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Research Article - Herbal Science

Antimicrobial activity of various extracts of *Plectranthus ambionicus* and *Phyllanthus amarus*

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Abstract

Hexane, chloroform, ethyl acetate and methanol extracts of leaves of *Plectranthus ambionicus* and *Phyllanthus amarus* were screened for their antimicrobial activity against human pathogenic bacterial and fungal strains. Antimicrobial activity was carried out by disc diffusion method, determination of minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) against four strains of Gram positive bacteria, three strains of Gram negative bacteria and three species of fungi. The antimicrobial activity of various extracts of *Plectranthus ambionicus* and *Phyllanthus amarus* showed varied levels of antimicrobial activity against the studied bacterial and fungal pathogens. The mean zone of inhibition produced by all the tested extracts ranged from 7.0 ± 0.50 mm to 29.5 ± 0.50 mm. The MIC, MBC and MFC values were between 15.62 and $1000 \,\mu\text{g/mL}$. The ethyl acetate extract of *Plectranthus ambionicus* showed good antimicrobial activity with the highest mean zone of inhibition ($29.5 \pm 0.5 \, \text{mm}$), lowest MIC ($15.62 \, \mu\text{g/mL}$) and MBC ($31.25 \, \mu\text{g/mL}$) values followed by ethyl acetate extract of *Phyllanthus amarus* ($23.6 \pm 0.50 \, \text{mm}$; MIC=62.5; MBC= $125 \, \mu\text{g/mL}$) against *Aspergillus fumigates*.

Key words: Antibacterial activity; Antifungal activity; Plectranthus ambionicus; Phyllanthus amarus; Solvent extracts.

Introduction

Plants are the rich resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants have played important role in the traditional and orthodox system of medicine in the curing of different types of diseases. Analysis of different species of medicinal plants for biological active components known to have pharmacological properties have been conducted and most of the

studied plants have shown antimicrobial property (Rabe et al., 1997; Ongsakul et al., 2009). The active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Sheriff and Modern, 2009). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of antibiotic prototypes (Afolayan, 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). The plants characteristics of the that inhibit microorganisms and are important for human health have been researched in laboratories since 1926. Traditional healing systems around the world that utilize herbal remedies are an important source of discovery of new antibiotics. All this has

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resulted in severe consequences including increased treatment failure and health care cost. This has urged the microbiologists all over the world to formulate new antimicrobial agents and evaluate the efficacy of natural plant products as the substitute for chemical antimicrobial agents (Pattnaik and Sharma, 2004; Alviano and Alviano, 2009). Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season, climate and particular growth phase. Leaf is one of the highest accumulatory plant parts of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Cowan, 1999).

Materials and methods

Collection of plant materials

Leaves of *Phyllanthus amarus*, *Plectranthus ambionicus* were collected from various places of Cuddalore district of Tamil Nadu, India. The voucher specimens were deposited at the Herbarium, Department of Botany, Annamalai University.

Preparation of crude extracts

The collected leaves were first washed with tap water and then surface sterilized in 10 per cent sodium hypochlorite to prevent the contamination of any microbes and again washed with distilled water. The leaf samples were shade dried followed by oven drying (at 60°C) and milled in an electrical blender. The powdered leaves (500g) were extracted separately one by one with hexane, chloroform, ethyl acetate and methanol in soxhlet apparatus. Each extract was extracted with respective solvent (1500 ml \times 4) for 72 hours. The extracts were pooled and the solvents were evaporated using a rotary evaporator (Hei-VAP advantage HB/HL/G1, Heidolph, Germany) under reduced pressure at 40°C. The crude extracts were kept at 4°C until further antibacterial assay.

