

Antimicrobial Activity of *Ipomoea pes-caprae* L. against Selected Microbial Species

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ABSTRACT

In the present investigation, petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves of Ipomea pes-caprae were studied for the antibacterial and antifungal activities against Gram-positive bacteria such as Bacillus pumilus, B. subtilis, Micrococcus luteus and Staphylococcus aureus, Gram-negative bacteria, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia fungal strains such as Aspergillus niger, A. fumigates and A. flavus and the results are presented. The mean zone of inhibition for bacteria ranged from 8.5 to 25.6 mm. The minimum inhibitory concentration and minimum bactericidal concentrations were from 15.62 to 500 μ g/mL and 31.25 to 1000 μ g/mL respectively. The standard drug, Ciprofloxacin (10 μ g/disc) was used for standard bacterial strains and recorded mean zone of inhibition ranged from 23.5 to 29.5 mm. The mean zone of inhibition for Aspergillus species ranged from 9.6 to 21.5 mm. The minimum inhibitory concentration and minimum fungicidal concentrations were from 31.25 to 500 μ g/mL and 62.5 to 1000 μ g/mL respectively. The standard drug, Ketoconazole (5 μ g/disc) was used for Aspergillus species and recorded mean zone of inhibition ranged from 23.5 to 1000 μ g/mL respectively. The standard drug, Ketoconazole (5 μ g/disc) was used for Aspergillus species and recorded mean zone of inhibition ranged from 23.5 to 1000 μ g/mL respectively. The standard drug, Ketoconazole (5 μ g/disc) was used for Aspergillus species and recorded mean zone of inhibition ranged from 23.5 mm to 26.5 mm.

Keywords : Ipomoea Pes-Caprae, Minimum Inhibitory Concentration, Minimum Bactericidal Concentrations.

I. INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and they are source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs. They possess varied medicinal properties, while some of these raw drugs are collected in small quantities by the local communities and traditional healers for local use [1]. The plants represent a rich source of antimicrobial agents [2, 3]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [4-6]. Medicinal plants not only provide health benefits as traditional medicines. In the last decade, there has been a nine fold increase in the export value of Indian herbal raw materials [7]. The aim of the present study was made to find out the biological properties of the medicinal plant, *Ipomoea pes-caprae* for antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis, B. pumilus , Micrococcus luteus* and *Staphylococcus aureus,* Gram-negative bacteria, *Escherichia coli ,Pseudomonas aeruginosa* and *Klebsiella pneumonia*, fungal strains such as *Aspergillus niger, A. fumigates* and *A. flavus.*

II. METHODS AND MATERIAL

A. DETERMINATION OF *In Vitro* ANTIMICROBIAL ACTIVITY

1) Microorganisms used

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The clinical isolates of Gram-positive bacteria such as Bacillus subtilis, B. pumilus, Micrococcus luteus and Gram-negative Staphylococcus aureus, bacteria, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia fungal strains such as Aspergillus niger, A. fumigates and A. flavus were obtained from the Department of Clinical Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar. The stock cultures were maintained on Muller Hinton Agar for bacteria and Sabouraud dextrose agar for fungus at 4ºC. In vitro antibacterial activity were determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) and antifungal activity were determined by using Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) obtained from Himedia Ltd., Mumbai.

2) Preparation of inocula

Twenty-four hours old culture of selected bacteria/fungi were mixed with physiological saline and the turbidity was corrected by adding sterile physiological saline until a Mac Farland turbidity standard of 0.5 (CFU) per mL was obtained. The mould fungi were sub cultured on SDA and incubated (at 30°C for 72-96 h for *Aspergillus* sps.). The growth was scraped aseptically, crushed and macerated thoroughly in sterile distilled water and the fungal suspension was standardized spectro-photometrically to an absorbance of 0.600 at 530 nm [8].

