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Research Article

PRELIMINARY PHYTOCHEMICAL EVALUATION OF WHOLE PLANT EXTRACT OF *DIPTERACANTHUS PROSTRATUS* NEES

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ABSTRACT
Dipteracanthus prostratus is an prostrate perennial herb. It is a small straggling, much branched herb it is purple at the nodes, internodes are long and hairy. It used as anti-cancer, hypoglycemic, anti-inflammatory and Diuretics. The present study was carried out to preliminary phytochemical evaluation of whole plant of *Dipeteracanthus prostratus* nees. The study includes preparation of different extracts by successive solvent extraction for detail analysis. Fluorescence analysis of different successive extract and powder were noted under UV light and normal ordinary light, which signifies there characteristics. Preliminary qualitative chemical test for different extract shows the presence of alkaloids, glycosides, fixed oil and fats, phenolic compounds, protein and amino acids, tannins, gum and mucilage, flavonoids and carbohydrates.
KEY WORDS: *Dipteracanthus prostratus,* preliminary phytochemical, successive solvent extraction.

INTRODUCTION

Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light modern scientific knowledge[1]. *Dipteracanthus prostratus* Nees (Acanthaceace) is an important medicinal plant and popularly known as black weed. It is found in It is a species growing widely in India and Australia. It is a prostrate perennial herb. It is a small straggling, much branched herb it is purple at the nodes, internodes are long and hairy. The Leaves are ovate or elliptic, acute hairy, entire with narrow base. The flowers bloom in August-September to October. The flowers are normally sessile, axillary's, solitary or few together. Pale blue to light violet and occasionally white in colour, braceoles like leaves but smaller. Calyx are par title and hairy. Corolla are infundibulate form with narrow tube. Capsules are many seeded[2-4]. It is believed to be Anti-cancer against the epidermis of the Naso-pharynx region and slightly hypoglycemic, Anti-Inflammatory. The weed may be potent Diuretics. It contains alkaloid, glycosides, fixed oil and fats, phenolic compounds, protein and amino acids, tannins, gum and mucilage, flavonoids and carbohydrates[5]. The present study is designed to explore the preliminary phytochemical and physicochemical analysis of *Dipteracanthus prostratus* whole plant, which is responsible for its pharmacological properties.

MATERIAL AND METHODS

The fresh whole plant of *Dipteracanthus prostratus* Nees were collected in the month of January 2011 from Salem district, Tamilnadu, India. The plant was identified and authenticated by the botanist Mr. A Balasubramanian (consultant central siddha research) Executive Director ABS botanical garden, Salem, Tamilnadu.

Preparation of crude drug for extraction

The authenticated fresh whole plant were dried under shade and used for the preparation of extract. These whole plant was coarsely powdered with the help of mechanical grinder and passed through sieve no.60. The powder was stored in an airtight container for further use.

Preparation of the Extracts[6-9]

Method of extraction

Continuous hot percolation (successive solvent extraction) process by using soxhlet apparatus and cold maceration method.

Materials

i. Soxhlet apparatus.
ii. Petroleum ether (60-80^{0}C)
iii. Chloroform
iv. Acetone
v. Ethanol (95 % v/v)
vi. Distilled water with chloroform (0.25%)
vii. Shade dried coarse powder of *Dipteracanthus prostratus* Nees.

Extraction Procedure

Petroleum ether extract

The shade dried coarsely powdered whole plant of *Dipteracanthus prostratus* Nees (1 kg) was extracted with petroleum ether (60-80°C) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark green colour residue was obtained. The residue was then stored in dessicator.

Chloroform extract

The marc left after petroleum ether extraction was dried and then extracted with chloroform (55-56°C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark greenish yellow colour residue was obtained. The residue was then stored in dessicator.

Acetone extract

The marc left after chloroform extraction was dried and then extracted with acetone (55-56°C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brownish green colour residue was obtained. The residue was then stored in dessicator.

Ethanol extract

The marc left after acetone extraction was dried and then extracted with ethanol 95% v/v (75-78°C), until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark brown colour residue was obtained. The residue was then stored in dessicator.

Aqueous extract

The marc left after ethanol extraction was dried and then extracted with chloroform water by cold maceration process for 7 days. At the end of 7th days, it was filtered through muslin cloth and the filtrate was concentrated. The remaining solution was evaporated by heating on a water bath. The brown colour residue was obtained. The residue was then stored in dessicator.

