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Physico-Chemical, Phytochemical and Bioactive Characterization of *Dipteracanthus Prostratus* (Poir) Nees – An Antidote Medicinal Plant

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Abstract

Dipteracanthus prostratus (Poir) Nees (Pottakanchi in tamil) belongs to Acanthaceae family and it is used as antidote for snakebite by Palliyar tribal settled in Sirumalai hills, Western Ghats, Tamil Nadu. The present investigation evaluates the physico-chemical, phytochemical, fluorescence analysis and antibacterial potential of this ethnomedicinal plant. The outcome of the study support and validate the traditional use of this plant in the treatment of various types of diseases.

Key Words: *Dipteracanthus prostratus*, Pottakanchi, *Palliyar*, Antibacterial, Phytochemical.

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1. Introduction

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.*, 2000). Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah *et al.*, 2007). These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design (Vijayalakshmi and Ravindran, 2012). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Doss, 2009). Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well (Pandey *et al.*, 2013). *Dipteracanthus prostratus* (Poir.) Nees very important traditional medicinal plant and the whole plant juice is used as a remedy for snakebite by *Palliyar* tribals in Sirumalai hills, Western Ghats, Tamil Nadu (Maruthupandian and Mohan, 2012). The aim of this study was to analysis physico-chemical, phytochemical, fluorescence and antibacterial activity of indigenous antidote medicinal plants of *Dipteracanthus prostratus* (Poir.) Nees.

2. Materials and Methods

The plant materials were collected from Sirumalai hills, Western Ghats, Tamil Nadu, India. The plant specimen was collected, air-dried and mounted on herbarium sheets and identified by using various standard floras (Fischer and Gamble, 1957; Gamble, 1957a,b; Matthew, 1983a, b, c; Nair and Henry, 1983; Henry *et al*., 1987; Henry *et al*., 1989; Sharma *et al*., 1993; Sharma and Balakrishnan, 1993; Sharma and Sanjappa, 1993; Sasidharan and Sivarajan, 1996; Hajra *et* al., 1997; Matthew, 1999a, b, c; Pallithanam, 2001), photographed and sample specimens were collected for the preparation of herbarium. The identified plant specimens were confirmed and deposited in the herbarium of Department of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu.

2.1 Physico-chemical and Fluorescence analysis

These studies were carried out as per the standard procedures (Lala, 1993). In the present study, the powdered whole plant was treated with various chemical reagents like aqueous 1N sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid, concentrated nitric acid, picric acid, acetic acid, ferric chloride and concentrated HNO_3+NH_3. These extracts were subjected to fluorescence analysis in day light and UV light (254nm and 366nm). Various ash types and extractive values were determined by standard methods (Anonymous, 1996).

2.2 Phytochemical analysis

Shade dried and powdered samples were successively extracted with chloroform, ethanol and petroleum ether. The extracts were filtered ad concentrated using vacuum distillation. The different extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure (Lala, 1993 and Brindha *et al.*, 1981).

2.3 Antibacterial Assay

2.3.1 Collection of microorganisms

Stock cultures of bacteria such as *Staphylococus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* were obtained from Research Laboratory, Department of Microbiology, School of Biosciences, Periyar University, Salem, Tamil Nadu.

2.3.2 Preparation of media

The growth media employed in the present study included Nutrient agar and Nutrient broth. The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.

2.3.3 Sub culturing of microorganisms

The pure culture of microorganism was maintained on nutrient agar slants by frequent sub culturing. The culture was stored at 4^0C.

2.3.4 Preparation of inoculum

Each organism was recovered for testing by sub culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5ml of nutrient broth and incubated overnight at 37^0C. These overnight cultures were used as inoculum.

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2.3.5 Antibacterial activity

Antibacterial activity was demonstrated by modification of the method described by Barry and Thornsberry, (1985). 0.1 ml of the diluted microbial culture was spread on sterile nutrient agar plate. The pre-soaked and dried discs of 6mm diameter of What man No.1 filter paper were then placed on the seeded plates and gently pressed down to ensure contact. At the same time standard antibiotic of Tetracycline (30µg/ disc) was used as reference or positive control. Respective solvents without plant extracts served as negative control. The plates were incubated at 37ºC for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extract saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone around the discs were measured and recorded as the difference in diameter between the disc (6mm) and growth free zone.

3. Results And Discussion

3.1 Physicochemical constants (Ash and Extractive values)

The result of the ash and extractive values of whole plant of *Dipteracanthus prostratus* are represents in table 1.