Antimicrobial assay

Microorganisms Anti bacterial activity was tested against four strains of Gram positive bacteria viz. *Bacillus subtilis*, *B. pumilus*, *Micrococcus luteus*, *Staphylococcus aureus* three

strains of Gram negative bacteria viz. *Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli* and three species of fungi viz. *Aspergillus niger, A. flavus* and *A. fumigates*. The microbial strains were collected from Raja Muthaiah Medical College, Annamalai Nagar. The stock cultures were maintained on nutrient agar medium (for bacteria) and Sabouraud dextrose agar medium (for fungi) at 4°C.

Disc diffusion assay

Antimicrobial susceptibility test of the crude extracts were tested against the above mentioned Gram positive, Gram negative bacteria and fungi by disc diffusion method (Bauer et al., 1966). Petri plates were prepared with 20ml of sterile Muller Hinton Agar (Himedia, Mumbai) for bacteria and 20ml of Sabouraud dextrose agar (SDA) for fungi. The twenty four hours prepared test inoculums were swabbed on the top of the solidified media and allowed to dry for 10 minutes. Previously prepared extracts were impregnated with discs at concentrations of 1000, 500, 250 µg/ml and were placed aseptically on plates with appropriate controls. The loaded discs were placed on the surface of the medium and left for 30 minutes at room temperature. Negative control was prepared using 10 % DMSO. For bacteria, Ciprofloxacin (5µg/disc) and for fungi, Ketaconozole (10µg/disc) were used as positive controls. Finally, the inoculated plates were incubated at 37 °C for 24 h (for bacteria) and 35 °C for 48 h (for Aspergillus). The inhibition zones were observed including the diameter of the disc (6 mm).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of the crude extracts were tested in Mueller Hinton broth for bacteria and Sabouraud dextrose broth for mycelial fungi to get the concentrations of 1000-15.2 μg/ml by the broth macro dilution method (Ericsson *et al.*, 1971). The culture tubes were incubated at 37°C for 24 h (bacteria) and at 35°C for 48 h (mycelial fungi).

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC of the crude extracts were determined 13 by plating 100 µl samples from each MIC assay with growth inhibition into freshly

Table. 1 Antimicrobial activity of Plectranthus amboinicus against bacterial and fungal strains

