A REVIEW OF PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF PLANT DESMODIUM GANGETICUM (L.) DC.

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ABSTRACT

In different countries approx. 20,000 medicinal plants are being used, out of which 10,000 (approx.) plants are used for phyto-therapy in Indian system of medicine, which has been compiled recently by World Health Organization (WHO). According to Biological Conservation Letter, more than 7,000 species of plants found in various ecosystems are said to be medicinal in the country. So, India is one of the world’s richest sources of medicinal and aromatic plants. Desmodium gangeticum (L) DC is an important medicinal plant. It is commonly used in ayurvedic formulations for the treatment of various disorders. Phytochemical evaluations, pharmacognostic evaluation, including determination of ash value, acid soluble ash, water soluble ash, extractive values, powder size, organoleptic characters, TLC profile and FTIR profile was carried out to set them as diagnostic indices for the identification/validation of the raw material and standardization of the formulations. Preliminary phytochemical analysis showed the presence of active constituents which is necessary for the pharmacological activity. Organoleptic properties, phyto-chemical studies, powder analysis., showed the presence of adulteration in the powder.

KEYWORDS: Desmodium gangeticum (L.) DC., Organoleptic properties, phytochemical studies, powder analysis, pharmacognostic studies, TLC, FTIR.
INTRODUCTION

Desmodium gangeticum (L.) DC. Often referred to as prsniparni by ayurvedic physicians in Kerala and Tamilnadu, is a member of the well-known group of plants Dasamoola (roots of ten plants). It has been mentioned to have high therapeutic value in Ayurveda. Desmodium gangeticum is a slender, sub erect diffusely branched under shrub growing about 2 – 3 feet high following a terrestrial life cycle.

The plant is an erect, diffusely branched under shrub, 90-120 cm in height with a short woody stem and numerous prostrate branches provided with soft grey hairs. Leaves simple, ovate lanceolate and membranous. Flowers white or purple or lilac in elongate lax terminal or axillary racemes. Fruits moniliform 6-8 glabrescent, pods sparsely pubescent with hooked hairs, joints separating when ripe into indehiscent one seeded segments, seeds compressed and reniform. It is distributed in all parts of India in dry conditions. In Kerala and Tamil Nadu the roots of this plant are used as an ingredient in more than 68 ayurvedic formulations.

Although in traditional medicine genus Desmodium is very well known, but still Desmodium gangeticum invites attention of researchers worldwide for its ethnomedicinal uses, phytochemistry and pharmacological activities ranging from antidiabetic to antiviral. To the best of our knowledge, very little information is available on phytochemical profile of Desmodium gangeticum. Hence, the present investigation was overviewed to explore the phytochemical profile and ethnomedicinal uses of valued endangered medicinal plant – Desmodium gangeticum (L.) DC.

Preliminary phytochemical analysis showed the presence of active constituents which is necessary for the pharmacological activity. Organoleptic properties, phytochemical studies, powder analysis, showed the presence of adulteration in the powder.

METHODOLOGY

1. Preparation of plant extract[1]

Whole plant parts of D. gangeticum were collected during the month of July-August from local herbal garden of kollam (kerala). The plant was taxonomically identified and authenticated by prof. DR. krishnaraj, Botany department, Baselious collage Kottayam (kerala).
Fresh whole parts of *D. gangeticum* were washed and shade dried than coarsely powdered in a grinder. Powder dried plant were extracted with three different solvents of increasing polarity (chloroform, water and ethanol) by the soxhlet extraction.

### 2. Organoleptic properties

Colour: greenish brown  
Texture: soft  
Odour: no characteristic odour  
Taste: no characteristic taste  
Solubility; Not soluble in water  
Bitterness value; negligible  
Particle size of power; moderately course

### 3. Phytochemical screening

Phytochemical screening for the identification of various phytoconstituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, flavonoids, carbohydrates, amino acids, phenolics and tannins according to standard methods were performed.

**Test for carbohydrate**

- **Molisch test**  
A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch’s reagent and formation of brick red colour confirmed the presence of reducing sugar.

