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Original Article

Phytochemical, Pharmacognostical, Antimicrobial activity of *Indigofera aspalathoids* vahl. (Fabaceae)

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Abstract

Our present study is aimed to detect the medicinal uses of the plant Indigofera *aspalathoides* Vahl. belonging to the family Fabaceae by performing various studies such as Phytochemical, Pharmacognostical and Antibacterial activity, using seven different bacterial strains, which are harmful to human beings. The *Indigofera aspalathiods* commonly known as 'Sivanar vembu' has been recognized in different system of traditional medicines for the treatment of different diseases and ailments of human beings. The leaves, flowers and tender shoots of the plant are said to be cooling and demulcent and are employed as decoction in leprosy and cancerous infections. The root is chewed as remedy for toothache. The whole plant rubbed up with butter is applied to reduce oedematous tumour. A preparation made from the ashes of the burnt plant is used to remove dandruff from the hair and oil for syphilitic and other skin diseases. A decoction of the entire plant is given as an alternative in secondary syphilis and psoriasis.

***Keywords*:** *Indigofera aspalathoids*, Sivanar vembu, Phytochemical, Pharmacognostical, Antibacterial activities

Introduction

Plants contain chemical compounds that may be in one way or another responsible for their healing properties and other functions. The chemical compounds are secondary metabolites of which at least twelve thousand have been isolated (Hasan *et al.*, 1988).

Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. These compounds are involved as well in allelopathy, dealing with the interactions between two plants, which process can change depending upon variations in the phytochemicals produced under particular environmental conditions (Zobel *et al*., 1999).

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subjects for very intense pharmacological studies; this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in the drug development. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care. There arises a need and therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies (Shailendra Gurav *et al.*, 2007). Up to 80% of the population depends directly on the traditional medicine for the primary health care (Kirby, 1996).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant (Prusti, 2008).

The present study is aimed to investigate the phytochemical, pharmacognostical and antibacterial activity of the medicinal plant *Indigofera aspalathoids* Vahl. which belongs to the family Fabaceae.

Materials and Methods

For the present study *Indigofera aspalathoids* Vahl. belongs to the family Fabaceae was subjected to study the phytochemical, pharmacognostic and antimicrobial bacterial activity. The plant was collected from Sivanthipatti hills in Tirunelveli District of Tamil Nadu during the month of January 2009.

Macroscopic (Taxonomic) Studies

The plant was collected during the month of January 2009 and well preserved. The observation was made by using student dissection microscope and the details were described in technical terms.

Microscopic (Anatomical) Studies

Fresh plant of *Indigofera aspalathoids* Vahl. (Fabaceae) was collected and fixed in FAA (Formalin Acetic acid and alcohol mixtures). Free hand section of stem, leaf and root were taken and kept in 70% ethanol. The sections were stained with saffranin and mounted according to the methods described by Johansen (1940). The photomicrographs were taken using Motic digital camera, and Phase Contrast Microscope, Japan.

Phytochemical Studies

Mature and healthy plants were collected and dried at room temperature (25–30˚C) for about two weeks. The dried plants were ground to powder. 5 grams of the powdered plant was put in to a bottle and shaken with a mechanical shaker for 12 hours. Then filtered with Whatman No. 1 filter paper to obtain petroleum ether, benzene, chloroform, ethanol and distilled water extracts. The qualitative phytochemical analysis was carried out on the extracts to determine the presence or absence of reducing sugar, protein, phenolic groups, alkaloids, steroid, triterpene, flavone, catachin, tannin and anthraquinone (Trease and Evans, 1996).

Pharmacognostic Studies

1. ***Fluorescence Analysis***

Fine powder and their extracts were obtained in various solvents namely petroleum ether, benzene, chloroform and ethanol. The aqueous extract was prepared by directly boiling the powder with distilled H_2O. They were examined under visible and U.V light. These powdered material were also treated with various reagents such as 50% $HN0_3$, acetone, ethyl alcohol, 50% H_2S0_4, 1N HCl and 1N aqueous NaOH and changes in colour were recorded.

2. ***Quantitative Determination***

The percentage of total ash, water soluble ash, acid insoluble ash, sulphated ash, were obtained by employing standard methods of analysis as described in Pharmacopoeia of India (1996).

3. ***Qualitative Determination***

Tests for detection of inorganic elements in plant ash.

Preparation of Ash Extracts

For detection of various elements in plant ash Ca, Fe, S, P and Cl, one gram ash material was dissolved in 25ml of 50% HCl for 12 hours and then filtered through filter paper. The filtrate was treated with suitable reagents to identify the presence of elements qualitatively.

Antibacterial Activity

A. ***Materials***

a. Microbial Strains

b. The different bacterial strains used for the study of antibacterial activities were collected from the Vivek laboratories, K.P.Road, Nagercoil and maintained by sub culturing. The bacteria's were sub-cultured in the Mullen-Hinton agar medium. The samples for bacterial strain were sub cultured in individual plates.

Solvant

The organic solvents such as petroleum ether, benzene, chloroform, ethanol and distilled water were used to extract bioactive compounds.

