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Antibacterial and Antifungal Activity of Leaf Extracts of *Pedalium murex L.*

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ABSTRACT

Pedalium murex Linn, commonly called Gokhru a member of family Pedaliaceae. The antimicrobial activity of Pedalium murex leaves were extracted successively with different solvents viz., petroleum ether, chloroform, ethyl acetate and methanol and screened for their antimicrobial activity against Gram positive bacteria: Staphylococcus aureus, Streptococcus pyogenes, and Enterococcus faecalis, Gram negative bacteria : Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Vibrio cholera and fungal species: Candida albicans, C. Guillurmondii and C.glabrata by using Disc diffusion method. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were also determined in the present research. The methanol leaf extract of Pedalium murex showed the highest antimicrobial activity against all the bacterial and fungal stains tested than the other extracts. The mean zones of inhibition produced by the extracts in Agar diffusion assays against the tested bacterial strains were ranged from 7.0 to 26.8 mm. The highest mean zone of inhibition (26.8 mm) was observed in the methanol extracts of Pedalium murex against the bacteria Staphylococcus aureus. The MIC values were between 7.81 and 250 µg/ml, MBC values:15.62 and 500 µg/ml and MFC values: 31.25 and 500 µg/ml were also recorded. From the above findings, we concluded that the methanolic leaf extract of Pedalium murex exhibited their antimicrobial potential which will be needed to isolate and characterize the compounds.

Keywords: Antimicrobial activity, Pedalium murex, MIC, MBC and MFC.

I. INTRODUCTION

Microbial infections are major public health problems in the developed countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotic, resistances in human pathogens is increasing. This has forced the scientists to search for new antimicrobial substances from various sources like medicinal plants. Medicinal plants constitute the main source of new pharmaceuticals and health care products (Ivanona et al., 2005). The use of traditional medicines was widespread in India (Jeyachandran and Mahesh, 2007). Infectious diseases caused by bacteria, viruses, fungi and parasites are still a major threat to public health, despite the tremendous progress in human medicine (Cosa et al., 2006). The past three decades have seen a dramatic increase in microbial resistances to antimicrobial agents (Chopra et al., 1996). Such situation stimulates the development of new antimicrobial agents in order to treat the infectious disease in an effective manner. So, this matter continued to an era to identify the potential antimicrobial agents from the natural resources. The edible plants that used for traditional medicine contain a wide range of substances that can be used to treat abundant of infectious disease with reduced side effects (Duraipandiyan et al., 2006). In Asia, the use of medicinal plants to cure specific illness has been use for many years (Bhattacharjee, 1998). Furthermore, Malaysia is rich in various edible plants with diverse biological & pharmacological properties (Yogalatha et al., 2005).

Pedalium murex Linn, commonly called Gokhru a member of family Pedaliaceae. It is commonly found

in Deccan and in some parts of Ceylon and Gujarat and in the coastal areas of southern India (Nadkarni, 1982). The leaf decoction is used to control white discharge due to excessive body heat. Root decoction is used as an anti bilious agent, while the juice of the fruit is used as an emmenagogue and to promote lochial discharge (Satyavathi et al., 1987). The decoction of the seeds and glycosides obtained from it showed mild diuretic activity and the alcoholic extract of the fruits reduced blood pressure in dog and rat (Harvey, 1996). The fruits are rich in flavonoids, saponin soluble proteins (Mukherjee, 2002). An infusion extract prepared using cold water from the leaves, stems and fruits of Pedalium murex is demulcent, diuretic and also found to be useful in the treatment of disorders of urinary systems such as gonorrhea, dysuria, incontinence of urine, etc., (Shukla and Khanuja, 2004). Pedalium murex is an important medicinal plant that contains several alkaloids like pedalitin, Diosmetin, Dinatin, Pedalin dinatin-7-glucuronide (Subramanian and Nair, 1972).Hence, present study was carried out to evaluate the antimicrobial activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves of Pedalium murex against bacterial and fungal strains.

II. MATERIALS AND METHODS

A. Collection of plant material and preparation of extracts

The fresh leaves of Pedalium murex were collected from Alapakkam region (11069'52 N 79076'55 E), Cuddalore District, Tamilnadu, India, during the months from March to April 2016. The specimens were deposited in the Herbarium of Department of Botany, Annamalai University, Annamalai Nagar. Collected leaves were initially washed with water, then surface sterilized with disinfectant solutions of 10% sodium hypochlorite solution and finally rinsed with sterile distilled water and shade dried under room temperature and grounded in to a course powder. One hundred grams of coarse powder was extracted with different organic solvents like non-polar to polar viz., Petroleum ether, chloroform, ethyl acetate and methanol for 8 hours using Soxhlet apparatus and the solvents were evaporated under vaccum in a rotary evaporator (Heidolph, Germany) and the dried powder was stored at 40C for further use.