Sl. No	Plant - extracts/ - solvents		Mean zone of in oncentration of	MIC	MBC			
		1000	500	250	Control (10 µg/disc)	(μg/mL)	(μg/mL)	
1.	Bacillus subtilis				(1 1 2 2 2 2 2 2 2			
	Hexane	16.5 ± 0.50	14.8 ± 0.28	11.3 ± 0.57	30.6 ± 0.86	125	250	
	Chloroform	18.1 ± 0.57	16.5 ± 0.50	13.5 ± 0.50	29.0 ± 0.50	125	250	
	Ethyl acetate	21.6 ± 0.76	19.0 ± 0.50	17.3 ± 0.57	28.3 ± 0.28	62.5	125	
	Methanol	19.0 ± 0.50	16.1 ± 0.28	14.1 ± 0.28	31.3 ± 0.57	62.5	125	
2.	Bacillus pumilus							
	Hexane	18.5 ± 0.50	15.1 ± 0.28	13.3 ± 0.57	28.0 ± 0.50	125	250	
	Chloroform	20.0 ± 0.50	17.5 ± 0.50	15.8 ± 0.71	30.3 ± 0.57	62.5	125	
	Ethyl acetate	22.1 ± 0.28	19.8 ± 0.76	16.6 ± 0.76	29.6 ± 0.76	62.5	125	
	Methanol	20.0 ± 0.50	17.1 ± 0.76	15.5 ± 0.50	29.6 ± 0.76	62.5	125	
3.	Micrococcus lute	us						
	Hexane	18.3 ± 0.76	16.0 ± 0.50	14.1 ± 0.28	29.3 ± 0.28	125	250	
	Chloroform	20.0 ± 0.50	17.8 ± 0.28	15.5 ± 0.50	29.8 ± 0.76	62.5	125	
	Ethyl acetate	21.7 ± 0.28	19. 3 ± 0.28	16.0 ± 0.50	30.0 ± 0.50	62.5	125	
	Methanol	18.3 ± 0.28	16.1 ± 0.28	14.5 ± 0.50	28.3 ± 0.57	125	250	
4.	Staphylococcus a	ureus						
	Hexane	21.5 ± 0.50	18.3 ± 0.78	16.5 ± 0.50	30.1 ± 0.28	31.25	62.5	
	Chloroform	23.0 ± 0.50	21.1 ± 0.28	19.5 ± 0.50	28.6 ± 0.76	31.25	62.5	
	Ethyl acetate	29.5 ± 0.50	26.3 ± 0.78	23.1 ± 0.28	28.6 ± 0.76	15.62	31.25	
	Methanol	26.0 ± 0.50	23.1 ± 0.28	20.0 ± 0.50	29.3 ± 0.57	31.25	62.5	
5.	Escherichia coli							
	Hexane	14.0 ± 0.50	12.6 ± 0.57	10.5 ± 0.50	29.8 ± 0.76	250	500	
	Chloroform	16.8 ± 0.76	13.5 ± 0.50	11.8 ± 0.76	28.3 ± 0.57	250	500	
	Ethyl acetate	19.6 ± 0.28	16.8 ± 0.76	14.5 ± 0.50	27.3 ± 0.57	125	250	
	Methanol	17.5 ± 0.50	15.3 ± 0.28	13.1 ± 0.28	27.3 ± 0.57	125	250	
5.	Pseudomonas aeruginosa							
	Hexane	14.6 ± 0.78	12.5 ± 0.50	10.0 ± 0.50	28.0 ± 0.50	250	500	
	Chloroform	15.2 ± 0.56	13.0 ± 0.50	$11.8 \pm .76$	26.6 ± 0.76	250	500	
	Ethyl acetate	18.0 ± 0.50	16.5 ± 0.50	$14.0 \pm .50$	27.3 ± 0.28	125	250	
	Methanol	16.6 ± 0.76	14.1 ± 0.28	$12.2 \pm .38$	30.3 ± 0.57	125	250	
7.	Methanol 10.0 ± 0.70 14.1 ± 0.28 $12.2 \pm .38$ 30.3 ± 0.57 12.5 2.50 <i>Klebsiella pneumoniae</i>							
•	Hexane	16.3 ± 0.28	14.0 ± 0.50	11.1 ±0.28	28.8 ± 0.76	125	250	
	Chloroform	17.0 ± 0.50	15.0 ± 0.50	13.3 ± 0.28	31.0 ± 0.50	125	250	
	Ethyl acetate	20.5 ± 0.50	17.5 ± 0.50	15.5 ± 0.50 15.5 ± 0.50	29.8 ± 0.76	62.5	125	
	Methanol	18.3 ± 0.28	16.0 ± 0.50	13.8 ± 0.28	27.6 ± 0.78	62.5	125	
3.	Aspergillus niger	10.5 ± 0.20	10.0 ± 0.50	13.0 ±0.20	27.0 ± 0.20	02.5	123	
0.	Hexane	14.5 ± 0.50	12.0 ± 0.50	10.1 ±0.28	18.3 ± 0.57	250	500	
	Chloroform	16.0 ± 0.50	13.6 ± 0.76	11.5 ± 0.50	19.8 ± 0.76	250	500	
	Ethyl acetate	18.3 ± 0.57	16.5 ± 0.70	13.8 ± 0.76	20.0 ± 0.70	62.5	125	
	Methanol	16.3 ± 0.37 16.1 ± 0.28	13.5 ± 0.50	11.6 ± 0.57	20.6 ± 0.76	125	250	
9.	Aspergillus flavus	10.1 ± 0.26	13.3 ± 0.30	11.0 ±0.37	20.0 ± 0.70	123	230	
	Hexane	13.7 ± 0.78	11.0 ± 0.50	9.1 ± 0.28	20.3 ± 0.28	250	500	
	Chloroform	13.7 ± 0.78 14.0 ± 0.50	12.6 ± 0.76	9.1 ± 0.28 9.5 ± 0.50	20.3 ± 0.28 22.8 ± 0.57	250	500	
	Ethyl acetate	14.0 ± 0.50 16.3 ± 0.57	12.0 ± 0.70 14.1 ± 0.50	12.8 ±0.76	19.3 ± 0.57	125	250	
	Methanol	10.3 ± 0.37 14.1 ± 0.28	14.1 ± 0.30 12.5 ± 0.50	12.8 ± 0.76 10.6 ± 0.57	19.3 ± 0.37 18.8 ± 0.76	250	500	
10	Aspergillus fumigat		14.5 ± 0.50	10.0 ±0.3 /	10.0 ± 0.70	230	300	
10.	Asperguus jumigai Hexane		12 2 ± 0 20	10 1 ±0 20	22.6 ± 0.76	125	250	
		14.0 ± 0.50	12.3 ± 0.28	10.1 ± 0.28	22.6 ± 0.76	125		
	Chloroform	16.5 ± 0.50	14.5 ± 0.50	12.5 ± 0.50	22.3 ± 0.57	125	250	
	Ethyl acetate	18.5 ± 0.50	16.6 ± 0.28	14.8 ± 0.76	21.8 ± 0.76	62.5	125	
	Methanol	17.0 ± 0.50	15.8 ± 0.28	13.1 ±0.28	20.3 ± 0.28	125	250	

