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# Antibacterial activity of selected Egyptian ethnomedicinal plants

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## ABSTRACT

**Aims:** Medicinal plants have recently received the attention of the antimicrobial activity of plants and their metabolites due to the challenge of growing incidences of drug-resistant pathogens. The aims of this study were to determine the antibacterial activities of plant extracts used as ethnomedicinal in Egypt.
**Methodology and Results**: Investigations were carried out to assess the antibacterial efficiency of 11 plant extracts used as ethnopharmacological among Egyptian native people against infectious diseases. Crude methanol, ethanol, chloroform, hexane, acetone and aqueous extract of plants were tested for antibacterial activity in vitro against ten bacterial isolates using the disc diffusion method test. Discs were impregnated with 2 mg/mL of different solvent extracts. Among all the crude extracts, the methanol extract showed the highest activity than other extracts. *P. harmala* and *S. officinalis* exhibited highest antibacterial activity against gram positive and negative bacteria while the remaining plants extracts showed less activity. All the plant extracts showed no significant effect against the *Bordetella bronchisepta* ATCC 4617 except the extracts of *M. fragrans* and *L. sativum*. *E. coli* is the most sensitive microorganism tested, with the lowest MIC value (0.5 mg/mL) in the presence of the plant extract of *P. harmala* and *S. officinalis*.
**Conclusion, significance and impact of study**: Results obtained herein, may suggest that the ethnomedicinal Egyptian plants possess antimicrobial activity and therefore, they can be used in biotechnological fields as natural preservative ingredients in food and/or pharmaceutical industry.

*Keywords:* Antibacterial, ethnomedicinal plants, Egypt

## INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). It has been estimated that 14–28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use of the plants (Baris *et al.*, 2006; Ncube *et al.*, 2008). Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses (WHO, 2005; Zhang, 2004; Ramzi *et al.*, 2008). Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases (Tanaka *et al.*, 2006) especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective ntimicrobial agents. Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases.

Traditional remedies have a long-standing history in many locations in Egypt and continue to provide useful and applicable tools for treating ailments. They use plants in treating diseases, such as hepatitis, skin infections, and rheumatic diseases. The curative plants vary between Egyptian native people in different localities. Despite the medicinal potential of plants in Egypt being considerable, knowledge of this area and studies on the biological activities of these plants remained scarce (Boulos and El-Hadidi, 1989; Batanouny, 1999; Khafagi and Dewedar, 2000). The aims of this study were to determine the antibacterial activities of plant extracts used as ethnomedicinal in Egypt.

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## MATERIALS AND METHODS

### Plant samples

Plant species are summarized in Table 1. The plants were selected on the basis of their ethnomedicinal use against infectious diseases (intestinal antiseptic, antidiarrhoeal, sore throat, cold and caught) in Egypt. For this study fresh plant material was used. The plants were collected during the vegetation period, botanically identified and immediately processed. Besides well-known species with documented antibacterial activity in direct contact assays, several species with unknown antibacterial effect were selected.

**Table 1:** List of plant species tested.

| Plant species | Family | Common (Nation) name | Plant part | % Yield[a] |
|---|---|---|---|---|
| *Ambrosia maritime* | Asteraceae | Ragweeds (Dimsesa) | Arial part | 8.9 |
| *Artimisia cina* | Asteraceae | Wormwood (Alsheeh) | Leaves | 6.4 |
| *Curcuma longa* | Zingiberaceae | Turmeric (Korkom) | Roots | 9 |
| *Cymbopogon proximus* | Poaceae | Lemon grass (Half Bar) | Arial part | 13.5 |
| *Cyperus rotundus* | Cyperaceae | Sedge (Alsaad) | Tubers | 6.9 |
| *Lepidium sativum* | Brassicaceae | Garden cress (Hab Rashad) | Leaves | 17.8 |
| *Myristica fragrans* | Myristicaceae | Nutmeg (Gozet Elteeb) | Seeds | 5.8 |
| *Origanum majorana* | Lamiaceae | Marjoram (Bardakoosh) | Leaves | 11.7 |
| *Peganum harmala* | Nitrariaceae | Harmel (Harmel) | Seeds | 7.7 |
| *Salvia officinalis* | Lamiaceae | Sage (Marmaria) | Leaves | 11.9 |
| *Senna alexandrina* | Fabaceae | Egyptian Senna (Sennameky) | Leaves | 17 |

[a]Percentage of methanolic extract yield (w/w) was estimated as dry extract weight/dry starting material weight x 100

Specimens were identified at Botany Department, Faculty of Science, Suez Canal University (Ismailia, Egypt) and voucher specimens were deposited at the Herbarium of the Department of Botany in the cited university.

