Phytochemical analysis and antimicrobial activity of medicinal plants against pathogenic microorganisms

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Abstract: The phytochemical content of five medicinal plants; namely, *Haloxylon persicum, Hammada elegans, Hammada scoparia, Juncus actus* and *Tamarix nilotica* and their antibacterial activities against pathogenic bacteria and fungi were investigated. The phytochemical analysis revealed the presence of tannins, flavonoids (free aglycone and glycoside), unsaturated sterols and/or triterpenoides, carbohydrates or glycosides and proteins and/or amino acids and absence of volatile oil, cardinolides, anthraquinones and oxidase enzyme in all plants. The ethanolic extracts of the investigated plants inhibited the growth of some pathogenic bacteria including *Enterococcus faecalis, Escherichia coli, Moraxella lacunata, Proteus merabiles, Pseudomons aeruginosa, Salmonella typhi, Serratia marcesens, Bacillus subtilis, Micrococcus luteus, Sarcina ventricull*, and *Staphylococcus aureus*. The highest activity was obtained by *Haloxylon persicum* against *Pseudomons aeruginosa* (18 mm) and *Proteus merabiles* (16 mm).

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1. Introduction

Medicinal plants have been used, from the ancient time, to prevent and treat various health problems. Plants are still an independent source of medication in the contemporary health care delivery system. Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs **(Asres et al., 2003).** Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases. Medicinal plants represent a rich source of antimicrobial agents **(Awaad et al., 2012).**

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Medical plants contain large varieties of chemical substances, which possess important therapeutic properties that could be utilized in the treatment of human diseases.

The potential of higher plants as source of new drugs is still largely unexplored. Among the estimated 250,000 – 500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics **(Mahesh and Satish, 2008; Kroschwitz and Howe-Grant, 1992; Gerhartz *et al*., 1985).**

The present study aimed at evaluating the antimicrobial activity of some medicinal plants against pathogenic Gram-positive and Gram-negative bacteria and pathogenic unicellular and filamentous fungi.

2. Materials and Methods

Plant Materials and Preparation of Extracts

The aerial pats of *Haloxylon persicum, Hammada elegans, Hammada scoparia, Juncus actus* and *Tamarix nilotica* were collected during flowering stage in March 2013 from desert around Riyadh. The plants were identified by Dr. Jacob T. Pandalayil, Assistant Professor of Plant Taxonomy, Botany and Microbiology Department, College of Science, King Saud University and by comparison with plant description **(Migahid, 1996).** A voucher specimen has been deposited in the herbarium of College of Science, King Saud University. The aerial parts of plant samples were washed 2-3 times with running fresh water and then air-dried under shade. After complete shade drying, the plant materials (100 g) was grinded with mechanical grinder and the powder was kept in tightly closed containers. The extract was

prepared by percolation in 90% ethanol **(Awaad et al., 2012)** at room temperature for two days. The ethanol extract was filtered and the residues were re-percolated for four times. The total ethanol extract was concentrated under reduced pressure at a temperature not exceeding 35°C.

Phytochemical Analysis

The freshly prepared extracts of *Haloxylon persicum*, *Hammada elegans*, *Hammada scoparia*, *Juncus actus* and *Tamarix nilotica*were chemically tested for thepresence of different phytochemical constituents such as carbohydrates and/or glycosides **(Majaw and Moirangthem, 2009),** flavonoides **(Jack and Okorosaye-Orubite, 2008)**, tannins, sterols and/or triterpenes **(Jack and Okorosaye-Orubite, 2008)**, proteins and/or amino acids**,** alkaloids and/or nitrogenous bases **(Kumar et al., 2009),** saponins **(Edeoga et al., 2005),** anthraquinones **(Majaw and Moirangthem, 2009),** cardinolides **(Sonibare et al., 2009)** and oxidase enzyme **(Wagner and Baldt, 2001).**

Antimicrobial Activity Assay

Eleven strains of pathogenic bacteria; *Enterococcus faecalis*, *Escherichia coli*, *Moraxella lacunata*, *Proteus merabiles*, *Serratia marcesens*, *Pseudomons aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Micrococcus luteus*, *Sarcina ventricull*, *Staphylococcus aureus* and five strains of pathogenic fungi; *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium chrysogenum*were obtained from the Microbiology Lab, Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt and used as test organisms.