3) Preparation of test solution and disc

The test solution was prepared with 100 mg of crude extracts was dissolved in 5 per cent dimethyl sulphoxide (DMSO). Sterile susceptibility test discs obtained from Himedia, Pvt. Ltd, Mumbai were impregnated with 20 μ L of the extract (corresponding to 50, 25 and 12.5 mg/mL of crude extracts) and allowed to dry at room temperature. All the extracts were streaked on the MHA and SDA plates to check their purity. After the

incubation period (24 h for bacteria and 24 to 48 h for fungi), the plates were observed.

B. Antimicrobial assay1) Disc diffusion method

The antimicrobial activities of crude extracts of Ipomoea pes-caprae was determined by disc diffusion method according to [9]. 20 mL of MHA/SDA was poured into petridishes and allowed to solidify. Plates were dried and 0.1 mL of standardized inoculum suspension (bacteria/fungi) was inoculated on the entire agar surface. The disc with different concentrations of crude extracts were prepared and aseptically applied on the surface of the petriplates with sterile forceps and gently pressed to ensure inoculated contact with the agar surface. Ciprofloxacin (5 µg/disc) for bacteria and Ketoconazole (5 µg/dics) for Aspergillus were used as positive controls. 10 per cent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37°C for 24 h for bacteria and at 30°C for 72-96 h for Aspergillus sp. The zones of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times.

2) Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined in MHB for bacteria and SDA for fungi described by broth macro dilution method [10]. The plant extracts were dissolved in 10 per cent DMSO obtained 2 mg/mL. 0.5 mL of stock solution was incorporated into 0.5 ml of MHB to get a concentration of 1000 to 15.62 µg/mL. 50 µL of standardized suspension of the test organism was transferred into each tube. The control tube contained only organism and devoid of plant extracts. The culture tubes were incubated at 37°C for 24 h for bacteria and 30°C for 72-96 h for Aspergillus sp. The lowest concentrations, which did not show any growth

of tested organism after macroscopic evaluation were determined as MIC.

3) Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)

The MBC and MFC of the extracts were determined [11] by plating 100 μ L of sample from each MIC assay tube with growth inhibition into freshly prepared MHB and SDA and the plates were incubated at 37°C for 24 h and 28°C for 48-72 h (fungi). The MBC and MFC values were recorded at the lowest concentration of the extracts that did not permit any visible bacterial/fungal colony growth on the agar plate during the period of incubation.

III. RESULTS AND DISCUSSION

RESULTS

The results of different extracts of antimicrobial activity of leaves of *Ipomoea pes-caprae* are presented in Table 1. For bacteria, the mean zone of inhibition for petroleum ether extracts ranged between 8.5 \pm 0.50 mm and 20.5 \pm 0.50 mm, the values of chloroform extracts were from 9.5 \pm 0.50 mm to 22.5 \pm 0.50 mm. On the other hand, mean zone of inhibition for ethyl acetate extracts, the values were from 10.1 \pm 0.76 mm to 22.8 \pm 0.76 mm and for methanol extracts were from 11.0 \pm 0.50 mm to 25.6 \pm 0.76 mm. The methanol extract produced the highest mean zone of inhibition of 25.6 \pm 0.78 mm (at

1000 µg/disc concentration), lowest MIC (15.62 μ g/ml) and the lowest MBC (31.25 μ g/ml) against Staphylococcus aureus. The highest values of MIC (500 μ g/mL) and MFC (1000 μ g/mL) were recorded in petroleum ether extracts against E. coli, and P. aeruginosa. With regard to Aspergillus strains tested, the mean zone of inhibition for petroleum ether extracts ranged between 9.6 \pm 0.26 mm and 16.5 \pm 0.50 mm, for chloroform extracts these values were from 10.5 ± 0.50 mm to 17.1 ± 0.26 mm. On the other hand, the values of ethyl acetate extracts were from 11.0 ± 0.50 mm to 18.8 ± 0.28 mm and for methanol extracts were from 11.8 ± 0.76 mm to 21.5 ± 0.50 mm. Among all the extracts tested, the methanol extract recorded the highest mean zone of inhibition of 21.5 ± 0.50 mm (at 1000 µg/disc concentration), the lowest MIC (31.25 μ g/mL) and MFC (62.5 µg/mL) values were recorded with methanol extracts against Aspergillus niger. The highest MIC and MFC of 500 and MFC of 1000 µg/mL were recorded in petroleum ether extract against A. flavus.

