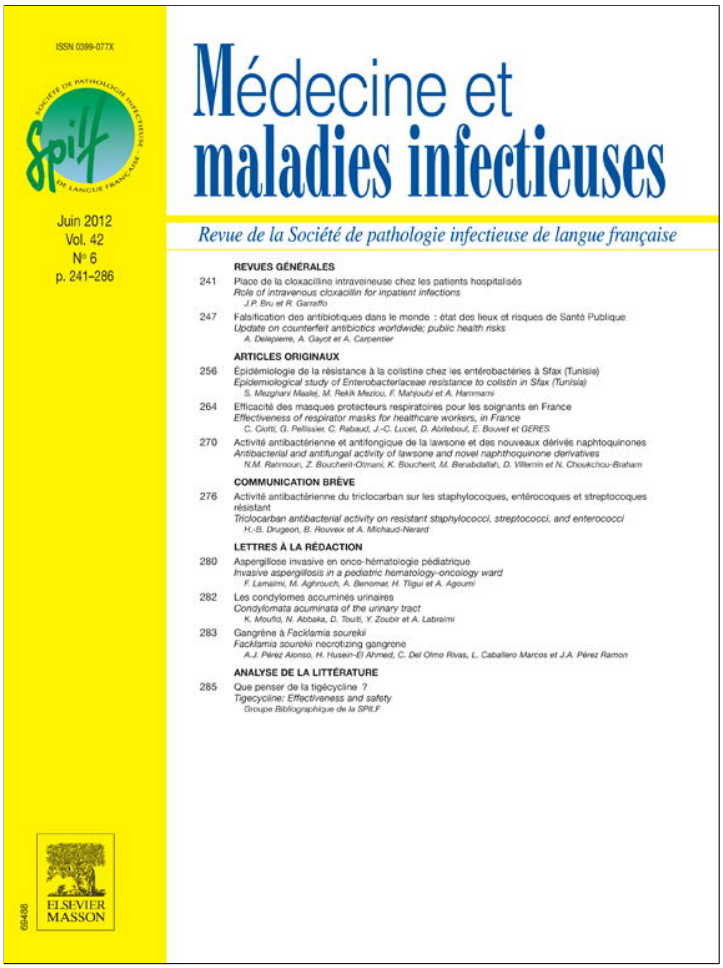


Disponible en ligne sur
SciVerse ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Médecine et maladies infectieuses 42 (2012) 270–275

Original article

Antibacterial and antifungal activity of lawsone and novel naphthoquinone derivatives

Activité antibactérienne et antifongique de la lawsone et des nouveaux dérivés naphtoquinones

N.M. Rahmoun [a,*], Z. Boucherit-Otmani [a], K. Boucherit [a], M. Benabdallah [b], D. Villemin [c], N. Choukchou-Braham [b]

[a] *Laboratoire Antibiotiques Antifongiques, physico-chimie, synthèse et activité biologique, Département de biologie, Faculté des Sciences, Tlemcen University, 13000 Tlemcen, Algeria*

[b] *Laboratoire de Catalyse et Synthèse en Chimie Organique, Department of Chemistry, Faculty of Sciences, BP119, Tlemcen University, 13000 Tlemcen, Algeria*

[c] *ENSICAEN, UCBN, LCMT-UMR 6507 CNRS, Caen University, 14050 Caen, France*

Received 26 August 2011; received in revised form 3 January 2012; accepted 1st May 2012
Available online 7 June 2012

Abstract

Introduction. – Naphthoquinone derivatives are under investigation as potential therapeutic agents. The antibacterial and antifungal activity of lawsone and of some novel naphthoquinone derivatives was assessed in vitro.

Methods. – The antimicrobial activity was determined using diffusion disk and the broth microdilution methods against seven bacteria and three *Candida* species, according to recommendations of the Clinical and Laboratory Standards Institute.

Results. – Two compounds (P05 et P06) presented a good antibacterial effectiveness against two gram-positive bacteria. No antifungal potency was observed against the three *Candida albicans* strains used in the test.

Conclusion. – Our results prove that the introduction of substituents on ketone function position 4 decreased the antimicrobial properties of the synthetized compounds.