The extractive values of various extracts of *Dipteracanthus prostratus* Nees were presented in **Table no.1**

Identification of phytochemical constituents

The therapeutic potentials of plant and animal origin are being used from the ancient times by simple process without isolation of pure compounds that is in the form of crude drugs. The pharmacological action of crude drug is determined by the nature of its constituents.

Thus plant species may be considered as a biosynthetic laboratory not only for chemical compounds, e.g. Carbohydrates, proteins and fats that are utilized as a food by humans and animals, but also for a multitude of compounds including alkaloids, flavonoids, glycosides etc. which exert definite pharmacological activity.

To obtained these pharmacological activities, plant materials were used as such in their crude form or may be extracted with suitable solvents to take out the desired components and the resulting principle being employed as therapeutic agents. The phytochemistry of herbal drug embraces a thorough consideration of these chemical entities that are termed as constituents. As the herbal drugs contain so many chemical compounds, it is essential to single out those responsible for therapeutic effect to be called as active constituents.

By considering the above facts, it is necessary to evaluate the nature of extract before evaluating the biological activity of same. We have been selected such extracts for pharmacological activity which contain large number of chemical constituents. Hence for this purpose, we have to go for following preliminary tests to evaluate chemical nature of extracts qualitatively.

Preliminary phytochemical tests[10-13]

All the extracts of *Dipteracanthus prostratus* nees were subjected to qualitative tests for the identification of various active constituents.

Test For Carbohydrates And Glycosides

A small quantity of various extracts were dissolved separately in 4ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molisch's test

The filtrate was treated with 2 - 3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Fehling's test

The filtrate was treated with each 1ml of Fehling's solution A and B and heated on a water bath. A reddish precipitate was obtained shows the presence of carbohydrates.

Another portion of extracts were hydrolyzed with dilute hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to the following tests to detect the presence of glycosides.

Legal's test

To the hydrolysate 1ml of pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Ammonia layer acquires pink colour shows the presence of glycosides.

Detection Of Fixed Oils And Fats

Filter paper test

Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicated the presence of fixed oils.

Saponification test

Few drops of 0.5M alcoholic potassium hydroxide was added to small quantities of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap indicates the presence of fixed oils and fats.

Detection Of Proteins And Free Amino acids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

Million's test

The above-prepared extracts were treated with Million's reagent. Red colour formed shows the presence of proteins and free amino acids.

Biuret test

To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids.

Ninhydrine test

The extracts were treated with Ninhydrine reagent. Purple colour produced shows the presence of proteins and free amino acids.

Detection Of Saponins

The extracts were diluted with 20ml of distilled water and it was agitated in a measuring cylinder for 15 minutes. The formation of 1cm layer of foam shows the presence of saponins.

Detection Of Tannins And Phenolic Compounds

Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1) 5% Ferric chloride solution - violet colour
2) 1% solution of gelatin containing 10% sodium chloride - white precipitate
3) 10% lead acetate solution - white precipitate

Above findings shows the presence of phenolic compounds and tannins.

Detection Of Phytosterols

Small quantities of various extracts were dissolved in 5ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

Salkowski test

To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

Libermann Burchard test

The above prepared chloroform solution was treated with a few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols.

Detection Of Alkaloids

Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

1) Mayer's reagent - cream precipitate
2) Dragendroffs reagent - orange brown precipitate
3) Hager's reagent - yellow precipitate
4) Wagner's reagent - reddish brown precipitate

Detection Of Gums And Mucilages

A small quantity of various extracts were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties. No swelling was observed indicates the absence of gums and mucilage.

Detection Of Flavonoids

1) Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.

2) To the small portion of each extract, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.

3) **Shinoda's test:-**Small quantities of the extracts were dissolved in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.

The phytochemical constituents present in various extracts were presented in **Table 2.**

Fluorescence Analysis

Many substances for example, quinine solution in dilute sulphuric acid when suitably illuminated emit light of a different wavelength or colour form that which falls on them. This emitted light (fluorescence) ceases when the exciting light is removed.

A very important generalization made by stokes in 1852 stated that "in fluorescence the fluorescence light is always of greater wavelength than the exciting light". Light rich in short wavelengths is very active in producing fluorescence and for this reason strong ultraviolet light produces fluorescence in many substances which do not visibly fluorescence in day light (Evans,2001) . The results are given in **table 3,4.**

RESULTS AND DISCUSSION

The phytoconstitutents were extracted by using different solvents of increasing polarity like petroleum ether, chloroform acetone, ethanol and water. The extractive values were given in Table 1 .