Table 1: Ash and extractive values of powdered whole plant of *Dipteracanthus prostratus*

Sl. No.	Type of ash	Ash value (%)
1	Total ash	6.78 ± 0.12
2	Water soluble ash	2.19 ± 0.05
3	Acid insoluble ash	2.41 ± 0.37
4	Sulphated ash	11.13 ± 0.22

Extractive Values

Sl.No.	Name of The extract	Extractive values (%)
1	Petroleum ether	8.16 ± 0.21
2	Benzene	11.21 ± 0.23
3	Chloroform	10.71 ± 0.15
4	Acetone	10.47 ± 0.31
5	Methanol	10.95 ± 0.24
6	Ethanol	12.46 ± 0.12
7	Water	11.30 ± 0.03

The total ash content of the powdered whole plant of *Dipteracanthus prostratus* is 6.78% and 6.40% respectively. The extractive value of ethanol is more than that in other solvents investigated in the present study. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (African pharmacopoeia, 1986). Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (Musa *et al.*, 2006). The above said ash values are indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. Samples have more acid insoluble ash than water soluble ash. The physicochemical standards were generally used for deciding the identity, purity and strength of the drug source.

3.2 Fluorescent analysis

The results of fluorescent analysis of whole plant of *Dipteracanthus prostratus* shows (Table 2) the characteristic fluorescent green colour treated with 1NHCl under long UV light and H_2SO_4 under short UV light.

Table -2 : Fluorescence analysis of the powdered in whole plant of *Dipteracanthus prostratus*

Sl.No.	Experiments	Visible / Day light	UV - light	
			254nm (short wave length)	365nm (long wave length)
1	Drug powder as such	Green	Dark green	Light green
2	Powder +1N NaOH (Aqueous)	Dark green	Green	Light green
3	Powder +1N NaOH (Alcohol)	Dark green	Green	Light green
4	Powder +1N HCL	Dark green	Green	Fluorescence green
5	Powder +50% H_2SO_4	Dark green	Fluorescence green	Light green
6	Powder + Nitric acid	Red	Red	Light brown
7	Powder +Acetic acid	Green	Dark green	Pink
8	Powder +5% Ferric chloride	Dark green	Dark green	Dark green
9	Powder + HNO_3 + NH_3	Light green	Green	Light green

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Table 3: Preliminary phytochemical screening of whole plant of *Dipteracanthus prostratus*

Pytochemical Test	Petroleum Ether	Chloroform	Ethanol
Alkaloid	+	+	+
Anthraquinone	-	-	-
Coumarin	+	-	-
Flavonoid	-	-	+
Phenol	-	-	+
Protein	-	-	-
Saponin	-	-	+
Steriod	+	+	-
Sugar	+	+	+
Tannin	+	+	+

Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al*., 2006).

3.3 Preliminary phytochemical analysis

The chloroform extract of the whole plant of *Dipteracanthus prostratus* shows the presence of alkaloid, steroid, sugar and tannins. The petroleum ether extracts of whole plant of *Dipteracanthus prostratus* shows alkaloid, coumarin, steroid, sugar and tannin. The ethanol extracts of whole plant of *Dipteracanthus prostratus* depicts alkaloid, flavonoid, phenol, saponin, steroid, sugar and tannin.

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds such as alkaloids, coumarin, flavonoid, phenol, protein, saponin, steroid, sugar and tannin were detected in *Dipteracanthus prostratus* extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound.

To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations was compared.The comparative analysis clearly showed a correlation between a compound present in it and their fluorescent behavior under different conditions. The major bioactive compounds present in these crude preparations are the coumarin, flavones, tannins, alkaloids and saponin. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavones which are light yellow in aqueous condition under UV light turns to bright yellow under alkaline conditions. Similarly the phytosterols when treated with 50% H_2SO_4 show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green fluorescence under short UV light (Horborne, 1976). Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all (Evans, 1996). Haydon (1975) studied the photophysical characters of coumarins. Hydroxy methyl coumarin fluoresced in the 420 - 440nm when observed in different solvents with increasing polarity (Chaltopudhyay, 2006). The fluorescence analysis of the crude drugs of *Dipteracanthus prostratus* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern. The physic-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The phytochemicals revealed that these plants contain bioactive substances such as alkaloids, coumarin, flavonoid, saponin, steroids, phenols, sugars and tannins. Flavonoids and phenols have been reported to expert multiple biological effects such as anti-inflammatory, antiallergic, antioxidant, antidiabetic and cancer activities. The curative properties of the medicinal plants are due to the presence of various secondary metabolites.

