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Antimicrobial activities of Saudi Arabian desert plants

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Abstract

The ethanol extracts of *Alhagi maurorum* Medic., *Chenopodium murale* L., *Convolvulus fatmensis* G. Kunze., *Conyza dioscoridis* (L.) Desf., *Cynanchum acutum* L., *Diplotaxis acris* (Forssk) Boiss, *Euphorbia cuneata* Vahl., *Origanum syriacum* L., *Solenostemma argel* (Del.) Hayne. and *Tamarix aphylla* L.(Karst) showed significant antimicrobial activity against Gram negative, Gram positive bacteria, unicellular and filamentous fungi. However, *Tamarix aphyla* showed remarkable activity against *Aspergillus flavus* and 16, out of 19, strain of the investigated test organisms. The highest MIC value was obtained by *Tamarix aphyla* against 8, including all the filamentous fungi, of the investigated test strains. However, the extract of *Cheno-podium mural* showed the best MIC against the unicellular fungi.

Keywords: medicinal plants; antibacterial activity; antifungal activity

Introduction

Natural products perform various functions, and many of them have interesting and useful biological activities (Harvey, 1999). There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. Researchers are incr-easingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Hoffmann *et al*., 1993; Harvey, 1999; Srinivasan *et al*., 2001).More than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicine in Asia repre-sents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infe-ctious diseases. A vast knowledge of how to use the plants accumulated in areas where the use of plants is still of great importa-nce (Diallo *et al*., 1999). The medicinal value of plants lies in some chemical substa-nces that body. The most important of these bioactive compoun-ds of plants are alkaloids, tannins and phenolic compounds (Edeoga *et al*., 2005).

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 (Gerhartz *et al*., 1985; Kroschwitz and Howe-Grant, 1992).

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal *et al*., 2006). The present study aimed at evaluati-ng the antimicrobial activity of ten plants (Table 1) against Gram-positive and Gram-negative bacterial strains and unicellular and filamentous fungi.

Materials and Methods

Plant material and preparation of extracts

The leaves of *Alhagi maurorum* Medic., *Chenopoidum murale* L., *Convolvulus fatmensis* G. Kunze., *Conyza dioscoridis* (L.) Desf., *Cynanchum acutum* L., *Diplotaxis acris* (Forssk) Boiss, *Euphorbia cuneata* Vahl., *Origanum syriacum* L., *Solenostemma argel* (Del.) Hayne. and *Tamarix aphylla* L.(Karst)were collected during flowering stage in March 2010 from the desert around Riyadh. The samples were kindly identified by Dr. M. Gebali, the former researcher of Botany and by comparison with plant description Flora of Saudi Arabia (El-Gohry, 2004). A voucher specimen of the titled plant has been deposited in the herbarium of Chemistry Department. The plant samples were air-dried in shade and grounded into fine powder. Three hundred grams from the air-dried powder of each plant was extracted by percolation in 90% ethanol (Awaad *et al*., 2008) 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.

Test organisms

Fourteen bacterial strains; Gram-negative bacteria, *Acinitobacter baumanni, Escherichia coli, Moraxella lacunata, Proteus merabiles, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi,* Gram-positive bacteria, *Bacillus subtilis, Micrococcus kristinae, Micrococcus luteus, Sarcina ventricull, Staphylococcus aureus, Staphylococcus haemolyticus, Stroptococcus byogenes*; and five fungal strains; unicellular fungi; *Candida albicans* and *Saccharomyces cerevisiae*; filamentous fungi; *Aspergillus flavus, A. fumigatus* and *Penicillium chrysogenum* were used for testing the activity in this study: The test microorganisms

Table 1. Uses and properties of medical plants collected for antimicrobial screening.

