Antibacterial, antifungal activity and phytochemical analysis of some desert plants against human pathogenic bacteria and fungi

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Abstract: Five desert plants; namely, *Bidens bipinnata, Cyperus alternifolius*, *Desmostachya bipinnata, Glossostemon bruguieri,* and *Schouwia thebica* were investigated for their antimicrobial activity. The ethanolic extract 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*, *Staphylococcus aureus* and pathogenic fungi including *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *A. fumigates* and *Penicillium chrysogenum*. The extracts of *Cyperus alternifolius* and *Desmostachya bipinnata* were similar in their phytochemical constituents, while the chemical groups of *Bidens bipinnata* were similar to those in *Schouwia thebica*. Traces of saponins were only present in *Glossostemon bruguieri*.

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1. Introduction

Plants are a source of great economic value all over the world. Naturehas given us avery rich botanical wealth and large number of diverse types of plants grow in different partsof the country. Interestingly, the use of medicinal plants as a source for relief fromillness can be traced back over five millennia to writtendocuments of the early civilization in China, India and theNear east, but it is doubtless an art as old asmankind.

Plants contain large varieties of chemical substances, which possessimportant therapeutic properties that can be utilized in the treatment ofhuman diseases. The studies of plants used in folklore remedies have attractedtheattention of many scientists in finding solution to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic microorganisms against their drugs **(Akinpelu et al., 2008).**

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 increasingly turning their attention to natural products looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (**Srinivasan *et al.*, 2001; Harvey, 1999;Hoffmann *et al.*, 1993).**

Now a day's maximum number of plants are being screened for their possible pharma-cological value. However, the plant kingdom still holdmany plant species containing substance of medicinal value which have yet to be discovered **(Tambe et al., 2013)**. The medicinal value of plants lies in some chemical substances that produce a definitephysiologic action on the human body. The most important of these phytocompounds of plantsare alkaloids, flavonoids, tannins and phenolic compounds **(Vedhanarayanan et al., 2013)**.The current study was mainly concerned was investigating the antibacterial and antifungal activities of some plants against representatives of human pathogenic bacteria and fungi.

2. Materials and Methods

Plant Materials

Plant samples of *Bidens bipinnata, Cyperus alternifolius*, *Desmostachya bipinnata, Glossostemon bruguieri,* and *Schouwia thebica* were collected during 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.

Plant Extracts

The plant samples were washed 2-3 times with running fresh water and then air-dried under shade. After complete shade drying, the plant materials (300 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.

Antimicrobial Activity Assay

Test organisms

Eleven strains of pathogenic bacteria; *Enterococcus faecalis*, *Escherichiacoli*, *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 use as test organisms.

Antibacterial activity

The antibacterial activity of the investigate plants was determined by well diffusion method according to National Committee for Clinical Laboratory Standards **(NCCLS, 1993).** Petri plates containing 20 ml of Nutrient Agar medium were seeded with 24 hrs. old cultures of bacterial inoculum (standardized inoculums 1-2 X 10^7 cfu/ml 0.5 Mcfarland standard). Wells (6 mm in diameter) were cut off from agar and 100 µl of plant extracts were tested in a concentration of 5 mg/ml and incubated at 37°C for 24 hrs. The antibacterial activity was determined by measuring the inhibition zone formed around the well.

Antifungal activity

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

Phytochemical Analysis

The extracts of*Bidens bipinnata, Cyperus alternifolius*, *Desmostachya bipinnata, Glossostemon bruguieri,* and *Schouwia thebica*were chemically tested for the presence of different phytochemical constituents such as carbohydrates and/or glycosides **(Majaw et al., 2009),** flavonoides, 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 et al., 2009), cardinolides (**Sonibare et al., 2009)**and oxidase enzyme **(Wagner and Baldt, 2001).**

3. Results

The antibacterial and antifungal activities of five desert plants were determined. Moreover, the chemical constituents of each plant were investigated. The ethanolic extract of each plant was investigated for its ability to inhibit the growth of human bacteria; *Enterococcus faecalis*, *Escherichiacoli*, *Moraxella lacunata*, *Proteus merabiles*, *Serratia marcesens*, *Pseudomons aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*, *Micrococcus luteus*, *Sarcina ventricull*, *Staphylococcus aureus* and pathogenic fungi; *Candida albicans*, *Candida tropicalis*, *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium chrysogenum*.