B. Microorganisms

Seven clinical bacterial isolates and three fungal species were obtained from Raja Muthaiah Medical Colleges Hospital, Annamalai University, TamilNadu. Gram positive bacteria: Staphylococcus aureus, Streptococcus pyogenes, and Enterococcus faecalis, Gram negative bacteria Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Vibrio cholera and fungal species: Candida albicans, C. Guillurmondii and C. Glabrata were tested in the present study. The stock cultures were maintained on Muller Hinton Agar medium and Sabouraud's Dextrose Agar (MHA) and Muller Hinton Broth(MHB). In vitro antibacterial activity of the isolates was determined by using Sabouraud's Dextrose Agar (SDA) and Sabouraud's Dextrose Broth (SDB) respectively.

C. Antibacterial and Antifungal assays Disc diffusion method

The agar diffusion method (Bauer et al., 1986) was employed for the initial assessment of antimicrobial potential of the extracts. Sterilized mediums of 20 ml MHA (Bacterial) and 20 ml of SDA (Fungi) were separately poured in each petri plate and kept untouched until it solidity. The standard inoculum of bacterial suspension containing 108CFU/ml and suspension of yeast containing 106 CFU/ml were swabbed on the top of the solidified media and the plates were dried and uniformly spread. After drying, the extracts were placed on the disc with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacine(5 µg/disc) for bacteria and Amphotericine –B (100 units/disc) for

yeast were used as positive control. Ten per cent DMSO was used as blind control. Finally, the incubated plates were incubated at 370 C for 24 hrs. The zone of inhibition was observed and measured in millimetres. Each experiment was carried out in triplicates.

D. Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of plant different extracts were tested in MHB for bacteria and SDB for fungi described by Ericsson and Sherris (2002). The extracts were dissolved in 10% DMSO to obtain 2 mg/ml and 0.5 ml of stock solution was incorporated into 0.5 ml of MHA to get concentrations of 1000, 500, 250, 125, 62.5, 31.2 and 15.6 µg/ml, 100µl as standard. Bacterial suspension of the test organism was transferred into each tube and incubated at 37^0 C for 24 hrs. The tube devoid of plant extracts kept as control and the MIC of the extracts were examined.

E. Minimum Bactericidal Concentration (MBC)

The MBC of the different extracts were determined by plating 100µl of samples from each MIC assay tube with growth inhibition into freshly prepared MHB and the plates were incubated at 37^0 C for 24 hrs bacteria. The MBC values were recorded as the lowest concentration of the extracts that did not permit any visible bacterial colony growth on the agar plate during the period of incubation.

F. Minimum Fungicidal Concentration (MFC)

The MFC of the different extracts were determined by plating 100 µl of samples from each MIC assay tube with growth inhibition into freshly prepared SDA and the plates were incubated at 37^0 C for 24 hrs yeast. The MFC values were recorded as the lowest concentration of the extracts that did not permit any visible bacterial colony growth on the agar plat during the period of incubation.

III. RESULTS

Table 1. Antibacterial and antifungal activity of Leaves of Pedalium murex

Sl. No	Bacterial / Fungal Strains/ Solvent Extracts	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)				MIC (µg/mL)	MBC/ MFC (µg/mL)
		1000	500	250	Ciprofloxacin (10µg/disc)/ Amphotericin B (100units / disc)		
1	Staphylococcus aureus						
	Petroleum Ether	19.8 ± 0.28	18.8 ± 0.78	16.5 ± 0.50	28.0 ± 0.50	31.25	62.5
	Chloroform	20.5 ± 0.28	19.1 ± 0.26	17.3 ± 0.28	28.5 ± 0.50	31.25	62.5
	Ethyl acetate	22.0 ± 0.50	20.6 ± 0.28	18.6 ± 0.28	27.5 ± 0.50	15.65	31.25
	Methanol	26.8 ± 0.76	23.5 ± 0.50	21.6 ± 0.76	28.5 ± 0.50	7.81	15.62
2	Streptococcus pyogenes						
	Petroleum Ether	18.0 ± 0.50	16.6 ± 0.57	14.8 ± 0.28	29.0 ± 0.50	62.5	125
	Chloroform	19.8 ± 0.28	17.5 ± 0.50	15.5 ± 0.50	27.5± 0.50	31.25	62.5
	Ethyl acetate	20.5 ± 0.50	18.2 ± 0.76	16.1 ± 0.76	29.0 ± 0.50	31.25	62.5
	Methanol	21.8 ± 0.28	19. 5± 0.50	17.8 ± 0.28	28.5 ± 0.50	62.5	125
3	Enterococcus faecalis						

