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Antimicrobial activities of the ethanol extracts of *Mirabilis jalapa* L. and *Euphorbia dendroide.*

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Abstract

Antimicrobial activity was examined from two selected plants: *Mirabilis jalap L.*(stem, leaves and seeds) and *Euphorbia dendroide* (stem, leaves and root) different parts were screened against seven bacterial strains; three Gram positive (*Staphlyococusaureus, Bacillus subtilis* and *Bacillus atropoeus and),* four Gram negative(*Escherichia coli, Pseudomonas aeruginosa, Salmonilatyphi* and *Kleibsiella pneumonia)*and one fungal strain *Candida albican*. Antibacterial activity was determined by the disc diffusion method; crude extracts were obtained by using ethanol as the extraction solvent. Two concentrations (6 mg/ml and 12 mg/ml) were used to check the antimicrobial activity of plant extracts. The result showed that *Euphorbia dendroide* different parts extracts were more active against gram negative bacteria and fungal strain then *Mirabilis jalap L.* while both plants showed good activity against gram positive bacteria. Azithromycin, Ciprofloxacin and Clotrimezole (50 µg/ml) were used as a standard drugs.

Keywords: Antimicrobial, Antibacterial, Disc diffusion method.

Introduction

In recent age the development of resistant bacterial drugs against important pathogens increasing the interest of researcher to develop more and more antimicrobial agents[1].Many scientist works on antimicrobial and other constituents of different parts of medicinal plants for the treatment of microbial infection[2]. According to World Health Organization (WHO),Medicinal plants have rich source of different types of drugs[3]. Antimicrobial drugs of medicinal plants are used for the treatment of various types of food borne disease[4]. Plant derived drugs remains important source, particularly in developing countries, to combat serious disease [5].

Mirabilis jalapa L. also known as four o'clock, one of the most medicinally used plant, belong to family *Nyctaginaceae*, used for the treatments various disease dysentery and as a laxative (purgative) by Mexican people [6; 7] also used for the treatment of diarrhea, muscular pain and abdominal colic [8-10].*Mirabilis jalapa* L. has great potential of biological activities like antibacterial, antiviral, antifungal and protein synthesis inhibition [11-13]. The leaves of *M. jalapa* L. traditionally used for inflammatory and painful diseases in barazil [14,15].

Euphorbia dendroide is traditionally used medicinal plant, belong to family Euphorbiaceae. The family has popular traditional medicinal herbs. In ancient Chinese medicine, 33species belonging to 17 genera of Euphorbiaceae used in herbal medicine [16].

Materials and Methods

Plant material

Disease free fresh leaves, stems, roots and seeds of*Mirabilis jalap* L. and *Euphorbia dendroide* were collected from the different parts of District Malakand, Khyber Pukhtunkhawa, Pakistan. The plants specimens were identified with the help of taxonomists, previous available literature [17 and 18] and flora of Pakistan [19; 20].

Preparation of extracts

Two hundred grams of each powdered air-dried plant material was soaked in ethanol. They were regularly shaken for maximum extraction at 80rpm for 7 days. After 7 days, the extract was filtered using Whattman filter paper (No. 1). The extracts solutions were evaporated to dryness under reduced pressure at temperature of 50 C using vacuum pump with the rotary evaporator. The paste obtained after rotary evaporation contained some water contents which was further dried in water bath at 60°C for one hour. The thick pastes obtained are known as the crude extract. The extracts were kept in sterile bottles at 4 C until use.

Test microorganisms

Seven bacterial strains and one fungal strain were used in this study in which three were Gram positive (*Staphlyococus aureus, Bacillus subtilis* and *Bacillus atropoeus*)and four Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonilatyphi* and *Kleibsiella pneumonia*)and one fungal strain *Candida albicans.* All the bacterial strains were clinical isolates obtained from the

Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawer, Pakistan. All the bacterial strains were cultivated in nutrient agar medium and incubated at 37 C for 24hrs.

Standard drugs

Three antibiotics were used in the experiment, Azithromycin (50 µg/ml) for Gram positive bacteria, Ciprofloxacin (50 µg/ml) for Gram negative and Clotrimezole (50 µg/ml) for fungal strain.

Stock solution

10 mg of extract was taken and dissolved in 1 ml of Dimethylsulphoxide (DMSO), which was used as stock solution with the concentration of 10,000 µg/ml. Further extracts concentrations were prepared from stock solution.