### Chemical extraction of plants

The extract was obtained by macerating 30 g of the different dried plants separately in cold methanol (300 mL/L) for 48 h. The resultant extract was filtered, concentrated to dryness in a rotary evaporator under reduced pressure at 40 °C, and then stored at -20 °C until use. Ethanol, chloroform, hexane, acetone or aqueous extracts were obtained by similar method as methanol extract. The resulting fractions were lyophilized. The amount of lyophilisate corresponding to 3 g extract was dissolved in 2 mL dimethyl sulfoxide (DMSO) and used as stock solution. The extracts were diluted in DMSO for approximately 2 mg/mL, and then sterilized by passage through 0.45 µm filter.

### Antimicrobial tests

The agar diffusion assay was performed according to modified Kirby-Bauer disc diffusion method (Collins *et al.*, 1998). One loopful of each test organism (*Bacillus cereus*; *Bacillus megaterium*; *Bacillus subtilis*; *Bordetella bronchisepta* ATCC 4617; vancomycin resistance *Enterococcus feacalis* (VRE); *Escherichia coli*; *Pseudomonas aeruginosa*; *Salmonella enteritidis*; *Serratia marcescens* and vancomycin resistance *Staphylococcus aureus* (VRSA) was suspended in 3 mL 0.9% NaCl solution separately. The bacterial strains used in this work (others than ATCC strains) were isolated from human beings and belong to the microbiological laboratory collection of the Department of microbiology from Szeged University, Hungary. Müller-Hinton agar (Oxoid) were inoculated with this suspension of the respective organism and poured into a sterile petri dish. Paper discs (Whatman, 6 mm) impregnated with 2 mg/disc of the extract dissolved in DMSO then placed on the seeded agar plates. The DMSO solvent was used as a negative control. A pre-diffusion for 3 h was guaranteed. Inhibition zones were measured after 18 h incubation at 37 °C. The inhibition zones were measured excepting the 6 mm paper disc. Every experiment was carried out 3 replicates. Similar antibacterial bioassays were also conducted for comparative analysis using plants extracts and 2 types of Oxoid antibacterial susceptibility discs (Table 2). Concentration used were according to NCCL levels; 30 µg per disc.

Minimal Inhibitory Concentrations (MICs) of the plants extract against the tested microorganisms were determined by the broth micro dilution method (NCCLS, 2006). Serial dilutions from 0.5 to 10 mg/ml for potent methanolic plant extracts were added to Müller-Hinton broth. Bacterial suspension of 1 mL containing approximately 1: $5\times10^5$ cells were added to each dilution of extract. Growth of bacteria was checked after overnight incubation at 37 °C. Minimum bactericidal concentration (MBC) is usually an extension from the MIC, where the organisms are quantitatively subcultured from MIC tubes on antibiotic free agar medium to indicate the minimum concentration was no viable organism appears in the culture.

### Statistical analysis

The variations between experiments were estimated by standard deviations and statistical significance of changes was estimated by student's t-test. Only the probability $P \leq 5\%$ was regarded as indicative of statistical significance.

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## RESULTS AND DISCUSSION

Antimicrobial activity of 11 different plants has been tested on different solvents based on their use in ethnomedicinal in Egypt. Out of the crude extracts, the methanolic extract showed the highest activity than other extracts (Data of other solvents not published). The crude yields of methanol were ranged from 5.8 to 17.8% in case of *M. fragrans* and *L. sativum* extracts respectively (Table 1). The results of the investigated plants showed variable degrees of antibacterial activity against one or more of the tested organisms (Table 2). *P. harmala* and *S. officinalis* exhibited highest antibacterial activity against *B. cereus*, *E. coli* and *S. enteritidis* while the remaining medicinal plants extracts showed less activity. Irrespective of the solvent systems, the selected plant extract showed an inhibition zone ranging from 2 to 16 mm on the tested bacterial strains.