	Petroleum Ether	16.8 ± 0.28	14.5 ± 0.50	12.6 ± 0.56	27.0 ± 0.76	62.5	125
	Chloroform	17.0 ± 0.50	15. 6 ± 0.76	13.5 ±0.50	28.1 ± 0.57	62.5	125
	Ethyl acetate	19.1 ± 0.26	17.3 ± 0.76	14.5 ± 0.50	28.0 ± 0.50	62.5	125
	Methanol	19.5 ± 0.50	17.8 ± 0.28	15.6 ± 0.76	27.5 ± 0.50	31.25	62.5
4	Escherichia coli						
	Petroleum Ether	13.5 ± 0.50	11.5 ± 0.50	9.8 ± 0.28	27.5 ± 0.50	250	500
	Chloroform	14.8 ± 0.28	12.6 ± 0.76	10.5 ±0.50	28.5 ± 0.50	250	500
	Ethyl acetate	17.0 ± 0.50	15.6 ± 0.28	13.8 ± 0.56	28.0 ± 0.50	62.5	125
	Methanol	19.5 ± 0.50	17.8 ± 0.26	15.5 ± 0.50	27.5 ± 0.50	31.25	62.5
5	Proteus vulgaris						
	Petroleum Ether	14.8 ± 0.28	12.0 ± 0.50	10.5 ± 0.50	28.5 ± 0.50	250	500
	Chloroform	15.5 ± 0.50	13.6 ± 0.28	11.8 ± 0.56	27.5 ± 0.50	125	250
	Ethyl acetate	17.5 ± 0.50	14.8 ± 0.76	12.5 ± 0.50	27.5 ± 0.50	62.5	125
	Methanol	19.0 ± 0.50	17.5 ± 0.50	15.8 ± 0.28	27.5 ± 0.50	62.5	125
6	Pseudomonas aeruginosa						
	Petroleum Ether	16.6 ± 0.28	15.5 ± 0.50	14.8 ± 0.28	28.0 ± 0.50	125	250
	Chloroform	18.2 ± 0.56	16.0 ± 0.50	15.1 ± 0.78	27.5 ± 0.50	62.5	125
	Ethyl acetate	20.8 ± 0.28	18.0 ± 0.50	16.5 ± 0.50	28.5 ± 0.50	31.25	62.5
	Methanol	21.8 ± 0.56	19.5 ± 0.50	17.6 ± 0.28	28.0 ± 0.50	31.25	62.5
7	Vibrio cholera						
	Petroleum Ether	15.5 ± 0.50	13.5 ± 0.50	11.8 ± 0.28	29.0 ± 0.50	125	250
	Chloroform	16.8 ± 0.28	14.6 ± 0.28	12.5 ± 0.50	27.0± 0.50	125	250
	Ethyl acetate	17.5 ± 0.50	15.8 ± 0.28	13.5 ± 0.50	28.0 ± 0.50	62.5	125
	Methanol	19.8 ± 0.28	17.6 ± 0.56	14.8 ± 0.28	27.0 ± 0.50	31.25	62.5
8	Candida albicans						
	Petroleum Ether	17.8 ± 0.28	16.1 ± 0.56	14.5 ± 0.50	23.5 ± 0.50	62.5	125
	Chloroform	18.0 ± 0.50	16. 5 ± 0.50	14.8 ±0.28	23.0 ± 0.50	62.5	125
	Ethyl acetate	20.3 ± 0.28	18.5 ± 0.50	16.8 ± 0.28	24.0 ± 0.50	31.25	62.5
	Methanol	21.0 ± 0.50	19.8 ± 0.76	17.6 ± 0.50	24.8 ± 0.28	15.62	31.25
9	C. guillurmondii						
	Petroleum Ether	15.5 ± 0.50	13.5 ± 0.50	11.8 ± 0.28	25.0± 0.50	125	250
	Chloroform	17.0 ± 0.50	15.5 ± 0.50	13.5 ± 0.50	25.5 ± 0.50	125	250
	Ethyl acetate	18.8 ± 0.28	16.1 ± 0.26	14.5 ± 0.50	24.0 ± 0.50	62.5	125
	Methanol	19.5 ± 0.50	17.6 ± 0.78	15.8 ± 0.26	24.5 ± 0.50	31.25	62.5

10	C. glabrata						
	Petroleum Ether	14.8 ± 0.28	12.0 ± 0.50	10.6 ± 0.78	24.0 ± 0.50	250	500
	Chloroform	15.5 ± 0.50	13.8 ± 0.28	11.5 ± 0.50	25.5 ± 0.50	125	250
	Ethyl acetate	16.1 ± 0.28	14.8 ± 0.28	12.5 ± 0.50	25.5 ± 0.50	125	250
	Methanol	18.5 ± 0.50	16.5 ± 0.50	14.0 ± 0.50	24.0 ± 0.50	62.5	125

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm

[b]-mean of three assays; ± - standard deviation.

