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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF DIFFERENT PARTS OF *STACHYTARPHETA URTICIFOLIA* (SALISB) SIMS

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ABSTRACT

Objective: The aim of the current study was to evaluate the antimicrobial activity of various organic solvent extracts of different parts (root, stem, leaves and inflorescence) of *Stachytarpheta urticifolia* (Salisb) Sims.

Methods: Antimicrobial activity was tested against Gram-positive, Gram-negative bacterial strains and fungal strains by observing the zone of inhibition. Methanol, ethyl acetate and hexane were the organic solvents used for preparing the extracts of different parts of *S. urticifolia.* The bacterial strains used in the study were *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumonia*, and *Enterobacter aerogenes*) and the fungal strains were *Aspergillus niger, Aspergillus flavus*, *Penicillium notatum*, *Candida albicans*, *Candida utilis* and *Metarhizium anisopliae.* Disc diffusion technique was used to assess the antimicrobial activity of plant extracts against bacterial and fungal strains. The diameter of zone of inhibition was taken as an indicator of antimicrobial effect.

Results: It was observed that ethyl acetate and methanol extracts of all the plant parts showed maximum inhibitory effect on all tested bacteria where as only ethyl acetate extract showed inhibitory effect on all fungal strains tested.

Conclusion: Low minimum inhibitory concentration (MIC) values were observed in ethyl acetate extract of leaf and inflorescence which were relatively low when compared with standard drug. The ethyl acetate leaf and inflorescence extracts exhibited high antimicrobial activity when compared to others.

Keywords: Antimicrobial activity, *Stachytarpheta urticifolia* (Salisb) Sims, Disc diffusion method, Minimum Inhibitory Concentration (MIC)

INTRODUCTION

Microorganisms such as bacteria, fungi, viruses, nematodes cause infections in human resulting serious damages in developing countries in the world. In recent years, multiple drug resistance in human pathogenic microorganism has been observed due to indiscriminate use of commercially available antimicrobial drugs used in the treatment of such diseases. Nature has bestowed with very rich botanical wealth and a large number of diverse types of plants grow in different parts of the world. Intensive efforts have been made by the various researchers to discover clinically useful antimicrobial compounds in the last century [1-3]. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. In developing countries plant based medicines are getting importance to prevent many dreadful diseases [4]. Plant extracts were used for the treatment of various diseases and this forms the basis for Indian system of Ayurvedic medicine. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [5-7]. It has been proved that various plants extracts posses bacteriostatic and bactericidal effects [8]. Much work has been done on ethno medicinal plants in India. These reports are restricted to mainly a few medicinal plant species. Most of them require a detailed study particularly with regard to the antimicrobial properties because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Therefore, there is a need to look for substances from other sources with proven antimicrobial activity.

MATERIALS AND METHODS

Collection and identification of plant material

The present plant *Stachytarpheta urticifolia* (Salisb) sims selected for investigation was collected from Damuku rai village (Araku valley), Ananthagiri mandal, Visakhapatnam (District), Andhra Pradesh (State). The plant was taxonomically identified and authenticated by the Department of Botany, Andhra University, Visakhapatnam Dist, Andhra Pradesh, India.

Preparation of extracts

Fresh root, stem, leaf and inflorescence of the plant were washed thoroughly under running tap water, shade dried and used for extraction. Dried root, stem, leaf and inflorescence were homogenized to a fine powder and stored in airtight bottles. 10 gm of root, stem, leaf and inflorescence powders were extracted separately with 100 ml of solvent (Methanol, Ethyl acetate and Hexane) for 24hours by using Soxhlet apparatus. The crude extracts were then concentrated in vacuum at 40°C by using *rota vapor* apparatus (PBU 6D model; Superfit). The crude concentrated extracts obtained were stored in deep freezer at -20°C until further use.