Keywords: Naphthoquinone; Lawsone; *Candida albicans*

Résumé

Introduction. – Les dérivés des naphthoquinones sont à l'étude en tant qu'agents ayant un potentiel thérapeutique. L'activité antibactérienne et antifongique de lawsone et d'une nouvelle série de dérivés naphthoquinones ont été évaluées in vitro.

Méthodes. – Le pouvoir antimicrobien était déterminé par la méthode de diffusion des disques et de microdilutions sur bouillon vis-à-vis de sept bactéries et trois espèces de *Candida*, selon les recommandations du Clinical and Laboratory Standards Institute.

Résultats. – Deux composés (P05 et P06) ont montré un bon pouvoir antibactérien vis-à-vis de deux bactéries gram-positives. Aucun pouvoir antifongique n'a été constaté vis-à-vis des trois souches de *Candida albicans* utilisées dans ce test.

Conclusion. – Nos résultats montrent que l'introduction des substituants en position 4 de la fonction cétone diminuait les propriétés antimicrobiennes des produits de synthèse.

Mots clés : Naphthoquinones ; Lawsone ; *Candida albicans*

1. Introduction

Treating microbial infections with chemotherapeutic agents began in the 1930s; it was one of great medical breakthroughs of the twentieth century. Most antimicrobial drugs in use today were discovered by empirical screening for inhibitors of

* Corresponding author.
E-mail address: nagrah113@hotmail.com (N.M. Rahmoun).

0399-077X/$ – see front matter © 2012 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.medmal.2012.05.002

microbial growth during the so-called "golden period" of antimicrobial drug discovery from the 1940s to the 1970s [1–3]. The subsequent development of these drugs, or agents dramatically reduced the burden of diseases caused by microorganism infection. Unfortunately, the emergence of resistance to antibiotics in pathogenic strains over the past 40 years has now become a serious threat to global public health and could undermine the major advances achieved in the treatment of infection [4–8]. Paradoxically, with the emergence of resistance to existing drugs, discovery and development of new antimicrobials has dwindled. The reasons for this are complex [1,3–6] but reflect the technical difficulty to identify new antimicrobial drugs.

Studying the mechanisms of action of compounds with naphthoquinone structure should prove interesting. They are functional constituents of several biochemical systems, natural colored substance [9], or can act as in the human defense system [10]. Several naphthoquinones have pharmacological properties as antibacterial, antifungal, antitumoral, or antiprotozoal agents [11,12].

The antibacterial activity of several well-known and widely used anthracyclin antibiotics such as daunomycin and doxorubicin is thought to be associated with the hydroxyquinone structure [13]. Moreover, equivalent active sites are also present in tetracyclin antibiotics as well as in myxopyronin [14,15]. The antibacterial effect is also related to naphthoquinones from vegetal origin [16], synthetic naphthoquinones [17], and isoxazolyl-naphthoquinones [18]. The fungitoxic effect of 1,4-naphtoquinones [19] was described.

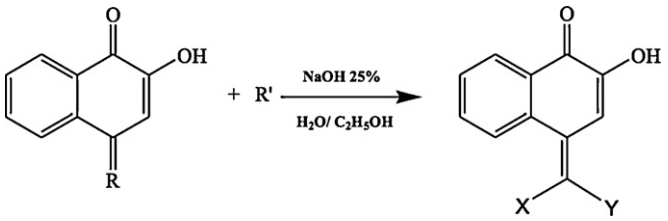
Hydroxynaphthoquinones such as lawsone have proved effective due to their chemical and pharmacological properties [20,21].

This is why we studied the antibacterial and antifungal properties of new 2-hydroxynaphthoquinone derivatives obtained by grafting acetonitrile groups on position 4 of the naphthoquinone moiety. These properties were compared to the structural analogue, 2-hydroxy, 1-4 naphthoquinone, or lawsone, an effective natural product compound, in the treatment of some diseases.