Bidens bipinnata

The obtianed results revealed that the ethanolic extract of *Bidens bipinnata*has antibacterial activity against the investigated human pathogenic bacteria (Table 1). The highest antibacterial activity (13 mm) was obtained against *Proteus merabiles*, *Bacillus subtilis*, and *Staphylococcus aureus*. However, good antibacterial activity (12, 11, and 10 mm) was obtained against *Serratia marcesens*, *Escherichia coli*, and *Enterococcus faecalis*, respectively. The lowest antiabacterial activity of *Bidens bipinnata* (6 mm) was obtained against *Salmonella typhi* and *Micrococcus luteus* (Table 1).

The antifungal activity of *Bidens bipinnata* (10 mm) was obtained against *Candida albicans* and *C. tropicalis*, (9 mm) against *Aspergillus flavus*, (8 mm) against *Aspergillus fumigates*, and (6 mm) against *Penicillium chrysogenum* (Table 1).

On the other hand, the obtained results of phytochemical analysis of *Bidens bipinnata* revealed the presence of tannins, flavonoids (free aglycone and glycoside), unsaturated and/or triterpenoides, carbohydrates or glycosides, and proteins and/or amino acids. The crystalline sublimate, volatile oil, alkaloikds and/or nitrogenous bases, cardinolides, saponins, anthraquinones, and oxidase enzymes were absent (Table 1).

Cyperus alternifolius

The ethanolic extract of *Cyperus*

alternifolius has antibacterial activity against the investigated human pathogenic bacteria (Table 2). The highest antibacterial activity (13 and 11 mm) was obtained against *Pseudomons aeruginosa* and *Serratia marcesens*, respectively. However, good antibacterial activity (10 mm) was obtained against *Moraxella lacunata*, *Bacillus subtilis*, and *Micrococcus luteus*; (9 mm) against *Proteus merabiles* and (8 mm) against *Enterococcus faecalis*, *Escherichia coli*, and *Sarcina ventricull*. The extract of *Cyperus alternifolius* showed no activity against *Salmonella typhi* and *Staphylococcus aureus* (Table 2).

On the other hand, the results of antifungal activity of *Cyperus alternifolius* revealed that the highest activity (15and 13mm) was obtained against *Candida albicans* and *C. tropicalis*, respectively (Table 2). Moreover, moderate antifungal activity (9 and 7 mm) was obtained against *Aspergillus flavus* and *Aspergillus fumigates*, respectively. The lowest antifungal activity (6 mm) was obtained against *Penicillium chrysogenum* (Table 2).

Table 1. The antibacterial, antifungal activities and chemical constituents of *Bidens bipinnata*.

Antibacterial activity		Antifungal activity		Phytochemical analysis	
Bacteria	**Zone of inhibition (mm)**	**fungi**	**Zone of inhibition (mm)**	**Test**	**result**
Gram-negative		**Unicellular**		Crystalline sublimate	-
Enterococcus faecalis	10	*Candida albicans*	10	Volatile oil	-
Escherichia coli	11	*Candida tropicalis*	10	Tannins	+
Moraxella lacunata	10	**Filamentous**		Flavonoids	
Proteus merabiles	13	*Aspergillus flavus*	09	a. free aglycone	+
Serratia marcesens	12	*Aspergillus fumigates*	08	b. glycoside	+
Pseudomons aeruginosa	09	*Penicillium chrysogenum*	06	Alkaloids and/or nitrogenous bases	-
Salmonella typhi	06			Cardinolides	-
Gram-positive				Unsaturated sterols and/or triterpenoides	+
Bacillus subtilis	13				
Micrococcus luteus	06			Saponins	-
Sarcina ventricull	09			Carbohydrates or glycosides	+
Staphylococcus aureus	13			Anthraquinones	-
				Proteins and/or amino acids.	+
				oxidase enzyme	-

(-), absent; (+), present

Table 2. The antibacterial, antifungal activities and chemical constituents of *Cyperus alternifolius*.

