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Full Length Research Paper

Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment

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Traditional medicine has a key role in health care worldwide. Obtaining scientific information about the efficacy and safety of the wild plants grown in western Mediterranean coast of Egypt is one of our research goals. In this study, 10 wild plants namely *Mesembryanthemum crystallinum*, *Blackiella aellen*, *Arthrocnemon glaucum*, *Atriplex halimus*, *Thymelaea hirsute*, *Carduus getulus*, *Nicotiana glauca*, *Alhagi maurorum*, *Atractylis carduus* and *Echinops spinosissimus* were collected from El-Hammam, Burg El Arab and Bahig regions located along the Western Mediterranean coast of Egypt. Hexane and methanol extracts of fresh aerial parts of the plants were screened *in vitro* for antimicrobial activity against 15 Gram positive and negative pathogenic bacteria. Both methanol and hexane plant extracts showed strong antibacterial activity against at least two pathogenic microorganisms tested. However, hexane extracts generally showed lower activity against microorganisms compared to methanol extracts. The microorganisms' susceptibility to different extracts did not correlate with the susceptibility or resistance to a particular antibiotic. The results of this study thus support the medical usage of the studied plants and suggest that some of these plants possess antimicrobial properties that can be used to cure infectious diseases.

Key words: Egyptian wild plants, antagonism, biological control, phytochemicals, multi-drug resistant.

INTRODUCTION

Plants could be described as a wonderful kitchen or chemical cabinets filled with attractive things. The ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in the treatment of various diseases (Abu-Shanab et al., 2004).

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. It has been observed however, that many plants contain one or more chemical substances with antimicrobial activities. For example, Fujii et al. (1991) reported that water and methanol extracts of some medicinal plants displayed antifungal activity against *Fusarium oxysporum,* which causes yellows of Japanese radish. Several other examples have emerged from research into the control of soil-borne plant diseases. Ushiki et al. (1996) reported that *Geranium pratense* L. strongly inhibited the growth of *Streptomyces scabies,* which causes common scab of potato. Powell and Ko (1986) also reported that root extract of *Allium sativum* inhibited germination of chlamydospores and encysted zoospores of *Phytophthora palmivora* in soil. These antimicrobial substances are of a natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred to as 'forgotten plants'. Taking into account the increasing demand for natural ingredients that might be used as

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Table 1. List of plant species screened for antimicrobial activity.

Voucher number/collection site	Plant name		
	Botanical name	**Vernacular name**	**Family**
UAE109/Burg el Arab	*Mesembryanthemum crystallinum*	Crystalline ice plant Slender-leaf ice plant	*Aizoaceae*
UAE409/Bahig	*Blackiella conduplicata (F. Muell.) Aellen*	*Blackiella aellen*	*Chenopodiaceae*
UAE609/El Hammam	*Arthrocnemon glaucum*	Shinaan, oshnan, Hatab ar ad, Khriyet, Khreiza	*Chenopodiaceae*
UAE709/El Hammam	*Atriplex halimus*	*Sea orach*	*Chenopodiaceae*
UAE509/Bahig	*Carduus getulus*	Hoshroof	*Compositae*
UAE809/El Hammam	*Atractylis carduus*	Shawk El-Gamal	*Compositae*
UAE209/Burg El Arab	*Echinops spinosissimus*	Kadaad	*Compositae*
UAE909/El Hammam	*Alhagi maurorum*	Aqool camel's thorn, Persian manna plant	*Fabaceae*
UAE309/Burg El Arab	*Nicotiana glauca*	Massas	*Solanaceae*
UAE1009/El Hammam	*Thymelaea hirsute*	Mithnaan *gy Sparrow-*	*Thymelaeaceae*

food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications, it is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods.