2. Materials and methods

2.1. Chemicals

Lawsone was purchased from Sigma-Aldrich and was used without further purification. The naphthoquinone derivatives were prepared according to Scheme 1 [22], in a single step,



Scheme 1. Synthesis of naphthoquinone derivatives tested in the present study.
Synthèse des dérivés naphthoquinones testés dans cette étude.

addition of Michael, based in 1,4-addition of a doubly stabilized carbon nucleophile to an α, β-unsaturated carbonyl compound, followed by elimination, between a sodium 1,2-naphtoquinone-4-sulphonate salt with various active methylene 1–8 in the presence of a base (soda, NaOH 25%), in a water-ethanol medium during 2 hours. The output was good. The results are presented in Table 1.

2.2. In vitro biological assay

2.2.1. Evaluation of the antibacterial activity

The in vitro antimicrobial activity of the examined compounds was assessed by the Kirby–Bauer disk diffusion method and by the microdilution method, according to recommendations of the National Committee for Clinical Laboratory Standards [23,24].

A panel of seven well-documented pathogenic bacteria obtained from our laboratory: Antibiotiques antifongiques: physico-chimie, synthèse et activité biologique, Département de biologie, Faculté des Sciences, (Tlemcen University) Algeria, was used in the study.

The in vitro activity of naphthoquinone derivatives (P1–8) against pathogenic bacteria was compared with that of lawsone. The following bacteria were used: *Pseudomonas aeruginosa*, ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 13311, *Acinetobacter baumannii* ATCC 19606 (gram-negative bacteria), *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Listeria monocytogenes* ATCC 15313 (gram-positive bacteria).

2.2.1.1. The disk diffusion method. Lawsone and all prepared compounds P1-8 were evaluated for their antibacterial activity by disk diffusion. The bacterial suspension was set at a concentration of 10^8 CFU (0.5 McFarland standard) with a

Table 1
Synthesis of naphthoquinone derivatives tested in the present study.
Synthèse des dérivés naphtoquinones testés dans la présente étude.

Entry	X	Y	Formula	Yield (%)
1	CO_2Et	CO_2Et	C16H18O6	56
2	CN	CO_2Et	C14H12O4	37
3	CN	Ph	C18H11O2	50
4	CN	4-methoxyphenyl	C18H15O3N	89
5	CN	4-chlorophenyl	C17H12O2NCl	60
6	CN	4-nitrophenyl	C17H12O2N	72
7	9*H*-fluoren-9-ylidene		C28H20O4N2	68
8	3Ph		C24H20O2	66

spectrophotometer (DO = 0.08-0.1/λ = 625 nm). This suspension was inoculated on Mueller–Hinton agar. After drying, Wattman paper disks N° 3, 6 mm in diameter and containing 2 mg/disk of examined compounds (dissolved in 100% of dimethyl sulfoxide), were applied in the Petri dish. An additional negative control disk without any sample but impregnated with the equivalent amount of DMSO solvent was also used in the assay. The activity was determined by measuring the inhibitory zone diameter in mm after incubation at 37 °C for 24 hou. The data used was the mean of three replicates. Gentamycin and Ciprofloxacin were used as positive controls. The antimicrobial activity was considered beyond a diameter of 6 mm or more, and was classified as follows: (–): 0–10 mm, (+): 11–16 mm, (++): 17–25 mm.

2.2.1.2. Broth microdilution method. The Minimum Inhibitory Concentration (MIC) of lawsone and prepared compounds was determined by the microdilution method. A series of two-fold dilutions from 512 to 2 μg/ml, (dissolved in DMSO up to 5% final DMSO concentration), were prepared in a 96 well-sterile microplate. The effect of DMSO at a concentration of 5% was checked and eliminated; at these concentrations DMSO has no apparent effect on the microbial growth. We introduced 100 μl of the dilution compound to be tested in each well. These dilutions were inoculated with 100 μl of a solution containing 10^6 CFU. The well used as a negative control was prepared using the inoculum alone. Gentamycin and ciprofloxacin were used as positive controls. The microplate was incubated at 37 °C for 24 hour. The MIC was considered as the weakest concentration for which there was no turbidity. The data was the mean of three replicates.

