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ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *INDIGOFERA ASPALATHOIDES* VAHL. EX. DC.

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ABSTRACT

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found in-vitro to have antimicrobial properties. The present study was conducted with a view to evaluate the therapeutic potentials *Indigofera aspalathoides* plant extracts against human pathogen. The leaves and aerial parts of *Indigofera aspalathoides* were dried and powdered leaf and stem material was extracted by soxhlation method using a non-polar (Hexane) solvent and polar solvents (Chloroform, Ethanol and Water), respectively and extracts were then subjected to phytochemical screening. Extract of *Indigofera aspalathoides* was evaluated for its antimicrobial activity against a wide variety of pathogenic bacteria such Escherichia coli, Klebsiella pneumonia and Aeromonas hydrophila by disc diffusion method. In the extract of I.aspalathoides maximum yield was obtained from in the leaf ethanol (20.64±1.02) than the chloroform (15.80±3.28) and hexane (6.52±1.78) and compare the stem extracts ethanol(10.23±3.55) was in high followed by chloroform(7.28±2.36) and hexane (3.52±1.35). Phytochemical analysis showed the presence of various phytoconstituents. Alkaloids, Phenolic compounds, Flavonoids, Tannins and Terpenoids were found to be present in all extracts of leaf and stem. Reducing sugar was absent both leaf and stem. Pholobatanins recorded in leaf and absent were in stem.Compare the solvent extracts of leaf, crude ethanol extracts of I. aspalathoides leaf caused more antibacterial activity against pathogens E. coli (24.4±2.5), B. subtilis (23.3±2.4) and A. hydrophila (20.6±3.1) followed by chloroform against E. coli (18.2±1.5) and hexane against A. hydrophila (11.2±1.2). Compare the stem extracts; hexane extracts caused more antibacterial activity against *K. pneumonia* (29.2±2.9) followed by chloroform against *B. subtilis* (25.2±0.4) and against *B. subtilis* (12.7±0.8). The results support that these plant extracts can be used for the treatment of bacterial diseases.

INTRODUCTION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern[1]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens[2]. Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another[3].

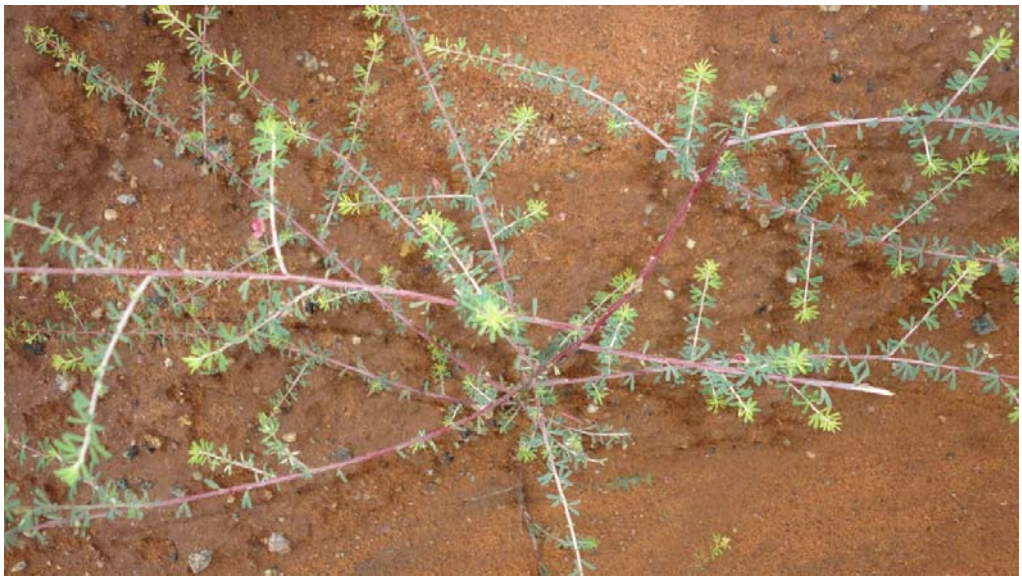
Civanarvembu/Iraivanvembu in Tamil is botanically equated as *Indigofera aspalathoides*Vahl.ex.DC. belonging to the family Legumsinosae. In Sanskrit it is Patakohomba/Sivanimba. Its leaves, flowers and tender shoots are cooling and demulcent, they are used in the form of decoction for leprosy and cancerous affections. The leaves are also applied to abscesses. Roots are used as dentrifice, and also in mouth ulcers. The root is chewed in toothache and apathies. The whole plant is an ingredient of an oily preparation used for dandruff[4,] syphilis and other skin affections. In Siddha system ofmedicine, the plant is prescribed for eczema, psoriasis, boils, burns, wounds, ulcers, and used also as an antidote to snake venom[5]. Sivanar vembu Thailam and Civanarvembuk kulit Thailam are two popular Siddha preparations used for various types of skin diseases including leprosy[6].It is used along with camphor for different kinds of wounds. This plant is regarded as one used in Kayakalpa drugs and in the discovery of anticancer elixir. Water soluble fraction of alcoholic extract of dried tender shoots of *I. aspalathoides* showed significant anti-inflammatory effect in experimental albino mice[7]. Phenolic compounds are secondary metabolites which synthesize in plants. They posse's biological properties such as: antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, antimicrobial agent, cardiovascularprotection[8]. In this study assessing the potential and antimicrobial activity of selected plant extracts against the human bacterial pathogens.