The antimicrobial activity was determined using well diffusion method according to National Committee for Clinical Laboratory Standards **(NCCLS, 1993**). Petri plates containing 20 ml of, nutrient (for bacteria) or malt extract (for fungi), agar medium were seeded with 1-3 day cultures of microbial inoculums (standardized inoculums 1-2 X 10^7 cfu/ml 0.5 Mcfarland standard). Wells (5 mm in diameter) were cut off into agar and 50 µl of plant extracts were tested in a concentration of 5 mg/ml and incubated at 37°C for 1- 4 days. The antimicrobial activity was determined by measurement of the inhibition zone formed around the well.

3. Results

Phytochemical Analysis

The ethanolic extract of the plants revealed the presence of tannins, flavonoids (free aglycone and glycoside), unsaturated sterols, carbohydrates or glycosides, and proteins and/or amino acids in *Haloxylon persicum*, *Hammada elegans*, *H. scoparia*, *Juncus actus* and *Tamarix nilotica*. However, the volatile oil, cardinolides, anthraquinones, and oxidase enzymes were absent in all the plants (Table 1).

On the other hand, crystalline sublimate, and alkaloids and/or nitrogenous bases were present only in *Haloxylon persicum*, *Hammada elegans*, and *H. scoparia*. Traces of saponins were present in *Haloxylon persicum*, *Hammada elegans*, *H. scoparia* and *Tamarix nilotica*(Table 1).

Table 1: Preliminary phytochemical screening of plants under investigation

Test	*Haloxylon persicum*	*Hammada elegans*	*Hammada scoparia*	*Juncus actus*	*Tamarix nilotica*
Crystalline sublimate	+	+	+	-	-
Volatile oil	-	-	-	-	-
Tannins	+	+	+	+	+
Flavonoids: **a.** free aglycone **b.** glycoside	 + +	 + +	 + +	 + +	 + +
Alkaloids and/or nitrogenous bases	+	+	+	-	-
Cardinolides	-	-	-	-	-
Unsaturated sterols and/or triterpenoides	+	+	+	+	+
Saponins	±	±	±	-	±
Carbohydrates or glycosides	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Proteins and/or amino acids.	+	+	+	+	+
oxidase enzyme	-	-	-	-	-

(-), absent; (+), present; (±), Traces.

Antimicrobial Activity

The ethanolic extract of the investigated plants; *Haloxylon persicum*, *Hammada elegans*, *H. scoparia*, *Juncus actus* and *Tamarix nilotica* showed antibacterial and antifungal activity (Tables 2 and 3). All the plant extracts inhibited the growth of pathogenic bacteria including *Enterococcus faecalis*, *Escherichia coli*, *Moraxella lacunata*, *Proteus merabiles*, *Serratia marcesens*, *Pseudomons aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Micrococcus luteus*, *Sarcina ventricull*, *Staphylococcus aureus* and pathogenic fungi including *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium chrysogenum* (Tables 2 and 3).

The obtianed results revealed that, among all the investigated plants, the best antibacterial activity was obtained by the extract of *Haloxylon persicum*. The highest antibacterial activity (18, 16 and 15 mm) was obtained by *Haloxylon persicum* against *Pseudomons aeruginosa*, *Proteus merabiles*, and *Enterococcus faecalis*, respectively (Table 2) .Moreover, strong antibacterial activity (15 mm) was obtained by extract of *Hammada scoparia* against *Escherichia coli* and extract of *Juncus actus* against *Moraxella lacunata* and good antibacterial activity (14 mm) was obtained by *Haloxylon persicum* against *Serratia marcesens*, *Hammada elegans* against *Enterococcus faecalis* and *Salmonella typhi*, and *Tamarix nilotica* against *Bacillus subtilis* (Table 2). The lowest antibacterial activity (7 mm) was obtained by extracts of *Hammada elegans* and *Juncus actus* against *Bacillus subtilis* and *Salmonella typhi*, respectively.

Table 2. Antibacterial activity of plant extracts against pathogenic Gram-negative and Gram-positive bacteria.