In the present study, petroleum ether, chloroform, ethyl acetate and methanol extracts leaves of *Ipomoea pes-caprae* were screened for antibacterial and antifungal activities. The highest mean zone of inhibition for bacteria (25.6 \pm 0.78 mm at 1000 µg/disc concentration) was observed with methanol extracts of leaves of *Ipomoea pes-caprae* against strain of *Staphylococcus aureus* when compared to all the extracts among tested strains.

Table 1. Antibacterial and	l antifungal activit	v of leaves of	Ipomoea pes-caprae
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			Mean zone of inhibition ^a (mm) ^b					
		Concentration of the disc (µg/disc)					MBC/	
Sl. No	Bacterial/Fungal Strains 1) Solvents/Extracts	1000	500	250	Ciprofloxacin (10 µg/disc)/ Ketoconazole (5 µg/disc)	MIC (µg/ml)	MFC (µg/ml)	
1	Bacillus subtilis							
	Petroleum ether	14.0 ± 0.50	12.5 ± 0.50	11.6 ± 0.78	26.0 ± 0.50	250	500	

	Chloroform	15.5 ± 0.50	13.8 ± 0.76	12.8 ±0.26	23.5 ± 0.50	250	500	
	Ethyl acetate	17.2 ± 0.28	14.5 ± 0.50	13.0 ± 0.50	25.0 ± 0.50	250	500	
	Methanol	18.5 ± 0.50	16.8 ± 0.76	14.5 ± 0.50	26.0 ± 0.50	125	250	
2	Bacillus pumilus							
	Petroleum Ether	15.5 ± 0.50	13.6 ± 0.28	11.8 ± 0.76	28.0 ± 0.50	250	500	
	Chloroform	17.6 ± 0.28	15.5 ± 0.50	13.5 ±0.50	26.5 ± 0.50	250	500	
	Ethyl acetate	18.8± 0.76	16.2 ± 0.26	15.0 ± 0.50	28.5 ± 0.50	125	250	
	Methanol	19.7 ± 0.75	17.5 ± 0.50	15.6 ± 0.28	25.8 ± 0.78	125	250	
3	Micrococcus luteus	1		1	1			
	Petroleum Ether	19.5 ± 0.50	17.8 ± 0.76	16.2 ± 0.26	26.0 ± 0.50	125	250	
	Chloroform	21.5 ± 0.50	19.8 ± 0.78	17.5 ± 0.50	26.5 ± 0.50	62.5	125	
	Ethyl acetate	22.8 ± 0.76	20.0 ± 0.50	18.5 ± 0.50	28.1 ± 0.50	31.25	62.5	
	Methanol	24.0 ± 0.50	22.5 ± 0.50	19.5 ± 0.50	27.5 ± 0.50	31.25	62.5	
4	Staphylococcus aureus							
	Petroleum Ether	20.5 ± 0.50	18.6 ± 0.76	17.1±0.26	29.5 ± 0.50	62.5	125	
	Chloroform	22.5 ± 0.50	20.3 ± 0.78	18.8 ±0.76	28.5 ± 0.50	31.25	62.5	
	Ethyl acetate	22.8 ± 0.76	20.6 ± 0.57	19.5 ± 0.50	28.5 ± 0.50	31.25	62.5	
	Methanol	25.6 ± 0.78	23.5 ± 0.50	21.8 ± 0.76	29.5 ± 0.76	15.62	31.25	
5	Escherichia coli							
	Petroleum Ether	12.5 ± 0.50	10.5 ± 0.50	9.5 ± 0.50	26.0 ± 0.50	500	1000	
	Chloroform	13.8 ± 0.76	11.5 ± 0.50	10.1 ± 0.28	25.0 ± 0.50	250	500	
	Ethyl acetate	15.0 ± 0.50	13.8 ± 0.26	11.5 ± 0.50	25.5 ± 0.50	250	500	
	Methanol	16.8 ± 0.26	14.5 ± 0.50	13.1±0.76	26.8 ± 0.76	250	500	

