Phytochemical and TLC Studies of Ethanolic Extract of *Sesbania grandiflora* (Fabaceae).

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**Abstract:** The aerial parts (fresh leaves, pods, flowers) of the plant Sesbania grandiflora were collected powdered and the powder was initially defatted with petroleum ether (60-80°C). The marc was further macerated with ethanol for 72 hrs. The extract was preserved at room temperature for further studies. Preliminary Phytochemical screening was done which revealed the presence of sugars, tannins, polyphenols, flavonoids, aminoacids, triterpenoids, saponins, flavoniods. The TLC techniques were used for qualitative determination of possible number of components from the ethanolic extract. A solvent system was optimized in order to get maximum separation on plate and presence of various phytochemicals present was confirmed by the use of different spraying reagents.

**Keywords:** Sesbania grandiflora, TLC of Sesbania grandiflora.

**Introduction**

*Sesbania grandiflora* (Fabaceae), commonly known as Agati is a widely available, fast growing plant, generally popular for its animal fodder use\(^1,2\). Traditionally the plant has been used for the treatment of head ache, in fever, as a tonic, in catarrh, as an astringent etc\(^3,4,5,6\). The leaves of the plant have been reported to have anxiolytic and anticonvulsant effect while the flowers have been reported to have anti microbial activity\(^7\). It shows hypolipemic, anti ulcer and anti-inflammatory properties as well.

**Experimental**

**Materials and Method:**

The aerial parts (fresh leaves, pods, flowers) of the plant Sesbania grandiflora were collected from Nagpur district; Maharashtra, India in the month of August-September (flowering season) and the plant was identified and authenticated from the botany department R.T.M Nagpur University, Nagpur. The voucher specimen of the plant (no.9088) has been kept in the department of botany for further references.

**Ethanolic Extract:**

The powder was initially defatted with petroleum ether (60-80°C). The marc was further macerated with ethanol for 72 hrs. The extract was preserved at room temperature for further studies.

**Preliminary Phytochemical Screening**

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids...
etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites all the extracts were subjected to battery of chemical tests.

**Thin Layer Chromatography of Ethanolic extract:**

Ethanolic extract of *Sesbania grandiflora* was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them.

**Preparation of the plates:**
The adsorbent used for thin layer chromatography was silica gel G. The precoated TLC plates (Merk, Germany) were heated in an oven for 30 minutes at 110°C for activation. 5μl of the test sample (1mg/ml in alcohol) was applied in the form of bands using LINOMAT IV applicator. Four bands using different volumes in increasing order were applied.

**Developing solvent system**
A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned in table. After development of plates, they were air-dried and numbers of spots were noted & Rf values were calculated. Spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Aluminum chloride(10 %in methanol) for flavonoids, ninhydrin solution (2% in methanol) for amino acids, amines and amino sugars, ferric chloride reagent, sulphuric acid reagent (5% in methanol) was used.

**Test extract**:
*Sesbania grandiflora* ethanolic extract

**Solvent system**
Ethyl acetate: Methanol: Water (5 : 1.1 : 1)

**HPTLC study:**
HPTLC precoated, silica gel G 60 F254 (Merck, Germany) plates were used for application of sample. A small quantity of extract was dissolved in methanol and sample was applied in precoated plate with the help of Linomat IV applicator. Solvent system optimized for TLC study was chosen for HPTLC study.

**Chromatographic conditions:**
Following are the chromatographic conditions required to get an effective resolutions by selected mobile phase.

- **Stationary phase** : HPTLC precoated, silica gel G 60 F254 (Merck, Germany)
- **Size** : 10 x 10 cm
- **Developing chamber** : Twin trough glass chamber
- **Mode of application** : Band
- **Band size** : 5 mm
- **Separation technique** : Ascending
- **Temperature** : 20 ± 5°C
- **Saturation time** : 30 min
- **Scanning wavelength** : 254 nm / 366 nm
- **Scanning mode** : Absorbance/Reflectance

**Result and Discussion**
The preliminary phytochemical screening of the ethanolic extract revealed the presence of chemical constituents like Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Amino acids which was confirmed by performing TLC separation technique and different spraying reagents. Presence of sterols and triterpenoids was detected visually by spraying with 5% Sulphuric acid in methanol, Tannins showed color reaction by ferric chloride solution where as flavonoids were confirmed by yellow fluorescence on spraying with aluminium trichloride solution. Amino acids were confirmed purple color on spraying with ninhydrin solution.
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant constituent</th>
<th>Test/Reagent</th>
<th>S. grandiflora</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pet ether extract</td>
</tr>
<tr>
<td>1.</td>
<td>Steroids</td>
<td>Salkowski reaction</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liebermann-Burchard test</td>
<td>–</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaloids</td>
<td>Dragendorff’s reagent</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s reagent</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hager’s reagent</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s reagent</td>
<td>–</td>
</tr>
<tr>
<td>3.</td>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>–</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>–</td>
</tr>
<tr>
<td>5.</td>
<td>Carbohydrates</td>
<td>Molish’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehling’s test</td>
<td>–</td>
</tr>
<tr>
<td>6.</td>
<td>Amino acids</td>
<td>Ninhydrin test</td>
<td>–</td>
</tr>
<tr>
<td>7.</td>
<td>Saponins</td>
<td>Foam test</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test extract</th>
<th>Solvent system</th>
<th>Number of Spots</th>
<th>Rf values</th>
</tr>
</thead>
<tbody>
<tr>
<td>For S. grandiflora alcoholic extract</td>
<td>Ethyl acetate: Methanol: Water 5: 1.1 : 1</td>
<td>12</td>
<td>0.09, 0.18, 0.29, 0.36, 0.47, 0.52, 0.58, 0.66, 0.75, 0.79, 0.84, 0.91.</td>
</tr>
</tbody>
</table>

Sulphuric acid UV light at 366 nm Aluminum chloride Ferric chloride
Chromatogram at 256 nm

Chromatogram at 366 nm

References:


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