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Phytochemical Screening and Antibacterial Activity of *Stachytarpheta indica*

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Abstract: Phytochemical screening and antibacterial activity of aqueous and methanolic root extracts of *Stachytarpheta indica* Vahl. was assessed. Phytochemical analysis revealed the presence of flavonoids, terpenoides, tannins and reducing sugars. The antibacterial properties of both the aqueous and methanolic extracts were studied against clinically important bacteria viz. *Escherichia coli, Bacillus cereus*, *Pseudomonas aeruginosa* and *Enterobacter aerogens* by disc diffusion method. The aqueous extract showed significant activity against all the presently investigated species of bacteria which is comparable with standard antibiotic streptomycin. At the concentrations of 50-100µg /disc, aqueous extract showed significant zone of inhibition against *E. coli*, (14 mm), *B. cereus* (13 mm), *P. aeruginosa*, (17 mm) and *E. aerogens* (7 mm). Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) have been determined. The MIC values observed was 20, 30, 5 and 25µg/ml (for aqueous extract) and 40, 35, 20 and 30µg/ml (for methanolic extract) against *E. coli*, *B. cereus, P. aeruginosa* and *E. aerogens* respectively. Further isolation of active compound responsible for the activity could be the potential sources of new antimicrobial agents.

Key words: *Stachytarpheta indica* · Antibacterial Activity · Phytochemicals · MIC · MBC

INTRODUCTION

The usage of herbal plants as traditional health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is reported to have minimal side effects [1]. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies including medicinal herbs [2]. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous system of medicine or indirectly in the pharmaceutical preparations [3]. Ayurveda and Siddha are the two Indian traditional systems of medicine practiced in India wherein many herbs were used as therapeutics. There are several reports of antibiotic resistance of human pathogens to available antibiotics [4,5]. Hence, there is an urgent need to identify novel substances active towards highly resistant pathogens [6]. Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics [7]. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity [8].

Stachytarpheta indica commonly known as snake weed is belonging to the family Verbenaceae. A well-branched herb 2-3 ft, high with very long narrow spikes; flowers deep blue with white centre; a weed. Drought-tolerant; suitable for xeriscaping. The plant has been used locally as an Abortifacient and in the management of Asthma, Headache, Alopecia, Bronchitis, Bruise, Chest Cold, Constipation, Itch, Diarrhea, Skin Sore, Vermifuge, Dysentery, Dysmenorrhea, Erysipelas, Fever, Inflammation, Liver Disease, Poisoning, Tumor, Venereal Disease, Cataract, Sedative, Anti-Fertility, Rheumatism [9]. In northern Nigeria a decoction of the leaves with natron is given for dysentery in humans and for similar conditions in horses [10].

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This study is an attempt to determine the antimicrobial activity of root extracts (Aqueous and methonolic) of *Stachytarpheta indica* on selected pathogenic bacteria.

MATERIAL AND METHODS

Collection of Plant Material and Extract Preparation: The roots of *Stachytarpheta indica* were collected from in and around Mysore district, Karnataka, India in April, 2009 and was authenticated by the Department of Studies in Botany, University of Mysore. The collected roots were thoroughly washed with water and shade dried. The dried roots were coarsely powdered with the help of a blender. 10g of powdered root was homogenized with 50ml of methanol for the preparation of methanolic extract and for aqueous extract 10g of root powder was mixed with 80 ml of distilled water. Then the samples were incubated at room temperature for 24 hrs with intermittent shaking. The extracts were filtered using Whatmann No.1 filter paper and then concentrated in vaccum at 40°C. The weight of each residue was recorded and percentage yield was calculated. The extracts were transferred to glass vials and kept at 4°C until used.

Phytochemical Analysis: Phytochemical screenings were performed by using standard procedures [11,12]. Both the Aqueous and methanolic extracts were evaluated for the alkaloids, saponins, tannins, flavonoids, terpenoids, reducing sugars, anthraquinones and cardiac glycosides.

Test Microorganisms: The root extracts were tested for possible antibacterial activity in the disk assay using four pathogenic bacteria viz. *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa and Enterobacter aerogens.* The bacterial strains were supplied by Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial cultures were maintained at 4^0C on nutrient agar.

Antimicrobial Assay: The antimicrobial activity of the aqueous and methanolic extract was determined based on inhibition zones in disc Diffusion method described by Bauer *et. al.*[13]. Mueller-Hinton agar media was used for preparing test plates. Filter paper discs (Whatman No. 1) of 5 mm diameter were loaded with different concentrations of crude extracts (25-100 µg). Discs were completely dried and sterilized. 100 µl of cultures were spread on sterilized agar media and impregnated discs were placed on it and incubated for 24 hrs at 37°C. Streptomycin discs (10µg/disc) were used as standard drug. The diameter of zone of inhibition in mm was recorded after incubation. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained. The disc soaked with the same extraction solvent was used as control.

Determination of Minimum Inhibitory Concentration (MIC): The extracts that showed antimicrobial activity by disc diffusion method were subjected to MIC assay. The root extracts were diluted to obtain concentration ranging from 10 -100 µg /ml. The test containing 3ml of Muller Hinton broth and 0.1 ml bacterial suspensions and 0.1 ml plant extract were incubated at 37°C for 24hrs. Bacterial turbidity was measured at 650 nm to determine minimum inhibitory concentration against test pathogens. The MIC value was determined as the lowest concentration of the crude extract in broth medium that inhibited the visible growth of the test organism [14]. To determine the Minimum Bactericidal Concentration (MBC), all the MIC tubes, which did not show any turbidity, were streaked over the Muller-Hinton agar plates. The plates were incubated at 37°C for 18-24 hrs. The MBC was recorded as the lowest concentration that did not permit any visible growth on the plates after the period of incubation [14].

