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Research Article

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Antibacterial activity of Sivanar Vembu (*Indigofera aspalathoides*) against some human pathogenic bacteria

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ABSTRACT

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects. The Indigofera Aspalathoides commonly known as Sivanar Vembu has been recognized in different system of traditional medicines for the treatment of different ailments of human beings. In this present study the antimicrobial activity of extracts of root, stem, leaf, and at different solvents in different concentration were evaluated against some human pathogenic gram positive and gram negative bacteria. The minimal inhibitory concentration of the extracts of root, stem and leaf in different solvents were also studied. The results were tabulated and discussed in this present communication.

INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases[1]. Over thousands of years Nature is an impressive source of medicines and a number of plant based drugs were isolated from natural sources [2]. Sivanar Vembu in Tamil is botanically equated as *Indigofera aspalathoides* belonging to the family Fabaceae. [3] In Sanskrit it is called as Patakohomba or Sivanimba, Manali in Malayalam, Pindi in Oriya, Redamandalam in Telungu, Shiva naaruballi in Kannada[4]. The whole plant is an ingredient of an oil preparation used for various disease of human beings .Sivanar vembu Thailam and Sivanarvembuk kulit Thailam are two popular Siddha preparations used for various types of skin diseases including leprosy [5]. This plant is regarded as one used in Kayakalpa drugs and historically it is the one among they which derived from the Lord Siva and as well as Sakthi name.[6]. In Siddha system of medicine, the plant is prescribed for eczema, psoriasis, boils, burns, wounds, ulcers, and used also as an drug against to snake venom. It possess biological activities such as antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, antimicrobial agent, cardiovascular protection[7]. Many efforts have been made to discover novel compounds from various sources such as microorganisms, animals, and plants; one such richest resource is folk medicines. Systematic screening of herbals may result in the discovery of novel effective compounds [8]. Sivanar vembu widely distributed in tropical and subtropical regions [9]. The whole plant is used as an effective antibacterial drug[10]. This present study is aimed to screen the antibacterial activity as well as the minimal inhibitory concentration of different plant part extracts in different solvents at different concentration of the plant extract Sivanar Vembu.

EXPERIMENTAL SECTION

Collection of Plant Material:
The plant sample was collected from the Inchikuzhi area of Kalakkadu Mundanthurai Tiger Reserve Forest, Tirunelveli District, Tamil Nadu. The plant sample was washed thoroughly 2-3 times with running water and rinse with sterile distilled water for removal of dust and soil particle, air dried, homogenised to a fine powder and stored in air tight containers.

Solvent Used
The organic solvents such as Chloroform, Methanol, Ethanol and distilled water were used for the extraction process.

Extraction of Bioactive Compounds
Dried powder form of plant sample was used to extract bioactive principles. Five grams of plant sample (Plant) was weighed and ground in to a part by using a laboratory in Chloroform, methanol, ethanol and water. The grounded material was made up to 50 ml using the respective solvent and it was maintained in refrigerator for 24 hours. Then it was centrifuged at 5000 rpm for 20 minutes. The supernatant extract was used for the analysis of sensitivity assay against the pathogens.

Culture Used
Six bacterial strains were used for testing the antimicrobial activity of the *Indigofera asphalathoides*. The list of the organisms were given in Table:1.

Maintenance of Bacterial Strains
Bacterial strains to be tested were streaked in selective agar plates to get pure cultures and stored at 4^0C to keep the bacterial strains viable.

Preparation of Inoculum
Ten ml of Nutrient broth was prepared in test tubes. These tubes were cotton plugged and autoclaved. The test tubes were labelled according to the type of the bacterial cultures to be inoculated. Then the broth were inoculated with the known bacterial strains under aseptic conditions and incubated at 37^0C for 24 hours.

In-vitro Antibacterial Assay
Agar Disc diffusion method was followed to determine the antimicrobial activity Muller Hinton Agar (MHA) plates were prepared and swabbed (sterile cotton swabs) with 24 hour old broth culture of respective bacteria. Discs were made from Whatman No. 1 filter paper. Stock solution of each plant extract was prepared using methanol, chloroform, ethanol and water as solvent. About 100µl and 200µl of different concentrations of plant solvent extracts was added using micropipette into the paper discs allowed to dry at room temperature. The discs were placed in the Muller Hinton Agar medium plates. The plates were incubated at 37^0C for 18-24 hours. At the end of incubation, inhibition zones formed around the disc were measured with the transparent ruler in millimeter. These studies were performed in triplicate and the values were recorded.

Minimum Inhibitory Concentration Assay
The antibacterial activity of the plant extracts were determined using sterile 96-well plates. The 12 wells of each row were filled with 0.1 ml sterilized Muller Hinton broth. Sequentially, each column wells 1-7 received an additional 0.055 ml of as mixture of culture medium and plant extract serially diluted to create a concentration sequence from 2000 µg/well to 30 µg/ well. Well 8 served as growth control, the deep-wells were incubated for 24 h at 37^0C. The resulting turbidity was observed, and after 24h MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600 nm after adding 0.1% alamar blue staining. The reading was observed with a Beckman DU-70 UV-Vis Spectrophotometer. At least two repetitions were run for each assay. Strong activity was defined as MIC<50 µg/well.