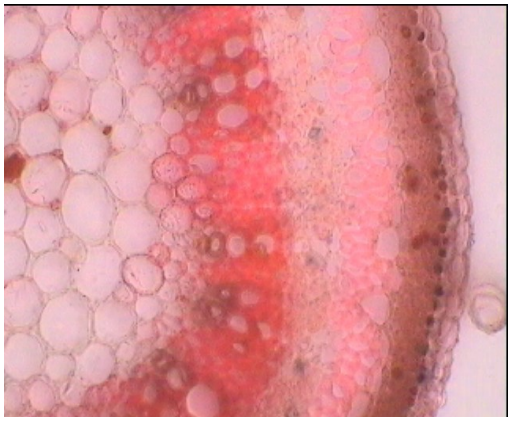
Methods

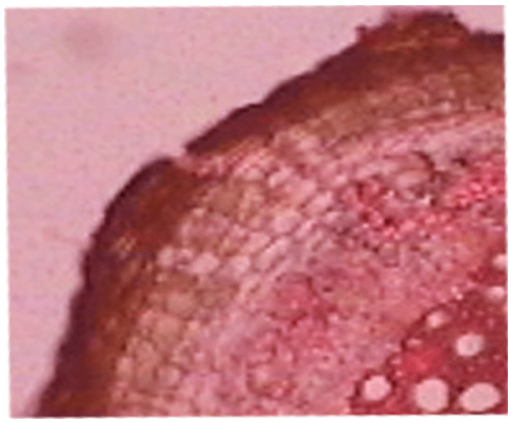
1. Extraction of antibacterial compounds
2. Maintenance of microbial strains
3. Nutrient media and nutrient broth
4. Preparation of sterile antibiotic discs
5. Plating and Assay of antimicrobial activity

Results and Discussion

The plant is an erect much branched stiff under shrub and grows erect. Stem is dark brown, when young, grayish white, branched 0.7cm to 1.5cm width. Young branches silvery pubescent and are characteristically purple with dense covering of minute trichomes; Roots are brown colored, woody, lateral roots present 0.5 to 2.0cm width. No characteristic odour. The

leaf is 1-3 trifoliate, pale green, oblanceolate, digitate, sessile and crowded on the young branches, stipules minute. Leaflets usually 3, thick, oblanceolate, folded; The flowers are purple, solitary and auxillary, pedicels slender, 2 cm in long; The pods are straight and cylindrical with sparse trichomes usually 6 cm in long. Seeds are cubical and smooth. The microscopic structures of stem and root was shown in photos.





The results of the phytochemical analysis shows that except napthoquinone all other secondary metabolites such as steroids, triterpenes, alkaloids, phenolic groups, flavone groups, saponin, tannin, sugar, catachin, aminoacids and reducing sugar are present in the plant.

Fluorescence analysis of the plant powder in various solvents have been studied and presented in table. It can be as a diagnostic tool for testing adulterations, if any. Under fluorescent light the plant powder showed different colors in various extracts.

The total ash content of *Indigofera aspalathoids*. is 18.06%. The acid insoluble ash content of *Indigofera aspalathoids* Vahl. Is 1.13%. The sulphated ash content of *Indigofera aspalathoids* Vahl. is 17.89%. The water soluble ash content in *Indigofera aspalathoids* Vahl. is 1.24%. Thus, the macroscopic characters, preliminary phytochemical screening and pharmacognostic studies can be used as a diagnostic tool in the correct identification of the plants and also to identify adulteration of these materials.

The plant *Indigofera aspalathoids* Vahl. shows the presence of almost all minerals such as sulphur, phosphorous, irons and calcium except chlorine.

Antibacterial activity of the extract of *Indigofera aspalathoids* Vahl. in different solvent extracts are shown in table.

The antibiotic disc ampicilin showed antibacterial activity against *Stapphylococcus aureus*, *Staphylococcus epidermidis*, *E.coli*, *Klebsiella* sps. and *Enterobacter* sps. It doesn't show any activity against *Proteus vulgaris* and Proteus mirabilis. It had the maximum inhibitory action against almost all the bacteria used for example *Streptococcus aereus* 14mm. Therefore it should be understand that by undergoing these studies various secondary metabolites present in the plant, its importance for used as drug, its activity to fight against certain bacteria which are harmful to human beings can be easily identified. Further integrated investigation using HPLC and GC - MS will lead to purification and structural elucidation of active principles against microorganisms.

Table-1: Flourescence analysis of the extracts of *indigofera aspalathoids* Vahl.

Sl.No	Treatment	Under visible Light	Under UV Light
1	Powder as such	Yellowish green	Light green
2	Petroleum ether extract	Light yellow	Light green
3	Chloroform extract	Yellowish green	Green
4	Ethanol extract	Light green	Dark green
5	Distilled water extract	Light orange	Green
6	Powder +1N aqueous NaOH	Brown	Green
7	Powder +acetone	Pale yellow	Light green
8	Powder +N HCl	Orange	Green
9	Powder +50%HNO_3	Reddish orange	Dark green
10	Powder +50%$H_2 SO_4$	Yellowish brown	Dark green

Table- 2: Antibacterial activity of *Indigofera aspalathoids*

Tested organism	Benzene	Ethanol	Petroleum ether	Chloroform	Control
S. aureus	11	11	11	25	25
S. epidermis	11	-	-	25	24
Escherichia eoli	-	-	-	25	27
Klebsiella sp	-	-	-	17	12
Proteus vulgaris	25	-	23	23	-
Proteus mirabilis	25	-	22	24	-
Enterobacter sp	11	10	-	13	24

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