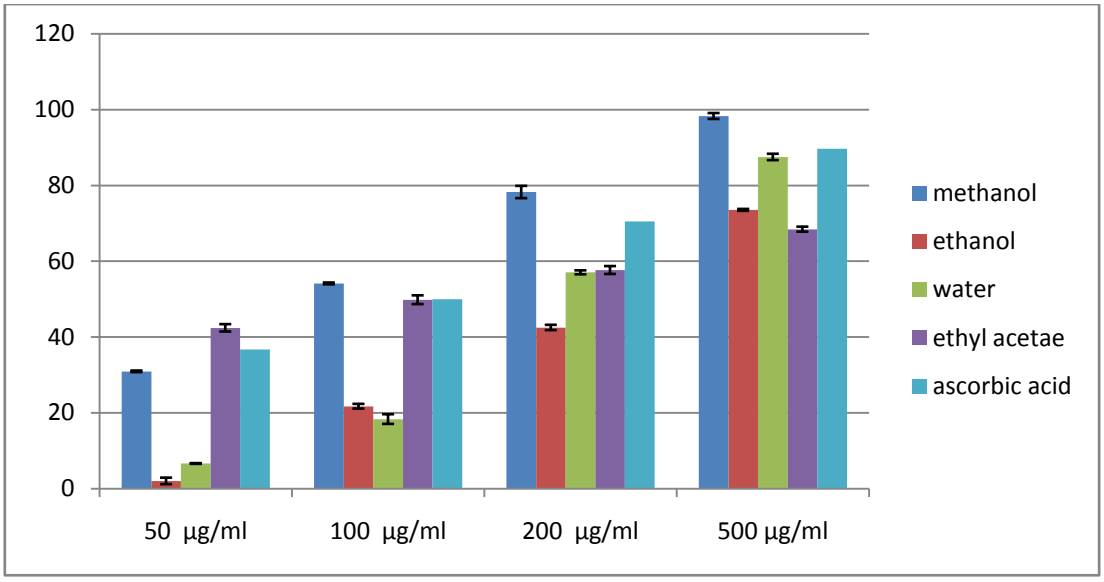

Figure 2: DPPH radical scavenging activity of the seeds extract of celery obtained using different solvents

The scavenging activity of the seeds extract of celery against hydrogen peroxide is shown in Figure3. The scavenging activities of the plant in various solvent were in the order: ethyl acetate > methanol >water > ethanol > hexane

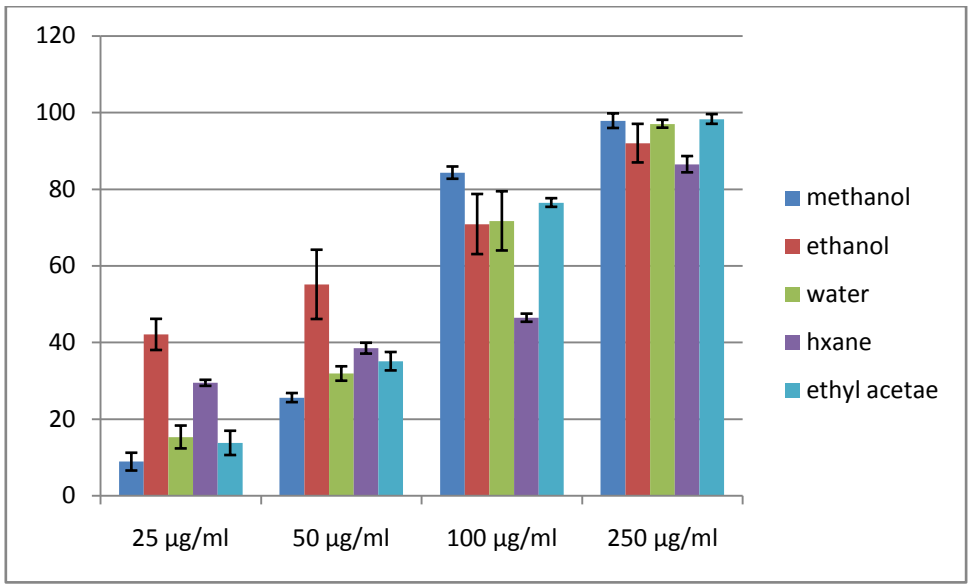

Figure 3:Hydrogen peroxide radical scavenging activity of the celery extract obtained using different solvents

The antioxidant capacity of seeds extracts of celery was found to decrease in the following order :methanol> ethyl acetate > hexane > water > ethanol as showed in (Figure 4)

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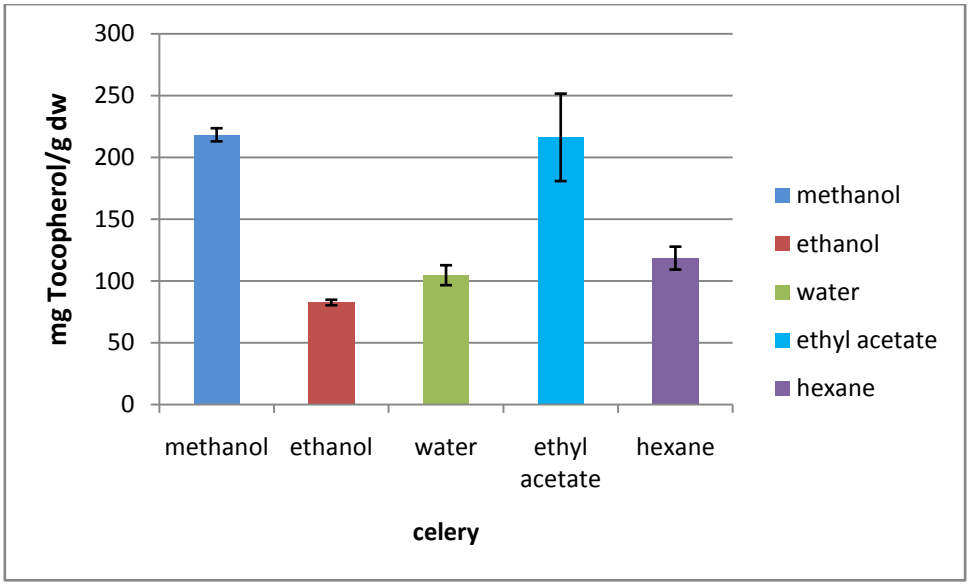

Figure 4: Total antioxidant capacity (TAOC) activity of the celery extract obtained using different solvents

DISCUSSION

Proteins play a central role in biological systems, the various biological function of protein can be categorized as enzyme catalysts, structural protein, contractile proteins, hormones, transfer proteins, antibodies,storage proteins, and protective proteins[27][. Our results showed that the concentration of protein and carbohydrate are good but they were low relatively to that reported byKhare[12].