2.2.2. Screening of antifungal activities

The in vitro antifungal assessment was performed according to NCCLS recommendations [25,26]. A panel of three well-documented pathogenic yeasts was obtained from our laboratory: Antibiotiques antifongiques: physico-chimie, synthèse et activité biologique, Département de biologie, Faculté des Sciences, (Tlemcen University) Algeria, and used for the antifungal assessment. The following yeasts were used: *Candida albicans* ATCC 444IPP, *Candida albicans* ATCC 10231, and *Candida albicans* ATCC 26790.

The disk diffusion method was performed as for bacteria but the culture medium used was Mueller–Hinton + 2% glucose + 0.5 μg/ml methylene blue/pH 7.4, and the fungal suspension was set at 0.12 – 0.15 (λ = 530 nm) at a concentration of 1–5 10^6 CFU. Amphotericin B was used as positive control. An additional negative control disk without any sample but impregnated with the equivalent amount of DMSO solvent was also used in the assay. The analysis was made as for bacteria.

The MIC of *C. albicans* was assessed as for bacteria but the culture medium used was RPMI 1640 (with glutamine and a pH indicator but without bicarbonate)/pH 7.0 supplemented with glucose to a final concentration of 2% (RPMI 2% G), and cell density was approximately 1–5 10^6 CFU.

3. Results

The antimicrobial activity of all naphthoquinone derivatives was evaluated by the disk diffusion method. Three of these compounds demonstrated an antimicrobial activity against the two gram-positive bacteria (Table 2). Two of the three tested compounds (P5 and P6) were highly effective against bacteria. Compounds P5 and P6 were significantly active against *S. aureus* ATCC 25923 (Table 2) with MIC values of 16-32 μg/ml and 32 to 64 μg/ml respectively (Table 3). Gram-negative bacteria were the most resistant in this study.

The data in Tables 2 and 3 prove that the presence of chloro- or nitrosubstituents positively influences the activity and significantly affected the activity of compounds P5 and P6 compared

Table 2
Antimicrobial activities of synthesized compounds using disk diffusion method.
Activité antimicrobienne des produits de synthèse par la méthode de diffusion des disques.

Microorganism code	Compound and inhibition zone (mm)											
	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]	L[a]	G	C	A
Escherichia coli ATCC 25922	–	–	–	–	–	–	–	–	++	++	++	ND
Pseudomonas aeruginosa ATCC 27853	–	–	–	–	–	–	–	–	–	++	++	ND
Acinetobacter baumannii ATCC 19606	–	–	–	–	–	–	–	–	–	+	++	ND
Salmonella typhimurium ATCC 13311	–	–	–	–	–	–	–	–	++	++	++	ND
Enterococcus faecalis ATCC 29212	–	–	–	+	–	+	–	–	+	++	++	ND
Staphylococcus aureus ATCC 25923	–	–	–	++	+	++	–	–	++	++	++	ND
Listeria monocytogenes ATCC 15313	+	–	–	+	–	–	–	–	–	++	++	ND
Candida albicans ATCC 444IPP	–	–	–	–	–	–	–	–	–	ND	ND	++
Candida albicans ATCC 10231	–	–	–	–	–	–	–	–	–	ND	ND	++
Candida albicans ATCC 26790	–	–	–	–	–	–	–	–	–	ND	ND	++

Results were interpreted in terms of the diameter of the inhibition zone: (–): 0–10 mm, (+): 11–16 mm, (++): 17–25 mm; Standards: G-Gentamycin in concentration of 15 μg/disk; C-Ciprofloxacin in concentration of 5 μg/disk; L-Lawsone; A-Amphotericin B: in concentration 2 mg/disk; ND: Not determined.

[a] all compounds are in concentration of 2 mg/disk.

Table 3
Antimicrobial activity expressed as MIC (μg/ml) of the active compounds.
Activité antimicrobienne exprimée en CMI (μg/ml) des produits actifs.

