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. *Dipteracanthus prostratus* is an important medicinal plant and popularly known as black weed. It is found 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. The flowers are normally sessile, axillary’s, solitary or few together. Pale blue to light violet and occasionally white in colour, bracteoles like leaves but smaller. Calyx are par title and hairy. Corolla are infundibulate in form with narrow tube. Capsules are many seeded. 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. 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**

The marc left after petroleum ether extraction was dried and then extracted with chloroform (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 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.

**Acetone extract**

The marc left after acetone extraction was dried and then extracted with ethanol (95 % v/v), 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.

**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.

**Extraction Procedure**

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

**Materials**

i. Soxhlet apparatus.
ii. Petroleum ether (60-80°C)
iii. Chloroform
iv. Acetone
v. Ethanol (95 % v/v)
vi. Distilled water with chloroform (0.25%)

**Preparation of the Extracts**

**Method of extraction**

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

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ii. Petroleum ether (60-80°C)
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**Extract of Dipeteracanthus prostratus**

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**Chloroform extract**

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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

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

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.

Detection Of Alkaloids
Small quantities of various extracts were separately treated with a few drops of concentrated sulphuric acid followed by few drops of dilute acetic acid, 3ml of acetic anhydride. A bluish green colour appeared indicates the presence of phytosterols.

Detection Of Flavonoids
A small quantity of various extracts were dissolved in 5ml of chloroform acetone, ethanol 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 2.

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

RESULTS AND DISCUSSION
The phytoconstituents 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 Dipteraeanthus prostates nees.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Part used</th>
<th>Method of extraction</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipteraeanthus prostates nees</td>
<td>Whole plant</td>
<td>Continuous hot percolation and Cold maceration process (successive solvent extraction)</td>
<td>2.4g 2.6g 3.1g 3.8g 4.2g</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Constituents</th>
<th>Petroleum Ether Extract</th>
<th>Chloroform Extract</th>
<th>Acetone Extract</th>
<th>Ethanol Extract</th>
<th>Aqueous Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td>Sterols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>03</td>
<td>glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>04</td>
<td>Fixed oil and fats</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>05</td>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>06</td>
<td>Protein and amino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>07</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>08</td>
<td>Gum &amp; mucilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>09</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

"+" 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 phytoconstituents, whereas the aqueous extract contains polar compounds.

Fluorescence Analysis

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

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

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

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

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, Carbohydrates, 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.

REFERENCES


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