- **Fehling’s test**  
Equal volume of Fehling A (coppersulphate in distilled water) and Fehling B (potassium tartrate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.

**Test for glycosides**

- **Borntrager’s test**  
200 mg crude extract was mixed with 2 ml of dilute sulphuric acid and 2 ml of 5% aqueous ferric chloride solution, boiled for 5 minutes which lead to oxidation to anthraquinones, indicating the presence of glycosides.
Test for Alkaloids

- **Mayer’s test**
  Crude extract was mixed with Mayer’s reagent (potassium mercuric iodide solution). Cream colour precipitate was formed, indicating the presence of alkaloids.

- **Dragendorff’s test**
  Crude extract was mixed with Dragendorff’s reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.

- **Wagner’s test**
  Filtrates were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for Flavanoids

- **Alkaline reagent test**
  Crude extract was mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned to colorless on addition of few drops of diluted acid, marked the presence of flavanoids.

- **Lead acetate test**
  To a solution of 0.5 g extract in water, about 1ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavanoids.

Test for Saponins

- **Froth test**
  0.5g extracts were dissolved in 10ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds.The test tube was allowed to stand in a vertical position and observed over 30 minutes period of time. If a “honeycomb” froth above the surface of liquid persists after 30 minutes the sample is suspected to contains saponin.

Test for Tannins

- **Ferric chloride test**
  Crude extract was mixed with ferric chloride. Blue green colour appeared, suggested the presence of tannins.
3. Pharmacognostic studies

**Determination of ash value**

**Determination of total ash**

2-4g of the sample was weighed in a crucible and was spread evenly and ignited slightly increasing the temperature to 500-600 degree Celsius until it turns white, it was cooled in a dessicator and weighed.

**Determination of acid insoluble ash**

25 ml of dilute hydrochloric acid was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

**Determination of water soluble ash**

25 ml of water was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

\[
\text{Percentage ash value} = \frac{\text{Initial weight taken}}{\text{Weight of ash}} \times 100
\]

**Determination of extractive value**

**Water soluble extractive value**

5 grams of the coarse drug was macerated with 100ml of water for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight. the percentage of water soluble extractive value was calculated with reference to the air dried drug.

**Alcohol soluble extractive value**

5 grams of the coarse drug was macerated with 100ml of alcohol for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight. the percentage of water soluble extractive value was calculated with reference to the air dried drug.
4. **Estimation of tannins**\[^6\]

The method of Okeke and Elekwa was used here for the determination using 5 g sample shaken with 50ml of water and left to stand for 30 minutes. The solution was filtered and 2ml of the filtrate was introduced into a test tube and 3ml of 0.1 M Ferric chloride and 2ml of potassium ferrocyanide were added. To this 46 ml of water was added. It was filtered again and 1ml of the filtrate was used to read the absorbance at 710nm within 10 min.

5. **Powder analysis**\[^7\]

Sieve size/powder fineness: 10 g of the sample was accurately weighed and passed through various sieves numbered 16,22,44,100 and shaken for 20 minutes successively and the powder remaining on each sieve was weighed and average particle size was determined.

6. **Thin layer chromatography**\[^8\]

100 gram of silica gel G was dissolved in sufficient amount of water and was coated on the glass plate. Solvent system chosen was toluene: chloroform :methanol (5:8:3) aqueous extract was dissolved in sufficient water to make up a concentration of 1mg/ml. similarly ethanolic extract and chloroform extract was dissolved in ethanol and chloroform.

