PRILIMINARY PHYTOCHEMICAL STUDIES OF HYDROALCOHOLIC EXTRACT OF SAUROPUS ANDROGYNUM LEAVES

Deepu Raveendran*¹, Jolly Samu¹ and Anju Thomas¹
Rajiv Gandhi Institute of Pharmacy Triakripur. Kasargod, Kerala, 671310, India.

ABSTRACT
Sweet leaf is one of the most popular, leaf vegetables from India to Malaysia. One such plant is Sauropus androgynus locally available has been used in traditional system of medicine, commonly consumed leafy vegetable. It is rich in Vitamin C, vitamin K, vitamin A, polyphenols and flavanoids. On the basis of use of leaves in Ayurveda for the treatment of CNS disorder and presence of bioactive flavonoids, Extraction and preliminary phytochemical studies of Sauropus androgynus revealed the presence of Alkaloids, Carbohydrates, Flavonoids, Saponins, Steroids and Proteins. Hence the present work is intended to study preliminary phytochemical studies.

KEYWORDS: Sauropus androgynus (SA) Leaves, phytochemical, traditional medicine, bioactive flavonoids.

INTRODUCTION
Sweet leaf is one of the most popular, leaf vegetables from India to Malaysia. It is a shrubby plant which grows up to 3m high. It merits attention and cultivation in Australia, as it is one of the most prolific, heavy yielding, nutritious and appetizing green leaves. In research trials in Malaysia, sweet leaf yields per hectare, surpassed all other greens. The bush has upright, multiple stems 1-2/1 meters high; dark-green, oval-shaped leaves 5-6cm long. Flat, round, orange/red flowers 1-2 cm across, form in the leaf axils. In tropical climates, a capsule forms, with small, black seeds. We have experienced, in our sub-tropical conditions; the bush thrives, flowers, but does not set seed.¹
Propagation is by seeds, suckers or cuttings. Sweet leaf will grow in most soils, including heavy clay. It will tolerate high rainfall and also dry conditions. The bush will grow in full sun, as well as 95% shade. Growth is rapid during the warm months, slowing down in leaf production in winter, or even going dormant. In cold climates, plant in a warm, sheltered, wind-protected position, or in a large pot, and can be moved to a warmer position overwintern. Fertilize regularly, and mulch to conserve moisture. An extract made of the plant has been found to have a strong activity against pine wood nematodes, and may have possibility against other species.

Leaf
Leaves are simple and alternatively arranged. Upper surface is dark green in colour and lower surface is light green. Petioles 2-4 mm long; leaf blade sub membranous or thin-papery, ovate-lanceolate, oblong-lanceolate or lanceolate, 3-10 cm long, 1.5-3.5 cm wide, base cuneate, rounded or truncate, apex acuminate; lateral veins 5-7 pairs, flattened adaxially, elevated abaxially, reticular nerves obscure.

Uses
The leaves are used as antitussive, brain tonic, soothing lungs and to relieve internal fever. It is a good anti-inflammatory and analgesic agent. It also helps to improve blood circulation. Stem has antimicrobial, anticancer and antioxidant properties.\(^1\)

A green dye obtained from rubbing and squeezing the leaves is used as food colour.\(^2\) The roots are used in treatment of cardiovascular diseases or its symptoms including vertigo, dizziness etc. Sauropus androgynus is highly nutritious and has many culinary uses. Fresh leaves are excellent source of provitamin A carotenoids, vitamins B and C, proteins and minerals.\(^2,4\)

MATERIAL AND METHODS
Collection of plant material and preparation of extract
The fresh leaves of Sauropus androgynus Linn used for the present studies were collected from Mangalore, in June 2014. The collected leaves material were cleaned to remove the adhered dust particles and were then shade dried. The dried plant materials were coarsely powdered, weighed and stored in an air tight container till use. The coarse powder was packed into Soxhlet column and extracted with 70% ethanol for about 48 h.\(^5\) The solvent
was evaporated using rotary flash evaporator to get syrupy consistency. Then the dried extract was stored in airtight container in refrigerator below 10°C.

1. **PRELIMINARY PHYTOCHEMICAL SCREENING**[^6,^7]

Preliminary phytochemical screening was carried out for hydroalcoholic extract of *Sauropus androgynus* Linn leaves as described below.

**A. Tests For Alkaloids**

0.5 gm extract was dissolved in 10 ml of dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids:

1. **Mayer’s test**

   To one ml of filtrate, 2 ml of Mayer’s reagent was added in a test tube. Formation of Yellow cream precipitate indicates the presence of alkaloids.