The mineral are essential for human body ,they are basic content of many of body tissues such as calcium and phosphor for bone and iron for blood and muscles [28].

These dietary fiber in food have been shown to be useful in reducing blood glucose levels in diabetes, in reducing blood cholesterol levels, for treatment of cardiovascular disease and also in preventing bowel cancer [29].

The phenolic compounds may contribute directly to the antioxidant action [30]. And they are known to exhibit strong antioxidant activities, which have direct antioxidant properties due to the presence of hydroxyl groups, which act as hydrogen donor [31].Additionally, they are found to be effective in scavenging free radicals as a result of their redox properties that allow them to act as reducing agents [32]. In this study TPC was in the range of 2549.9-166. 19 mg/100 g of celery seeds. They amounts of total phenolic compounds were higher in water extract 2549.9 mg/100g while lowest for hexane. studies show that celery seeds, leaves and roots have total phenolic range 5100-1637. 1mg/100g in leaves [33], 233.1 in roots [34]While total phenolic content was 2486mg/100g in seeds [35].

The ability of a substance to act as an antioxidant depends on its strength to reduce ROS by donating hydrogen atom [36]. The reducing power ability of the plant extract was found out by measuring the transformation of Fe^{3+} into Fe^{2+}. The reducing power ability of a compound usually depends on the existence of reductions, which mainly act by braking the free radical chain reaction by donating a proton [37]. The results of reducing power in our

study were 0.21645 nm, 0.4454 nm, 0.85825 nm at concentration 45µg/ml, 90µg/ml, 180µg/ml respectively were higher in compare with that of Muthuswamy[37]. who studied reducing power ability of methanolic extract for celery seeds, and obtained that the reducing power was 0.0279 nm , 0.0946 nm, 0.1440 nm, 0.1971 nm at the concentrations 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml respectively. The reducing power of water extract in this study was the higher among the other extracts, these results are in relative with that of total phenols and total flavonoids where water extract had five fold that of the other extracts.

DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom, this method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH the DPPH solution [38]. The weak activity observed in the DPPH radical scavenging assay in hexane extract may be as a result of the low level of total phenolic and flavonoid content in the plant extract of hexane, statistic result of each TPC and TFC with DPPH by T test showed that they are negative correlation between each of TPC and TFC with DPPH of all extract, but there no significant different.

Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects [39].

Celery activity against hydrogen peroxide was good in all of plant extracts, and there were correlation between these results and the results of reducing power of plant extracts, that may refer to the similarly in reaction mediums and the conditions of these methods, where the water is the main solvent in both reactions and the pH was ranged from 6.6-7.4.

The antioxidant capacity of the fractions was measured spectrophotometrically throughphosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by thesample analyte and the subsequent formation of green phosphate/Mo (V) compounds with amaximum absorption at 695 nm [40].

With regard to the result of the phytochemical structure of celery and each of reducing power, DPPH, Hydrogen peroxide in comparing with total capacity of celery we found that there are accordance with all the extracts in particularly methanol, ethyl acetate, and ethanol extract which showed the high activity in all methods of antioxidant activity that used in this study.

CONCLUSION

Based on our study we can conclude that Celery seeds is a potent as source of nutrients as protein, lipids, sugars, ash and fiber. It also contains important quantities of polyphenols, flavonoids, and vital trace minerals, in addition Celery seeds seems to be respectable antioxidant, therefor Celery seeds might be used as food supplement, and to contribute in health improvement and maintenance, it will be useful in particularly with the disease that related with the free radicals such as diabetes mellitus, cancer, and cardiovascular disease.

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Research Article

CHEMICAL AND PHYTOCHEMICAL ANALYSIS OF SOME ANTI DIABETIC PLANTS IN YEMEN

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ABSTRACT

There are many hypoglycemic plants known through the folklore in Yemen and some of Arabic Countries. The study was carried out to analyze the nutritional composition, mineral content and phytochemicals of the seeds of *Trigonella foenum* L, *Apium graveoleus* L and risen of *Commiphora myrrha* which are used traditionally in Yemen to treat diabetic patients. For different plant species the crude fat content ranged between (9.61 to 0.54 g/100 g) and crude fiber (14.77 to 1.51 g/100 g). The crude protein content was determined high in the seeds of *T. foenum* L (26.78 %), seeds *A. graveoleus* L (16.37 %) and risen *C. myrrha* (10.45 %) while the carbohydrate content was highest in the *C. myrrha* (71.21 %). The nutritive value ranged from 362.35 - 306.49 cal/100 g in the various plants. Calcium was present in the highest quantity (14000 ppm) in the *C. myrrha*. The content of total phenol varied from 1422.622 mgGAE/100 g to 505.286 mgGAE/100 g in the extracts.