Sl. No	Bacterial/Fungal Strains Solvents/Extracts	Mean zone of inhibition ^a (mm) ^b				MIC	MBC/
		Concentration of the disc (µg/disc)					
		1000	500	250	Ciprofloxacn (10 µg/disc)/ Ketoconazoe (5 µg/disc)	MIC (µg/ml)	MFC (µg/ml)
6	Pseudomonas aeruginosa	-	-				
	Petroleum Ether	11.6 ± 0.78	9.5 ± 0.50	8.5±0.50	26.5 ± 0.50	500	1000
	Chloroform	12.0 ± 0.50	10.8 ±0.76	9.5±0.50	26.0 ± 0.50	250	500
	Ethyl acetate	12.5 ± 0.50	11.5 ±0.50	10.1±0.76	26.5 ± 0.50	250	500
	Methanol	14.0 ± 0.50	12.6 ±0.78	11.0±0.50	24.5 ± 0.50	250	500
7	Klebsiella pneumoniae	•	•		•		

	Petroleum Ether	13.0±0.28	11.6 ± 0.28	10.5± 0.50	25.6 ± 0.28	250	500
	Chloroform	14.1 ± 0.21	12.5 ± 0.50	11.2 ± 0.28	24.0 ± 0.50	250	500
	Ethyl acetate	15.5 ± 0.50	13.8±0.76	12.5 ± 0.50	24.0 ± 0.50	250	500
	Methanol	17.0 ± 0.50	14.5 ± 0.50	12.5 ± 0.50	25.5 ± 0.50	250	500
8	Aspergillus niger						
	Petroleum Ether	16.5 ± 0.50	14.8 ± 0.76	12.5 ± 0.50	25.5 ± 0.50	250	500
	Chloroform	17.1 ± 0.26	15.5 ± 0.50	13.5 ±0.50	26.0 ± 0.57	250	500
	Ethyl acetate	18.8 ± 0.78	16.5 ± 0.50	14.8 ± 0.76	26.5 ± 0.50	125	250
	Methanol	21.5 ± 0.50	18.5 ± 0.50	16.8 ± 0.76	26.5 ± 0.50	31.25	62.5
9	Aspergillus fumigates						
	Petroleum Ether	14.1±0.26	12.5 ± 0.50	10.6 ± 0.28	26.5 ± 0.50	250	500
	Chloroform	15.5 ± 0.50	13.5 ± 0.50	12.0 ±0.50	25.0 ± 0.50	250	500
	Ethyl acetate	16.0 ± 0.50	14.3 ± 0.28	12.5 ± 0.50	24.5 ± 0.50	125	250
	Methanol	18.2 ± 0.78	15.8 ± 0.76	13.5 ± 0.50	25.5 ± 0.50	62.5	125
10	Aspergillus flavus						
	Petroleum Ether	12.5 ± 0.50	10.8 ± 0.76	9.6±0.26	24.6 ± 0.26	500	1000
	Chloroform	13.0±0.50	11.5 ± 0.50	10.5 ±0.50	25.5 ± 0.50	250	500
	Ethyl acetate	13.5 ± 0.50	12.0 ± 0.50	11.0 ± 0.50	24.8 ± 0.26	250	500
	Methanol	14.8 ± 0.76	13.0 ± 0.50	11.8 ± 0.76	23.5 ± 0.50	250	500