The Mediterranean coastal land of Egypt is one of the eight phytogeographical territories, extending for about 970 km between Sallum and Rafah (El Hadidi, 1993). According to Zahran et al. (1985), the Mediterranean coastal land can be divided into three sectors: western (the Mareotis, extending for 550 km between Sallum and Alexandria), middle (Deltaic, extending for 180 km between Alexandria and Port Said), and eastern (Sinaitic, extending for 220 km between Port Said and Rafah). The Mareotis region (including El-Hammam, Burg El-Arab and Baheg) is vegetationally and floristically the richest part of Egypt (Ayyad, 1996). Its flora includes about 52.5% of the 2,085 species recorded in Egypt (Boulos, 1995). The regions of southwest Alexandria, Egypt has a rich and poorly valorized wild vegetal heritage, including a remarkable diversity of medicinal plants. This area has regular winter rainfall. Plants germinate in cooler months and reach maturity in spring or early summer, while others may continue to grow in the following year.

Considering that there are not many data on biotic activity of the identified medicinal plants growing naturally in El Hammam area, the objective of this study was to investigate the antibacterial properties of 10 plant namely *Mesembryanthemum crystallinum, Blackiella aellen, Arthrocnemon glaucum, Atriplex halimus, Thymelaea hirsute, Carduus getulus, Nicotiana glauca, Alhagi maurorum, Atractylis carduus* and *Echinops spinosissimus*, as well as to estimate whether some extracts of these plants could potentially be used in the control of selected pathogenic microorganisms.

MATERIALS AND METHODS

Plant collection

The aerial parts of the ten selected plants were collected from Burg El-Arab, Bahig and El- Hammam regions in the Western Mediterranean coastal part of Egypt during spring season of 2009. The taxonomic identities of these plants were confirmed by Dr. S. M. Abdel Rahman, a taxonomist at the Botany Department, Faculty of Science, Alexandria University, Egypt (Tackholm, 1974). The ethno-botanical information is reported in Table 1.

Preparation of plant extracts

After collection, the fresh aerial parts were cleaned with water, air-dried and coarsely powdered using a mortar and pestle, then further reduced to powder using electric blender. To obtain fractions

Table 2. List of Gram positive and negative bacterial pathogens used in this study.

Gram positive	Source	Gram negative	Source
Bacillus cereus	Locally isolated organisms	*Echericha coli*	Locally isolated organisms
Clostridium perfringens ATCC 13124	Culture collection of Cairo Mircen	*Yersinia enterocolitica ss. Enterocolitica* ATCC 23715,	Culture collection of Cairo Mircen
Listeria innocua ATCC 33090	Culture collection of Cairo Mircen	*Salmonella enterica* ATCC 25566	Culture collection of Cairo Mircen
Listeria monocytogenes ATCC 19116	Culture collection of Cairo Mircen	*Klepsiella pneumonia*	Locally isolated organisms
Listeria ivanovii Li4 (pVS2)	Provided by Dr. Lars Axelsson (Matforsk, Norwegian Food Research Institute)	*Klepsiella oxytoca*	Locally isolated organisms
Staphylococcus aureus 72	Locally isolated organisms		
Staphylococcus aureus 132	Locally isolated organisms		
Staphylococcus aureus 224	Locally isolated organisms		
Staphylococcus epidermis	Locally isolated organisms		

with different polarities, powdered plant material was extracted with hexane and methanol. Extraction with each solvent was done along a period of two weeks under occasional shaking. Each sample extract was collected and concentrated to dryness using rotary evaporator at 40°C. The dried extracts were weighed and then stored in air tight container and kept at 4°C until it was used for further analysis.

Microbial strains

Fifteen microbial species were used to test the antimicrobial activities of medicinal plants tested (Table 2). Five of the tested bacterial pathogeneses were taken from the culture collection of Cairo Mircen (Faculty of Agriculture, Ain Shams University, Egypt). The species *Staphylococcus aureus* 132,and 72 were isolated from patients in Ain Shams University Hospital Cairo, Egypt. *Klepsiella pneumonia* and *Klepsiella oxytoc* were isolated from urine specimen. *Listeria ivanovii* Li4 (pVS2) was provided by Dr. Lars Axelsson (Matforsk, Norwegian Food Research Institute).

