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Antibacterial Properties of Selected Medicinal Plants from Saudi Arabia

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The present study sought to investigate the antibacterial activity of the methanol and acetone leaf extracts of three different medicinal plants selected from Saudi Arabia. The three medicinal plants are *Aerva javanica, Ocimum basilicum* and *Artemisia absinthium.* The microorganisms assayed for antibacterial activity were: gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, and the gram-negative *Escherichia coli* and *Pseudomonas aeruginosa.* Antibacterial activity was evaluated through measuring the zones of inhibition. All leaf extracts showed significant broad-spectrum antibacterial activity. The acetone extracts have in general produced larger bacterial inhibition zones than the methanol extracts. The study concluded that all three medicinal plants exhibited antibacterial properties with *Artemisia absinthium* showing the most potent effect, followed by *Aerva javanica* and finally *Ocimum basilicum.*

Key words: Medicinal Plants, Antibacterial Properties, Folk medicine, Antibacterial Assay.

According to the WHO, over 80% of the world's population rely on plant-derived medicines especially those located in developing countries[1-2]. Developing countries in particular mainly rely on the use of herbal medicines or native plants in the treatment of the majority of illnesses, as most communities lack an adequate access to modern medicine. There are many advantages attributed to the use of herbal medicines over synthetic medications including safety and fewer side effects. Culture also plays an important role in the acceptability of herbal medicines and their use. It has been observed that in developed countries, nearly a quarter of all prescribed pharmaceuticals include compounds that are derived from plants, whether directly or indirectly[3].

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Medicinal plants are said to represent a vital health and economic component of biodiversity. The Arabian Peninsula is the birth place of herbal drugs according to Al-Yahya[4] and the use of folk medicine has existed in the region since time immemorial. The Kingdom of Saudi Arabia has a wide range of flora that comprises a large number of medicinal herbs, shrubs and trees[5]. Saudi Arabia is estimated to have a great medicinal species diversity that is expected to be over 1200 (above 50%) out of its 2250 species. Today, there is a wide interest in the production of plant-derived drugs considering the rise in multi-drug resistant pathogen strains. Plants are naturally subjected to a plethora of inimical microorganisms and so have developed a range of defences to microbial attacks[6]. In addition to the existence of general defence elements, there are also large numbers of new compounds that are synthesized at the post-infection stage containing a range of antimicrobial composites[7]. Plant extracts and products have been observed to be effective against a large

number of pathogens[8]. Similarly, viral, fungal and bacterial pathogens have been mainly controlled through the use of plant seed oils[9-12].

The present study aimed to highlight the potential use of plant derived products as antibacterial agents. *Aerva javanica* is an erect perennial herb that belongs to the *Amaranthaceae* family and is widely distributed worldwide. The herb is used in the treatment of diarrhoea and kidney stones and is also used as a diabetic demulcent and an astringent[13]. In concentrated amounts, the plant is used in the treatment of swellings as well as urinary disorders[13]. In powder form, the plant is applied externally to treat ulcers in domestic animals.

The *Lamiaceae* plant family (mint family) represents a large and valuable pool of plant species that contain biologically active molecules[14]. *Ocimum basilicum* belongs to the *Lamiaceae* plant family and is commonly known as Basil. *Ocimum basilicum* is a widespread plant that is cultivated in numerous regions globally. The plant grows to a height of nearly 50-80cm and has leaves that are oval and slightly toothed. The plants' flowers are either white or purple. *Ocimum basilicum* has been used in cosmetics, liqueurs, medicines and perfumes[15]. The *Ocimum basilicum* plant is used as aromatic, antimicrobial, astringent in dysentery, whilst the leaves are antipyretic. The seeds of the plant are laxative and are particularly used in habitual constipation. For the treatment of cough, the juice of the leaves and the flowers may be used. There are many other medicinal uses for basil[15].

On a similar note, *Artemisia* is a large and diverse genus of plants consisting of nearly 400 species that belong to the *Asteraceae* daisy family. The genus is well known for powerful chemical constituents in their essential oils. The *Artemisia* species grow in the temperate climates of both hemispheres, commonly in dry or semiarid habitats. White hairs cover the leaves of many species. Most *Artemisia* species possess strong aromas and bitter tastes as a consequence of the terpenoids and sesquiterpene lactones present in the plant. Plants in the *Artemisia* genus grow best in free-draining sandy soil that is unfertilised and under full sun[16]. The use of compounds for pharmaceutical purposes derived from *Artemisia* has been generally on the rise[17-22].

MATERIALS AND METHODS

Plant materials

Fresh leaves from the respective plants were rinsed using distilled water in order to remove impurities and insects, separately. Following this, the washed leaves were allowed to dry at room temperature. The leaves were then weighed separately and dried overnight in an oven at 40°C. The dried leaves were separately weighed again to determine the percentage yield of dry plant material. The dried leaves of the respective plants were then divided into two equal amounts from which the methanol and acetone extracts were prepared.

Extraction Method

The plant powders for each of *Aerva javanica, Ocimum basilicum* and *Artemisia absinthium* were extracted using the solvents methanol and acetone via the sequential extraction method[23]. 100g of dried plant powder material (leaf) was soaked in 250ml of the respective solvent in an air tight bottle separately and kept in an electric shaker for 72 hours at room temperature. The suspensions were then filtered using a double layered muslin cloth followed by a Whatman No.1 filter paper. Separately, each filtrate was collected into a conical flask. The extraction procedure was repeated three times and the extracts were concentrated using a rotary evaporator under reduced pressure and low temperature. The dried extracts were kept in the refrigerator until used for the assay.