Keywords: nutritional composition, diabetic, mineral content, total phenol

INTRODUCTION

The use of medicinal and aromatic plants species in Yemen goes back thousands of years, and form an important part of the culture, some of the plants traditionally still play important role in the health and body care system. Fenugreek commonly used in Yemen, it is an annual herb native to the Mediterranean region, North Africa, India, and Yemen, It is now widely cultivated in these areas. The material of commerce comes exclusively from cultivated plants mainly from Morocco, Turkey, India, and China. (Wichtl *et al*, 1994; Bruneton, 1995; Bhp, 1996; Leung *et al*, 1996, Al-Mamary *et al*., 1997), Fenugreek is stated to possess mucilaginous demulcent, Laxative, nutritive , expectorant and orexigenic and vulnerary properties[1]. In addition to its use in flavoring foods, the antifungal and antibacterial properties of fenugreek are now being applied to food preservation, some studies also show that serum cholesterol levels in diabetics and perhaps in others are reduced by fenugreek. There is some evidence that internal use of fenugreek seed can decrease some stone-forming substances in the kidney, particularly calcium oxalate. Fenugreek may encourage a flagging appetite. Cancer researchers are also studying fenugreek for its potential effectiveness as a cancer preventive. It is thought shows that fenugreek may help to prevent cancer by raising the levels of vitamin C, vitamin E, and other antioxidants in the bloodstream[2]. Myrrh (*Commiphora. myrrha*) is a close relative and member of the Burseraceae family, Myrrh is an Arabic word meaning bitter, the highly valued aromatic gum resin of myrrh has a bitter, pungent taste and a sweet, pleasing aroma. Myrrh grows to a height of about 9 ft (2.7 m). The light gray trunk is thick and the main branches are knotted with smaller branches protruding at a right angle and ending in sharp spines. The hairless, roughly toothed leaves are divided into one pair of small, oval leaflets with a larger, terminal leaflet. The yellow-red flowers grow on stalks in an elongated and branching cluster. The small brown fruit is oval; tapering to a point.[3] It is native to the eastern Mediterranean, Ethiopia, the Arabian peninsula, in particularly Yemen and Somalia. This plant is used in folk medicine as an expectorant, anti-inflammatory and antispasmodic for the treatment of oro-dental in infections, it is used also as and amulet, analgesic, treat emotional and psychological.[3] It is employed as a mouth wash and gargle as emmenagogue. In West Africa the gum of Myrrh resin is boiled for treatment of inflammation of the eyes by holding the face over the steaming pot; the myrrh resin has antimicrobial properties and acts to stimulate macrophage activity in the blood stream. The herb is being studied for its potential as an anticancer medication[2], Anesthetic, Antiemetic, Antioxidant, Fungicide[4], antidiabetic.[5] Celery is a biennial vegetable, although grown as an annual crop. it is a member of the Apiaceae family.[6] Its seeds are used as an antispasmodic, also it is to treat asthma, bronchitis, disease of the liver, spleen, kidney failure, bladder and kidney calculi, edema, arthritis, dizziness, gout, weight loss, lowering blood pressure, relief of anxiety, insomnia, and reducing blood sugar. In the European tradition, the seeds have been used as carminative, stomachic, emmenagogue, diuretic, and laxative, also for glandular stimulation, rheumatic complaints, nervous unrest, loss of appetite and exhaustion.[7-9]

MATERIALS AND METHODS

Plant sources and preparation

Fenugreek seeds were collected in 2011 from region (Bit Al Ashowal –Ibb-Yemen), Myrrhresin were obtained in 2011 from (island Soqatra–Yemen) and celery seeds were collected also in 2011 from (Lahj–Yemen). Plant samples were collected and transferred in separated sterilized plastic sacks). Fresh plant material was allowed to air dry at ambient temperature (25°C) in the laboratory for approximately 15 days. The completely dried seeds and resin were crushed to powder by a hammer mill and stored at 4◦C until analysis.

Chemical analysis

Determination of the lipid content

The lipid content was determined using petroleum ether in Soxhlet reflux extractor.[10]

Determination of crude fibre content

Defatted sample (1 g) was placed in a glass crucible and attached to the extraction unit (InKjel, D-40599, behr Labor-Technik GmbH, Dusseldorf, Germany). 150 ml boiling 1.25 % sulphuric acid solution was added. The sample was digested for 30 minutes and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25 % sodium hydroxide solution (150 ml) was added. The sample was digested for 30 minutes, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace (MF-1-02, PCSIR Labs., Lahore, Pakistan) for 2 h, cooled in a desiccator and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula[11]

Crude fibre (%) = Digested sample (W1)-Ashed sample (W2) / Weight of sample x 100

Determination of crude protein

The crude protein was determined using micro Kjeldahl method.[12] Two grams of oven-dried material was taken in a Kjeldahl flask and 30 ml conc. H_2SO_4 was added followed by the addition of 10 g copper sulphate. The mixture was heated first gently and potassium sulphate and 1 g then strongly once the frothing had ceased. When the solution became colorless or clear, it was heated for another hour, allowed to cool, diluted with distilled water and transferred to 800 ml Kjeldahl flask, washing the digestion flask. Three or four pieces of granulated zinc, and100 ml of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N is receiving flask and distilled. When two-thirds of the liquid sulphuric acid was taken in; had been distilled, it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content.

Determination of moisture

Approximately 2 g of the material under test is accurately weighed (to 0.001 g) into a small dish. This is then placed in the oven for 1 hour, removed from the oven and put in the desiccator to cool. It is then weighed. The dish is replaced in the oven for 30 minutes and the process repeated to constant weight.[13] The moisture content is found using the following formula

$$\%\ \text{moisture} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weigh}} \times 100$$

Determination of total ash

For determination of ash content, 10 g of each sample was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3–5 h at 600°C. It was cooled in a desiccator and weighed to ensure completion of ash. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or greyish white). Weight of ash gave the ash content.[12]

Determination of minerals

Weigh 2.0000 g of the dried and milled (to 1 mm) sample into a silica crucible and place in a cold muffle furnace with the chimney vent open, and allow to heat up to 450°C. Close the vent and maintain at this temperature overnight. Remove from the furnace and allow to cool, then add 15 drops HCl from a polythene Pasteur pipette, being careful to moisten the entire sample. Using a fume cupboard, gently evaporate off all the HCl on a hotplate at moderate heat, then remove and cool. Dissolve the residue in 0.1 M HCl, and transfer quantitatively to a 10-ml volumetric flask. Make up trace element standards in 0.1 M HCl covering the expected ranges in the sample solutions and analyses by (spectrophotomètre d' absorption atomique.AI 1200.Aurora Canada) according to the instrument manufacturer's instructions Calculation. The sample solution is of 2 g in 10 ml, therefore the concentrations in µg ml–1 of the trace element should be multiplied by 5 to give the concentration in µg g–1 of the trace element in the dried sample.[14]

Determination of total carbohydrate

Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein.[15]

Determination of alkaloids

alkaloids contents of the plants were determined using the method that described by Harborne (1998)[16] by soxhlet, ten gramme of the powdered sample was extracted with 250 mL of ethanol period five hours, extracted of ethanol was evaporated to dryness with a rotary evaporator, under reduced pressure at 40◦C. dry residue repeat by 150 mL of chloroform and acidify by HCl 5 % pH 3, it let pillow during 30 minutes in the room temperature, the phase acid aqueous were extracted by 150 ml of chloroform, basify by the $NaHCO_3$ 5 % pH 9 and lit it during 15 minutes in the room temperature. The chloroform phase was evaporated to dryness with a rotary evaporator under reduced pressure. The dry residue is the total alkaloids.