Antimicrobial disk susceptibility testing

Antibiotic susceptibility testing for *S. aureus* species were performed using Kirby-Bauer disk diffusion method according to the guidelines of NCCLS (NCCLS, 2000) on Muller-Hinton agar plates at 37°C using antibiotic disks containing penicillin, gentamicin, teteracycline, methicillin, and vancomycin. Isolates showed 100% resistant to penicillin G, methicillin and erythromycin, 53% resistant to tetracycline, 44% resistant to gentamycin, while multi-resistance to these antibiotics was observed in 44%. Methicillin-resistant *S. aureus* are persistent and increasingly causes nosocomailly acquired infections in many hospitals (Hafez et al., 2006). *K. pneumonia* and *K. oxytoca* are known to cause urinary tract infection. Antibiotic susceptibility testing indicated that these strains were resistant to many antibiotics tested. Strains of *Klepsiella* were identified by biochemical characterization, API20 and molecular techniques.

Preparation and standardization of bacterial inoculums

The bacterial strains were maintained on nutrient agar slants. All strains were kept at 10°C, sub-cultured and checked for purity every 4 to 5 weeks. For bacterial cultures, single colony from each strain was inoculated in nutrient broth and incubated for 24 h. On the second day, 100 µL of the fresh bacterial culture of each strain was added to 7 ml soft agar.

Antibacterial activity assay

Antibacterial activity was determined by agar well diffusion method on nutrient agar (Jorgensen et al., 1999). Different plant extracts were prepared by dissolving 6 mg of each plant extract in 2 ml sterile dimethyl sulfoxide (DMSO) serving as stock solution. Nutrient agar (40 to 50 ml) was poured into sterile Petri plates and then

allowed to solidify. The inoculum suspension of each strain of bacteria was inoculated into 7 ml soft agar. Soft agar tubes inoculated with fresh bacterial cultures were poured on agar plates. After solidifying, wells were cut in the hardened media using a cooled-flamed 8 mm cork borer. Plant extracts were tested in triplicates by adding 100 µL of each extract solution to each of three wells. Other wells were supplemented with DMSO serving as negative control and Imipenum (Jiaruite; China; purity > 98% by HPLC) serving as reference antibacterial drug. Test and control Petri dishes inoculated with bacteria were incubated at 37°C for 24 h. Results of antimicrobial activities of the tested plants extracts were expressed as inhibition diameter zones in millimeters (mm) as follows: N.A. (no activity) ≤ 4 mm; + (weak) = 5 - 10 mm; ++ (moderate) = 10 - 16 mm; +++ (strong) ≥ 17 mm. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

Phytochemical screening

The fresh aerial parts extracts of the tested plants were analysed for the presence of alkaloids, saponins, tannins, anthraquinone, steroid, flavonoids, glycosides and glucosides according to standard methods (Harborne, 1973; Odebiyi and Sofowora, 1978; Sofowora, 1982, 1993; Williamson et al., 1996; Banso and Negbede, 2006).

Determination of protein content and effect of proteolytic enzymes on antibacterial activity

Protein concentration of plant extracts was measured according to bicinchoninic acid method (Smith et al., 1985) using bovine serum albumin (BSA) as standard. Proteolytic enzymes including pepsin, trypsin and proteinase k were dissolved in 0.05 M citric acid at pH 2.0, and 0.05 M sodium phosphate at pH 7.0 and 7.5, respectively. Enzymes solutions were sterilized by passing them through Millipore membrane filter (0.2 µm). Plant filtrates were added to a final concentration of 1 mg/ml and incubated for 2 h at 37°C. All the vials after the enzyme treatment were subsequently heated in boiling water bath for 5 min to inactivate the enzymes and finally assayed for antimicrobial activity with *S. aureus* 224.

RESULTS

A total of 20 extracts from 10 various plants species belonging to 6 families were tested. The botanical name, common name and families of the collected plant species are shown in Table 1.

Antibacterial activity of extracts

The antibacterial activity of the plant extracts were evaluated *in vitro* against both Gram-positive and Gram-negative bacteria known to cause infectious diseases in humans. The results of both methanol and hexane extracts displaying antibacterial activity are presented in Tables 3 to 8.