The spots were made 1 cm from the bottom of the glass slide. The glass plate was kept in to the chamber after chamber saturation and allowed to run 2-3\(^{rd}\) of the glass plate. \(R_f\) value was calculated.

\[
R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}
\]

**RESULTS**

**RESULT OF PHYTOCHEMICAL ANALYSIS**

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Phytochemical constituents</th>
<th>Tests</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Chloroform extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorffs test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagners test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayers test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>3</td>
<td>Phenolic compounds</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>Molischs Fehlings</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavanoids</td>
<td>Alkaline test</td>
<td>_</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>Froth formation test</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>
RESULT OF PHARMACOGONOSTIC ANALYSIS

Table 2.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water soluble extractive value</td>
<td>1.24%</td>
</tr>
<tr>
<td>2</td>
<td>Alcohol soluble extractive value</td>
<td>1.12%</td>
</tr>
<tr>
<td>3</td>
<td>Determination of total ash values.</td>
<td>6.45%</td>
</tr>
<tr>
<td>4</td>
<td>Determination of acid – insoluble ash.</td>
<td>0.50%</td>
</tr>
<tr>
<td>5</td>
<td>Determination of water soluble ash.</td>
<td>2.71%</td>
</tr>
</tbody>
</table>

RESULT OF PARTICLE SIZE

Table 3.

<table>
<thead>
<tr>
<th>Sieve size</th>
<th>Pore size</th>
<th>Weight of powder(n)</th>
<th>n/gx100</th>
<th>Cumulative frequency</th>
<th>Mean pore diameter(d)</th>
<th>nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1000</td>
<td>5.0490</td>
<td>50.49</td>
<td>50.49</td>
<td>855</td>
<td>4316.89</td>
</tr>
<tr>
<td>22</td>
<td>710</td>
<td>1.9240</td>
<td>19.24</td>
<td>69.73</td>
<td>532.5</td>
<td>1024.53</td>
</tr>
<tr>
<td>44</td>
<td>355</td>
<td>1.1025</td>
<td>11.025</td>
<td>80.75</td>
<td>252.5</td>
<td>278.38</td>
</tr>
<tr>
<td>100</td>
<td>150</td>
<td>0.6753</td>
<td>6.753</td>
<td>87.50</td>
<td>75</td>
<td>50.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2052</td>
<td>2.052</td>
<td>89.56</td>
<td></td>
<td>Total=5670.4535</td>
</tr>
</tbody>
</table>

Average particle size=$\frac{\sum nd}{\sum n}$ = $\frac{5670.4535}{8.8785}$ = 638.67.

RESULT OF THIN LAYER CHROMATOGRAPHY

Table 4.

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Aqueous extract toluene : chloroform :methanol (5:8:3)</th>
<th>Ethanol extract toluene : chloroform :methanol (5:8:3)</th>
<th>Chloroform extract toluene : chloroform :methanol (5:8:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance traveled by the solute</td>
<td>3 cm</td>
<td>3.1 cm</td>
<td>4.2 cm</td>
</tr>
<tr>
<td>Distance traveled by the solvent front</td>
<td>4.5 cm</td>
<td>4.2 cm</td>
<td>4.8 cm</td>
</tr>
<tr>
<td>R_f value</td>
<td>0.6 6</td>
<td>0.73</td>
<td>0.87</td>
</tr>
</tbody>
</table>
DISCUSSION
Preliminary quantitative analysis of the drug showed the presence of the alkaloid, flavonoids, and carbohydrates.

- The water soluble extractive value was found to be 1.24%.
- The alcohol soluble extractive value was found to be 1.12%.
- The total ash value was found to be 6.45%.
- The acid –insoluble ash value was found to be 0.50%.
- The water soluble ash value was found to be 2.71%.
- Rf value of aqueous extract was found to be 0.66.
- Rf value of ethanolic extract was found to be 0.73.
- Rf value of chloroform extract was found to be 0.87.

CONCLUSIONS
Preliminary phytochemical screening showed the presence of active constituents necessary for the pharmacological activity. Pharmacognostic study revealed the Ash values, extractive value and Particle size and helped to determine the average particle size, level of contamination and adulterants.

In conclusion, the pharmacognostic features examined in the present study that includes organoleptic characters, phyto-chemical, particle size distribution, and TLC, may serve as a tool for identification/ validation of the raw material and standardization of its formulations in fixing quality control parameters. Rf values 1,2,3 ranges with in the standard values of alkaloids, tannis, and flavonoids.
REFERENCE


2. Trease and Evans, Pharmacognosy, Page No: 425.