Culture media

Nutrient agar media and nutrient broth media (without agar) is best growth media for bacteria. The Media was composed of Beef extract 3.0g, Agar 15.0g and Peptone 5.0g. One liter media was prepared by dissolving 40g of nutrient agar in 700ml of distilled water. After complete dissolution, the final volume of the media was raised to 1000ml by adding more distilled water. The media was boiled using a hot plate. The PH was adjusted to 7.0 at 25ºC, using 0.1M NaOH and 0.1M HCl. The needed media and all glassware were sterilized through autoclaving at 15psi at 121 for 20 minutes.

Antibacterial activity

The antibacterial activities of the extracts were determined using the disc diffusion method. About 20 ml of molten nutrient agar was poured into the sterile petri dishes and allowed to set. About 50 µl of a 24 h old culture of each test organism was inoculated into the nutrient agar plate by sterile pipette. 12 µl and 6 µl of extracts were applied to the sterile perforated filter paper disc and placed on the nutrient agar plates seeded with the test organisms. Antibiotics Azithromycin for Gram positive strains and Ciprofloxacin for Gram negative strains were used as standard drugs .The plates were then incubated at 37ºC for 24 h and the zone of inhibitions were measured.

Antifungal activity

Suspension of microorganisms were added to sterile Sabouraud dextrose agar medium at 45ºC and the mixture was transferred to sterile petridishes and allowed to solidify. Sterile discs dipped in various concentrations (12 mg/ml and 6 mg/ml) of *Mirabilis jalap* L. and *Euphorbia dendroide* and Clotrimezole (50 µg/ml) were placed on the surface of agar plates. The plates were left for 1h at room temperature and incubated at 37 ◦C for 24 h. The diameter of zone of inhibition of extracts and standard was measured.

Statistical analysis

All the tests were conducted in triplicates. The data were statistically analyzed and expressed as mean ± S.D.

Results

Different parts of ethanolic extract of *M.jalapa*L. and *E.dendroide* were tested against four Gram negative bacteria,*Escherichia coli, Pseudomonas aeruginosa, Salmonellatyphi, Kleibsiella pneumonia*,three Gram positive bacteria,*Staphylococcusaureus, Bacillus subtilis, Bacillus atropoeus*and one fungal strain,*Candida albicane*by disc diffusion method, results were tabulated in table 1, 2 and 3 respectively.

Antibacterial activity against Gram Negative Bacteria (Table 1).

Table 1: Antibacterial Activity of *Euphorbia dendroide* and *Merabilis jalapa* L. Ethanol Extract Against Gram negative Bacteria.

		Diameter of zone of inhibition (mm)						
		Euphurbia dendroide			*Merabilis jalapa* L.			Antibiotic
Bacterial strain	Conc	Stem	Leaves	Root	Stem	Leaves	Seed	Ciprofloxacin (50 µg/ml)
E. coli	6 mg/ml	12.1±0.5	0.0±0.0	0.0±0.0	10.0±0.2	0.0±0.0	11.1±0.7	43.2±0.8
	12 mg/ml	15.0±0.0	0.0±0.0	7.2±0.2	14.1±0.3	0.0±0.0	13.1±0.3	
P. aeruginosa	6 mg/ml	12.0±0.3	13.1±0.6	13.2±0.2	0.0±0.0	12.2±0.2	11.1± 0. 3	42.4±0.0
	12 mg/ml	17.1±0.1	17.2±0.4	16.0±0.1	0.0±0.0	17.1±0.1	19.8±0.3	
S. typhi	6 mg/ml	16.9±0.1	7.2±0.2	4.3±0.03	6.5±0.5	0.0±0.0	0.0±0.0	34.0±0.0
	12 mg/ml	21.8±0.9	18.0±0.2	9.2±0.3	11.1±0.3	0.0±0.0	0.0±0.0	
K. pneumonia	6 mg/ml	0.0±0.0	16.2±0.2	0.0±0.0	9.2±0.2	0.0±0.0	8.7±0.2	29.0±0.0
	12 mg/ml	22.2±0.2	21.2±0.3	11.4±0.4	14.2±0.2	0.0±0.0	14.2±0.2	

The ethanolic extracts of different parts of *E. dendroide* showed good activity against all the Gram negative bacterial strains, the stem extract showed highest activity was observed against *K. pneumonia* of 22.2 mm, while root extract showed least activity against *S. typhi* of 4.3 mm. *Merabilis jalapa* L ethanolic extracts showed moderate level of activity against gram positive bacterial strains, the highest activity was noted against *P. aeruginosa* by seed extract of 19.8 mm, whereas stem extract showed lowest activity of 6.5 mm against *S. typhi*, the leaves of *Merabilis jalapa* L. showed activity against *P. aeruginosa*.
Antibacterial activity against Gram Positive Bacteria (Table 2).