**Table 2:** Antimicrobial susceptibility of tested methanolic plant extracts (2 mg) against studied bacteria

| Plant species extract | Microbial species / Inhibition zone (mm)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | B. c | B. m | B. s | B. b | E. f | E. c | P. a | S. e | S. m | S. a |
| *Ambrosia maritime* | 5 | 5 | 6 | - | 5 | 3 | - | - | 7 | - |
| *Artimisia cina* | 2 | 2 | - | - | 6 | - | - | 2 | - | - |
| *Curcuma longa* | 3 | 2 | 3 | - | 3 | 3 | 2 | 3 | 2 | - |
| *Cymbopogon proximus* | 5 | 6 | 9 | - | 4 | 3 | 2 | 4 | 4 | 3 |
| *Cyperus rotundus* | 3 | 4 | 3 | - | - | - | 2 | - | 6 | 2 |
| *Lepidium sativum* | 5 | 3 | 6 | 10 | - | 3 | - | - | 3 | - |
| *Myristica fragrans* | 2 | 2 | 3 | 2 | 3 | 6 | 2 | - | 2 | 2 |
| *Origanum majorana* | 4 | 3 | 6 | - | 7 | - | 2 | 4 | 4 | 6 |
| *Peganum harmala* | 14 | 13 | 16 | - | 3 | 7 | - | 5 | 5 | 3 |
| *Salvia officinalis* | 6 | 9 | 9 | - | - | 9 | 4 | 8 | 6 | 5 |
| *Senna alexandrina* | 4 | 4 | 6 | - | - | 6 | 3 | 2 | 2 | - |
| Ceftazidime 30µ/disc | NT | NT | NT | NT | NT | 8 | - | - | - | NT |
| Vancomycin 30µ/disc | NT | NT | NT | NT | - | NT | NT | NT | NT | - |

All tests were performed in induplicate and repeated 3 replicates.
[a]The diameter (6mm) of the disc is not included.
**Key:** B. c: *Bacillus cereus*; B. m: *Bacillus megaterium*; B. s: *Bacillus subtilis*; B. b: *Bordetella bronchisepta* ATCC 4617; E. f: *Enterococcus feacalis*; E. c: *Escherichia coli*; P. a: *Pseudomonas aeruginosa*; S. e: *Salmonella enteritidis*; S. m: *Serratia marcescens*; S. a: *Staphylococcus aureus*; - : no activity and $_{NT}$: Not Tested**.**

*P. harmala* showed a maximum inhibition effect on *B. subtilis* in methanol extract with an inhibition zone of 16 mm. *A. maritime* and *L. sativum* showed an inhibitory effect on six bacterial strains with the inhibition zone ranging from 3 to 7 mm. From this, it may be inferred that these plant extracts have a potent antimicrobial activity to control bacterial pathogens. A similar result was also reported by Okeke *et al.*, (2001), when evaluating the extracts of the root of *Landolphia owerrience* reported that, the antibacterial activity of root extracts varied with the kind of solvents used for the extraction. Crude preparations of whole plant parts containing both the active and non-active components have been suggested to have a higher efficiency than semi-crude or pure plant substances (Shahidi Bonjar, 2004). In a study on the antibacterial activity of extracts from some edible plants commonly consumed in Asia, Alzoreky and Nakahara (2003) reported that the buffered methanol and acetone were proved to be good solvents in extracting inhibitory substances from the plant materials. In the present study, the antibiotic assay was also performed on the studied strains and the results are provided in Table 2. The results on sensitivity test indicated that, all gram negative bacteria were resistant to ceftazidime antibiotic. The pathogen *S. aureus* and *E. feacalis* were resistant to vancomycin.

