EVALUATION OF PHARMACOGNOSTIC, PHYSICO-CHEMICAL AND PRELIMINARY PHYTOCHEMICAL PROPERTIES OF SCLERIA LITHOSPERMA LINN (CYPERACEAE) WHOLE PLANT.

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ABSTRACT
Scleria lithosperma L. belonging to the family Cyperaceae is a perennial plant grown in shady trails, fertile, pine rock land, common along the streams, tanks, hill slopes and rocky areas. It is found in India in the areas of Tirumala, Chittoor District, A.P. The Pharmacognostic studies of the plants revealed the microscopic, macroscopic and physiochemical features of the whole plant. The Preliminary Phytochemical examinations showed an evidence for the presence of chemical constituents - steroids, saponins, glycosides and flavanoids. Methanolic, Ethanol and Chloroform extracts showed the presence of Glycosides, Flavanoids, Saponins and Steroids while the aqueous extract showed only positive result for the presence of Saponin glycoside. It was also observed that methanol gave the highest percentage yield.

KEYWORDS: Pharmacognostic, phytochemical, physico-chemical, evaluation, preliminary tests.

INTRODUCTION[1-4]
Scleria lithosperma L. is a common perennial plant with rhizomes short, nodulose, aromatic when fresh belongs to family cyperaceae. It mainly contains steroids, saponins, glycosides. It is traditionally used in the treatment of skin diseases, as abortifacient, in correcting menstrual cycle.
Table No. 1: Taxonomical classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kingdom</td>
<td>Plantae – Plants</td>
</tr>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta – Vascular plants</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta – Seed plants</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta – Flowering plants</td>
</tr>
<tr>
<td>Class</td>
<td>Liliopsida – Monocotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Commelinidae</td>
</tr>
<tr>
<td>Order</td>
<td>Cyperales</td>
</tr>
<tr>
<td>Family</td>
<td>Cyperaceae – Sedge family</td>
</tr>
<tr>
<td>Genus</td>
<td>Scleria P.J. Bergius – nutrush</td>
</tr>
<tr>
<td>Species</td>
<td>Scleria lithosperma (L.) Sw. – Florida Keys nutrush</td>
</tr>
</tbody>
</table>

Common name

English: Florida keys nutrush, slender nutrush, Scirpus lithosperma
Tamil: Kathipul
Telugu: Kondashaka thunga

Ecology and Distribution

Habitat
Scleria lithosperma is a perennial plant grown in shady trails, fertile, pine rockland, hammock growth, common along the streams, tanks, hill slopes, rocky areas and under the growth of forest areas, roadside areas.

Geographical distribution: Mexico, West Indies, Central America, South America, tropical Asia, Africa. In India it is found majorly in the areas of Japalitheertham, microwave station and papanasanam in Tirumala, Kailasakona, Kambakkam hills. It is grown in the most part of the year.

Plants

Perennial herb, erect tufted, stems slender, rhizomes short, nodulose, aromatic when fresh.

Culms in tufts, slender, 30–90(–115) cm, glabrous or slightly scabrous.

Leaves

Sheaths purplish, leaves clustered about the middle of stem, wingless, weakly ribbed, hairy, finely pilose or nearly glabrous; contra-ligules reddish, triangular, rigid, distinctly ciliate; blades distinctly grayish green and revolute when dry, linear, attenuate, keeled, 1–3(–5) mm wide, shorter than culms.

Inflorescences

Axillary 1–3, terminal 1, quite lax; stalked spikes or panicles 2–4, terminal one 3–4.5(–8.5) cm with 2–7 open fascicles 2–6(–9) mm wide, of 1–4 spikelets; bracts subtending and overtopping inflorescence leaflike, broadly attenuate, scabrous. Spikelets bisexual (an occasional terminal staminate spikelet), few flowered, 3–5 mm; stamine scales lanceolate, pistillate scales ovate-acuminate, with prominent green keel. Achenes whitish or gray between angles, obscurely trigonous, ovoid or globose, 2–2.5(–3) mm, smooth, base broadly attenuate, somewhat depressed between angles, trigonous, not porose, apex umbonate; hypogynium obsolete, reduced to distinct brown band at base of achene.