Test microorganisms and microbial culture

Antibacterial activity of crude plant extracts was tested against Gram negative bacteria (*Escherichia coli* (MTCC 448), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 741), *Serratia marcescens* (MTCC 8708), *Enterobacter aerogenes* (MTCC 111) and Gram-positive bacteria (*Bacillus subtilis* (MTCC 2394)). Antifungal activity was tested on selected fungal cultures (*Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 2456), *Penicillium notatum* (MTCC 5108), *Candida albicans* (MTCC 227) and *Candida utilis* (MTCC 773)). These microorganisms were procured from Microbial Type Culture Collection centre, IMTECH- Chandigarh and purity of the cultures was confirmed by Dept. of Microbiology, GITAM Institute of Science, GITAM University, Visakhapatnam. One entomopathogenic fungal culture *Metarhizium anisopliae* (ARSEF 8736) isolated from infected silk worm was included as out group in the current investigation. Bacterial strains were cultivated at 37°C and maintained on nutrient agar slants at 4°C. Fungal strains were cultivated at 25°C and maintained on potato dextrose agar slants at 4°C. Isolated single colonies of microbial cultures were stored on agar slants at 4°C until further use. Then all the bacterial strains were incubated at 37°C for 24h and fungi at 27°C for 48h.The test organisms were grown overnight in respective broth media.

Screening of antimicrobial activity

Antimicrobial activity of different parts of S.*urticifolia* in various organic extracts were evaluated by the paper disc diffusion method

[9].For the determination of antibacterial activity, over night grown bacterial cultures were adjusted to 0.5McFarlnad turbidity standards [10].For the determination of antifungal activity, all the fungal isolates were first adjusted to the concentration of 10^6 cfu/ml respectively using spread plate technique. About 10 µl of each plant extract (100 mg/ml) were loaded on sterile Whatman No. 1 filter paper disc (9 mm) and placed on the nutrient agar and potato dextrose agar plates spread with the respective microorganism. The pure solvents in equal volume served as negative control and streptomycin (10µg/ml) for bacteria and Albendazole (10 µg/ml) for fungi antibiotic disc was used as positive control. Then the bacterial culture plates were incubated at 37°C for 18-24 hrs while the fungal cultures were incubated at 25°C-27°C for 48 hours. Antibacterial activity was determined by measurement of zone of inhibition of growth around each paper disc (mm). For each extract (test) three replicate trials were conducted against each organism. Each zone of inhibition was measured with a ruler and compared with standard [11].

Minimum inhibitory concentration (MIC) for bacteria

The minimum inhibitory concentration (MIC) of the solvent extracts was estimated against bacterial strains. It was carried out by the method as described [12]. The determination of the MIC was done with different concentrations of solvent extracts (10, 25, 50, 75,100 µg/ml). To 10ml of nutrient broth 0.5ml of the extract with different concentration was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity units' bacterial isolates was inoculated into the test tubes. The procedure was repeated on the test organisms using the standard antibiotic streptomycin. Tubes containing only nutrient broth seeded with the test organism serve as control. Tubes containing bacterial cultures were then incubated at 37°C for 24h. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth [13, 14].

RESULTS

Antibacterial assay

The results of antibacterial activities of the different parts of *S.urticifolia* are given in **Table1**. Among the three solvent extracts of different parts of the plant tested, ethyl acetate extract had greater antibacterial potential followed by methanol extract. Hexane extract had no antibacterial potential. The highest zone of inhibition was observed for ethyl acetate leaf extract against the bacteria, *Pseudomonas aeruginosa*, (17±0.5mm) and *Serratia marcescens* (17±0.9mm) followed by other microorganism (**Table 1**).