Scientific name	Family	Local name	Used part	Medical use
Alhagi maurorum Medic.	Leguminosae	Al-Agool	Aerial parts	Antioxidant, antinociceptive
Chenopoidum murale L.	Chenopodiacea	Al-Zorbaih	Whole plant	Cytotoxic, hypotensive
Convolvulus fatmensis G. Kunze	Convulvulaceae	Al-Oleeq	Aerial parts	Anti-inflammatory
Conyza dioscoridis (L.) Desf	Asteraceae	Ain alkatkot	Aerial parts	Epilepsy in children
Cynanchum acutum L.	Asclepiadaceae	Al-Modeed	Leaves and stems	Insecticide, parasiticide
Diplotaxis acris (Forssk) Boiss	Cruciferae	Fegl Algabal	Aerial parts	Antidiabetic, wound healing
Euphorbia cuneata Vahl.	Euphorbiaceae	Al-Baky	Leaves	Anti-inflammatory analgesic
Origanum syriacum L.	Labiatae	Al-Bardakosh	Aerial parts	Antitussive, anti-inflammatory
Solenostemma argel (Del.) Hayne.	Solanaceae	Al-Argal	Leaves	Rheumatic pains, cough
Tamarix aphylla L.(Karst)	Tamrecaseae	Al-Athl	Leaves	Antioxidant

were obtained from the Microbiology Laboratory, Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Antimicrobial activity

Antimicrobial activity was determined by the well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 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 (6 mm in diameter) were cut off into agar and 50 µl of plant extracts were tested in a concentration of 100 mg/ml and incubated at 37°C (bacterial strains) and at 25°C (fungal strains) for 24-48 h. The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the well.

Determination of Minimum Inhibitory Concentrations (MIC)

MIC was determined by micro-dilution method using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000). MIC of the extracts was determined by dilution of *Alhagi maurorum, Chenopoidum murale, Convolvulus fatmensis, Conyza dioscoridis, Cynanchum acutum, Diplotaxis acris, Euphorbia cuneata, Origanum syriacum, Solenostemma argel* and *Tamarix aphylla* of concentrations of 0.0-100 mg/ml.equ al volume of each extract and nutrient broth were mixed in a test tube. Specifically 0.1 ml of

Table 2. Antimicrobial activity of the methanolic extracts of plants against different bacterial and fungal strains

Test organism	Zone of Inhibition (mm)									
	Alhagi maurorum	*Chenopoidum murale*	*Convolvulus fatmensis*	*Conyza dioscoridis*	*Cynanchum acutum*	*Diplotaxis acris*	*Euphorbia cuneata*	*Origanum syriacum*	*Solenostemma argel*	*Tamarix aphylla*
Bacteria										
Gram Negative										
Acinetobacter baumanni	00	00	13	00	10	11	00	12	00	08
Escherichia coli	10	00	00	10	09	08	08	10	00	15
Moraxella lacunata	13	00	00	00	00	11	08	13	11	08
Proteus merabiles	12	00	13	10	00	00	00	00	09	11
Proteus vulgaris	10	12	08	15	00	10	11	12	00	13
Pseudomons aeruginosa	09	15	11	00	00	12	00	11	09	08
Salmonella typhi	08	00	11	00	00	10	08	00	00	00
Gram Positive										
Bacillus subtilis	00	10	11	12	10	00	12	00	11	14
Micrococcus kristinae	00	10	10	08	08	00	00	11	00	00
Micrococcus luteus	08	08	08	08	08	08	00	08	08	08
Sarcina ventricull	12	00	07	15	11	09	11	11	00	11
Staphylococcus aureus	00	00	13	09	11	00	12	14	00	11
Staphylococcus haemolyticus	00	00	00	00	00	00	00	00	00	00
Streptococcus byogens	10	00	00	10	09	08	08	08	00	15
Fungi										
Unicellular										
Candeda albicans	09	15	11	00	00	12	12	11	09	08
Saccharomyces cirivisae	11	13	12	00	00	12	00	11	09	08
Filamentous										
Aspergillus flavus	00	00	00	11	00	08	08	00	00	12
Aspergillus fumigates	00	00	00	11	00	08	08	09	10	17
Penicillium chrysogenum	00	00	00	10	09	12	11	09	00	12

Table 3. Minimum Inhibitory Concentration (MIC) of the methanolic extracts of plants against different bacterial and fungal strains (in mg/ml).