Determination of Tannin

Five grams of each part (seeds resin) was milled into powder. The powder was extracted with 100 ml acetone–water (70/30, V/V), and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40^0C to remove acetone. The washed with 30 ml dichloromethane to remove lipid soluble remaining solution was substances. After that, the solution was further extracted with ethyl acetate at a ratio of 30/30 (V/V). The water layer was separated and extracted twice more similarly. Then the resulting water layer was evaporated to dryness, and the resulting substance was weighed.[17]

Determination of total phenols

The powdered plant material (2 g) was extracted with methanol, at room temperature overnight. The methanol extracts were combined and concentrated under reduced pressure on a rotary evaporator. Total phenolic content of each plants extract was determined with the Folin–Ciocalteu's reagent (FCR) according to the published method.[18] Each sample (0.5 ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na_2CO_3 (7.5 %, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30°C for 90 minutes. Results were expressed as Gallic acid equivalent (mg Gallic acid /g dried extract).

Determination of flavonoid

The total flavonoid content of plants extracts was determined by a colorimetric method as described in the literature[19]. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a $NaNO_2$ solution (15 %). After 6 minutes, 0.15 ml of aluminum chloride ($AlCl_3$) solution (10 %) was added and allowed to stand for 6 minutes, then 2 ml of NaOH solution (4 %) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as Catechin equivalent (mg Catechin/100 g dried extract).

RESULT

Results show that Fenugreek seeds contain a highest percentages of proteins and calories and lowest percentage of moisture and ash, while the highest content of carbohydrate and moisture appears in myrrh, and it contains the lowest percent of Protein, Crude fat and Crude Fiber. Whereas Celery was superior in its content of ash, fats and fibers, Table 1.

Table 1: Nutritive Values of Experiment's Plants Parts

Plant Sample	Moisture %	Total Ash %	Protein % N × 6.25	Lipid (%)	Crude Fiber %	Carbohydrate %	Nutritive value (Cal/100 g)
Celery	7.60	13.2	16.37	9.61	14.77	38.63	306.49
Myrrh	10.09	6.20	10.45	0.54	1.51	71.21	331.5
Fenugreek	6.57	4.03	26.78	6.35	6.75	49.52	362.35

The concentration of Ca, Na, Zn, Ag, Cu, Fe and Pb in Celery, Myrrh and Fenugreek samples is given in Table 2. The highest concentrations of Zn, Ag, Cu, Fe and Pb found in Celery, while that of Ca and Na were in Myrrh and Fenugreek respectively. And the lowest concentrations of Na, Zn, Ag and Fe were in Myrrh, and they were of Ca, Ag, Cu, and Pb in Fenugreek. In each plant samples Ca present in great amount followed by Na, Fe, Zn, Cu, and Pb respectively. While Ag present in slightest amount in Celery and weren't found in both of Myrrh and Fenugreek.

Table 2: Mineral Composition of Plants Samples

Plant Sample	Ca (ppm)	Na (ppm)	Zn (ppm)	Ag (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)
Celery	6425	1425	45.5	0.025	18.75	162,25	6,25
Myrrh	14000	900	6,5	0.00	16	432.5	5,25
Fenugreek	3225	1500	25.5	0.00	15	101.25	3,5

Table 3 Expose the photochemical structure of experiment's plant samples (Mg/ 100 g). Tannin was quite low in myrrh it was higher in celery seeds, while total flavonoids were higher in myrrh and celery. Alkaloids were least amount in all plants. Fenugreek possesses the upper value of total phenols followed by Myrrh and Celery respectively.

Table 3: Photochemical Content of Experiment's Plants

Plant sample	Total phenol mg/100 g	Alkaloids g/100 g	Tannin g/100 g	Flavonoids g/100 g
Celery	720.879	0,1645	5,216	9,15
Fenugreek	505.286	0,7555	2,032	4,99
Myrrh	1422.622	0,1645	0,84	9,4

DISCUSSION

The aqueous extracts of seeds and leaves of fenugreek have been shown to possess hypoglycemic activity and are nontoxic.[20] Fenugreek seed contains 45-60 % carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30 % proteins high in lysine and tryptophan; 5-10 % fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.36 %), choline (0.5 %), gentianine and carpaine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; free amino acids, such as 4- hydroxyisoleucine (0.09 %); arginine, histidine and lysine; calcium and iron; saponins (0.6-1.7 %); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol; vitamins A, B1,C and nicotinic acid; coumarin compounds and 0.015 % volatile oils (nalkanes and sesquiterpenes).[21] Our results showed that the concentration of proteins, lipids and carbohydrates of fenugreek were in the same ranges of that of the previous study. But protein contents in this study Higher than the results of Randhir.[22] The lipid content of experimental plant samples was low relatively, especially in Myrrh which is in the recommended value by the British Pharmacopoeia.[23] The presence of Ash in these plant in such quantities are satisfying, because of the high importance of mineral for health maintenance and development. Where the minerals are essential for human body, they are basic content of many of body tissues, such as calcium and phosphor for bone and iron for blood and muscles. They consider basic element of biomolecules (proteins, enzymes, phospholipids) in addition to their roles in connectivity process, and in all of biochemical reactions.[24] The content of fiber and ash in the plants of our experiment was adequate particularly in Celery. These dietary fiber in food have been shown to be useful in reducing blood glucose levels in diabetes, in reducing blood cholesterol levels, for treatment of cardiovascular disease and also in preventing bowel cancer.[25,26] Some literatures suggested that abnormal zinc metabolism play a role in the pathogenesis diabetes and/or its complications.[27] The complexes of zinc and insulin in varying ratios are stored in pancreatic β-cells and released into the circulation via the portal vein.[28] Enzymes that do not contain a trace element as an integral part but are activated by metals such as Cu, Fe,