Methanol extracts

The antibacterial activity of the two different concentrations (6 and 23 mg/ml) of various medicinal plants tested are presented in Tables 3 to 6. All plant extracts showed anti-microbial activity against at least three microorganisms tested. In some cases, increasing the concentration of specific plant extract resulted in higher antimicrobial activity against specific indicator microorganism. For example *k. oxytoca* was inhibited by *M. crystallinum* and *A. carduus; S. aureus* 72 was inhibited by *C. getulus; B. cereus* was inhibited by *A. carduus* and *E. spinosissimus*; *Candida albicans* was inhibited by *M. crystallinum, A. glaucum, N. glauc* and *A. carduus;* while *L. ivanovii* Li4 (pVS2) was inhibited by *M. crystallinum, A. glaucum, A. halimus,* and *A. maurorum.* In all cases previously mentioned, the plant extracts were more potent compared to antibiotics used against the microorganisms. Furthermore, some of the extracts tested (for example; *C. getulus*) exhibited antimicrobial activity only at higher concentration (23 mg/ml), while little or no activity was observed at low concentrations (6 mg/ml). Among the ten methanol plant extracts, only two extracts (*C. getulus* and *E. spinosissimus*) showed significant antibacterial activity against *E. coli* whereas, *Carduus getulus* was the only extract that showed antibacterial activity against *Listeria innocua*. It was also noted that *K. oxytoca* and *S. aureus* were the most susceptible bacteria to all plant extracts. On the contrary, *Staphylococcus epidermis* and *Listeria monocytogenes* were the most resistant microorganisms. Since several *Staphylococcus* strains were reported as drug resistance, plant natural extracts were shown to be effective anti-*Staphylococcus*. In this study, the methanolic extract showed significant inhibition against *S. aureus.* The extracts of *A. carduus* and *E. spinosissimus* possessed maximum activity against *Bacillus cereus.* However, *C. getulus* and *N. glauca* showed high inhibitory activity against *Yersinia enterocolitca* ss*. enterocolitica*. Both *M. crystallinum* and *A. halimus* were very active against *L. ivanovii* Li4 (pVS2) giving large inhibition zone (40 mm). On the other hand, the highest antibacterial activity of *Blackiella conduplicata* and *A. maurorum* were recorded against *Salmonella enterica ss. enterica* and *Clostridium perfringens*, respectively. The susceptibility of any microorganism to different extracts had no correlation with the susceptibility to a particular antibiotic. It was obvious that the microbial strains of a specific species susceptible to specific drugs, showed higher susceptibility to extracts than those of resistant species. This fact was evident for *S. aureus* 224 and 72. In general, the entire methanol plant extracts showed strong antibacterial activity against at least one of the tested pathogenic bacteria.

Hexane extracts

The hexane extracts of various tested plants were screened for their possible antibacterial activity (Tables 7 and 8). Some of the tested extracts showed potent activity against Gram positive and negative bacteria,

Table 3. Antibacterial activity of methanol extracts (6 mg/ml) of medicinal plants tested against Gram positive pathogenic bacteria.

Plant	Zone of inhibition (mm)								
	B cereus	***C. perfringens* ATCC 13124**	***L. innocua* ATCC 33090**	***L. ivanovii* Li4 (pVS2)**	***L. monocytogensis* ATCC 19116**	***S. aureus* 72**	***S. aureus* 132**	***S. aureus* 224**	***S. epidermis***
Mesembryanthemum crystallinum	8	0	0	12	0	9	12	21	0
Blackiella conduplicata	12	0	0	6	0	0	15	0	0
Arthrocnemon glaucum	20	0	0	10	0	6	9	18	0
Atriplex halimus	10	0	0	18	0	5	1	20	0
Thymelaea hirsute	10	0	0	0	0	6	12	30	0
Carduus getulus	15	0	0	0	0	7	10	17	0
Nicotiana glauca	15	0	0	7	0	5	11	22	0
Alhagi maurorum	10	0	0	7	0	5	12	20	0
Atractylis carduus	25	0	0	0	0	5	7	30	0
Echinops spinosissimus	12	0	0	0	0	12	10	32	0
Positive control[1]	10	20	0	30	0	0	0	0	30

[1]Antibacterial drug (Imipenum) served as positive control, DMSO served as negative control; Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria.