Table 1: Data showing the extractive values of *Dipteracanthus prostatus* nees.

Plant Name	Part used	Method of extraction	Percentage yield				
			Petroleum ether	Chloroform	Acetone	Ethanol	Aqueous
Dipteracanthus prostatus nees	Whole plant	Continuous hot percolation and Cold macerationprocess (successive solvent extraction)	2.4g	2.6g	3.1g	3.8g	4.2g

The phytoconstitutents were identified by chemical tests which showed the presence of various phytoconstitutents (Table2) mainly in the following extracts.

Table 2: Data showing the preliminary phytochemical screening of the various extracts of *Dipteracanthus prostates.* nees

Sr. no.	Constituents	Petroleum Ether Extract	Chloroform Extract	Acetone Extract	Ethanol Extract	Aqueous Extract
01	Alkaloids	-	-	-	+	+
02	Sterols	-	-	-	-	-
03	glycosides	-	-	-	+	+
04	Fixed oil and fats	+	+	+	+	+
05	Phenolic compounds	+	+	+	+	+
06	Protein and amino acids	+	+	+	+	+
07	Tannins	-	-	-	+	+
08	Gum & mucilage	+	+	+	+	+
09	Flavonoids	-	-	+	+	+
10	Carbohydrates	-	-	-	+	+
11	Saponins	-	-	-	-	-

'+' Presence, '-'absence

Ethanol extract

Alkaloids, Fixed oil and fats, Carbohydrate, proteins, amino acids, tannins, glycosides, phenolic compounds and flavonoids, Gum and mucilage.

Aqueous extract

Alkaloids, Fixed oil and fats, Carbohydrate, proteins, amino acids, tannins, glycosides, phenolic compounds and flavonoids, Gum and mucilage.

In the above stated extracts, aqueous and ethanol extracts showed the same types of constituents. Hence ethanol and

aqueous extracts were selected for pharmacological studies. Ethanol extract was selected for the isolation of the available active constituents, because ethanol being a bipolar solvent, which can dissolve a wide range of phytoconstitutents, whereas the aqueous extract contains polar compounds.

Fluorescence Analysis

Table 3: Fluorescence characteristic of whole plant powder of *Dipteracanthus prostratus* Nees.

S.No	Particulars of the treatment	Under ordinary light	Under UV light (366nm)
1	Powder as such	Dark green	Brick red
2	Powder + 1N NaOH (aqueous)	Green	Brick red
3	Powder +1N NaOH (alcoholic)	Dark green	Reddish green
4	Powder + 1N HCL	Blackish green	Chocolate brown
5	Powder + H2SO4 (1:1)	Green	Brown
6	Powder + HNO3 (1:1)	Yellow	Orange
7	Powder + Ammonia	Greenish yellow	Greenish yellow
8	Powder + Iodine	Dark brown	Brown
9	Powder + 5% FeCl3	Dark-yellowish brown	Dark brown
10	Powder + Acetic acid	Light green	Orange

Table 4: Fluorescence characteristic of whole plant extract of *Dipteracanthus prostratus* nees.

S.No	Particulars of the treatment	Under ordinary light	Under UV light (366nm)
1	Petroleum ether (40-60°C)	Green	Yellowish green
2	Chloroform	Dark green	Red
3	Acetone	Dark green	Red
4	Ethanol	Dark green	Brown
5	Water	Brownish green	Blackish brown

CONCLUSION

Based on the traditional uses and literature review of earlier studies the plant was selected. The preliminary phytochemical and physicochemical evaluation of studies on *Dipteracanthus prostratus* nees were done the Phytochemical constituents were extracted by successive solvent extraction and identified by chemical tests. These tests showed the presence of various phytochemical constituents like Alkaloids, Fixed oil and fats, Carbohydrate, proteins, amino acids, tannins, glycosides, phenolic compounds and flavonoids, Gum and mucilage.

Ethanolic and aqueous extracts shows the presence of majority of phyto constituents. Hence it was selected for the pharmacological studies.

The ethanol extracts which has the polarity in between the acetone and aqueous has been selected for isolation of the available active constituents.

The present study on preliminary phytochemical and physicochemical evaluation of *Dipteracanthus prostratus* whole plant could be used as the diagnostic tool for the standardization of medicinal plant. There are controversial identities of many plants. Thus, our study is an important landmark in correct identification of *Dipteracanthus prostratus*.

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