MATERIALS AND METHODS

Collection and Extraction of the Plant

The leaf and stem of *Indigofera aspalathoides*Vahl.Plants (Fig 1.) were collected from in and around Old Courtallam (8.9342°N 77.2778°E) Tamil Nadu, India; during the spring season (April to July 2012). Collected plant materials were washed twice with tap water and once with distilled water. The plant material were shade-dried and partially powdered using domestic blender and stored in air tight container for further use.

Figure 1.Field image of medicinal *plant Indigofera aspalathoides*Vahl. Ex. DC. (SIVANAR VEMBU).



For extraction, 250gm of powdered leaf and stem material was extracted by soxhlation method using a non-polar (Hexane) solvent and polar solvents (Chloroform,and Ethanol) by hot continuous extraction for about 12 hours at room temperature (27±2^{o}C) in 750ml capacity Soxhlet apparatus. The collected crude extracts were reduced 10ml, transferred to clear glass vial (15mL) and was evaporated and dried over sodium sulphate in desiccator under vacuum. The concentrated crude extracts were stored in the refrigerator for further use[9].

Preliminary phytochemical screening

Qualitative analysis

The qualitative phytochemical screening of the plants was carried out according to the standard procedure[10, 11].

Bacterial culture collection

The human pathogenic bacteria such as *Escherichia coli, Klebsiella pneumonia* and *Aeromonas hydrophila*were obtained from Vivek Laboratory, Nagercoil, Tamilnadu. The bacterial cultures were maintained in nutrient broth (Himedia, M002)at 37^{o}C and maintained on nutrient agar (Himedia, MM012) slants at 4^{o}C.

Screening for antibacterial activity

The antibacterial assay was performed by agar disc diffusion method[12]. All the microbiological media used in this experiment were obtained from Hi-media Laboratories, Mumbai. Overnight cultures were prepared by inoculating approximately in 2ml nutrient broth with 2–3 colonies of each organism taken from nutrient agar. Broths were incubated overnight at 30°C with shaking. The suspension of tested bacterial strains (0.1ml) was spread on the nutrient agar plates.The stock

solutions of corresponding fractions and crude extract were prepared in Dimethyl sulphoxide (DMSO) [13]; steriledisc (sigma-Aldrich) were impregnated in 50µl of the stock solution plant extracts and dried aseptically. The discs were placed on the bacterial lawn of agar plates and incubated at 30ºC for 24h. The diameters of the inhibition zones were measured using a scale in millimetres (mm). Experiments were performed in triplicates to obtain standard results and the maximum zones of inhibition against the pathogens were noted.

Statistical analysis

Since the readings of control (DMSO) experiments in the *in vitro* antibacterial studies of those plants were zero, the data were analysed by simple arithmetic means of the different extracts, and the standard error were compared with the control.