RESULTS

In this present study the Antibacterial inhibitory studies shows that out of four solvent namely methanol, Chloroform, ethanol and water extracts of the root, stem and leaf, the methanolic and chloroform extract showed

more than 50% inhibitory effect when compared to leaf extract control. The results are given in the Tables:2-4 and figure No. 1-3.The minimal inhibitory concentration test results were given in the Table No.5-7. From this result it is inferred that these extracts can be used as an effective antibiotic. The result of the other two extracts namely ethanol and water are insignificant.

Table:1 List of the organisms in the present study

S. No	List of Organisms
1	*Bacillus subtilis*
2	*Escherichia coli*
3	*Enterococcus faecalis*
4	*Pseudomonas aueroginosa*
5	*Staphylococcus aureus*
6	*Vibrio cholera*

Table: 2 Antimicrobial activity of *Indigofera asphalathoides* Leaf extracts

Organisms	LEAF Zone of inhibition in diameter in mm				
	Methanol	Chloroform	Ethanol	Water	Positive control
Bacillus subtilis	8	9	-	-	24
Escherichia coli	10	12	-	-	26
Enterococcus faecalis	9	11	-	-	21
Pseudomonas aueroginosa	14	12	-	-	13
Staphylococcus aureus	10	10	-	-	28
Vibrio cholera	9	12	-	-	30

Table: 3 Antimicrobial activity of *Indigofera asphalathoides* Stem extracts

Organisms	STEM Zone of inhibition in diameter in mm				
	Methanol	Chloroform	Ethanol	Water	Positive control
Bacillus subtilis	-	-	-	-	24
Escherichia coli	11	14	-	-	26
Enterococcus faecalis	-	-	-	-	21
Pseudomonas aueroginosa	14	11	-	-	13
Staphylococcus aureus	11	9	-	-	28
Vibrio cholera	8	12	-	-	30

Table: 4 Antimicrobial activity of *Indigofera asphalathoides* Root extracts

Organisms	ROOT Zone of inhibition in diameter in mm				
	Methanol	Chloroform	Ethanol	Water	Positive control
Bacillus subtilis	-	-	-	-	24
Escherichia coli	-	-	-	-	26
Enterococcus faecalis	-	-	-	-	21
Pseudomonas aueroginosa	-	-	-	-	13
Staphylococcus aureus	8	9	-	-	28
Vibrio cholera	-	-	-	-	30

Table 5 Minimum inhibitory concentration of *Indigofera asphalathoides* Leaf extracts

Organisms	LEAF Minimum inhibitory concentration in µg				
	Methanol	Chloroform	Ethanol	Water	Positive control
Bacillus subtilis	1100	1000	-	-	25
Escherichia coli	1000	800	-	-	25
Enterococcus faecalis	700	900	-	-	25
Pseudomonas aueroginosa	500	700	-	-	50
Staphylococcus aureus	950	1050	-	-	20
Vibrio cholera	850	650	-	-	20

Table 6 Minimum inhibitory concentration of *Indigofera asphalathoides* Stem extracts

Organisms	STEM Minimum inhibitory concentration in µg				
	Methanol	**Chloroform**	**Ethanol**	**Water**	**Positive control**
Bacillus subtilis	-	-	-	-	25
Escherichia coli	950	750	-	-	25
Enterococcus faecalis	-	-	-	-	25
Pseudomonas aueroginosa	600	800	-	-	50
Staphylococcus aureus	900	950	-	-	20
Vibrio cholera	1000	750	-	-	20

Table 7 Minimum inhibitory concentration of *Indigofera asphalathoides* Root extracts

Organisms	ROOT Minimum inhibitory concentration in µg				
	Methanol	**Chloroform**	**Ethanol**	**Water**	**Positive control**
Bacillus subtilis	-	-	-	-	25
Escherichia coli	-	-	-	-	25
Enterococcus faecalis	-	-	-	-	25
Pseudomonas aueroginosa	-	-	-	-	50
Staphylococcus aureus	900	800	-	-	20
Vibrio cholerae	-	-	-	-	20

DISCUSSION

The plant *Indigofera aspalathoides* is used as a effective medicinal plant to cure the chronic diseases like as Leprosy, Skin diseases, Food poisoning, Urinary Tract infection, and Tumor, as per the Indian System medicine. The antibacterial evolution study supports the previous literature of the usage of the plant as an effective antibiotic to control some of the dreadful diseases like Soriasis, Leukemia, Intoxication, Syphilis which is caused by human pathogenic bacteria.

CONCLUSION

From this study we can authentically say that Sivanar Vembu can be used as a Antibacterial medicinal herb against human pathogenic bacteria. The earlier phytochemical studies reveals that there are fourteen major phytochemical compound which places an important role as antibiotic and Antioxidant properties. As per Siddha literature this plant is an immune-modulatory drug. The Sivanar Vembu can be used as a natural antibiotic and anti- cancerous and anti-oxidant activity drug without any side effect. It can rescue the human from chronic ailments.

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