Antibacterial assay

The bacterial strains used in the present study were obtained from Ain–Shams University, Faculty of Agriculture, Cairo, Egypt. The strains comprised gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, and the gram-negative *Escherichia coli* and *pseudomonas aeruginosa*. The bacteria were maintained in Mueller-Hinton Agar. Inocula were prepared by adding to the nutrient broth an overnight culture of the organisms to achieve an OB600 of 0.1. The cultures were allowed to develop until a McFarland standard of 0.5 was achieved (approximately 108 CFU/ml). To obtain 106 CFU/ml, the suspensions were diluted in nutrient broth to 1:100. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to McFarland's standard), at a temperature of 40°C, and poured aseptically in

sterile Petri dish. This was allowed to solidify at room temperature. 0.2 ml of the extracts at 100mg/ml concentration in DMSO was added to bores made on the agar medium using a sterile borer. For the control, 0.2 ml of standard streptomycin at a concentration of 100 µg/ml. To facilitate the diffusion of the extracts and the control, the Petri dishes inoculated with the bacteria and containing the extracts and control were refrigerated for 2 hours at 4°C. Following diffusion, the Petri dishes were incubated at 37°C for 24 hours in an incubator. The zones of inhibition were measured using a scale[24]. The diameter of the resulting zones of inhibition in the replicates were expressed as mean ± standard deviation (SD).

RESULTS

The results obtained from the present study demonstrate that the three tested plant extracts possess promising antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The acetone leaf extract of *Artemisia absinthium* showed the highest antibacterial activity, followed by the acetone leaf extract of *Aerva javanica* and finally *Ocimum basilicum*. *Escherichia coli* and *Bacillus subtilis* appeared to be the most susceptible bacteria as they exhibited the largest inhibition zones compared to the remaining two bacteria tested. The highest antibacterial activity of (21mm) inhibition zone was measured for *Escherichia coli* in the presence of Artemisia absinthium and the lowest antibacterial activity was for *Pseudomonas aeruginosa* (8mm) in the presence of *Ocimum basilicum*. Treatment with the acetone extract of *Artemisia absinthium* produced better results than treatment with the streptomycin control. Likewise, treatment with *Aerva javanica* produced comparable results to the treatment with the streptomycin control.

The results also demonstrate that the acetone plant leaf extracts all had a higher antibacterial activity than the methanol plant leaf extracts of the respective plants. Again, *Artemisia absinthium* appeared to have the highest

Table 1. Antibacterial activity of the acetone extracts of *Aerva javanica, Ocimum basilicum* and *Artemisia absinthium* (100µg/ml) and antibiotic (100µg/ml) against bacterial species tested by disc diffusion assay

Bacterial	Zone of inhibition (mm)			
	Plant Extracts			Streptomycin
Species	*Aerva javanica*	*Ocimum basilicum*	*Artemisia absinthium*	Sulphate
Staphylococcus aureus	15±0.26	9±0.58	17±0.71	16±0.52
Bacillus subtilis	17±0.34	10±0.31	18±0.80	18±0.43
Escherichia coli	19±0.83	13±0.70	21±0.87	19±0.49
Pseudomonas aeruginosa	13±0.40	8±0.76	15±0.49	16±0.48

Values are mean inhibition zone (mm) ± S.D of three replicates

Table 2. Antibacterial activity of the methanol extracts of *Aerva javanica, Ocimum basilicum* and *Artemisia absinthium* (100µg/ml) and antibiotic (100µg/ml) against bacterial species tested by disc diffusion assay

Bacterial	Zone of inhibition (mm)			
	Plant Extracts			Streptomycin
Species	*Aerva javanica*	*Ocimum basilicum*	*Artemisia absinthium*	Sulphate
Staphylococcus aureus	11±0.68	5±0.47	13±0.81	16±0.52
Bacillus subtilis	13±0.74	5±0.34	16±0.68	18±0.43
Escherichia coli	14±0.67	11±0.34	17±0.54	19±0.49
Pseudomonas aeruginosa	10±0.67	4±0.42	11±0.49	16±0.48

Values are mean inhibition zone (mm) ± S.D of three replicates

antibacterial activity, followed by *Aerva javanica* and finally *Ocimum basilicum*. Despite the low antibacterial activity of Ocimum basilicum against the tested bacterial species, it displayed greater antibacterial activity against *Escherichia coli* (11mm) compared to (5mm) for both *Staphylococcus aureus* and *Bacillus subtilis* and (4mm) for *Pseudomonas aeruginosa*. As with the acetone experiment, *Escherichia coli* and *Bacillus subtilis* again appeared to be the most susceptible bacteria as they exhibited the largest inhibition zones compared to the remaining two bacteria tested.

DISCUSSION

Plants are an important source of potentially useful agents that could be effectively utilised in the search and development of new antibacterial agents. Both the acetone and methanolic extracts of *Aerva javanica*, *Ocimum basilicum* and *Artemisia absinthium* proved to possess good antibacterial properties against the gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, and the gram-negative *Escherichia coli* and *Pseudomonas aeruginosa.* The results of this study demonstrated that although all the extracts for the plants tested exhibited antibacterial activity, *Artemisia Absinthium* had the strongest activity. The study also demonstrated that the acetone plant extracts were more effective than the methanolic ones. In both the acetone and methanol experiments, *Escherichia coli* and *Bacillus subtilis* were found to be more susceptible to the extracts than the other tested bacteria. *Pseudomonas aeruginosa* displayed the smallest inhibition zones when tested with the acetone and methanol extracts in comparison to the other bacteria. For future research, in order to better understand how the antibacterial properties of the three plants operate, there would be a need to examine the phytochemical composition of each plant separately, and then examine the effect of each constituent on the selected bacteria. A comparison of the phytochemical composition of the three plants would also prove useful in determining the plant with the highest antibacterial activity. It remains difficult at this stage to predict which chemical constituent shows the best antibacterial activity and against which organism.

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