Table 4. Antibacterial activity of methanol extracts (6 mg/ml) of medicinal plants tested against Gram negative pathogenic bacteria.

Plant name	Zone of inhibition (mm)				
	E. coli	***Y. enterocolitica* ATCC 23715**	***K. oxytoca***	***K. pneumonia***	***S. enterica* ATCC 25566**
Mesembryanthemum crystallinum	0	0	7	7	0
Blackiella conduplicata	0	0	9	7	0
Arthrocnemon glaucum	0	0	9	6	0
Atriplex halimus	0	0	10	8	0
Thymelaea hirsute	0	0	0	11	0
Carduus getulus	0	0	0	6	0
Nicotiana glauca	0	0	0	6	0
Alhagi maurorum	0	0	0	7	0
Atractylis carduus	0	0	5	0	0
Echinops spinosissimus	11	0	12	0	0
Positive control [1]	3	0	7	0	30

[1]Antibacterial drug (Imipenum) served as positive control, while DMSO served as negative control. Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria.

Table 5. Antibacterial activity of methanol extracts (23 mg/ml) of medicinal plants tested against Gram positive pathogenic bacteria.

Plant name	Zone of inhibition (mm)								
	B. cereus	***C. perfringens* ATCC 13124**	***L. innocua* ATCC 33090**	***L. ivanovii* Li4 (pVS2**	***L. monocytogensis* ATCC 19116**	***S. aureus* 72**	***S. aureus* 132**	***S. aureus* 224**	***S. epidermis***
Mesembryanthemum crystallinum	8	0	0	40	8	10	14	25	0
Blackiella conduplicata	12	0	0	6	0	20	17	6	5
Arthrocnemon glaucum	18	12	0	35	0	10	9	18	0
Atriplex halimus	11	0	0	40	0	10	3	25	0
Thymelaea hirsute	11	20	0	0	0	7	12	32	0
Carduus getulus	18	20	23	0	0	20	12	27	0
Nicotiana glauca	15	20	0	7	0	5	12	27	0
Alhagi maurorum	10	24	0	23	0	6	13	20	0
Atractylis carduus	40	0	0	0	0	5	9	33	0
Echinops spinosissimus	42	0	0	0	0	12	11	35	0
Positive control [1]	10	20	0	30	0	0	0	0	30

[1]Antibacterial drug (Imipenum) served as positive control while DMSO served as negative control. Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria.

Table 6. Antibacterial activity of methanol extracts (23 mg/ml) of medicinal plants tested against Gram negative pathogenic bacteria.

Plant name	Zone of inhibition (mm)				
	E. coli	***Y. enterocolitica* ATCC 23715**	***K. oxytoca***	***K. pneumonia***	***S. enterica* ATCC 25566**
Mesembryanthemum crystallinum	0	15	30	8	22
Blackiella conduplicata	0	0	30	8	32
Arthrocnemon glaucum	0	0	9	5	0
Atriplex halimus	0	0	10	9	0
Thymelaea hirsute	0	0	0	15	0
Carduus getulus	25	35	27	7	21
Nicotiana glauca	0	34	0	4	0
Alhagi maurorum	0	0	18	7	0
Atractylis carduus	0	0	24	0	0
Echinops spinosissimus	13	0	20	15	0
Positive control [1]	3	0	7	0	30

[1]Antibacterial drug (Imipenum) served as positive control while DMSO served as negative control. Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria.

Table 7. Antibacterial activity of hexane extracts (6 mg/ml) of medicinal plants tested against Gram positive pathogenic bacteria.