RESULTS AND DISCUSSION

The solvents with an increasing order of polarity were used for the extraction of *I.aspalathoides* leaf and stem. The Hexane, Chloroform and Ethanol solvent extracts yield results were given Table 1. The yields of the extracts for *I.aspalathoides* were: 20.64±1.02 (Leaf-Ethanol), 15.80±3.28 (Leaf- Chloroform) and 10.23±3.55 (Stem-Ethanol). Ethanol extracted the most materials from the plant followed chloroform and hexane. The extracts of hexane yielded the lowest amount.

Table 1. Yield of the solvent extracts of *Indigofera aspalathoides*

Plant parts	Solvents	Yield (mg/100gm)
Leaf	Hexane	6.52±1.78
	Chloroform	15.80±3.28
	Ethanol	20.64±1.02
Stem	Hexane	3.52±1.35
	Chloroform	7.28±2.36
	Ethanol	10.23±3.55

The preliminary qualitative phytochemical screening results are summarized in Table 2. Alkaloids, Phenolic compounds, Flavonoids, Tannins and Terpenoids were found to be present in all extracts of leaf and stem. Reducing sugar was absent both leaf and stem. Pholobatanins recorded in leaf and absent were in stem.

Table 2. Qualitative analysis of phytochemical constituents *Indigofera aspalathoides*

Indigofera aspalathoides						
Phytochemical constituents	Leaf			Stem		
	Hexane	Chloroform	Ethanol	Hexane	Chloroform	Ethanol
Alkaloids	+	+	+	+	+	+
Steroids	+	+	-	+	-	+
Reducing sugar	-	-	-	-	-	-
Tannins	+	+	+	+	+	+
Pholobatanins	-	-	+	-	-	-
Saponins	+	+	+	-	+	+
Flavonoids	+	+	+	+	+	+
Phenolic groups	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Xanthoprotein	+	-	-	+	-	-

The antimicrobial activity of three solvent extracts of leaf and stem of *Indigofera aspalathoides* was studied by the disc diffusion method and the results are shown (Table 3.). All the solvent extracts showed a significant inhibitory activity against all pathogenic bacterial strains. Compare the solvent extracts of leaf, crude ethanol extracts of *I. aspalathoides* leaf caused more antibacterial activity against pathogens *E. coli* (24.4±2.5), *B. subtilis* (23.3±2.4) and *A. hydrophila* (20.6±3.1) followed by chloroformagainst *E. coli* (18.2±1.5) and hexane against *A. hydrophila* (11.2±1.2). Compare the stem extracts; hexane extracts caused more antibacterial activity against *K. pneumonia* (29.2±2.9) followed by chloroform against *B. subtilis* (25.2±0.4) and against *B. subtilis* (12.7±0.8).

Table 3. Antibacterial activities of hexane (HE), chloroform (CH) and ethanol (CH) extracts (100mg/ml) of medicinal plant *Indigofera aspalathoides*.

Indigofera aspalathoides							Positive control	Negative control
Phytochemical constituents	Leaf			Stem				
	HE	CH	ET	HE	CH	ET		
E. coli	10.0±0.6	18.2±1.5	24.4±2.5	20.7±3.2	19.5±2.1	12.4±0.7	23.7±2.5	-
K. pneumonia	10.0±0.4	15.2±2.1	20.4±2.6	29.2±2.9	12.6±2.3	10.5±0.3	26.5±2.4	-
A. hydrophila	11.2±1.2	16.1±0.6	20.6±3.1	22.1±2.5	13.5±0.6	10.7±0.5	22.6±3.9	-
B. subtilis	10.5±0.3	11.4±1.2	23.3±2.4	31.3±3.8	25.2±0.4	12.7±0.8	27.2±1.3	-

HE- Hexane, CH- Chloroform and ET- Ethanol

CONCLUSION

The present study justifies the traditional use of medicinal plant to treat various infectious diseases caused by the microbes. Therefore, it has been concluded that the crude extracts of medicinal plants may be used enough as a drug to treat diseases caused by those bacteria, which are sensitive to the above mentioned samples. The toxicological and clinical trials of pure compounds should be carried out in model animals before use in human being. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

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