Table 1: Anti bacterial activity of *Stachytarpheta Urticifolia* (Salisb) Sims plant parts (root, stem, leaf and inflorescence) in methanol, ethyl acetate and hexane extracts

Plant part	Extract	Zone of inhibition of crude plant parts in mm (Mean± SD) [b] against bacterial cultures[a]					
		A	B	C	D	E	F
Root	Methanol	8 ± 0.7	5 ± 0.6	9 ± 0.2	4 ± 0.5	0	4 ± 0.6
	Ethyl acetate	12 ± 0.4	8 ± 0.4	12 ± 0.6	14 ± 0.7	13 ± 0.2	0
	Hexane	0	0	0	0	8 ± 0.4	0
Stem	Methanol	3 ± 0.5	0	0	6 ± 0.2	0	6 ± 0.4
	Ethyl acetate	12 ± 0.1	12 ± 0.3	0	10 ± 0.7	8 ± 0.5	14 ± 0.5
	Hexane	0	0	0	0	0	0
Leaf	Methanol	9 ± 0.3	0	10 ± 0.1	0	0	0
	Ethyl acetate	14 ± 0.3	14±0.2	17 ± 0.5	17 ± 0.9	14 ± 0.3	16±0.5
	Hexane	0.00	0	0	0	0	0
Inflorescence	Methanol	6 ± 0.7	7 ± 0.1	8 ± 0.7	0	0	0
	Ethyl acetate	16 ± 0.5	17 ± 0.8	10 ± 0.6	15 ± 0.4	8 ± 0.5	13 ± 0.8
	Hexane	0	0	0	0	0	0
Antibiotic [standard]	Streptomycin	15±0.7	18±0.5	19±0.5	19±1.0	16±0.2	15±0.4

[a]A= *Escherichia coli*; B= *Klebsiella pneumoniae*; C = *Pseudomonas aeruginosa*; D = *Serratia marcescens*; E = *Enterobacter aerogenes*; F= *Bacillus subtilis*

[b]Values are mean of three replicates ±SD

Table 2: Minimum inhibitory concentration of *Stachytarpheta Urticifolia* (Salisb) Sims plant parts (root, stem, leaf and inflorescence) in methanol, ethyl acetate and hexane extracts

Bacteria[a]	plant extract	Minimum Inhibitory Concentration (MIC) Value[b]																			
		Root					Stem					leaf					Inflorescence				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
A	Methanol	++	++	++	+	+	++	+ +	+ +	+	+	++	++	+	+	+	++	++	+	+	+
	Ethyl acetate	+	+	+	*	-	+	+	*	-	-	+	*	-	-	-	+	*	-	-	-
	Hexane	++	++	++	++	+	++	++	++	++	++	++	++	++	++	+	++	+ +	++	+ +	+
B	Methanol	++	++	++	+	+	++	++	++	++	+	++	++	++	++	+	++	++	++	++	+
	Ethyl acetate	+	+	+	*	-	+	+	*	-	-	+	*	-	-	-	+	*	-	-	
	Hexane	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
C	Methanol	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++
	Ethyl acetate	++	+	+	+	*	+	+	*	-	-	+	*	-	-	-	+	*	-	-	-
	Hexane	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
D	Methanol	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	Ethyl acetate	++	+	+	*	-	++	++	+	*	-	+	*	-	-	-	+	*	-	-	-
	Hexane	++	++	++	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
E	Methanol	++	++	++	+	+	++	++	++	++	+	++	++	++	++	+	++	++	++	++	+
	Ethyl acetate	++	+	+	*	-	++	+	+	*	-	++	+	+	*	-	+	*	-	-	-
	Hexane	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
F	Methanol	++	++	++	++	+	++	++	++	++	+	++	++	++	++	+	++	++	++	++	+
	Ethyl acetate	+	+	*	-	-	+	+	*	-	-	+	*	-	-	-	+	*	-	-	-
	Hexane	++	++	++	++	+	++	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	++	++

[a]A= *Escherichia coli*; B= *Klebsiella pneumonia*; C = *Pseudomonas aeruginosa*; D = *Serratia marcescens*; E = *Enterobacter aerogenes*; F= *Bacillus subtilis*

[b]1=10 µg/ml, 2=25 µg/ml, 3= 50 µg/ml, 4= 75 µg/ml, 5= 100 µg/ml

++ = dense growth; + =little growth; * = MIC; - = No growth

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism [15]. Due to high antibacterial activity of the plant extracts, the MIC was determined only for bacteria (**Table 2**). The MIC values are ranging between 25µg/ml to100µg/ml. Lowest MIC value 25µg/ml was observed in the case of ethyl acetate leaf extract followed by methanol extracts.