Microorganism code	Compounds				Standard		
	L	4	5	6	G	C	A
Escherichia coli ATCC 25922	≥ 512	≥ 512	≥ 512	≥ 512	0.5	0,008	ND
Pseudomonas aeruginosa ATCC 27853	≥ 512	≥ 512	≥ 512	≥ 512	0.5	0,5	ND
Acinetobacter baumannii ATCC 19606	≥ 512	≥ 512	≥ 512	≥ 512	≥ 512	0.5	ND
Salmonella typhimurium ATCC 13311	≥ 512	≥ 512	≥ 512	≥ 512	≥ 512	0.008	ND
Enterococcus faecalis ATCC 29212	≥ 512	≥ 512	≥ 512	256	8	0.5	ND
Staphylococcus aureus ATCC 25923	256–512	≥ 512	16–32	32–64	0.25	0.25	ND
Listeria monocytogenes ATCC 15313	≥ 512	≥ 512	≥ 512	≥ 512	≥ 512	0.25	ND
Candida albicans ATCC 444 IPP	≥ 512	≥ 512	≥ 512	≥ 512	ND	ND	2
Candida albicans ATCC 10231	≥ 512	≥ 512	≥ 512	≥ 512	ND	ND	4
Candida albicans ATCC 26790	≥ 512	≥ 512	≥ 512	≥ 512	ND	ND	8

L: lawsone; Standards: G: gentamycin; C: ciprofloxacin; A: amphotericin B; ND: not determined.

to the non substituted one. This activity was up to 32 fold that of lawsone (512 μg/ml) for compound P5 and up to 16 fold that of lawsone for compound P6. Finally, compound P6 had a four-fold higher activity than lawsone (256 μg/ml) against *E. faecalis*. However the antibacterial effectiveness (MIC) of P4 and P1 was decreased against *Enterococcus faecalis* and *Listeria monocytogenes*.

No antifungal activity was recorded for lawsone or any of the synthesized compounds against the three Candida strains.

Finally, there was no inhibition zone on DMSO disks for all strains used in this study.

4. Discussion

Many of the 1,4-naphthoquinone derivatives are found in plants and fungi, and have also been used by humans. For example, the plant metabolite lawsone (2-hydroxy-1,4-naphthoquinone) is the material responsible for the yellow-to-orange dyeing properties of henna [27]. More recently, some naturally occurring naphthoquinones, or closely related substances were used or considered for use as antibiotics, e.g., axenomycins [28], kalafungin, nanaomycins [29], and naphthocyclinones [30]. We investigated the influence of acetonitrile substituents on 1,4-naphthoquinone antimicrobial activity against bacteria and yeasts, to consider the use of such compounds in humans.

Compounds P5 and P6 exhibited the most potent in vitro antibacterial activity with a MIC ranging between 16 to 32 μg/ml and 32 to 64 μg/ml respectively against *S. aureus* ATCC 25923 (oxacillin susceptible strain). The different electronic distribution observed on the naphthoquinone ring is a direct consequence of the particular characteristic of substituents (neutral, electron-donor, or electron-withdrawing). This could explain the various antibacterial activities of these compounds [31–33].

Replacing hydrogen with a chloro- or nitrosubstituents (or another halogen group) induces a considerable change of the naphthoquinone ring electronic distribution in these compounds (compared to compound P4). The binding properties of these compounds in various oxidation states, its electron reduced form, semiquinone, or its two electron reduced form, catechol, can explain their ability for DNA intercalation, alkylation, or inhibition of special proteins or enzymes such as topoisomerases [34,35].

These two compounds could be considered for the development of new antibacterial agents. *Staphylococcus aureus* is one of the most frequent microorganisms worldwide implicated in nosocomial infections. Methicillin resistant strains account for 15 to 45% of all *Staphylococcus aureus* strains [36].

The structural activity of lawsone and naphthoquinones derivatives P1–8 proves they have unreliable levels of microbial inhibition. The antibacterial and antifungal activity seemed to depend on the ketone function in position 2 and 4, according to results obtained with lawsone that has a broader spectrum of activity than most of our new compounds.