^aDiameter of zone of inhibition (mm) including the disc diameter of 6 mm; ^bMean of three assays; ± - Standard deviation

Table 2. Antimicrobial activity of *Phyllanthus amarus* against bacterial and fungal strains

Sl. No	Plant - extracts/ - solvents		Mean zone of in oncentration of	MIC	MBC			
		1000	500	250	Control (10 µg/disc)	MIC (μg/mL)	MBC (μg/mL)	
1.	Bacillus subtilis				(10 µg/uise)			
	Hexane	13.2 ± 0.38	10.1 ± 0.78	8.0 ± 0.50	27.3 ± 0.57	250	500	
	Chloroform	14.9 ± 0.33	11.5 ± 0.50	9.4 ± 0.76	28.0 ± 0.50	125	250	
	Ethyl acetate	17.1 ± 0.86	15.0 ± 0.50	13.6 ± 0.68	27.1 ± 0.28	125	250	
	Methanol	16.5 ± 0.50	14.7 ± 0.28	11.8 ± 0.58	28.6 ± 0.76	125	250	
2.	Bacillus pumilus							
	Hexane	14.9 ± 0.36	12.6 ± 0.28	10.3 ± 0.57	30.0 ± 0.50	250	500	
	Chloroform	16.0 ± 0.50	14.8 ± 0.33	12.0 ± 0.50	30.3 ± 0.57	125	250	
	Ethyl acetate	19.1 ± 0.78	16.5 ± 0.50	14.8 ± 0.83	29.6 ± 0.76	62.5	125	
	Methanol	17.0 ± 0.50	15.6 ± 0.36	12.8 ± 0.33	28.6 ± 0.76	125	250	
3.	Micrococcus lute	us						
	Hexane	14.0 ± 0.50	12.6 ± 0.28	10.8 ± 0.33	27.3 ± 0.28	250	500	
	Chloroform	15.4 ± 0.43	13.1 ± 0.28	10.3 ± 0.28	28.8 ± 0.76	250	500	
	Ethyl acetate	17.5 ± 0.50	15. 8 ± 0.33	13.4 ± 0.78	29.0 ± 0.50	125	250	
	Methanol	16.1 ± 0.28	13.5 ± 0.50	11.8 ± 0.26	26.3 ± 0.57	250	500	
1.	Staphylococcus a							
	Hexane	19.5 ± 0.50	16.8 ± 0.28	15.3 ± 0.78	25.1 ± 0.28	62.5	125	
	Chloroform	20.7 ± 0.38	18.3 ± 0.76	16.5 ± 0.50	24.6 ± 0.76	62.5	125	
	Ethyl acetate	23.0 ± 0.50	20.6 ± 0.38	18.9 ± 0.33	27.6 ± 0.76	62.5	125	
	Methanol	21.0 ± 0.50	18.5 ± 0.50	16.8 ± 0.28	29.3 ± 0.57	62.5	125	
5.	Escherichia coli	21.0 = 0.00	10.0 = 0.00	10.0 = 0.20	27.0 = 0.07			
	Hexane	12.8 ± 0.68	10.5 ± 0.50	9.3 ± 0.38	28.8 ± 0.76	250	500	
	Chloroform	13.5 ± 0.50	11.1 ± 0.83	8.8 ± 0.38	26.3 ± 0.57	250	500	
	Ethyl acetate	18.0 ± 0.50	14.6 ± 0.28	12.0 ± 0.50	27.3 ± 0.57	125	250	
	Methanol	15.0 ± 0.50	13.8 ± 0.86	11.9 ± 0.86	29.3 ± 0.57	250	500	