Test organism	*Alhagi maurorum*	*Chenopoidum murale*	*Convolvulus fatmensis*	*Conyza dioscoridis*	*Cynanchum acutum*	*Diplotaxis acris*	*Euphorbia cuneata*	*Origanum syriacum*	*Solenostemma argel*	*Tamarix aphylla*
Bacteria										
Gram Negative										
Acinetobacter baumanni	-	-	02	-	04	02	-	03	-	-
Escherichia coli	03	-	-	03	04	04	05	03	-	02
Moraxella lacunata	02	-	-	-	-	02	05	03	04	05
Proteus merabiles	03	-	02	04	-	-	-	-	05	03
Proteus vulgaris	03	04	-	02	-	03	03	03	-	02
Pseudomons aeruginosa	04	03	03	-	-	02	-	-	05	-
Salmonella typhi	04	-	03	-	-	03	06	-	-	-
Gram Positive										
Bacillus subtilis	-	03	03	03	03	-	04	-	03	02
Micrococcus kristinae	-	03	03	04	04	-	-	05	-	-
Micrococcus luteus	04	05	-	04	04	03	-	-	-	-
Sarcina ventricull	05	-	04	02	03	04	03	04	-	03
Staphylococcus aureus	-	-	02	05	04	-	03	02	-	02
Staphylococcus haemolyticus	-	-	-	-	-	-	-	-	-	-
Streptococcus byogens	05	-	-	03	05	04	04	05	-	02
Fungi										
Unicellular										
Candeda albicans	-	02	02	-	-	05	03	04	04	-
Saccharomyces cirivisae	05	02	03	-	-	02	-	04	04	-
Filamentous										
Aspergillus flavus	-	-	-	03	-	02	05	-	-	02
Aspergillus fumigates	-	-	-	04	-	03	05	-	03	02
Penicillium chrysogenum	-	-	-	03	-	03	04	-	-	02

standardized inoculum (1-2 X 10^7 cfu/ml) was added in each tube. The tubes were incubated at 25°C and 37°C for 24-48 h. Two control tubes, tube containing the growth medium, saline and the inoculum, were maintained for each test batch. The lowest concentration (highest dilution) of the extract that produced no visible microbial growth (no turbidity) when compared with the control tubes were regarded as MIC.

Results and Dissection

The recent intensive work revealed that the plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al*., 1998). In this study, the antibacterial and antifungal activities of extracts of ten plants (table 1) were carried out by the well diffusion method are shown in tables 2 and 3. All the investigated plant extracts exhibited different degrees of antibacterial and antifungal activities. The extracts of *Diplotaxis acris* and *Tamarix aphyla* showed activity against all the investigated fungal test organisms (Table 2). On the other hand, with the exception of *Salmonella typhi*, *Micrococcus kristinae*, and *Staphylococcus haemolyticus*, *Tamarix aphyla* showed activity against the bacterial test organisms. The highest activity of *Tamarix aphylla* was obtained against *Aspergillus fumigatus* followed by *Escherichia coli* and *Streptococcus byogens*. On the other hand, among all the methanolic extracts of the investigated plants, the highest antimicrobial activity was obtained by *Conyza dioscoridis* against both of *Proteus vulgaris* and *Sarcina ventricull*; *Chenopodium murale* against *Pseudomonas aeruginosa* and *Candida albicans*; and *Origanum syriacum* against *Staphylococcus aureus*. There are many reports available on the biological activities of natural products (Kumaraswamy *et al*., 2002; Stepanovic *et al*., 2003; Bylka *et al*., 2004; Behera and Misra, 2005; Govindarajan *et al*., 2006; Zain *et al*., 2009).

The minimum inhibitory concentration (MIC) of plant extracts against the bacterial and fungal strains varied from plant extract to the other. Moreover, the MIC value of the same plant extract has changed according to the test organism (Table 3). The highest MIC value, 2 mg/ml, was obtained by *Tamarix aphylla* against 8, including all the filamentous fungi, of the investigated test strains. However, the extract of *Chenopodium murale* showed the best MIC against the unicellular fungi. A similar study of screening natural plant extracts against different fungal pathogens was well recorded in literature (Ahmad *et al*., 2000; Rani and Murty, 2006; Parekh and Chanda, 2008). Also, the lowest concentration of extracts, 2 mg/ml, was obtained by some plants against specific bacterial strains. On the other hand, the extract of *Alhagi maurorum* showed inhibitory concentration against all, with the exception of *Acinetobacter baumanni*, the investigated Gram negative bacteria. However, the extract of *Conyza dioscoridis* showed inhibitory concentrations against all, except *Staphylococcus haemolyticus*, the investigated Gram positive bacteria (Table 3).