and Ni respond to *in vitro* addition of several transition elements with a dose-dependent activation.[29] Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting, and the regulation of cell permeability. It also plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system. Inorganic elements like Zn, Cr, V, Fe, Cu, and Ni play basic role in the improvement of impaired glucose tolerance and their indirect role in management of diabetes mellitus are being increasingly recognized .[30] The results of minerals in our study for fenugreek were approximately similar with Choudhury[31] with exception of Ca and Fe where it was the double in our results for Ca and the infers for Fe. And Ca content in fenugreek in turkey was higher in comparing with the same plant in Yemen, while the other minerals contents were similar found lower concentration of Zn and Cu in Myrrh.[32,33] That can be referred to the difference of soil and water structures, the climate and weather conditions. Phenolic in plants are usually found in conjugated forms through hydroxyl groups with sugar as glycosides.[34] The phenolic compounds may contribute directly to the antioxidant action[35]. It has been suggested that phenolic are secondary metabolites, and in part, are produced as a result of the plant's interaction with the environment.[36] In addition, a wealth of other classes of compounds, such as polyphenols including Flavonols glycosides are also suggested to contribute to the health promoting properties of these species.[37] Studies show that fenugreek seeds have antioxidant Properties.[38] Some studies have suggested a possible protective effect of flavonoids against vascular diseases.[39] The presence of tannins may be responsible for ability to cure diseases such as diabetes.[40] The alkaloids may be responsible for the anticancer, anti diabetics, anti-aging and antiviral activities of this herbal plant.[41] The tannins have been reported to inhibit digestive enzymes , affect the utilization of vitamins and minerals and are capable of binding and precipitating protein causing a reduction in nutritional value.[42] They have therefore been regarded as antinutrients and considered nutritionally undesirable,[42] however, these compounds are also believed to have some favorable effects on human health, such effects as the lowering of human low-density lipoprotein, reduction of heart diseases and cancer.[42] Total phenolic was 171, 3 of the fenugreek extract, this result agreed with the results of previous study.[36,43] While the result of Flavonoid content in fenugreek in this study is agreed with the earlier study by Gupta and[44] which had shown that the fenugreek seeds are rich in flavonoids (>100 mg/g). and it was higher comparing with that of Bukhari.[36] Celery leaves have total phenolic range between 5100-1637.1 mg/100g,[45] 233.1 in roots,[46] while total phenolic content was 2486 mg/100 g in seeds.[47] In this study total phenolic was 720.879 mg/100 g.

CONCLUSION

The plants of this experiment contain elevated concentrations of Fe, Cu and Zn, that essential element of importance in diabetes. The components of Celery, Myrrh and fenugreek, such as phenolic compounds, especially phenolic acid and flavonoid derivatives, carotenoids, tocopherol and vitamin C, minerals, fibers possess Antioxidant and Antidiabetic activities.

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Mineral Content of Some Medicinal Plants Used in the Treatment of Diabetes Mellitus in Yemen

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There are many hypoglycemic plants known through the folklore in Yemen and some of Arabic countries, some findings of several studies indicate that some of minerals as potassium, copper, zinc, sodium, and iron have hypoglycemia activity, in this study some of medical plants (*Aloe vera*, *Eugenia aramatica*, *Commiphora myrrha, Punica graneotum L*., *Nigelle sativa L., Portulaca oleracea L., Trigonella foenum L*., *Morus alba*, *Lupinus termis*, *Apium graveoleus L.and Cinnamomum verum*) which are used traditionally in Yemen to treat diabetic patients, these plants were tested to determine total ash and the content of these plants from Ca, Na, Zn, Ag, Cu, Fe and Pb. using atomic absorption spectroscopy. A total Ash content was 15.05 % as maximum level in *Trigonella foenum L* while the minimum level was 1.45 % in *Commiphora myrrha*, the limited concentration of Ca was 22000 ppm in *Nigelle sativa L. and* 3225 ppm in *Aloe vera*, and Na was high 3025 ppm in *Punica graneotum L*. and low 800 ppm in *Morus alba*, and Zn concentration ranged from 45,5 ppm in *Cinnamomum verum* to 6,5 ppm in *Apium graveoleus L*., Ag was 0,175 ppm in *Portulaca oleracea L.* and 0,025 ppm in *Nigelle sativa L.* and *Cinnamomum verum*. And it was absent in *Apium graveoleus L*., *Morus alba*, *Eugenia aramatica and Aloe vera*, The major value of Cu 20.75 ppm found in *Nigelle sativa L*. and the minor value 8.25 ppm in *Trigonella foenum L*. and Fe was raised in *Apium graveoleus L* 432.5ppm and increased in *Lupinus termis* 47,52 ppm. While Pb had the higher concentration 7 ppm in *Trigonella foenum L*. And the lower concentration 3ppm in Eugenia *aramatica*. These results indicate to the affectivity of these plants as antidiabetic plants where they will decrease serum glucose level when consumed in adequate quantities.

Keywords: *minerals; medicinal plants, Atomic absorption, spectroscopy, hypoglycemia activity.*

Introduction

Diabetes mellitus is a chronic disease which is a medical disorder characterized by persistently variable high blood sugar levels causing hypoglycemia and other complications of the kidney eye, and a variety of neuropathies. It arises either from inadequate biosynthesis, secretion, or action of the hormone insulin, an inadequate response by the body's cells to insulin, or a combination of these factors (Choudhury R. Paul et al. 2007).

It arises either from inadequate biosynthesis, secretion, or action of the hormone insulin, an inadequate response by the body's cells to insulin, or a combination of these factors (Krentz AJ, Bailey C. 2006).

Plant based drugs have been in use against various diseases since time immemorial. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues (Mushtaq et al. 2009).

There are many hypoglycemic plants known through the folklore but their introduction into the modern therapy system awaits the discovery of animal test system that closely parallel to the pathological course of diabetes in human beings. Hypoglycemic activity has been reported in many plants during the last twenty years (Anon ymous 1992).

Since time immemorial, diabetes has been treated with plant medicines such as Neem (*Azadirachta indica*) and Curry leaves (*Murraya koenigi*i), Bitter gourd or Karela (*Momordica charantia*) fruit, Fenugreek (*Trigonella foenum*), Gurmaar (*Gymnema sylvestre*). And Jaamun (*Syzygium cuminii*) seeds (Sivarajan VV and Balachandran I 1996).