2. **Wagner’s test**

   One ml of filtrate was treated with few drops of Wagner’s reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

3. **Dragendorff’s test**

   One ml of filtrate was treated with few drops of Dragendorff’s reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

4. **Hager’s test**

   One ml of filtrate was treated with few drops of Hager’s reagent. Formation of yellow precipitate indicates the presence of alkaloids.

**B. Tests for glycosides**

1. **Bromine water test**

   Test extract was dissolved in bromine water. Formation of yellow precipitate indicates the presence of glycosides.

2. **Baljet Test**

   Test extract was treated with sodium pirate. Formation of yellow to orange colour indicates the presence of glycosides.

3. **Keller-Killiani Test**

   0.5g of dried extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solutions. This was then under laid with 1 ml of concentrated H₂SO₄. It forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green, indicates the presence of glycosides.
4. Legal’s test
Test extract was treated with pyridine (made alkaline by adding sodium nitroprusside solution). Formation of pink to red colour indicates the presence of glycosides.

C. Tests For Tannins
1. Ferric chloride test
Few drops of 5% w/v FeCl3 solution was added to 1-2ml of the extract. Formation of brown colour indicates the presence of pseudo tannins.

2. Vanillin hydrochloride test
Extract was treated with vanillin hydrochloride reagent. Formation of purplish red colour indicates the presence of tannins.

3. Gelatin test
Extract was treated with gelatine solution. Formation of white precipitate indicates the presence of tannins.

D. Tests For Saponins
1. Sulphur test
Sulphur was added to the extract solution. Sulphur sinks at bottom indicates the presence of saponins.

2. Froth’s test
The extract was diluted with distilled water and shaken for 15 min. Formation of foam indicates the presence of saponins.

3. Liberman Buchard’s test
The extract was treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. The formation of brown ring at the junction indicated the presence of steroidal saponins.

E. Tests For Carbohydrates
Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

1. Molisch’s Test:
Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml concentrated sulphuric acid was added carefully along the sides of the test tubes. Formation of violet ring at the junction indicates the presence of carbohydrates.
2. **Benedict’s Test**
Filtrates were treated with Benedict’s reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

3. **Fehling’s Test**
Filtrates were hydrolyzed with dilute hydrochloric acids, neutralized with alkali and heated with Fehling’s A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

4. **Barfoed’s test**
1ml Barfoed’s reagent was added to 1ml of extract and heat for 2 min. Formation of red precipitate indicates the presence of carbohydrate.

5. **Seliwanoff’s test**
Plant Extract was treated with Seliwanoff’s reagent and heat strongly. Formation of a characteristic cherry red colour indicates the presence of keto sugar.

**F. Tests For Flavonoids**

1. **Lead acetate test**
Lead acetate solution was added to small amount of extract. Formation of yellow precipitate indicates the presence of flavonoids.

2. **Shinoda test**
A little quantity of extract was dissolved in alcohol with few fragments of Mg turnings and con: HCl drop wise. Formation of pink or crimson-red colour indicates the presence of flavonoids.

3. **Alkaline reagent test**
Increasing amount of sodium hydroxide was added to the sample extract. Formation of yellow colouration observed which disappears upon addition of acid indicates the presence of flavonoids.

4. **Ferric chloride test**
Extract was treated with ferric chloride solution. Formation of Intense green to black colour indicates the presence of flavonoids.

**G. Tests For Steroids**

1. **Salkowski reaction:** 2mg of dry extract was shaken with CHCl₃, to the CHCl₃ layer, H₂SO₄ was added slowly by the sides of test tube. Formation of red colour indicates the presence of steroids.
2. **Lieberman Burchard’s test:** 2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of conc. H$_2$SO$_4$. Formation of red violet or green colour indicates the presence of Steroids.

RESULT AND DISCUSSION

EXTRACTION OF PLANT MATERIAL

The percentage yield of SA was found to be 9.32% as shown in Table 1.

Table 1: Percentage yield of crude extract of SA leaves.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Color</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroalcoholic</td>
<td>Dark Greenish</td>
<td>9.32%</td>
</tr>
</tbody>
</table>

**Preliminary phytochemical screening**

Preliminary phytochemical analysis of extract is shown in Table No. 9 revealed the presence of following phytochemicals: Carbohydrates, Proteins Flavonoids, Glycosides, Saponins and Steroids.

Table 2: Preliminary phytochemical screening.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Chemical Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>-</td>
</tr>
</tbody>
</table>

( + = Present in test, - = Absence in test)

**CONCLUSION**

The hydroalcoholic extract prepared was subjected to phytochemical tests and the outcome of these tests revealed the presence of carbohydrate, Alkaloids, Glycosides flavonoids, steroids and saponins. Further investigations are required to find out active component of the extract.

**REFERENCE**