6.	Pseudomonas aeruginosa							
	Hexane	14.0 ± 0.50	12.7 ± 0.33	10.0 ± 0.50	24.0 ± 0.50	250	500	
	Chloroform	16.3 ± 0.28	14.8 ± 0.28	12.5 ± 0.50	27.6 ± 0.76	125	250	
	Ethyl acetate	17.8 ± 0.38	15.6 ± 0.33	13.5 ± 0.50	27.3 ± 0.28	125	250	
	Methanol	16.2 ± 0.28	14.5 ± 0.50	12.3 ± 0.86	30.3 ± 0.57	125	250	
' .	Methanol 16.2 ± 0.28 14.5 ± 0.50 12.3 ± 0.86 30.3 ± 0.57 125							
•	Hexane	15.0 ± 0.50	13.6 ± 0.28	11.8 ±0.38	28.8 ± 0.76	250	500	
	Chloroform	16.1 ± 0.36	14.5 ± 0.50	12.4 ± 0.57	27.0 ± 0.76	125	250	
	Ethyl acetate	18.5 ± 0.50	16.5 ± 0.50 16.5 ± 0.50	12.4 ± 0.57 14.3 ± 0.68	28.8 ± 0.76	125	250	
	Methanol	17.1 ± 0.78	15.8 ± 0.78	13.6 ± 0.28	29.3 ± 0.28	125	250	
8.	Aspergillus niger	17.1 ± 0.76	13.6 ± 0.76	13.0 ±0.26	27.3 ± 0.26	123	230	
	Hexane	14.0 ± 0.50	12.5 ± 0.50	10.8 ±0.28	19.3 ± 0.57	250	500	
	Chloroform	15.2 ± 0.68	12.3 ± 0.30 13.8 ± 0.35	10.8 ± 0.28 11.5 ± 0.50	18.8 ± 0.76	250	500	
	Ethyl acetate	17.8 ± 0.78	15.8 ± 0.53 15.5 ± 0.50	13.5 ± 0.50	18.0 ± 0.70 18.0 ± 0.50	125	250	
	Methanol	17.8 ± 0.78 16.1 ± 0.78	13.5 ± 0.50 14.5 ± 0.50	13.3 ± 0.50 12.3 ± 0.58	17.6 ± 0.76	125	250	
9.	Aspergillus flavus	10.1 ± 0.78	14.5 ± 0.50	12.3 ±0.36	17.0 ± 0.70	123	230	
9.	Hexane	12.0 ± 0.50	9.5 ± 0.50	8.0 ± 0.50	16.3 ± 0.28	500	1000	
	Chloroform	12.0 ± 0.30 12.6 ± 0.86	9.3 ± 0.30 10.2 ± 0.35	9.3 ± 0.86	16.3 ± 0.28 19.8 ± 0.57	500	1000	
	Ethyl acetate	12.0 ± 0.80 15.0 ± 0.50	10.2 ± 0.35 13.0 ± 0.50	9.3 ± 0.86 10.3 ± 0.78	19.8 ± 0.57 20.3 ± 0.57	250	500	
	Methanol			9.8 ± 0.28		500	1000	
10	Aspergillus fumigat	13.1 ± 0.76	11.4 ± 0.76	9.8 ±0.28	18.8 ± 0.76	300	1000	
10.	Asperguus jumiga Hexane		12.5 - 0.50	11.0.10.60	10 () 0 7(250	500	
		15.3 ± 0.67	13.5 ± 0.50	11.8 ± 0.68	18.6 ± 0.76	250	500	
	Chloroform	16.6 ± 0.45	14.1 ± 0.86	12.1 ± 0.28	17.3 ± 0.57	125	250	
	Ethyl acetate	18.0 ± 0.50	16.1 ± 0.28	13.5 ± 0.50	18.8 ± 0.76	62.5	125	
	Methanol	16.5 ± 0.50	14.3 ± 0.86	12.8 ± 0.68	20.3 ± 0.28	125	250	