TRADITIONAL USES

Uses

Sebacious cyst (vakkathipullu) Scleria lithosperma tuber is washed, baked, powdered and mixed with coconut oil is applied on the infection which alleviates the problem. It is also used traditionally in correcting menstrual cycle, during pregnancy and as anti-aborifacients. It is used to treat enlarged stomach in children and also used an antiseptic. The plants or herbs belonging to the family cyperaceae exhibited the properties like anti-diabetic, anti-oxidant and hypolipidemic.

Collection

The plant is collected from the forests of Chittoor district of Andhra Pradesh and certified from the professor Dr.
Madhava Shetty who is a professor in the department of Botany.

Drying and size reduction of the plant
The whole plant is subjected to shade drying for 5 weeks. The dried plant material was further crushed to powder mechanically and sieved and stored in air-tight container for further analysis.

Macroscopic characteristics
Colour: leaves are green in colour
Odour: Characteristic
Taste: Bitter

MICROSCOPIC CHARACTERISTICS[5-12]
Physicochemical parameters
The shade dried plant of Scleria lithosperma was subjected for determination of physicochemical parameters such as foreign organic matters, methanol extractives, water soluble extractives, ethanol extractives, chloroform extractives, total ash content, acid-insoluble ash, water soluble ash, percentage moisture content were determined according to standard method(Trease-1983).

Determination of total ash
About 2-3 g accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 4500oC until free from carbon. It was then cooled and weighed. The %w/w of ash with reference to the air dried drug was calculated (I.P. 1996).

Calculations
Weight of empty dish = x
Weight of the drug taken = y
Weight of dish+ ash (after incineration) = z
‘y’ gms of the crude drug gives(z-x) gms of the ash
Therefore 100gms of the crude drug gives 100/y× (z-x) gms of the ash
Total ash value of the sample= 100(z-x)/y%

Determination of acid-insoluble ash
After the determination of total ash value further steps were followed:
1) 25ml of dilute hydrochloric acid was taken and washed the ash from the dish that is used for total ash into a 100ml beaker.
2) Boiled for 5 minutes by placing over bunsen burner.
3) Filtered through ash less filter paper washed the residue twice with hot water.
4) Ignited the crucible in the flame, cooled and weighed.
5) Kept the filter paper and residue together into the crucible, heated gently until vapours ceased to be evolved and then more strongly until all carbon has been removed.
6) It was cooled in a dessicator.
7) Then the residue was weighed and calculated acid-insoluble ash of the crude drug with reference to the air-dried sample of crude drug.

Determination of water soluble ash
Similar procedure was performed as acid insoluble ash but instead of dil hydrochloric acid 25ml of water was used.

Determination of moisture content
1) About 1.5 gms of the powdered drug was weighed and placed into a flat thin porcelain dish which was previously weighed.
2) Dried in the oven at 100oC.
3) Then cooled in the desiccator and weighed.
4) The loss of weight is recorded as moisture.

Determination of extractive values
Extractive values
1) Useful for the evaluation of a crude drug.
2) Give the idea about the nature of the chemical constituents present in a crude drug.
3) Useful for the estimation of specific constituents, soluble in that particular solvent used for extraction.

Determination of alcohol soluble extractive
Procedure
1) 5 gm of powered drug was weighed and transferred to a 250 ml dry conical flask.
2) 100 ml of solvent (90% alcohol) was transferred into the conical flask.
3) The flask was corked and set aside for 24hrs with frequent shakings.
4) Filtered into a 50ml cylinder when sufficient filtrate was collected 25 ml of it was transferred into a thin porcelain dish which was previously weighed.
5) Evaporated to dryness on a water bath and completed the drying in an oven at 100 oC which was then cooled in dessicator and weighed.
6) Calculated the percentage w/w of extractive with reference to the air dried drug.

Calculation
25 ml of alcoholic extract gives = x gms of residue
100ml of alcoholic extract gives=4x gms of residue
Since 5gms of air dried drug gives 4x gms of alcohol soluble residue
100gms of air dried drug gives 80x gms of alcohol soluble residue
Alcohol (90%) soluble extractive value of the sample=80x
(Practical pharmacognosy-2006)

Determination of water soluble extractive
Procedure was the same as for alcohol soluble extractive using water instead of ethanol. Following the same
procedure the extractive values for methanol and chloroform are also found.