Minerals are considered one of the more important components which play an important role in diabetes treatment.

In this study seven eleven Yemeni plant which used traditionally in the treatment of diabetes will be examined to determine their contents of total minerals (ash) and the concentration of seven important minerals(Ca, Na, Zn, Ag, Cu, Fe, Pb) by atomic absorption technique in these plants to determine their antidiabetic properties.

MATERIALS AND METHODS

Sample Collection

Plants were purchased from local markets in Sana'a -Yemen. The common and scientific names and the used part of the plants are given in Table 1.

Table 1. Common and botanic name and used parts of plants

Common name	Botanical name	Used parts
Aloe	Aloe vera	Leaf
Clove	Eugenia aramatica	Buds
Myrrh	Commiphora myrrha	oleo-gum resin from stem
Pomegrante	Punica graneotum L	Rind of fruit
Black cumin	Nigelle sativa L	Seed
Common purslane	Portulaca oleracea L	Seed
Fenugreek	Trigonella foenum L	Seed
White plnlberry	Morus alba	Leaf
Lupin	Lupinus termis	Seed
Celery	Apium graveoleus L	Seed
Cinnamon	Cinnamomum verum	The outer bark

Preparation of plant samples for analysis

Tow grams (2.0000 g) of the dried and milled (to 1 mm) sample weighed into a silica crucible and placed in a cold muffle furnace with the chimney vent open, and allowed to heat up to 450 °C. The vent was closed and maintained at this temperature overnight. The crucible then removed from the furnace and allowed to cool, then 15 drops HCl was added from a polythene Pasteur pipette. Using a fume cupboard, after that all of HCl gently evaporated off on a hotplate at moderate heat, and then the crucible and their content was removed and cooled (Faithful N.T. 2002).

Determination of mineral contents

The residue from the previous step dissolved the in 0.1 M HCl, and transfer quantitatively to a 10-ml volumetric flask. Make up trace element standards in 0.1 M HCl covering the expected ranges in the sample solutions and analyzed by atomic absorption spectrometer (AAS) technique using (spectrophotomètre d'absorption atomique. AI 1200. Aurora. Canada) according to the instrument manufacturer's instructions (Faithful N.T. 2002).

Calculation of mineral concentration

The sample solution is of 2 g in 10 ml, therefore the concentrations in μg/ml of the trace element should be multiplied by 5 to give the concentration in μg/g of the trace element in the dried sample (Faithful N.T. 2002).

RESULTS AND DISCUSSIONS

Mineral compositions of experiment plants are shown in Table (2).

A total Ash content was 15.05 % as maximum level in *Trigonella foenum L* while the minimum level was 1.45 % in *Commiphora myrrha*., the limited concentration of Ca was 22000 *ppm* in *Nigelle sativa L. and* 3225 ppm in *Aloe vera*, and Na was high 3025 *ppm* in *Punica graneotum L.* and low 800 ppm in *Morus alba*, and Zn concentration ranged from 45,5 ppm in *Cinnamomum verum* to 6,5 ppm in *Apium graveoleus L*., Ag was 0,175 *ppm* in *Portulaca oleracea L.* and 0,025 *ppm* in *Nigelle sativa L.* and *Cinnamomum verum*. And it was disappeared in *Apium graveoleus L*., *Morus alba*, *Eugenia aramatica and Aloe vera*, The major value of Cu 20.75 ppm found in *Nigelle sativa L*. and the minor value 8.25 *ppm* in *Trigonella foenum L*. and Fe was raised in *Apium graveoleus L.* 432.5 ppm and increased in *Lupinus termis* 47,52 *ppm*. While Pb had the higher concentration 7 *ppm* in *Trigonella foenum L*. and the lower concentration 3 *ppm* in *Eugenia aramatica*.

Table 2. The mineral compositions of plants

Plant	Ca (ppm)	Na (ppm)	Zn (ppm)	Ag (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)	ASH %
Aloe vera	3225	1500	25.5	0.00	15	101.25	3.50	3.40
Eugenia aramatica	4600	1000	38.5	0.00	12	139.75	3.00	4.34
Commiphora myrrha	7115.6	1918	7.72	0.11	9.9	68.22	25.05	1.45
Punica graneotum L	4100	3025	9	0.075	9.25	174.75	5.00	5.10
Nigelle sativa L	22000	1225	36.5	0.025	20.75	239.25	5.00	12
Portulaca oleracea L	4000	1450	11.75	0.175	8.75	210.5	4.25	7.10
Trigonella foenum L	16250	1175	19.5	0.125	8.25	85.75	7.00	15.05
Morus alba	3600	800	10	00.00	11,75	65,5	6,5	2.20
Lupinus termis	6525	1053	32.67	0.27	9.18	47.52	4.86	3
Apium graveoleus L	14000	900	6.5	0.00	16	432.5	5.25	8
Cinamomum verum	6425	1425	45.5	0.25	18.75	162.25	6.25	6.85

The concentration of elements in the plants which are analyzed in the study decreases in the following order: Ca, Pb, Na, Cu, Zn, and Ag.

The order of plant according their minerals contents is as follows *Nigelle sativa L*, *Trigonella foenum L*, *Apium graveoleus L*, *Commiphora myrrha*, *Cinnamomum verum*, *Lupinus termis*, *Punica graneotum L*, *Eugenia aramatica*, *Portulaca oleracea L*, *Aloe vera* and *Morus alba*.

The results of the present study showed that the concentration of Ca, Cu and Fe. In Nigelle sativa L. was more than that in previous study in Turkey while the concentration of Zn was less than that in that study. And we note decreasing of Zn, CU and Fe concentration in Trigonella foenum L. and increasing of Ca concentration comparing with the pervious study (Ozcan M. 2004).

The role of inorganic elements like Zn, Cr, V, Fe, Cu, and Ni in the improvement of impaired glucose tolerance and their indirect role in management of diabetes mellitus are being increasingly recognized (NARENDHIRAKANNAN RT et al. 2005). Levine A. S et al (1983) has observed that there is tissue zinc deficiency in genetically obese, insulin-resistant diabetic mice. Failla M. L et al (1983) suggested that abnormal zinc metabolism play a role in the pathogenesis diabetes and/or its complications. The complexes of zinc and insulin in varying ratios are stored in pancreatic β-cells and released into the circulation via the portal vein (Scott D. A. and Fisher A. M. 1938). Enzymes that do not contain a trace element as an integral part but are activated by metals such as Cu, Fe, and Ni respond to in vitro addition of several transition elements with a dose-dependent activation (Speck J. F. 1949).