^aDiameter of zone of inhibition (mm) including the disc diameter of 6 mm; ^bMean of three assays; ± - Standard deviation

prepared Mueller Hinton agar (for bacteria) and Sabouraud dextrose agar (for mycelial fungi). The plates were incubated at 37°C for 24 h (bacteria) and at 35°C for 48 h (mycelial fungi).

Statistical Analysis

All the data of microbial activities were examined as mean \pm SD. One-way analysis of variance (ANOVA) was carried out to determine the significant differences (P <0.05) between the means. The analyses were carried out using SPSS package software, 11.5 (SPSS Inc., Chicago, IL).

Results

In the present investigation the antimicrobial activity of different solvent extracts of leaves of Plectranthus amboinicus and Phyllanthus amarus were screened against bacterial and fungal stains and results are presented in Table 1. All the extracts produced the mean zone of inhibition ranged between 9.1 ± 0.28 mm and 29.5 ± 0.50 mm, MICs were from 15.62 to 250 µg/mL and MBC/MFC were from 31.25 to 500 µg/mL. Among all the extracts tested, the ethyl acetate extract recorded the highest mean zone of inhibition (29.5 \pm 0.50 mm) at 1000 µg/disc concentration. The lowest MIC (15.62 μg/mL) and MBC (31.25 μg/mL) values were recorded with ethyl acetate against Staphylococcus aureus at 1000 µg/disc concentration. With regard to fungal strains tested, Aspergillus fumigatus showed the highest susceptibility against ethyl acetate extract of leaves of P. amboinicus with a mean zone of inhibition of 18.5 ± 0.50 mm at 1000μg/disc concentration. The lowest values of MIC = 62.5 μ g/mL and MFC = 125 μ g/mL were produced in ethyl acetate extract against Aspergillus fumigatus at 1000 µg/disc concentration. The lowest activity of bacteria (11.1 \pm 0.28 mm) was observed in hexane extract against Klebsiella pneumoniae at 250 µg/disc concentration. The lowest mean zone of inhibition for fungi (9.1 \pm 0.28 mm) was recorded in hexane extract against Aspergillus flavus at 250 µg/disc concentration.