Quinone (lawsone) cellular toxicity has been extensively studied, and the consensus is that it depends on (a) the quinone capacity to produce reactive oxygen species (ROS), and (b) the electrophilicity of quinones, which enables them to form

adducts with cellular macromolecules [38]. This was confirmed by the huge influence of structural modification on the biological activity of lawsone. However, naphthoquinone derivatives P1-8 can be less cytotoxic than naturally occurring lawsone. Thus, naphthoquinone compounds, thanks to their redox cycling capacity, have broad antimicrobial as well as antitumoral activities [11,37,38]. However, the potential antimicrobial activity of naphthoquinone compounds, particularly those isolated from natural sources, remains unexplored. In our study, lawsone had a significant antimicrobial activity. The reported tuberculostatic and antimicrobial activity of *Lawsonia inermis* Linn. [20,21] is probable due to lawsone, the major bioactive constituent of this plant.

The results of our study do not allow concluding on the possible antimicrobial mechanism of naphthoquinone activity or identifying cation specific structural features associated with this activity. Newer analogue naphthoquinone derivatives must be designed to solve the structure – activity relationship in this series, leading to the development of more effective antimicrobial agents while trying to keep free the ketone function in position 4, and considering grafting an active substituent on position 2 or 3.

5. Conclusion

We assessed the antimicrobial activity of some novel naphthoquinone derivatives and compared their activity to that of lawsone. Two compounds presented significant antibacterial effectiveness against gram-positive bacteria, due to the presence of either chloro- or nitrosubstituents. But, introduction of substituents on the ketone function in position 4 decreased the antimicrobial properties, compared to lawsone. This suggests that the quinone systems of naphthoquinone play a positive role in the antimicrobial effectiveness of this class of compounds.

Disclosure of interest

The authors declare that they have no conflict of interest concerning this article.

References

[1] Chopra I, Hesse L, O'Neill AJ. Exploiting current understanding of antibiotic action for discovery of new drugs. J Appl Microbiol 2002;92:S4–15.

[2] Knowles DJC. New strategies for antibacterial drug design. Trends Microbiol 1997;5:379–83.

[3] Odds FC, Brown AJP, Gow NAR. Antifungal agents: mechanisms of action. Trends in Microbiology 2003;11:272–9.

[4] Spellberg B, et al. The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 2008;46:155–64.

[5] Talbot GH, et al. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis 2006;42:657–68.

[6] Chopra I, et al. Treatment of health-care-associated infections caused by Gram-negative bacteria: a consensus statement. Lancet Infect Dis 2008;8:133–9.

[7] Overbye KM, Barrett JF. Antibiotics: where, did we go wrong? Drug Discov Today 2005;10:45–52.

[8] Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nature Rev Drug Discov 2007;6:29–40.

[9] Ollinger K, Brunmark A. Effect of hydroxyl substituent position on 1,4 naphthoquinone toxicity to rat hepatocytes. J biol Chem 1991;266:21496–503.

[10] Floreani M, Carpenedo F. The catecholaminemediated positive inotropic effect of simple quinones is related to superoxide anion generation. J Pharmacol Exp Ther 1992;260:468–73.

[11] Morello A, Pavani M, Garbarino JA, Chamy MC, Frey F, Mantilla J, et al. Effects and mode of action of 1,4-naphthoquinones isolated from *Calceolaria sessilis* on tumoral cells and Trypanosoma parasites. Comp Biochem Physiol C: Pharmacol Toxicol Endocrinol 1995;112C:119–28.

[12] Zhang L, Arnold LD. Natural Products: Drug Discovery and Therapeutic Medicine. Humana Press Inc; 2005.

[13] Young RC, Ozols RF, Myers CE. The anthracycline antineoplasic drugs. New Engl J Med 1981;305:139–53.

[14] Smilack JD, Wilson WR, Cockerill FR. Tetracyclines, chloramphenicol, erythromycin, clindamycin, and metronidazole. Mayo Clinic Proc 1990;66:1270–80.

[15] Chopra I, Hawkey PM, Hinton M. Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother 1991;29:245–57.

[16] Machado TB, Pinto AV, Pinto MCF, Leal ICR, Silva MG, Amaral ACF, et al. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. Int J Antimicrob Agents 2003;21:279–84.

[17] Oliveira CGT, Miranda FF, Ferreira VF, Freitas CC, Rabelli RF, Carballido JM, et al. Synthesis and antimicrobial evaluation of 3-hydrazinonaphthoquinones as analogs of lapachol. J Braz Chem Soc 2001;12:339–45.