Antibacterial activity		Antifungal activity		Phytochemical analysis	
Bacteria	**Zone of inhibition (mm)**	**fungi**	**Zone of inhibition (mm)**	**Test**	**result**
Gram-negative		**Unicellular**		Crystalline sublimate	+
Enterococcus faecalis	08	*Candida albicans*	15	Volatile oil	-
Escherichia coli	08	*Candida tropicalis*	13	Tannins	+
Moraxella lacunata	10	**Filamentous**		Flavonoids	
Proteus merabiles	09	*Aspergillus flavus*	09	a. free aglycone	+
Serratia marcesens	11	*Aspergillus fumigates*	07	b. glycoside	+
Pseudomons aeruginosa	13	*Penicillium chrysogenum*	06	Alkaloids and/or nitrogenous bases	-
Salmonella typhi	00			Cardinolides	-
Gram-positive				Unsaturated sterols and/or triterpenoides	+
Bacillus subtilis	10				
Micrococcus luteus	10			Saponins	-
Sarcina ventricull	08			Carbohydrates or glycosides	+
Staphylococcus aureus	00			Anthraquinones	-
				Proteins and/or amino acids.	+
				oxidase enzyme	-

(-), absent; (+), present

The phytochemical constituents of *Cyperus alternifolius* contained crystalline sublimate, tannins, flavonoids (free aglycone and glycoside), unsaturated and/or triterpenoides, carbohydrates or glycosides, and proteins and/or amino acids. However, volatile oil, alkaloikds and/or nitrogenous bases, cardinolides, saponins, anthraquinones, and oxidase enzymes were absent (Table 2).

Desmostachya bipinnata

The obtained results revealed that the ethanolic extract of *Desmostachya bipinnata*has antibacterial activity against the investigated human pathogenic bacteria (Table 3). The highest antibacterial activity (13 and 12 mm) was obtained against *Micrococcus luteus* and *Bacillus subtilis*, respectively. However, good antibacterial activity (10 mm) was obtained against *Proteus merabiles*, *Pseudomons aeruginosa*, and *Salmonella typhi*; and (9 mm) against *Sarcina ventricull* and *Staphylococcus aureus*. The lowest antibacterial activity of *Desmostachya bipinnata*was obtained against *Salmonella typhi* and *Micrococcus luteus* (Table 3).

Table 3. The antibacterial, antifungal activities and chemical constituents of *Desmostachya bipinnata*.

Antibacterial activity		Antifungal activity		Phytochemical analysis	
Bacteria	**Zone of inhibition (mm)**	**fungi**	**Zone of inhibition (mm)**	**Test**	**result**
Gram-negative		**Unicellular**		Crystalline sublimate	+
Enterococcus faecalis	07	*Candida albicans*	10	Volatile oil	-
Escherichia coli	07	*Candida tropicalis*	11	Tannins	+
Moraxella lacunata	08	**Filamentous**		Flavonoids	
Proteus merabiles	10	*Aspergillus flavus*	07	a. free aglycone	+
Serratia marcesens	07	*Aspergillus fumigates*	08	b. glycoside	+
Pseudomons aeruginosa	10	*Penicillium chrysogenum*	07	Alkaloids and/or nitrogenous bases	-
Salmonella typhi	10			Cardinolides	-
Gram-positive				Unsaturated sterols and/or triterpenoides	+
Bacillus subtilis	12				
Micrococcus luteus	13			Saponins	-
Sarcina ventricull	09			Carbohydrates or glycosides	+
Staphylococcus aureus	09			Anthraquinones	-
				Proteins and/or amino acids.	+
				oxidase enzyme	-

(-), absent; (+), present

The highest antifungal activity of *Desmostachya bipinnata* was (11 and 10 mm) was obtained against *Candida tropicalis* and *C. albicans*, respectively. While the antifungal activity (8 mm) was obtained against *Aspergillus fumigates*. The lowest antifungal activity (7 mm) was obtained against *Aspergillus flavus* and *Penicillium chrysogenum* (Table 3).