The results of antimicrobial activity of different solvent extracts of leaves of *Phyllanthus amarus* is presented in Table 2. The mean zone of inhibition for all extracts ranged between 8.0 ± 0.50 mm and 23.0 ± 0.50 mm, MIC were from 62.5 to 500 µg/mL and MBC/MFC were from 125 to 1000 µg/mL. Among all the extracts tested, the ethyl

acetate extract showed the highest mean zone of inhibition (23.0 \pm 0.50 mm) at 1000 μ g/disc concentration. The lowest values of MIC (62.5 μg/mL) and MBC (125 μg/mL) were observed in all the extracts against Staphylococcus aureus and ethyl acetate extract against Bacillus pumilus at 1000 µg/disc concentrations. With regard to Aspergillus species tested, the highest mean zone of inhibition (18.0 \pm 0.50 mm), the lowest MIC (62.5 μg/mL) and MFC (125 μg/mL) were recorded in ethyl acetate extract of leaves of P. amarus against Aspergillus fumigatus at 1000 µg/disc concentration.

Discussion

The hexane, chloroform, ethyl acetate and methanol extracts of Plectranthus amboinicus and Phyllanthus amarus showed broad spectrum of antimicrobial activity against all microorganisms tested. In the present study, Gram positive bacteria were more susceptible than Gram negative and fungal pathogens. The differences in the antimicrobial activity of crude extracts may be due to the amount of antimicrobial agent present in the extract (Barbour et al., 2004). The present study showed the highest antimicrobial activity with lower MIC values and the same trend was observed by Kannathasan et al., 2011. The methanol extract of V. peduncularis showed the highest mean zone of inhibition (22.670 ±0.667 mm) and the lowest MIC (62.5 μ g /mL) and MBC values (125.0 μ g /mL) against S. aureus followed by M. luteus $(21.670 \pm 0.667 \text{ mm}; \text{MIC } (62.5 \mu \text{g/mL}). \text{ Bakht } et$ al., 2012 investigated antibacterial activity of different solvent extracts of Nicotiana tabacum extracts at different concentrations. Ethyl acetate extract was more effective to control B. cereus and E. carotovora followed by butanol extract against S. aureus and A. tumefaciens. However, Abrimi et al., 2012. studied the antimicrobial activity of chloroform, acetone and methanol extracts of Enicostemma littorale leaf, stem and root against various Gram positive, Gram negative and fungal pathogens. The chloroform extract of E. littorale showed the highest antibacterial activity (20 mm) against B. subtilis with the MIC values of >8.5 mg/mL. This value is much higher than that of the present study. But, all the extracts of E. mlittorale did not inhibit the growth of the studied fungal pathogens, A. fumigatus and A. flavus. Moreover, varying degree of sensitivity of test microorganisms

may be due to inherent tolerance of microorganism (Agil and Ahmad 2003). Muthukumaran et al. (2011) screened the crude extract of Cassia auriculata for antimicrobial activity and recorded differential zone of inhibition against Escherichia coli (17.9 mm), Staphylococcus aureus, (18 mm), Bacillus subtilis, (21 mm), Pseudomonas aeruginosa (18 mm) and fungi, Candida albicans (24 mm), Candida tropicalis, (23 mm) and Aspergillus niger (22.3 mm) at 100 µg/mL concentration. Similarly, Doshi et al. (2011) recorded with 80% aqueous methanol extract of flowers of Cassia auriculata against S. aureus (16 mm), S. typhi (16 mm), E. coli (15 mm) and B. subtilis (12 mm). The greater resistance of Gram negative bacteria to plant extracts has been documented previously for Plectranthus sp. (Rabe and van Staden, 1994). In the present study, the ethyl acetate extract of Plectranthus amboinicus was identified for its potential antimicrobial activity against all the microorganisms tested. Based on this preliminary study, isolation and identification of antimicrobial molecule from ethyl acetate extract of *Plectranthus amboinicus* is in progress.

Conclusion

The ethyl acetate extract of *Plectranthus* amboinicus had a potential antimicrobial activity against all the microorganisms tested. Based on this preliminary study, isolation and identification of antimicrobial molecule from ethyl acetate extract of *Plectranthus* amboinicus is in progress.

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