Copper is widely distributed in biological tissues, where it occurs largely in the form of organic complexes, many of which are metalloproteins and function as enzymes. Copper enzymes are involved in a variety of metabolic reactions, such as the utilization of oxygen during cell respiration and energy utilization (Aras NK. and Ataman OY .2006). Calcium is the major component of bone and assists in teeth development (Brody T. 1994). Iron is also required for the activity of certain enzymes involved in energy production and about 10 % of the body pool of iron is used in this way (Aras NK. and Ataman OY .2006).

Mean dietary intakes of children have been reported to be in the range 9–278 μg of lead per day and for adults 20–282 μg day_1. A typically high dietary intake (e.g. _500 μg of lead per day) was found in one Indian investigation (Aras NK. and Ataman OY .2006). According to our results the lead contains in the plants are adequate and in the save range.

Our results demonstrate that he concentration of Major elements and Micronutrients in all of experimented plants are in Sufficient or normal ranges according to reference and standards values (kalra Y P 1998). The importance of these elements cannot be overemphasized because many enzymes require them as cofactors (Akpanabiatu MI. et al 1998).

From the present study, it is concluded that the presence of various inorganic trace elements such as, zinc, copper, iron, potassium, and sodium in the plants could account for the hypoglycemic nature of these plants. Further, the data obtained on individual element concentration in each plant will be useful in deciding the dosage of herbal drugs prepared from these plant materials for the management of diabetes related metabolic disorders. And the present of these minerals in this plant in good balance to improve and maintenance of human health and to help meet daily mineral needs. In addition to these findings the results from the traditional use of these plants in Yemen gives affirmative assurances to the benefits of these plants in the treatment of diabetes.

Finally we conclude in this study that the plants which are examined in this work have potential antidiabetic activity when consumed in adequate quantities.

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ملخص

يعود استخدام النباتات الطبية والعطرية في اليمن الى آلاف السنين ، حيث تشكل جزء هام من الثقافة العلاجية. بعض النباتات مازالت تلعب دور مهم في الصحة ونظام العناية بجسم الانسان. تعتبر النباتات مصدر رئيسي للكثير من الأدوية المستخدمة لعلاج مختلف الامراض . ترجع قدره العقاقير النباتية ضد الامراض بشكل رئيسي الى مكوناتها الكيميائية. والعقاقير النباتية اليوم تعتبر اكثر امانا مقارنه بالعقاقير المصنعة كيميائيا.

تهدف دراستنا الى الكشف عن التركيب الكيمائي وتقدير مركبات الايض الثانوية لثمانية من النباتات اليمنية، وتحديد قدرتها كمضادات للأكسدة ، وكذا قدرتها على تثبيط نمو الميكروبات الممرضة وتشمل الصبر السقطري ، الكرفس، المر، الترمس المر، التوت، حبه البركة ، الرجلة واخيرا الحلبة .

تم استخلاص النباتات بعدة مذيبات مختلفة وتم تقدير المركبات الفينولية الكلية والفلافونيدات والتانينات والقلويدات. اظهر مستخلصا الايثانول والميثانول لنبات الصبر السقطري اعلى محتوى من الفينولات الكلية (20184.16 ملغGAE / 100 غ وزن جاف، 10771 ملغ GAE/ 100غ وزن جاف) على التوالي.

تقييم فعالية النباتات كمضادات للأكسدة أجري مخبريا باستخدام طريقه تنظيف DPPH و فوق اكسيد الهيدروجين ،و اختزال الحديديك والقدرة الكلية المضادة للأكسدة. وبينت النتائج ان مستخلص اسيتات الايثيل لنبات الصبر السقطري يملك اعلى قدره على تنظيف جزيئات DPPH (IC50 = 5.34 مكغ / مل)، كما أن له قدرة على اختزال الحديديك (EC50 = 3.273 مكغ /مل)، بينما امتلك مستخلص الميثانول لنبات الصبر السقطري اعلى قدره كمضاد للأكسدة الكلية (478.0535 ملغ الفا توكوفيرول/غ وزن نبات جاف).

أجريت دراسة قدره المستخلصات على تثبيط الميكروبات بطريقه الانتشار بالأقراص، حيث تم استخدم تسع سلالات من الميكروبات الممرضة (سبع سلالات بكتيرية وسلاله واحدة من كلا الفطريات والخمائر). بينت النتائج ان مستخلصي اسيتات الاثيل والميثانول لنبات الصبر السقطري اظهرا اعلى تثبيط لنمو بكتيريا الكلبسيلة الرئوية ، اشريشيا المعويه، بكتيريا العنقودية الذهبية(18 ملم، 11ملم ، 18 ملم على التوالي). بينما المستخلص الميثانولي لكل من اوراق التوت والصبر السقطري كان لديهما اعلى قدره على تثبيط نمو البكتيريا العصوية الدقيقة (20ملم)، بينما مستخلصات اسيتات الاثيل والايثانول لأوراق التوت كانت هي الاعلى في تثبيط نمو بكتيريا ميكروكوكاس لاتيوس وبكتيريا الليستيرية المستوحدة (11 ملم و 28 ملم على التوالي).

جميع مستخلصات الهكسان لم تظهر أي فعالية ضد انواع البكتيريا المختلفة ماعدا نبات الكرفس حيث اظهر مستخلصه قدرته على تثبيط البكتيريا العصوية الدقيقة (11 ملم). وبينت النتائج ان كل مستخلصات الكرفس لها القدرة على تثبيط الخميرة المبيضية، بينما كان مستخلص الايثانول لأوراق التوت ومستخلص الهكسان لنبات الصبر السقطري لديهما قدره جيده في ثبيط نمو فطر اسبرجلس فلافوس.