The obtained results of phytochemical analysis of *Desmostachya bipinnata* revealed the presence of crystalline sublimate, tannins, flavonoids (free aglycone and glycoside), unsaturated and/or triterpenoides, carbohydrates or glycosides, and proteins and/or amino acids. The volatile oil, alkaloikds and/or nitrogenous bases, cardinolides, saponins, anthraquinones, and oxidase enzymes were absent (Table 3).

Glossostemon bruguieri

The ethanolic extract of *Glossostemon bruguieri* has antibacterial activity against the investigated human pathogenic bacteria (Table 4). The highest antibacterial activity (15, 12 and 11 mm) was obtained against *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus merabiles*, respectively. However, good antibacterial activity (10 mm) was obtained against *Moraxella lacunata* and *Serratia marcesens*; (9 mm) against *Escherichia coli* and (8 mm) against *Enterococcus faecalis*, *Micrococcus luteus*, and *Sarcina ventricull*; (7 mm) against *Pseudomons aeruginosa*. The extract of *Glossostemon bruguieri* showed no activity against *Salmonella typhi* (Table 4).

On the other hand, the results of antifungal activity of *Glossostemon bruguieri* revealed that the highest activity (11 mm) was obtained against *Aspergillus flavus* and *Aspergillus fumigates*; (10 mm) against *Penicillium chrysogenum* (Table 4). There was no activity against *Candida albicans* and *C. tropicalis*.

The phytochemical analysis of *Glossostemon bruguieri* showed the presence of tannins, flavonoids (free aglycone and glycoside),

unsaturated and/or triterpenoides, traces of saponins, carbohydrates or glycosides, and proteins and/or amino acids. The crystalline sublimate, volatile oil, alkaloikds and/or nitrogenous bases, cardinolides, saponins, anthraquinones, and oxidase enzymes were absent (Table 4).

Schouwia thebica

The obtianed results revealed that the ethanolic extract of *Schouwia thebica* has antibacterial activity against the investigated human pathogenic bacteria (Table 5). The highest antibacterial activity (11 mm) was obtained against *Enterococcus faecalis* and *Bacillus subtilis*. A good antibacterial activity (10 mm) was obtained against *Moraxella lacunata*, *Pseudomons aeruginosa* and *Staphylococcus aureus*; (9 mm) against *Escherichia coli*, *Proteus merabiles* and *Sarcina ventricull*. The lowest antiabacterial activity of *Schouwia thebica* (7 mm) was obtained against *Salmonella typhi* and *Micrococcus luteus*. There was no activity against *Serratia marcesens* (Table 5).

Table 4. The antibacterial, antifungal activities and chemical constituents of *Glossostemon bruguieri*.

Antibacterial activity		Antifungal activity		Phytochemical analysis	
Bacteria	**Zone of inhibition (mm)**	**fungi**	**Zone of inhibition (mm)**	**Test**	**result**
Gram-negative		**Unicellular**		Crystalline sublimate	-
Enterococcus faecalis	08	*Candida albicans*	00	Volatile oil	-
Escherichia coli	09	*Candida tropicalis*	00	Tannins	+
Moraxella lacunata	10	**Filamentous**		Flavonoids	
Proteus merabiles	11	*Aspergillus flavus*	11	a. free aglycone	+
Serratia marcesens	10	*Aspergillus fumigates*	11	b. glycoside	+
Pseudomons aeruginosa	07	*Penicillium chrysogenum*	10	Alkaloids and/or nitrogenous bases	-
Salmonella typhi	00			Cardinolides	-
Gram-positive				Unsaturated sterols and/or triterpenoides	+
Bacillus subtilis	12				
Micrococcus luteus	08			Saponins	±
Sarcina ventricull	08			Carbohydrates or glycosides	+
Staphylococcus aureus	15			Anthraquinones	-
				Proteins and/or amino acids.	+
				oxidase enzyme	-

(-), absent; (+), present

Table 5. The antibacterial, antifungal activities and chemical constituents of *Schouwia thebica*.