DISCUSSION

In the present investigation, petroleum ether, chloroform, ethyl acetate and methanol extracts of *Ipomoea pes-caprae* leaves were screened for antibacterial and antifungal activities against gram positive bacteria, such as *Bacillus subtilis, B. pumilus, Micrococcus luteus* and *Staphylococcus aureus,* Gram-negative bacteria, *Escherichia coli,* *Pseudomonas aeruginosa* and *Klebsilla pneumonia* fungal strains such as *Aspergillus niger, A. fumigates* and *A. flavus* and results exhibited varied level of antimicrobial activity. The mean zone of inhibition for bacteria ranged from 7.0 to 25.6 mm and for *Candida* species ranged from 7.1 to 21.5 mm. The differences in the antimicrobial activity of crude extracts may be due to the amount of antimicrobial agent present in the extract and their mode of action on different test microorganisms [12]. However differences were observed between antibacterial activities of the extracts. These differences could be due to the differences in the chemical composition of these extracts as the secondary metabolites of plants have many effects including antibacterial properties [13, 14]. We observed in this study the variation of antimicrobial activity. This may be attributed to the nature of the plant material or its origin [15].

Among the extracts tested, the methanol extract of *I. pes-caprae* leaves was exhibited the highest mean zones of inhibition of 25.6 ± 0.78 mm (at 1000 µg/disc concentration) against Staphylococcus aureus. This may suggest that the antimicrobial activity of individual plants or their extracts may involve distinct active principles with distinctive mechanisms of action [16]. The same plant was exhibit antimicrobial properties but the results are differed. [17] Recorded the methanol extracts of I. pes-caprae leaves exhibited highest inhibition zone of 17, 18 and 21 mm against Klebsiella pneumoniae, Escherichia coli and Bacillus subtilis respectively. The methanolic crude extract as well as its aqueous soluble fractions of stem bark of Ipomoea pes-caprae exhibited moderate antimicrobial activity with average zone of inhibition ranging from 8-12 mm each as compared to standard (40-42 mm) [18]. The results presented here were accordance to some previous studies. The same methanol/other solvents extracts of many plants produced different zone of inhibition. [19] Recorded the similar trend in the chloroform and the methanol extracts of Boesenbergia rotunda and the chloroform extracts of Alpinia galanga, Piper betle, Spilanthes acmella, and Zingiber zerumbet with the inhibition zones ranged from 8.6 to 29.1 mm. The maximum zone (29.1 mm) of antibacterial effect against Staphylococcus aureus was produced from the chloroform extract of Alpinia galanga. [20] Recorded the similar observation in Ocimum gratissimum and O. sanctum showed maximum zone of inhibition with that of 30 and 25.5 mm respectively against Salmonella typhi.

In the present study, the minimum inhibitory concentration and minimum bactericidal/fungicidal concentrations were from 15.62 to 500 μ g/mL and 31.25 to 1000 µg/mL respectively. The lowest minimum inhibitory concentration (15.62)µg/mL) and minimum bactericidal concentrations $(31.25 \,\mu\text{g/mL})$ were observed with methanol extracts of leaves of Ipomoea pes-caprae against strain of S. aureus. With regard to Aspergillus strains tested, the methanol extract of leaves showed the lowest MIC (31.25 µg/mL) and MFC (62.5 µg/mL) respectively against Aspergillus niger. In the present study, lowest MIC values were recorded in Grampositive bacteria only but in results of [21] showed a few exceptions *i.e.*, the MIC values of all extracts of Ipomoea pes-caprae were found to be less for gram-positive bacteria.

IV.CONCLUSION

The results indicated that methanol solvent extracts exhibit highest antimicrobial activities as compared with the other extracts. So, use of natural products, especially of *I. pes-caprae* may be considered as a new source of antibacterial and antifungal agents. The findings of this work are useful for further research to identify, isolate and characterize the specific compound which is responsible for higher antimicrobial properties.

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