This result is consistent with the present finding that *P. harmala* and *S. officinalis* extracted with methanol showed a better inhibitory effect on eight out of ten bacterial strains tested. Eloff (1998) and Cowan (1999) found that methanol was more efficient than acetone in extracting phytochemicals from plant materials and these results agreed with our data. Also, Otshudi *et al.*, (1999) considered that diethyl ether extracts of plants were inactive against bacteria compared with aqueous methanol extracts. Chakraborty *et al.*, (1999) have also reported a similar view when studying the antibacterial steroid alkaloids of the stem bark of *Holarrhena pubescens*. In their observations, the crude methanolic extracts exhibited the antibacterial activity to different degrees (10 to 16 mm). However, the plant extracts of *P. harmala* and *S. officinalis*, failed to show antibacterial activity toward *Bordetella bronchisepta* ATCC 4617. Also *S. officinalis* showed antimicrobial activity against *Pseudomonas* and *Staphylococcus,* these results in contrast with results were obtained by Delamare Longaray *et al.*, (2007), when they studied *S. officinalis*, cultivated in south Brazil, showed no activity against several *Pseudomonas* and *Staphylococcus* strains. All the plant extracts showed no significant effect against the *Bordetella bronchisepta* ATCC 4617 except the extracts of *M. fragrans* and *L. sativum*. This antimicrobial spectrum obtained with the *S. officinalis*, is comparable in most cases, to that reported by Marino *et al.*, (2001). This discrepancy, between used plant extracts, in inhibiting the tested strains can be explained by the fact that the activity depends on the type, composition and concentration of the plant extract, and the type of target microorganism (Marino *et al.*, 2001). Many other factors could also be involved such as insolubility in aqueous media, the effects of solvents and the extraction method on the phenolic contents and/or seasonal and intraspecific variation of plant extract composition (Hayouni *et al.*, 2007; 2008).

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Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate. The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. Polyphenolic compounds such as flavones and most other reported bioactive compounds are generally soluble in polar solvents such as methanol. Most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent (Parekh *et al.*, 2006). In a study by Masoko and Eloff (2006) where they investigated the antifungal activity of *Combretum* species, from the extracts used, which included hexane, dichloromethane, acetone and methanol, they discovered that acetone and methanol extracted more chemical compounds from the leaves than the other solvents.

MIC was assessed for potent selected plant extracts (*P. harmala* and *S. officinalis*) on the isolated bacterial strains by giving due consideration to bacterial growth inhibition by the respective plant products (Table 3). Accordingly, the MIC of *P. harmala* was 4 mg $mL^{-1}$ on *S. aureus* and 0.5 mg/ml on all *Bacillus* species. Likewise, the MIC *S. officinalis* on *E. coli* was 0.5 mg $mL^{-1}$. The MIC of *P. harmala* on *S. aureus* was at the maximum concentration of 4 mg/ml. The MBCs of the extracts were in general significantly higher than the corresponding MIC values (Table 3). MBC of 3 mg $mL^{-1}$ was reached by the extracts of *P. harmala* and *S. officinalis* against *P. aeruginosa*. Results obtained from disc diffusion method, followed by measurements of MIC values, indicated that *E. coli* is the most sensitive microorganism tested, with the lowest MIC value (0.5 mg $mL^{-1}$) in the presence of the plant extract of *S. officinalis*.

**Table 3:** MIC and MBC (mg/ml) of selected potent methanolic extracts of *P. harmala* and *S. officinalis* plants on bacterial strains.

| | MIC(mg/ml) | | MBC(mg/ml) | |
|---|---|---|---|---|
| | *P. harmala* | *S. officinalis* | *P. harmala* | *S. officinalis* |
| *B. cereus* | 0.5 | 2 | 0.5 | 4 |
| *B. megaterium* | 0.5 | 2 | 0.5 | 4 |
| *B. subtilis* | 0.5 | 2 | 4 | 4 |
| *E. feacalis* | 2 | - | 2 | - |
| *E. coli* | 0.5 | 1 | 0.5 | 0.5 |
| *P. aeruginosa* | - | 3 | - | 3 |
| *S. enteritidis* | 1 | 2.5 | 2 | 3 |
| *S. marcescens* | 1 | 1 | 1 | 2 |
| *S. aureus* | 4 | 3 | 7 | 3 |

## CONCLUSION

In conclusion, the results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants estimated. The obtained results could form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. *P. harmala* and *S. officinalis* could be a source for antibacterial drugs against Gram-positive bacteria, especially against multi-resistant microorganisms.

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