Plant name	Zone of inhibition (mm)								
	B. cereus	***C. perfringens* ATCC 13124**	***L. innocua* ATCC 33090**	***L. ivanovii* Li4 (pVS2**	***L. monocytogensis* ATCC 19116**	***S. aureus* 72**	***S. aureus* 132**	***S. aureus* 224**	***S. epidermis***
Mesembryanthemum crystallinum	15	0	0	11	20	10	13	12	0
Blackiella conduplicata	0	0	0	0	12	9	0	10	10
Arthrocnemon glaucum	0	0	0	0	0	0	0	0	15
Atriplex halimus	9	0	0	14	0	0	0	0	20
Thymelaea hirsute	5	0	0	0	0	0	0	7	25
Carduus getulus	9	0	0	15	0	7	0	11	0
Nicotiana glauca	12	0	0	17	10	17	17	0	45
Alhagi maurorum	7	0	0	7	10	10	15	0	25
Atractylis carduus	30	0	0	12	0	11	15	16	0
Echinops spinosissimus	0	0	0	0	0	0	0	0	16
Positive control [1]	10	20	0	30	0	0	0	0	30

[1]Antibacterial drug (Imipenum) served as positive control while DMSO serving as negative control. Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria.

Table 8. Antibacterial activity of hexane extracts (6 mg/ml) of medicinal plants tested against Gram negative pathogenic bacteria.

Plant name	Zone of inhibition (mm)				
	E. coli	***Y. enterocolitica* ATCC 23715**	***K. oxytoca***	***K. pneumonia***	***S. enterica* ATCC 25566**
Mesembryanthemum crystallinum	12	20	0	13	0
Blackiella conduplicata	0	17	0	0	0
Arthrocnemon glaucum	0	12	0	6	0
Atriplex halimus	0	0	0	0	0
Thymelaea hirsute	0	9	0	10	0
Carduus getulus	0	10	0	0	0
Nicotiana glauca	12	25	0	19	0
Alhagi maurorum	12	13	11	15	0
Atractylis carduus	7	10	10	22	0
Echinops spinosissimus	0	20	0	0	0
Positive control [1]	3	0	7	0	30

[1]Antibacterial drug (Imipenum) served as positive control while DMSO serving as negative control. Zone of inhibition was 0.00 in negative control in all the concentrations against all the test bacteria.

including, *M. crystallinum, B. conduplicata (F. Muell.) Aellen, N. glauca, A. maurorum* and *A. carduus.* Of the different hexane plant extracts used in the investigation, the least active were *A. glaucum, T. hirsute, C. getulus* and *E. spinosissimus.* On the other hand, the most active hexane extracts were *N. glauca* (73.3%), *M. crystallinum* (60.0%), *A. maurorum* and *A. carduus* (53.3%). The hexane extracts from *N. glauca* and *M. crystallinum* presented the highest activities, since they were able to inhibit 11 and 9 types of microorganisms of interest, respectively. Moreover, *N. glauca* and *M. crystallinum* also had the highest activity rate against antibiotic resistant bacteria. *L. innocua, S. enterica ss. enterica* and *C. perfringens* were the most resistance bacteria to any of the hexane extracts tested. On the contrary, *S. epidermis* and *Yersinia enterocolitca* ss*. enterocolitica* were the most susceptible bacteria to most hexane plant extracts.

The results therefore indicate that the methanolic extract gave a well defined response, while hexane extracts gave to some extent a lesser reaction. Since methanolic extracts of the different tested plants were active and was present in larger amount than other active extracts, it was further subjected to phytochemical and protein content analysis.

Phytochemical screening

The results of the phytochemical analysis of the different methanol plant extract are shown in Table 9a and b. The extracts mostly contained higher quantities of flavonoides, glycosides and alkaloids, followed by steroids and saponins which are active phytochemicals. Tannins and anthroquinone were entirely absent in most of the tested extracts except for *N. glauca* and *A. mauroru,* whereas *A. carduus* and *E. spinosissimus* contained anthroquinone. Although, a high concentration of flavonoides and glycosides were found in all methanol extracts, the results of hexane extracts showed absence, lesser or moderate concentration (Table 9a and b).