Antifungal assay

The results of antifungal activities of different parts of *S.urticifolia* are given in **Table 3**. Among the three solvent extracts of different plant parts tested, ethyl acetate extract had greater antifungal potential. Methanol and hexane extracts had no antifungal activity. The highest zones of inhibition were observed for ethyl acetate inflorescence and leaf extracts against fungi, *Candida utilis* (15±0.8mm) and (12±0.1mm) respectively. The antifungal activity of inflorescence was almost equal to standard antifungal compound albendazole.

Table 3: Anti fungal activity of *Stachytarpheta Urticifolia* (Salisb) Sims plant parts (root, stem, leaf and inflorescence) in methanol, ethyl acetate and hexane extracts

Plant part	Extract	Zone of inhibition of crude plant parts in mm (Mean± SD) [b] against fungal cultures[a]					
		A	B	C	D	E	F
Root	Methanol	0	0	0	0	0	0
	Ethyl acetate	10 ± 0.4	9±0.8	12 ± 0.3	9±0.4	6 ± 0.3	0
	Hexane	0	0	0	0	0	0
Stem	Methanol	0	0	0	0	0	0
	Ethyl acetate	6 ± 0.5	0	7 ± 0.1	8 ± 0.2	9 ± 0.5	13 ± 0.2
	Hexane	0	0	0	0	0	0
Leaf	Methanol	0	0	0	0	0	0
	Ethyl acetate	11 ± 0.7	14 ± 0.9	15 ± 0.3	11 ± 0.5	12 ± 0.1	14 ± 0.1
	Hexane	0	0	0	0	0	0
Inflorescence	Methanol	0	0	0	0	0	0
	Ethyl acetate	5 ± 0.9	7 ± 0.6	12 ± 0.8	14 ± 0.7	15 ± 0.8	10 ± 0.8
	Hexane	0	0	0	0	0	0
Antibiotic [standard]	Albendazole	12±1.4	11±1.5	16±0.9	14±0.5	14±1.6	18 ± 0.6

[a]A= *Aspergillus niger*; B= *Aspergillus flavus*; C = *Penicillium notatum*; D = *Candida albicans*; E = *Candida utilis*; F= *Metarhizium anisopliae*

[b]Values are mean of three replicates ±SD

DISCUSSION

The ethnobotanical approach assumes that the traditional uses of plants can offer strong clues to the biological activity of plants and their usage in medical treatment. The results of the current investigation reveals that the organic solvent extracts (Methanol, ethyl acetate and hexane extracts) exhibited good antimicrobial activity because the antimicrobial compounds were either polar or non-polar and they were extracted only with the organic solvents[16,17]. Antimicrobials from natural sources have received much attention and efforts have been put into identify compounds that can act as suitable antimicrobial agents to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [18,19]. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [20,21]. The present study justifies the claimed uses of different parts *S.urticifolia* in the traditional system of medicine to treat various infectious diseases caused by the microorganisms. When the results of ethyl acetate extract of leaf are compared with controls it is more effective towards pathogenic microbes. The preliminary results of the crude extracts in the current investigation indicate that *S.urticifolia* leaf have high potential of antimicrobial activity along with the other parts of the plant.

CONCLUSION

The current investigation supports the traditional use of *S.urticifolia* to treat microbial infections. Among the different parts and different solvents tested, ethyl acetate extract of leaf showed high antibacterial activity with low MIC values. Highest antifungal activity was exhibited by ethyl acetate extract of leaf and inflorescence.

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