3.5 Antibacterial activity

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The present investigation, the antibacterial activity evaluated chloroform, ethanol and petroleum ether extracts of *Dipteracanthus prostratus* against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) used with antibiotic tetracycline. Chloroform extracts of whole plant of *Dipteracanthus prostratus* showed maximum inhibitory activity was observed against *Staphylococcus aureus* (4mm). Moderate activity was observed against *Bacillus subtilis* (3.6mm) and *Staphylococcus aureus* (3mm). Ethanol extracts of whole plant powder of *Dipteracanthus prostratus* showed maximum inhibitory activity was observed against *Staphylococcus aureus* (5.2mm). Moderate activity was observed against *Bacillus subtilis* (5.1mm). Petroleum ether extracts of whole plant powder of *Dipteracanthus prostratus* showed maximum inhibitory activity was observed against *Staphylococcus aureus* (5mm) moderate activity observed against *Bacillus subtilis and Pseudomonas aeruginosa* (4.6mm).

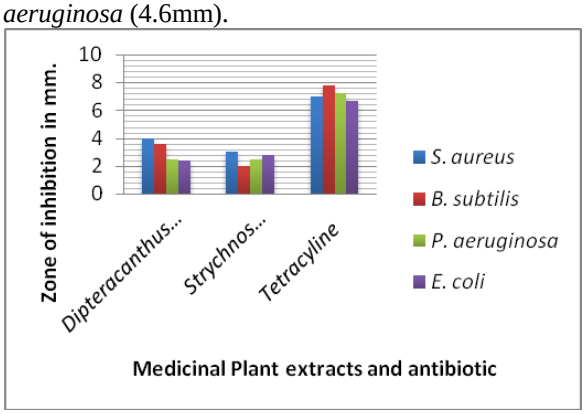

Fig. 1: Antibacterial activity of chloroform extract of *Dipteracanthus prostratus*

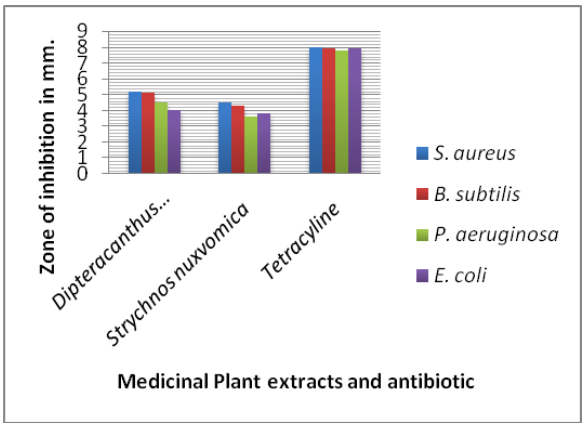

Fig. 2: Antibacterial activity of ethanol extract of *Dipteracanthus prostratus*

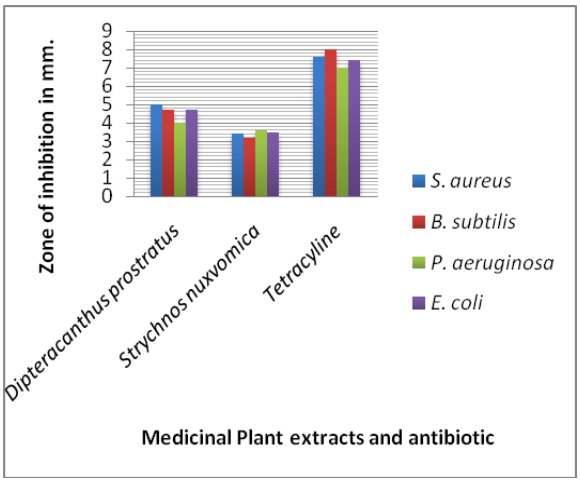

Fig.3: Antibacterial activity of Petroleum ether extract of *Dipteracanthus prostratus*

Among the bacterial organisms tested, the chloroform, petroleum ether and ethanol extracts of whole plant of *Dipteracanthus prostratus* exhibit maximum activity with showed higher inhibitory activity with *Staphylococcus aureus*, a gram positive bacterium. The three extracts of above said plants inhibited the growth of this organisms compared with tetracycline as positive control, a standard antibiotic used. The results obtained are encouraging as the chloroform and ethanol extracts have shown considerable antibacterial activity against tested organisms. The antibacterial activities of the plants may be attributed to the presence of bioactive principles such as phenols, steroids, alkaloids and flavonoid as suggested by several literatures.

3.6 Conclusion

The results were recommend in this plant of *Dipteracanthus prostratus* (Poir) Nees can be act as a potential source of bioactive compounds which could be used as effective drug to cure various diseases after completion of further pharmacological studies and prepare active principles with drug standardization.

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