Antibacterial activity		Antifungal activity		Phytochemical analysis	
Bacteria	**Zone of inhibition (mm)**	**fungi**	**Zone of inhibition (mm)**	**Test**	**result**
Gram-negative		**Unicellular**		Crystalline sublimate	-
Enterococcus faecalis	11	*Candida albicans*	09	Volatile oil	-
Escherichia coli	09	*Candida tropicalis*	09	Tannins	+
Moraxella lacunata	10	**Filamentous**		Flavonoids	
Proteus merabiles	09	*Aspergillus flavus*	07	a. free aglycone	+
Serratia marcesens	00	*Aspergillus fumigates*	11	b. glycoside	+
Pseudomons aeruginosa	10	*Penicillium chrysogenum*	08	Alkaloids and/or nitrogenous bases	-
Salmonella typhi	07			Cardinolides	-
Gram-positive				Unsaturated sterols and/or triterpenoides	+
Bacillus subtilis	11				
Micrococcus luteus	07			Saponins	-
Sarcina ventricull	09			Carbohydrates or glycosides	+
Staphylococcus aureus	10			Anthraquinones	-
				Proteins and/or amino acids.	+
				oxidase enzyme	-

(-), absent; (+), present

The highest antifungal activity of *Schouwia thebica* (11 mm) was obtained against *Aspergillus fumigates*. While the antifungal activity (9 mm) was obtained against *Candida tropicalis* and *C. albicans*; (8 mm) against *Penicillium chrysogenum*. The lowest antifungal activity (7 mm) was obtained against *Aspergillus flavus* (Table 5).

The obtained results of phytochemical analysis of *Schouwia thebica* revealed the presence of tannins, flavonoids (free aglycone and glycoside), unsaturated and/or triterpenoides, carbohydrates or glycosides, and proteins and/or amino acids. The crystalline sublimate, volatile oil, alkaloikds and/or nitrogenous bases, cardinolides, saponins, anthraquinones, and oxidase enzymes were absent (Table 5).

4. Discussion

Natural sources including plants are important source of potentially useful structures, which could be used for the development of new chemotherapeutic agents. However, to achieve this goal, *in vitro* antibacterial and/or antifungal activity assay are required **(Tona et al., 1998).** Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants **(Zain et al., 2014, 2012, 2009; Awaad et al., 2012; Chanda and Baravalia, 2010; Ahameethunisa and Hooper, 2010; Chattopadhyay et al., 2009; Alam et al., 2009; Govindarajan et al., 2006; Behera and Misra, 2005; Bylka et al., 2004).**

In the present study five extracts of desert plants; *Bidens bipinnata, Cyperus alternifolius*, *Desmostachya bipinnata, Glossostemon bruguieri,* and *Schouwia thebica* inhibited the growth of human pathogenic bacterial and fungi. The antimicrobial activity of plants and their use in traditional medicine have been investigated and approved long time ago **(Vedhanarayanan et al., 2013; Zain et al., 2012; Kambizi and Afolayan, 2008; Uniyal et al., 2006; Newman et al., 2000; Thomson, 1978).**

The results of the current study revealed the presence of the investigated plants revealed the presence of different chemical 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. Isolation and identification of bioactive compounds from natural resources including plants were carried out by many studies **(Zain et al., 2014; Awaad et al., 2013; El-Meligy et al., 2013; Malu et al., 2009; Ahmad et al., 2008;Raghavendra et al., 2006; Bylka et al., 2004; Girish et al., 2006;Mothana and Lindequist, 2005; Karaman et al., 2003).**

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