Protein content and effect of proteolytic enzymes on antimicrobial activity

Methanolic extracts from *C. getulus, A. halimus, N. glauca,* and *E. spinosissimus* exhibited remarkable protein content of 5.9, 4.3, 4.3 and 4.1 mg/ml, respectively while five extracts displayed moderate crude protein content between 2.3 and 3.2 mg/ml. However, the lowest protein content of 1.1 mg/g was observed in *M. crystallinum* (Table 10).

We experimentally deactivated antimicrobial effects referred to plant proteins by adding three different proteases, and then the effect of both methanol and extracts on the bacterial growth was evaluated. Measurements of antibacterial activity proved the positive correlation between activity and crude protein content in different methanol extracts (Table 10).

DISCUSSION

Traditional medicinal plants are important sources of natural products in treating common infectious bacteria. The emergence of multidrug-resistant infectious bacteria, high cost of synthetic compounds, as well as undesirable side effects of certain drugs, insist on pharmaceutical companies to look for new therapeutic agents from alternative sources including medicinal plants. The antibacterial activities significantly differed depending on taxonomic characteristics of the plant species as well as biological characteristics of the tested bacteria which may explain the variations in the antibacterial activity of plant extracts tested. In classifying the antibacterial activity as Gram-positive or Gram-negative, it would be generally expected that a much greater number would be active against Gram-positive than Gram-negative bacteria (Cutcheon et al., 1992). In this findings, 10 samples (100%) extracts showed activity against Gram-positive bacteria (mainly *S. aureus)*, supporting the aforementioned view. However, the growth of Gram-negative bacteria namely *E. coli* was controlled by the extract of *C. getulus* and *E. spinosissimus*, thus, indicating that they could inhibit the activity of these bacteria that causes diarrhea and dysentery. Similar results were obtained with extracts of *Paris polyphylla* and *Zanthoxylum armatum* (Panthi and Chaudhary, 2006). Although, Samy (2005) showed methanolic extracts of *Zingiber officinale* did not present antimicrobial effect against *S. aureus* and *E. coli*, Indu et al. (2006) using a different method of *Zingiber officinale* extract preparation, however, verified an inhibitory action against *E. coli* as well as high antimicrobial activity of *Allium sativum* extracts against *E. coli* and *Salmonella*. Such behavior of the antibacterial action was also verified by Adonizio et al. (2006), who used *Cymbopogon citratus* extracts and did not observe antibacterial effects. Success in isolating compounds from plant material is highly dependent on the type of the solvent used in the extraction procedure (Masoko et al., 2007). In this study, all extracts showed varying degrees of antimicrobial activity on the microorganisms tested. Some of these plants were more effective than traditional antibiotics to combat the pathogenic microorganisms studied.

The plant materials were extracted using two different solvents (methanol and hexane). Among all solvents, methanol was quantitatively the best extractant, extracting a greater quantity of plant material than hexane (data not shown). Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol

Table 9a. Phytochemical screening of methanolic extract of medicinal plants tested.

Plant extract	Phytochemical constituent*						
	Alkaloid	Tannin	Steriod	Saponin	Anthraquinone	Flavonoide	Glycoside
Mesembryanthemum crystallinum	+	-	+	+	-	++++	++++
Blackiella conduplicata	++	-	+	-	-	++++	++
Arthrocnemon glaucum	++	-	++	+	-	++++	++++
Atriplex halimus	+	-	++	+	-	++++	++++
Thymelaea hirsute	+++	-	+	+	-	++	ND
Carduus getulus	+++	-	+	+	-	++++	++++
Nicotiana glauca	+++	+	++	+	+	++++	++++
Alhagi maurorum	+++	+	+	++	+	++++	++++
Atractylis carduus	+++	-	++	+	+	++++	++++
Echinops spinosissimus	+++	-	+	+	+	++++	++++

*+ = Positive; ++ = strongly positive; ++++= extremely positive; - = Not detected; ND = not determined.

Table 9b. Flavonoids and glycosides content of hexane extract of medicinal plants tested.