Table 2: Antibacterial Activity of *Euphorbiadendroide* and *Merabilis jalapa* L. Ethanol Extract Against Gram Positive Bacteria.

		Diameter of zone of inhibition (mm)						
		Euphurbia dendroide			*Merabilis jalapa* L			Antibiotic
Bacterial strain	Conc	Stem	Leaves	Root	Stem	Leaves	Seed	Azithromycin (50 µg/ml)
S. aureus	6 mg/ml	12.5±0.2	17.9±0.5	17.1±0.1	16.2±0.2	6.4±0.2	20.4±0.3	23.0±0.0
	12 mg/ml	16.1±1.1	21.0±0.1	21.5±0.4	20.6±1.0	11.3±0.1	25.2±0.4	
B. subtilis	6 mg/ml	16.3±1.5	16.9±0.4	17.3±0.2	20.1±0.1	15.1±0.2	19.6±0.4	25.0±0.0
	12 mg/ml	22.1±1.8	20.0±0.0	22.2±0.2	25.1±0.3	17.1±0.1	26.7±0.3	
B. atropoeus	6 mg/ml	15.0±2.0	15.4±0.2	15.0±0.3	16.3±0.2	14.3±0.2	10.3±0.2	30.1±0.1
	12 mg/ml	22.2±2.4	16.8±0.3	16.8±0.1	24.2±0.1	20.2±0.2	11.4±0.4	

The ethanolic extracts of different parts of *E. dendroide* showed excellent activity against all the tested gram positive bacterial strains, the highest activity was noted against *B. subtilis* and *B. atropoeus* of 22.2 mm by root and stem extracts respectively, while stem extract shoed least activity against S. aureus of 12.5 mm. whereas *Merabilis jalapa* L. different parts extracts showed excellent activity against all the tested gram positive bacterial strains, the maximum zone of inhibition was recorded against *B. subtilis* of 26.7 mm by root extract, whereas leaves extract showed minimum activity of 6.4 mm.
Antifungal activity (Table 3).

Table 3: Antifungal Activity of *Euphorbia dendroide* and *Merabilis jalapa* L. Ethanol Extract Against Fungal strain.

		Diameter of zone of inhibition (mm)						
		Euphurbia dendroide			*Merabilis jalapa* L			Antibiotic
Fungal strain	Conc	Stem	Leaves	Root	Stem	Leaves	Seed	Clotrimezole (50 µg/ml)
C.albican	6 mg/ml	9.1±0.1	16.3±0.1	7.3±0.1	13.4±0.3	0.0±0.0	0.0±0.0	32.4±0.5
	12 mg/ml	10.3±0.2	22.3±0.2	9.1±0.1	19.2±0.1	0.0±0.0	0.0±0.0	

The *E. dendroide* different parts ethanolic extracts showed excellent antifungal activity against *C. albican*, the leaves extract showed maximum zone of inhibition of 22.3 mm, whereas root extract showed minimum activity of 7.3 mm. Among *Merabilis jalapa* L. different parts extracts only stem showed excellent activity of 13.4 – 19.2 mm.

Discussion

Plants contain useful important sources for the development of new therapeutic agents on the basis of their antibacterial, antifungal and antiviral activities[21]. Mostly these observations helped toidentify and developed new drugs for the use of human health[22].The current study demonstrated in-vitro antimicrobial activity of ethanolic extract of *Euphorbia dendroide* and *Mirabilis jalapa L.* against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Kleibsiella pneumonia, Staphylococcus aureus, Bacillus subtilis, Bacillus atropoeus* and fungal strain *Candida albican.* From the present study observed that the ethanolic extract of *Euphorbia dendroide* and *Mirabilis jalapa L.* possess potential ability to inhibit the growth of all zone of bacteria and fungi. The ethanolic extract of *Euphorbia dendroide* showed better result as compared to *Mirabilis jalapa L.* ethanolic extract of both plants aim to finding the inhibition

effect at different concentration in the treatment of bacterial and fungal infection. Further worked should be need to discover new active constituent for these plant against bacterial and fungal infections.

Conclusion

In developing countries (including Pakistan) as whole and generally worldwide, where infectious diseases are common, it is worthy to search out and promote plants based medicines. The present study concluded that the selected plants have good antimicrobial activities against the clinically isolated pathogenic bacterial strains and fungal strain; further study is needed on the selected plants.

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