RESULTS AND DISCUSSION

Phytochemical analysis of the aqueous extract revealed the presence of Flavonoids, Terpenoids, Tannins and Reducing sugars. Methanolic extract shows presence of Terpenoids, Tannins and Reducing sugars (Table 1). The antimicrobial activities of various plants have been reported by many researchers [15,16]. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism.

In vitro antibacterial assay was performed to assess the efficacy of *Stachytarpheta indica* to inhibit the growth of pathogenic bacterial organisms. Both the aqueous and methanolic extract showed predominant inhibition against all the presently investigated species of bacteria (Table 2). Aqueous extract of *S. indica* exhibited strong antibacterial potential compared to methanolic extract. The efficiency was shown to be concentration dependent. Lower concentration (25 µg /disc) of aqueous

Table 1: Showing phytochemical constituents of root extracts of *S.indica.*

Sl.No.	Test	Methanolic extract	Aqueous extract
1	Flavonoids	--	++
2	Terpenoids	++	++
3	Tannins	++	++
4	Saponins	--	--
5	Alkaloids	--	--
6	Reducing sugars	++	++
7	Anthraquinones	--	--
8	Cardiac glycosides	--	--

++ Presence of constituent

-- Absence of constituent

Table 2: Antibacterial efficacy of the root extracts of *S. indica*. (Zone of inhibition measured in mm)

		Aqueous extract (µg/disc)				Methanolic extract (µg/disc)				
Sl. No.	Test organisms	25	50	75	100	25	50	75	100	Streptomycin (10 µg/disc)
1	*E.coli*	3±0.12	8±0.25	14±0.32	19±1.06	2±0.12	4±0.43	7±0.44	9±0.43	13 ± 0.76
2	*B.cereus*	2±0.21	5±0.15	8±0.45	13±0.33	1±0.02	3±0.21	4±0.21	6±0.32	12 ± 0.53
3	*P. aeruginosa*	8±0.24	17±0.61	21±0.78	23±0.73	4±0.21	7±0.32	12±0.67	14±0.95	18 ± 0.68
4	*E.aerogens*	4±0.11	7±0.22	12±0.14	16±0.51	2±0.13	3±0.12	5±0.32	6±0.52	5 ± 0.58

Values are shown in mean ± SE

Table 3: Antibacterial activity (MIC and MBC in µg/ml) of the root extracts of *S.indica*

		Aqueous extract		Methanolic extract	
Sl. No.	Test organisms	MIC	MBC	MIC	MBC
1	*E.coli*	20	40	40	80
2	*B.cereus*	30	60	35	70
3	*P. aeruginosa*	5	10	20	40
4	*E.aerogens*	25	50	30	60

extract exhibited 3, 2, 8 and 4 mm of inhibition zone against *E. coli*, *B. cereus, P. aeruginosa* and *E. aerogens* respectively. The methanolic extract showed 2, 1, 4 and 2 mm of inhibition zone at the concentration of 25µg /disc against *E. coli*, *B. cereus, P. aeruginosa* and *E. aerogens* respectively. At the higher concentrations (50-100µg /disc), aqueous extract showed significant activity when compared to methanolic extract against *E. coli*, (14 mm) followed by *B. cereus* (13 mm), *P. aeruginosa*, (17 mm) and *E. aerogens* (7 mm) which is comparable with standard antibiotic streptomycin (Table 2). The MIC and MBC of extracts of *S. indica* was given in Table 3. The MIC values observed was 20, 30, 5 and 25µg/ml (for aqueous extract) and 40, 35, 20 and 30µg/ml (for methanolic extract) against *E. coli*, *B. cereus, P. aeruginosa* and *E. aerogens* respectively. The minimal inhibitory concentration of *S. indica* against pathogens is less in methanolic extract compared to aqueous extract. The MBC values observed for aqueous extract was 40, 60, 10 and 50µg/ml against *E. coli*, *B. cereus, P. aeruginosa* and *E. aerogens* respectively. The present work clearly indicates that the aqueous extract effectively control the growth of bacteria. Further work is warranted to isolate and characterize the active principles available in the extracts of *S. indica.* It is quite sure that such components could be useful in developing drugs. Recently the attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [17,18]. The literature indicates that the antibacterial activity is due to different chemical agents present in the extract, Including essential oils (especially thymol), flavonoids and triterpenoids and other natural phenolic compounds or free hydroxyl groups. These are classified as active antimicrobial compounds [19]. Devi *et al.* [20] reported Antibacterial Activity of leaf extracts of 4 coastal living medicinal plants Viz. *Ocimum canum, Acalypha indica, Eclipta alba* and *Lawsonia inermis*. Hatil Hashim El-Kamali and Ehsan Musa Awad EL-Karim [21] studied the ethanolic, petroleum ether, ethyl acetate, methanolic and water

extracts of some medicinal plants (*Acacia nilotica* ssp. *nilotica* pods, *Lawsonia inermis* leaves, *Azadirachta indica* leaves, *Trigonella foenumgraecum* seeds and *Cordia sinensis* stem bark) for their antibacterial activity.

The present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human and plant systems. The phytochemical analysis revealed that the active principle responsible for the antibacterial activity is a phenolic compound.

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