Flavonoid and glycoside content	Plant Extract*									
	Mesembryanthemum crystallinum	***Blackiella conduplicata***	***Arthrocnemon glaucum***	***Atriplex halimus***	***Thymelaea hirsute***	***Carduus getulus***	***Nicotiana glauca***	***Alhagi maurorum***	***Atractylis carduus***	***Echinops spinosissimus***
Falvonoides	+++	-	++	-	++	-	++++	++	++++	+++
Glycosides	++	-	-	-	-	-	++	+++	++	-

*+ = Positive; ++ = strongly positive; +++= extremely positive; – = not detected.

extraction (Cowan, 1999). The obtained results indicated that most of the tested plants contained a large proportion of methanol soluble polar compounds than hexane. It has been documented that different solvents have diverse solubility capacities for different phytochemical constituents (Marjorie, 1999).

Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. These literatures indicated that the biological activity is due to different chemical agents in the extract, including essential oils, steroids, alkaloids, flavonoids, triterpenoids and phenolic compounds or free hydroxyl groups. Antibacterial role of seed protein (Dahot, 1998) and antibacterial and antifungal roles of leaf protein (Terras et al., 1995) has been previously documented.

Phytochemical constituents such as tannins, flavonoids, alkaloids, glycosides, cyanogenetic glycosides, reducing sugar and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Lutterodt et al., 1999; Marjorie, 1999). Secondary metabolites and all other active principles of plants have been shown to be responsible for the antimicrobial activities shown by these extracts (Cowan, 1999; Nweze et al., 2004). From the phytochemical analysis of plant extracts tested, some of these secondary metabolites were absent example, tannins, anthroquinone, while other metabolites were found

Table 10. Antibacterial activity of enzymatic deactivated plant extracts against *S. aureus* 224.

Plant extract[1]	Untreated plant extract (inhibition zone, mm)	Deactivated plant extract by protease[2] (inhibition zone, mm)			Protein content (mg/ml)
		Trypsin	Protinase k	pepsin	
Mesembryanthemum crystallinum	21	8.0	-ve	-ve	1.1
Blackiella conduplicata	0	2.0	-ve	-ve	2.3
Arthrocnemon glaucum	18	-ve	-ve	-ve	3.2
Atriplex halimus	20	-ve	-ve	-ve	4.3
Thymelaea hirsute	30	-ve	5.0	-ve	2.7
Carduus getulus	17	-ve	25	-ve	5.9
Nicotiana glauca	22	15	1.0	2.0	4.2
Alhagi maurorum	20	8.0	-ve	-ve	3.0
Atractylis carduus	30	5.0	5.0	7.0	2.2
Echinops spinosissimus	32	18	1.0	1.0	4.1

[1]Methanol extract of medicinal plants (6 mg/ml);
[2]Treated methanolic plant extract by proteases (1 mg/ml) for 2 h at 37°C.

in low and moderate concentrations.

On the other hand, the antimicrobial activities shown by the extracts would likely be due to one or more of phytochemical constituents shown to be present in the plant extracts. Flavonoides and glycosides are a special class of phytochemical which have antimicrobial characteristics. The antibacterial activity of flavonoids against both Gram-positive and Gram-negative bacteria including antibiotic resistance bacteria have been reported (Bylka et al., 2004). The antimicrobial activity of flavonoids and polyophenolic compounds might be due to their ability to form complex with bacterial cell wall and therefore inhibiting the microbial growth (Sivapriya et al., 2011).

The demonstration of antibacterial activity against both Gram positive and Gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds (Srinivasan et al., 2001). Plant based antimicrobials represent a vast untapped source for medicines. The use of plants to heal diseases, including infectious one, has been extensively applied by people. Data from these literatures as well as our results revealed the great potential of plants for therapeutic treatment in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds. Once extracted, and before being used in new therapeutic treatments, they should have their toxicity tested *in vivo*. Bioassays (Carvalho et al., 1988; Nascimento et al., 1990) have demonstrated the toxicity of extracts from different plants. Therefore, our results reveal the importance of plant extracts to control resistant bacteria, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

Conclusion

The results of this study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation.

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