Bacteria	Zone of Inhibition (mm)				
	Haloxylon persicum	***Hammada elegans***	***Hammada scoparia***	***Juncus actus***	***Tamarix nilotica***
Gram-negative					
Enterococcus faecalis	15	14	13	10	10
Escherichia coli	13	08	15	11	12
Moraxella lacunata	11	11	12	15	09
Proteus merabiles	16	09	12	09	12
Serratia marcesens	14	11	10	10	11
Pseudomons aeruginosa	18	13	09	10	08
Salmonella typhi	10	14	12	07	09
Gram-positive					
Bacillus subtilis	11	07	11	08	14
Micrococcus luteus	09	08	12	12	11
Sarcina ventricull	09	11	09	09	10
Staphylococcus aureus	13	10	10	12	12

On the other hand, the results of antifungal activity revealed that the highest activity (14 and 12 mm) was obtained by extract of *Tamarix nilotica* against *Aspergillus flavus* and *Aspergillus fumigates*, respectively (Table 3). Moreover, strong antifungal activity was obtained by extracts of *Haloxylon persicum* against *Candida tropicalis* and *Tamarix nilotica* against *Penicillium chrysogenum*. Despite that the extract of *Hammada scoparia* showed no activity against *Candida tropicalis*, the lowest antifungal activity (7 mm) was obtained by extracts of *Haloxylon persicum* and *Hammada elegans* against *Aspergillus fumigates*, and Juncus actus against *Aspergillus flavus* (Table 3).

Table 3. Antifungal activity of plant extracts against pathogenic unicellular and filamentous fungi.

Fungi	Zone of Inhibition (mm)				
	Haloxylon persicum	***Hammada elegans***	***Hammada scoparia***	***Juncus actus***	***Tamarix nilotica***
Unicellular					
Candida albicans	09	09	10	09	09
Candida tropicalis	11	10	00	08	10
Filamentous					
Aspergillus flavus	09	09	09	07	14
Aspergillus fumigates	07	07	08	10	12
Penicillium chrysogenum	09	10	10	09	11

4. Discussion

The obtained results of the current study revealed that the ethanolic extracts of *Haloxylon persicum*, *Hammada elegans*, *Hammada scoparia*, *Juncus actus* and *Tamarix nilotica* showed antibacterial and antifungal activity. The antimicrobial activity of plant extracts have been extensively reported **(Awaad et al., 2012; Zain et al., 2012; Chanda and Baravalia, 2010; Ahameethunisa and Hooper, 2010; Chattopadhyay et al., 2009; Alam et al., 2009; Ahmad and Beg, 2001).**

The results of phytochemical analysis of the investigated plants revealed the presence of different bioactive compounds including tannins, flavonoids (free aglycone and glycoside), unsaturated sterols, carbohydrates or glycosides, and proteins and/or amino acids, crystalline sublimate, and alkaloids and/or nitrogenous bases. Many The antimicrobial activity of chemical constituents of plants were confirmed in different studies **(Emad et al., 2009; Malu et al., 2009; Ahmad et al., 2008; Parekh et al., 2005; Mothana and Lindequist, 2005; Karaman et al.,2003; Darout et al., 2000; Cowan, 1999; Lin et al., 1999; Eloff, 1998).**