The results of different extracts of antimicrobial activity of leaves of P. murex are presented in Table 1. For bacteria, the mean zone of inhibition for petroleum ether extracts were ranged from 9.8 ± 0.28 mm to 19.8 ± 0.28 mm, the values of chloroform extracts were from 10.5 ± 0.50 mm to 20.5 ± 0.28 mm. On the other hand, mean zone of inhibition for ethyl acetate extracts, the values were from 13.8 ± 0.56 mm to 22.0 ± 0.50 mm and for methanol extracts were from14.8 ± 0.28 mm to 26.8 ± 0.76 mm.The methanol extract produced the highest mean zone of inhibition of 26.8 ± 0.76 mm (at 1000 µg/disc concentration), lowest MIC (7.81µg/ml) and the lowest MBC (15.62 µg/ml) against Staphylococcus aureus. The highest values of MIC (250 µg/mL) and MFC (500 µg/mL) were recorded in petroleum ether extracts against E. coli and Proteus vulgaris and chloroform extracts against E.coli with regard to Candida species tested, the mean zone of inhibition for petroleum ether extracts were ranged from 10.6 ± 0.78 mm to 17.8 ± 0.28 mm, for chloroform extracts the values were from 11.5 ± 0.50 mm to 18.0 ± 0.50 mm. On the other hand, the values of ethyl acetate extracts were from 12.5 ± 0.50 mm to 20.3 ± 0.28 mm and for methanol extracts were from 14.0 ± 0.50 mm to 21.0 ± 0.50 mm. Among the extracts tested, the methanol extract recorded the highest mean zone of inhibition of 21.0 ± 0.50 mm (at 1000 µg/disc concentration) against C. albicans. The lowest MIC (15.62 µg/mL) and MFC (31.25 µg/mL) values were recorded with methanol extracts against Candida albicans. The highest MIC of 250 µg/mL and MFC of 500 µg/mL were recorded in petroleum ether extract against C. glabrata.

IV. DISCUSSION

In the present study, different solvent viz., petroleum ether, chloroform, ethyl acetate and methanol extracts of Pedalium murex leaves were showed varied level of inhibitory activities against the bacterial and fungal stains tested.All the extracts of Pedalium murex possessed significant antibacterial and antifungal activity against all the bacterial and fungal strains.The methanol leaf extract of Pedalium murex showed the highest antibacterial activity against S.aureus followed by other stains. The highest mean zone of inhibition (26.8 mm) and the lowest MIC value (7.81 µg/ml) and MBC value (62.5 µg/ml) were observed in methanol leaf extracts. In antifungal activity, the methanol leaf extract of Pedalium murex showed highest antifungal activity and it was followed by petroleum ether, chloroform and ethyl acetate.

Similar results were also observed in previous studies, methanol extracts have been shown to result in high extraction yields with strong antibacterial activities (Jo et al., 2012). Prashanth et al., 2001) proved that methanolic extracts of pomegranate peels were more active than water extracts against Echerichia coli, Staphylococcus aureus and Bacillus subtilis. (AshikMossadik et al., 2000) reported that the methanol extracts of Alangium salviifolium flowers showed a wide spectrum of antibacterial activity against Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus and gram negative bacteria viz.,

Echerichia coli, Shigella sonnei, Shigella shiga, Shigella boydii, Salmonella typhi and Klebsiella sp. Gram positive bacteria were more susceptible than that of gram negative bacteria in responseto the plant extract observed in the present study. It is in agreement with the previous reports (Karou et al., 2005; Nar et al., 2005).

A possible explanation for these observation may lie in the significant difference in the outer layers of gram negative and positive bacteria. The cell wall in the gram positive bacteria is single layer whereas the gram negative cell wall in multi layered structure; the passage of the active compound through the gram negative cell wall may be inhibited (Yao and Moellering, 2011). The basis of varying degree of sensitivity of the tested organisms may be due to the intrinsic tolerance of microorganism, the nature and combination of phytochemicals present in the crude extract (Seasotiya and Dalal, 2014).

V. CONCLUSION

Pedalium murex is a valuable plant source of medicinally useful compounds that have been traditionally used for several traditional ailments. Leaves extract in organic solvents exhibited the presence of many bioactive compounds whose presence were proved that they could be used for making antimicrobial drugs. Finally, it can conclude that the methanol extract of Pedalium murex can be used as an antimicrobial substance for the treatment of microbial infections.

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