كلمات مفتاحيه : النباتات الطبية اليمنية، مركبات الايض الثانوية، ، مضادات الأكسدة، تثبيط الميكروبات، التركيب الكيميائي، المعادن

ABSTRACT

The use of medicinal and aromatic plants species in Yemen goes back thousands of years, and form an important part of the culture. Some of the plants still play important role in the health and body care system. Plants are the major sources for various therapeutic agents in the treatment of a wide variety of diseases. The potency of herbal drugs against diseases is mainly due to their chemical compounds. The herbal products today symbolize safety in contrast to the synthetics.

Our study aimed at detection and determination the chemical and phytochemical composition, estimation antioxidant and antimicrobial activities of eight Yemeni plants "*Aloe perryi*, *Apium graveoleus L*, *Commiphora myrrha*, *Lupinus termis*, *Morus alba L*, *Nigelle sativa L*, *Portulaca oleracea L*, *Trigonella foenum L*".

Plants extracted by different solvents, Total phenol, Flavonoids, Tannins, and alkaloids of plants extracts were measured. The ethanol and methanol extracts of *Aloe perryi* revealed highest total phenols (20184.16 mg GAE /100g , 10771.78mg GAE /100g) respectively.

Estimation of Antioxidant activity was carried out in vitro by DPPH, hydrogen peroxide, reducing power and total antioxidant capacity methods. The results showed that ethyl acetate extracts of *Aloe perryi* has a strong scavenging of DPPH (IC50 = 5.34 µg/ml), and better reducing capacity (EC50 = 3.273 µg/ml), and methanol extract of *Aloe perryi* has highest total antioxidant capacity (478.0535mg Tocopherol /g.dw).

Evaluation of Antimicrobial study was carried out using disk diffusion method, it used 7 bacteria, 1 fungi, and 1 yeast pathogenic strains. Based on the results obtained, it was found that ethyl acetate and methanol extracts of *Aloe perryi* show the highest effective action against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* (18 mm,11 mm and 18mm) respectively. The methanol extracts of *Morus alba L* and *Aloe perryi* showed highest antibacterial activity against *Bacillus subtilis* (20 mm), while ethyl acetate and ethanol extracts of *Morus alba L* showed significant activity against *Micrococcus luteus* and *Listeria monocytogenes* (11 mm and 28 mm) respectively. Hexane extracts of all plants didn't show activity against all the tested bacterial strains except *Apium graveoleus* which showed efficacy to inhibit *Bacillus subtilis* (11 mm) .

All extracts of *Apium graveoleus* displayed significant antifungal activity against *Candida albicans*, the ethanol extract of *Morus alba* and hexane extract of *Aloe perryi* exhibited significant antifungal activity against *Aspergillus flavus*.

Key words: Yemeni Medicinal plants, Phytochemicals, Antioxidant , Antimicrobial, Chemical composition, minerals.

RESUME

L'utilisation des plantes aromatiques et médicinales au Yémen remonte depuis des millénaire, et fait partie intégrantes de la culture locale. Certaines de ces plantes jouent encore un rôle important dans le système de santé locale. Les plantes médicinales sont la source principale de différents agents thérapeutiques impliqués dans le traitement d'une grande variété de maladies. Le pouvoir thérapeutique des drogues d'origine végétale contre les maladies est principalement dû à leurs composés chimiques. Les produits issus des plantes symbolisent, aujourd'hui, la sûreté contrairement aux produits synthétiques.

Notre étude a visé la détection et la détermination de la composition chimique et phytochimique, les activités antioxydantes et antimicrobiennes de huit plantes du Yémen, à savoir : "*Aloe perryi, Apium graveoleus L, Commiphora myrrha, Lupinus termis, Morus alba L, Nigelle sativa L, Portulaca oleracea L, Trigonella foenum L*.

Les plantes médicinales choisies, ont été extraites par différents solvants, les phénols totaux, les flavonoïdes, les tannins et les alcaloïdes. Des extraits de plantes ont été mesurées, les extraits alcooliques (éthanolique et méthanolique), d'*Aloe perryi* ont révélé des teneurs élevées en phénols totaux (20184.16 mg GAE /100g, 10771.78mg GAE /100g, respectivement).

L'activité antioxydante a été mesurée *in vitro* par les méthodes suivantes, piégeage du radical libre DPPH, piégeage du peroxyde d'hydrogène, la réduction du fer et l'activité antioxydante totale. Les résultats obtenus montrent que les extraits d'acétate d'éthyl du *Aloe perryi*, exerce un grand effet sur le radical DPPH (IC_{50} = 5.34 µg/ml) et une grande capacité à reduire le fer (EC_{50} = 3.273 µg/ml). Les extraits alcooliques (méthanolique et éthanolique) d'*Aloe perryi*, ont montré une capacité antioxydante totale la plus élevée (478.0535 mg Tocopherol /g.ps).

L'étude de l'activité antimicrobienne, a été effectuée en utilisant la méthode de diffusion de disque. 10 bactéries, un moisissure, et un souche de levure pathogènes ont été utilisées. A partir des résultats obtenus, les extraits méthanolique et acétate d'éthyle d'*Aloe perryi*, montrent une activité antimicrobienne très élevée contre *Klebsiella pneumoniae*, *Escherichia coli* et *Staphylococcus aureus (*18 mm,11 mm and 18mm) respectivement. Les extraits éthanolique de *Morus alba* et *Aloe perryi*, ont montré une forte activité antibactérienne vis-à-vis de *Bacillus subtilis* (20 mm), alors que les extraits méthanolique et d'acétate d'éthyle ont révélé une activité relativement significative contre *Micrococcus luteus* et *Listeria monocytogenes* (11 mm and 28 mm) respectivement.

Les extraits hexaniques de toutes les plantes choisies dans cette étude, n'ont pas exercé une activité antibactérienne contre les différentes souches bactériennes testées, exception faite pour *Apium graveoleus*, qui a montré une activité contre *Bacillus subtilis* (11 mm). Tous les extraits d'*Apium graveoleus*, ont exercé une activité antifongique significative vis-à-vis de *Candida albicans*. L'extrait éthanolique de *Morus alba* et l'extrait hexanique d'*Aloe perryi*, ont montré une activité antifongique significative vis-à-vis d'*Aspergillus flavus.*

Mots clés : Plantes médicinales de Yémen, Phytocomposés, Antioxydants, Antimicrobiens, Composition Chimique, Minéraux