Results of the current study revealed the antifungal activity of *Tamarix aphylla* against *Aspergillus flavus*, *A. fumigatus* and *Penicillium chrysogenum*. However, there are many plant extracts could be used for treatment of certain bacterial infections.

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References

Ahmad I, Mehmood Z, Mohammad P, Ahmed S (2000). Antimicrobial potency and synergistic activity of five traditionally used Indian medicinal plants. *Journal* of Medicinal and *Aromatic Plant Sciences* 22, 173-176.

Awaad S, Amani, Nawal H. Mohamed, Derek. J. Maitland and Gamal A. Soliman (2008). Anti-ulcerogenic Activity of Extract and Some Isolated Flavonoids from Desmostachia bipinnata (L.) Stapf. *Records of Natural Products* 3; 76-82.

Behera SK, MK Misra (2005). Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. *Journal of Ethnopharmacology* 102: 319-325.

Bylka W, M Szaufer-Hajdrych, I Matalawskan and O Goslinka (2004). Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris* L. *Letters* in *Applied Microbiology* 39, 93-97.

Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A (1999). An ethnobotanical survey of herbal drugs of Gourma district, Mali. *Pharmaceutical Biology*. 37, 80–91.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *African* Journal of *Biotechnology* 4, 685–688.

El-Gohary HM (2004). Study of essential oils of the tubers of *Cyperus rotundus* L. and *Cyperus alopecuroides Rottb. Bulletin of Faculty of Pharmacy, Cairo University* 42, 157–164.

Gerhartz W, Yamamota YS, Campbell FT, Pfefferkorn R, Rounsaville JF (1985). Ullmann's Encyclopedia of Industrial.

Govindarajan R, M Vijayakumar, M Singh, CHV Rao, A Shirwaikar, AKS Rawat and P Pushpangadan (2006). Antiulcer and antimicrobial activity of *Anogeissus latifolia. Journal of Ethnopharmacology* 106, 57-61.

Harvey AL (1999). Medicines from nature: are natural products still relevant to drug discovery? Trends in Pharmacological Sciences.20, 196-198.

Hoffmann JJ, N Timmerman, R Mclaughlin, H Punnapayak (1993). Potential anti-microbial activity of plants from the South Western United States. International Journal of Pharmacology 31, 101-115.

Kroschwitz JI, M Howe-Grant (1992). Kirk-Othmer. Encyclopedia of chemical technology, 2, 893.

Kumaraswamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD. (2002). Screening seeds of Scottish plants for antibacterial activity. *Journal of Ethnopharmacology* 83, 73-77.

NCCLS, (1993). Performance Standards Antimicrobial Disc Susceptibility Tests. Approved Standard Fifth Edition. NCCLS Document M2-A5, Villanova, PA, USA.

NCCLS, (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Fifth Edition. NCCLS Document M7-A5, NCCLS: Wayne, PA, USA.

Paerkh J, Chanda S (2008). *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African Journal of Biotechnology* 7, 4349-4353.

Rani SA and Murty SU (2006). Antifungal potential of flower head extract of *Spilanthes acmella* Linn. *African Journal of Biomedical Research* 9, 67-69.

Srinivasan D, Sangeetha N, Suresh T, Perumalsamy PL (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmac-*

ology 74, 217-220.

Srivastava J, Lambert J, Vietmeyer N (1996). Medicinal plants: An expanding role in development. *World Bank Technical Paper. No. 320*.

Stepanovic S, Antic N, Dakic I, Svabicvlahovic M. (2003). *In vitro* antimicrobial activity of propilis and antimicrobial drugs. *Microbiology Research* 158, 353-357.

Tona L, K Kambu, N Ngimbi, K Cimanga and AJ Vlietinck (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. *Journal of Ethnopharmacology* 61, 57-65.

Uniyal SK, Singh KN, Jamwal P, Lal B (2006). Traditional use of medicinal plants among the tribal communities of chhota Bhangal, Western Himalayan. *Journal of Ethnobiology and.Ethnomedicine* 2, 1-14.

Zain ME, Amani S Awaad**,** AA Razak, DJ Maitland, NE Khamis and MA Sakhawy (2009). Secondary Metabolites of *Aureobasidium pullulans* Isolated from Egyptian Soil and Their Biological Activity. *Journal of Applied Sciences Research* 5, 1582-1591.

