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Antibacterial properties of leaf extracts of Strobilanthes cusia (Nees) Kuntze, a rare ethno-medicinal plant of Manipur, India

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Abstract : In the present study a rare ethno-medicinal plant species *Strobilanthes cusia* (Nees) Kuntze. ('Kum' in Manipuri) was tested for its antimicrobial properties. Leaf extracts in various solvents namely ethanol, methanol, acetone and petroleum ether was tested against five bacterial pathogens. The extracts showed variable antibacterial efficacy. The maximum antimicrobial activity was noted in the methanol extract against *Staphylococcus aureus* followed by *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella pneumonia*.
Key words: *Strobilanthes cusia, Antimicrobial activity, Agar well diffusion method.*

Introduction

Plants have been used as a source of medicine since the pre-histroric time. In India, Ayurvedic system of medicine has existed for over four thousand years. From ancient literature it is evident that the various parts of the plants were used in Siddha, Ayurvedha and Unani medicine for the treatment of disease of human beings [1].

The medicinal value of plants lies in some chemical substrates that produce a definitive physiological action on the human body. The use of plant extracts and phytochemicals, with established antimicrobial properties, could be of great significance in preventive and/or therapeutic approaches. The most important antimicrobial compounds of plants are alkoloids, flavonoids, tannins and phenolic compounds[2-5] .The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies [6-7]. Therefore, the search for new drugs from plants continues to be a major source of commercially consumed drugs. Contrary to synthetic drugs, antimicrobials of plant origin usually are not associated with many side effects and have an enormous anti-infective potential in numerous infectious diseases. In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as important source for discovery of new agents for treating various ailments related to bacterial infections.

Strobilanthes cusia (Nees) Kuntze (Assam indigo, Vern. 'Kum' in Manipuri), belongs to the family Acanthaceae, is an important medicinal plant species used traditionally in the state of Manipur, India. It is a glabrous shrub growing up to 5-6 ft tall in wild habitat. It is often cultivated for the dye in Manipur valley. It is reported that the leaf of *S. cusia* is used to treat influenza, epidemic cerebrospinal meningitis, encephalitis B, viral pneumonia, and mumps. It is also used for soar throat, apthae, inflammatory diseases with redness of skin, etc[8]. The entire fresh plant of *S. cusia* has anti-fungal activity and is used to treat athletes foot[9]. Recently, this

crude drug has also been used in Severe Acute Respiratory Syndrome(SARS). Some compounds obtained from *S. cusia* were tested for anti-herpes simplex virus type-1 (HSV-1) activity[10].

The present authors have collected fragmentary information on traditional medicinal uses of this species in Manipur. Considering the preliminary information, the plant species was investigated for antimicrobial activities against several selected pathogenic microorganism namely *Staphylococcus aureus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli* and *Klebsiella pneumonia*.

Materials And Method

Collection and identification of the plant materials

Fresh leaves of *Strobilanthes cusia* (Nees) Kuntze. were collected from different parts of Manipur during August-October 2012. The plant species was initially identified based on information of local medicine man and finally authenticated by consulting 'Assam' herbarium at Botanical Survey of India, Eastern Circle, Shillong. The voucher specimens were deposited in the Department of Biotechnology, Gauhati University, Assam, India for future reference.

Extraction of plant materials

The fresh plant leaves of *S. cusia* were separated from the stalks and thoroughly washed under tap water followed by rinsing with distilled water. The cleaned leaves samples were air dried at room temperature (26º C) for 2 weeks and then grounded to a uniform powder in order to increase the surface area of the sample. The powdered samples were soaked separately with different solvents ranging from non polar to polar in glass containers (Borosil Beaker). At the end of extraction it was passed through Whatmann filter paper No.42 (125mm). The extracts were concentrated using a rotary evaporator with the water bath set at 40°-60°C. The samples were stored at 4ºC until use[11].

Test Microorganisms

The cultures were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), housed at the Institute of Microbial Technology (IMTECH), Chandigarh, India. The cultures include two gram positive bacteria such as *Staphylococcus aureus* (MTCC 96), *Baccilus subtilis* (MTCC 441),and three gram negative bacteria as *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (MTCC 739), *Klebsiella pneumonia* (MTCC 432). The cultures were maintained on Nutrient agar slants. The cultures were revived with nutrient broth again by streaking to nutrient agar for pure culture and then to agar slants keeping in duplicate one for working culture and the other for storing at 4ºC.

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37ºC for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately1-2 x 10^8 .

Agar well diffusion Assay

Cultures were inoculated separated on the surface of Mueller Hinton agar plates (MHA) by surface spreading using sterile cotton swab and each bacterium evenly spread over the entire surface of agar plates to obtain uniform inoculums. The sensitivity test of the plants extracts was done using the agar well diffusion method[12] whereby well of 6mm diameter and 5mm depth were made on the solid agar using glass borer. Different concentration (50mg/ml,30mg/ml,10mg/ml) of the extract was dispensed into the respective wells and 10µg/ml amoxicillin was used as a positive control. Physiological saline / Dimethyl sulfoxide (DMSO) was used as a control. All the tests were run in triplicates for quality results. The set up was incubated for 24hrs at 37ºC. Twenty for hours (24) later, the zone of inhibition was measured using a ruler then results reported in millimeter (mm). Control well did not show any activity against microorganism.