[18] Bogdanov PM, Albesa I, Sperandeo NR, Luna C, Bertorello MM. Antibacterial effect of 2-hydroxy-N-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine on *Staphylococcus aureus*. Experientia 1996;52:600–4.

[19] Sasaki K, Abe H, Yoshizaki F. In vitro antifungal activity of naphthoquinone derivatives. Biol Pharm Bull 2002;25:669–70.

[20] Sharma VK. Tuberculostatic activity of henna (*Lawsonia inermis* Linn.). Tubercle 1990;71:293–5.

[21] Rahmoun MN, Benabdallah M, Villemin D, Boucherit K, Mostefa-Kara B, Ziani-Cherif C, et al. Antimicrobial screening of the Algerian *Lawsonia inermis* (henna). Der Pharma Chemica 2010;2:320–6.

[22] Villemin D, Benabdallah M, Rahmoun N, Jouannic C, Choukchou-Braham N, Mostefa-Kara B. A Green Route for Synthesis of New 1,2-Naphthoquinomethane Acetonitriles in Water. Synthetic Communications 2010;40:3514–21.

[23] National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eight Edition; 23. M2-A8. 2003.

[24] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, PA 2000.

[25] Epsinel-ingroff A. Standardized disk diffusion method for yeasts. Clin Microbiol Newslett 2007;29:97–100.

[26] Clinical Laboratory Standards Institute –, CLSI., Reference method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard- Third Edition, M27-A3, Wayne, PA 2008.

[27] Taylor A. All about: henna for hair with a shine, p. CIO. The New York Times; 1977.

[28] Bauch HJ, LeAistner E. Bioproduction of axenomycins in batch cultures of Streptomyces lisandri. Z Naturforsch Teil C 1980;35:936–44.

[29] Omura S, Tanaka H, Okada Y, Marumo H. Isolation and structure of nanaomycin D, an enantiomer of the antibiotic kalafungin. J Chem Soc Chem Commun 1976:320–1.

[30] Zeeck A, Zihner H, Mardin M. Stoffwechselprodukte von Mikroorganismen. 129. Antibiotica Pund-y-Naphthocyclinon. Justus Liebigs Ann Chem 1974:1100–25.

[31] Salunke-Gawali S, Rane SY, Puranik VG, Guyard-Duhayon C, Varret F. Three dimensional hydrogen-bonding network in a copper complex

of 2-hydroxy-1,4-naphthoquinone: structural, spectroscopic and magnetic properties. Polyhedron 2004;23:2541–7.

[32] Yan Y, Xiao FS, Zheng G, Zhen K, Fang C. Selective catalytic oxidation of naphthol to 2-hydroxyl-1, 4-naphthoquinone by hydrogen peroxide over metalloporphyrin catalysts. J Molecular Catalys A: Chemical 2000;157:65–72.

[33] Rostkowska H, Nowak MJ, Lapinski L, Adamowicz L. Molecular structure and infrared spectra of 2-hydroxy-1,4- naphthoquinone; experimental matrix isolation and theoretical Hartree-Fock and post Hartree-Fock study. Spectrochimica Acta Part A 1998;54:1091–103.

[34] Pierpont CG. Unique properties of transition metal quinone complexes of the MQ3 series. Coord Chem Rev 2001;219–221:415–33.

[35] Plyta ZF, Li TH, Papageorgiou VP, Mellidis AS, Assimopoulou AN, Pitsinos EN, et al. Inhibition of topoisomerase I by naphthoquinone derivatives. Bioorg Med Chem Lett 1998;8:3385–90.

[36] Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin Microbiol Rev 1993;6: 428–42.

[37] Medina LFC, Hertz P, Stefani V, Henriques JAP, Zanotto-Filho A, Brandelli A. Aminonaphthoquinone induced oxidative stress in *Staphylococcus aureus*. Biochem Cell Biol 2006;84:720–7.

[38] Shaukat A, Tanveer H, Rakhshanda N. Optimization of alkaline extraction of natural dye from Henna leaves and its dyeing on cotton by exhaust method. J Clean Production xxx 2008:1–6.