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References

1. Ahameethunisa, A. R. and Hooper, W. (2010). Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. BMC Complemen. and Alternat. Med., 10: 1- 6.
2. Ahmad, I. and Beg, A. I. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol., 74: 113–123.
3. Ahmad, I.; Zahin, M.; Aqil, F.; Hasan, S.; Khan, M.S.A.; Owais, M. (2008). Bioactive compounds from *Punica granatum*, *Curcuma longa* and *Zingiber officinale* and their therapeutic potential. Drugs Fut. 33, 329.
4. Alam, M.T.; Karim, M.M.; Shakila, K.N. (2009). Antibacterial activity of different organic extracts of *Achyranthes aspera* and *Cassia alata*. J. Sci. Res., 1: 393-398.
5. Asres, K.; Taddese, S.; Gebremariam T. (2033). In vitro antimicrobial activities of some selected topically applied medicinal plants of Ethiopia. Ethio Pharm J. 2003; 21: 34-45.
6. Awaad, Amani S.; Soliman, G. A.; El-Sayed, Dalia F.; El-Gindi, Omimah D., and Alqasoumi, S. I. (2012). Hepatoprotective activity of *Cyperus alternifolius* on carbon tetrachloride–induced hepatotoxicity in rats. Pharmaceutical Biology, 2012; 50(2): 155–161.
7. Chanda, S. and Baravalia, Y. (2010). Screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms, Afr. J Biotech., 9: 3210-3217.
8. Chattopadhyay, R. R.; Bhattacharya, S.K.; Medda, C.; Chanda, S. and Anvesa, B. (2009). A comparative evaluation of antibacterial potential of some plants used in Indian traditional medicine for the treatment of microbial infection, Braz. Archives of Bio. and Technol., 52: 1123-112 8.
9. Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. (12): 564–582.
10. Darout, I.A.; Christy, A.A.; Skaug, N.; Egeberg, P.K. (2000). Identification and quantification of some potentially antimicrobial anionic components in Miswak extract. Ind. J. Pharmacol. 32, 11–14.
11. Edeoga, H.O.; Okwu, D.E.; Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. Afri. J.Biotechnol. 4:685–688.
12. Eloff, J.N. (1998). Which extract should be used for the screening and isolation of antimicrobial components from plants? J. Ethnopharmacolol. (60): 1–8.
13. Emad, M.A.; Amna, S.K.; Nazlina, I. (2009). Antibacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* against methicillin resistant *Staphylococcus aureus* (MRSA). Sci. Res. Essays, 4 (4): 351–356.
14. Gerhartz, W.; Yamamota, Y. S.; Campbell, F. T.; Pfefferkorn R. and Rounsaville J. F. (1985). Ullmann's Encyclopedia of Industrial Chemistry. Vol. A1 5th ed. Weineim: VCH Verlagsgesellschaft mbH, pp. 527-541.
15. Jack, I.R. and Okorosaye-Orubite, K. (2008). Phytochemical analysis and antimicrobial activity of the extract of leaves of fleabane (*Conyza sumatrensis*). J. Appl. Sci. Eeviron. Manage, 12 (4): 63-65.
16. Karaman, I.; Sahin, F.; Gulluce, M.; Ogutcu, H.; Sengul, M.; Adiguzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 231–235.

17. Kroschwitz, J.I. and Howe-Grant, M. (1992). Kirk-Othmer encyclopedia of chemical technology, (2): 893.
18. Kumar, A.; Llavarasan, R.; Jayachandran, T.; Decaraman, M.; Aravindhan, P.; Padmanabhan, N. and Krishnan, M.R.V. (2009). Phytochemicals investigation on atropical plant, syzygium cumini from kattuppalayam Erode District, Tamil Nadu, South India. Pakistan Journal of Nutrition, 8 (1): 83-5.
19. Lin, J.; Opoku, A.R.; Geheeb-Keller, M.; Hutchings, A.D.; Terblanche, S.E.; Jager, A.K.; van Staden, J. (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J. Ethnopharmacol. 68, 267–274.
20. Mahesh B. and Satish S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences 4 (S): 839-843.
21. Majaw,S., and Moirangthem,J. (2009): "Qualitativ and Quantitative Analysis of *Clerodendroncolebrookianum* Walp. Leaves and *Zingiber cassumunar* Roxb, Rhizomes". Ethnobotani Cal Leaflets, 13: 578-89.
22. Malu, S.P., Obochi, G.O., Tawo, E.N., Nyong, B.E., 2009. Antibacterial activity and medical properties of ginger. (*Zingiber officinale*). Global J. Pure Appl. Sci. 15, 365–368.
23. Migahid A.M. (1996). Flora of Saudi Arabia. 4th edition. *King Saud University Press*. Volume 1, 127.
24. Mothana, R.A.A.and Lindequist, U. (2005). Antimicrobial activity of some medicinal plants of the island soqotra. J. Ethnopharmacol. 96: 177–181.
25. NCCLS, (1993). Performance Standards Antimicrobial Disc Susceptibility Tests. Approved Standard Fifth Edition. NCCLS Document M2-A5, Villanova, PA, USA.
26. Parekh J. and Chanda S. (2008). *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. African Journal of Biotechnology Vol. 7 (23), pp. 4349-4353.
27. Sonibare, M.A.; Soladoye, M.O.; Esan O.O. and Sonibare, O.O.(2009). Phytochemical and Antimicrobial studies of four species of *cola schott* and endi (sterculiaceae)".Afr J Tradit Complement Altern Med, 6, 4, p.518-25.
28. Wangner, H. and Baldt, S. (2001). Plant drug analysis. 2nd edt., Springer-Verlag, Berlin, Heidelberg, New York.
29. Zain M. E.; Awaad, Amani S.; Al-Outhman Monerah R.; El-Meligy, Reham M. (2012). Antimicrobial activities of Saudi Arabian desert plants. *Phytopharmacology*, 2(1): 106-113.

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