Results and Discussion

The antimicrobial activity of various solvents extracts of *Strobilanthes cusia* was investigated in the present work. It was found that the antimicrobial activities of all active extracts were concentration dependant. The extracts showed antibacterial activity against most of the tested bacteria mainly at higher concentrations.The methanol extract exhibited highest to moderate inhibition against all test bacteria with maximum against *S. aureus* (50 mm) and minimum against *E.coli* and *K. pneumoniae* (10 mm) at highest concentration (50 mg/ml). The activities decreased with decrease in concentration. This extract was found to possess maximum concentration dependent antibacterial effects showing moderate inhibition even at the lowest concentration of 10 mg/ml against *S. aureus* (38mm) and *B. subtilis* (15 mm).The ethanol extract showed comparatively high to moderate inhibitory activity against all test bacteria with maximum against *S. aureus* (40mm), *B. subtilis* (20 mm) and minimum against *E. aerogenes* (10 mm) at 50 mg/ml (highest) concentration. Here also activities decreased with concentration but were not detected up to the lowest concentration employed.The petroleum ether extract also showed moderate to feeble activity in comparison with the methanol and ethanol extract, against all bacteria tested with maximum inhibition (35mm) against *S. aureus* and minimum against *K. pneumoniae* (12 mm), while it did not exhibit any inhibition on *E. aerogenes* and *Escherichia coli*. The effects were observed in the highest and next lower concentration (10 mg/ml) of extract against some bacteria.The acetone extract also exhibited negligible inhibition in the gram negative bacterias *E.coli* and *E.aerogenes*. However, it showed very postive result in gram positive bacteria against *S. aureus*(27 mm) and *B. subtilis* (17mm) at 50 mg/ml (highest) concentration. It showed the weakest activity as compared to other three extracts against other five bacteria mostly in the highest (50 mg/ml) concentration. The activity of different solvent extracts for *Strobilanthes cusia* in term of inhibition zone diameter in decreasing order can be stated as methanol > ethanol > petroleum ether > acetone. The results depicted that most of the antimicrobial activity linearly increased with concentration of extracts.From the experiment it is clear that the *Strobilanthes cusia* can be a potent drug against gram positive bacterias specially *S. aureus* and *B. subtilis*.

Table 1.Antimicrobial activity of leaf extracts of *Strobilanthes cusia* (Nees) Kuntze. with different concentration

Bacteria	***Concentration mg/ml***	***Petroleum ether***	***Acetone***	***Methanol***	***Ethanol***
		Zone of inhibition in (mm)			
Staphylococcus aureus	50	35	27	50	40
	30	32	25	42	38
	10	30	15	38	32
Bacillus subtilis	50	18	17	23	20
	30	16	14	19	17
	10	12	12	15	15
Enterobacter aerogenes	50	-	-	10	10
	30	-	-	-	-
	10	-	-	-	-
Escherichia coli	50	-	7	10	-
	30	-	-	10	-
	10	-	-	-	-
Klebsiella pneumonia	50	12	7	10	-
	30	9	-	-	-
	10	-	-	-	-

Values are mean of three triplicates

It is not surprisingly that there are difference in the antibacterial activities of the different extracts of the same plant. Out of the solvents employed for extraction the methanol extracts exhibited higher activity against the test organisms. Different solvents have the capacity to extract different antimicrobial constituents from the plants.These findings correlate with the observations of Anupam *et al*.,(2008). Two reasons accounting for the higher antibacterial activity of methanol extracts may be: the nature of the biological active compounds (alkaloids, flavonoids, essential oil , biterpenoids etc.) which could be enhanced in the presence of methanol;

the stronger capacity of methanol may have produced a greater number of active constituents responsible for antibacterial activity than the ethanol,acetone and petroleum ether[13].

Among the five bacterial strains investigated, highest inhibition was recored in *S. aureus* followed by *B.subtilis* and least inhibition was recored in *E. coli*, *K. pneumonia* and *E. aerogenes*. Susceptibility difference in gram positive and gram negative bacteria may be due to cell wall structural difference in between these classes of bacteria. The gram negative bacterial cell wall outer membrane appears to acts as a barrier to many substances including antibiotics[14].

Conclusion

The results of the present investigation indicated that *Strobilanthes cusia* leaf extract has a good source of antibacterial compound. However, methanol extract showed highest antibacterial activity against the tested bacteria which surpassed the remaining extract. Our results support the use of crude drug of such plants as an agent to control microbial pathogen needs further extensive research for their better economic and therapeutic utilization. Further phytochemical analysis are required to identify the compounds responsible for the antibacterial activities of this plant which could serve as a useful sources of new antimicrobial agents rather than used as a dye in Manipur,NE,India.

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