**TABLE No. 2: PHYSICOCHEMICAL EVALUATION OF CRUDE DRUG OF SCLERIA LITHOSPERMA**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values obtained (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign organic matter</td>
<td>3.8±0.2</td>
</tr>
<tr>
<td>Total ash value</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>0.11±0.02</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Moisture content</td>
<td>4.2±0.2</td>
</tr>
</tbody>
</table>

Values expressed as mean of triplicate determination ± SEM.

**TABLE No. 3: EXTRACTION**

<table>
<thead>
<tr>
<th>Solvent for extraction</th>
<th>Extractive value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>15.76</td>
</tr>
<tr>
<td>Ethanol</td>
<td>11.2</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.6</td>
</tr>
<tr>
<td>Water</td>
<td>8.8</td>
</tr>
</tbody>
</table>

**Phytochemical screening**

Different chemical tests were performed on the extracts. These tests were performed to determine the presence of compounds like Carbohydrates, Proteins, Amino acids, Fats, Steroids, Glycosides, Alkaloids, Tannins and Phenolic compounds, Enzymes etc.

**TESTS FOR CARBOHYDRATES**

1) **Molisch test**: To 2-3ml of extract added few drops of α-naphtol solution in alcohol, shaken well and added conc. sulphuric acid from sides of test tube. Violet ring is formed at the junction of two liquids.
2) **Fehling’s test**: 1ml Fehling A and 1ml Fehling B solution was mixed and boiled for 1min. Equal volumes of test solution was added. Heated in boiling water bath for 5-10 mins. First yellow then brick red precipitate should be observed.
3) **Benedict’s test**: Mixed equal volumes of Benedict’s reagent and test solution in test tube. Heat in water bath for 5mins. Solution should appear green, yellow or red depending on reducing sugar present in the solution.

**TESTS FOR PROTEINS**

1) **Biuret test**: To 3ml test solution add 4% NAOH and few drops of 1% CuSO4 solution. Violet or pink color appears.
2) **Million’s test**: Mix 3ml test solution with 5ml Millions reagent. White precipitate appears. Warm precipitate turns brick red or the precipitate dissolves giving red colored solution.
3) **Xanthoprotein test**: Mix 3ml test solution with 1ml conc. sulphuric acid. White precipitate is formed. Boil precipitate turns yellow; add ammonium hydroxide precipitate turns orange. Solution turns black or brownish due to PbS formation.

**TESTS FOR AMINO ACIDS**

1) **Ninhydrin test**: Heat 3ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10mins. Purplish or Bluish colour appears.

**TESTS FOR STEROIDS**

1) **Salkowski reaction**: To 2ml chloroform and 2ml conc. H2SO4 Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
2) **Liebermann-Burchard reaction**: Mix 2ml extract with chloroform. Add 1-2ml acetic anhydride and 2 drops conc. H2SO4 from the sides of test tube. First red, then blue and finally green color appears.

**TESTS FOR GLYCOSIDES**

1) **Legal’s test (test for Cardenoloids)**: To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitro prusside. Pink to red color appears.
2) **Keller-Killiani test (test for Deoxy sugars)**: To 2ml extract add glacial acetic acid and 2 drops conc. H2SO4. Reddish brown color appears at junction of two liquid layers and upper layer appears bluish green.

**TEST FOR SAPONIN GLYCOSIDE**

1) **Foam test**: Shake the drug extract or dry powder vigorously with water. Persistent foam observed.

**TEST FOR FLAVANOIDS**

1) **Shinoda test**: To dry powder or extract, add 5ml 95% ethanol, few drops conc. HCL and 0.5gms magnesium turnings. Pink color observed.

**TEST FOR ALKALOIDS**

1) **Dragendroff’s test**: To 2-3 ml of extract, add few drops of dragendroff’s reagent. Orange brown precipitate is formed.
2) **Mayer’s test**: 2-3ml filtrate with reagent gives precipitate.
3) **Hager’s test**: 2-3ml filtrate with Hager’s reagent gives yellow precipitate.

**TESTS FOR TANNINS AND PHENOLIC COMPOUNDS**

To 2-3ml of aqueous or alcoholic extract, add few drops of following reagents:

1) **5% FeCl3 solution**: Deep blue-black color
2) **Acetic acid solution**: Red color solution
3) **DiluteHNO3**: Reddish to yellow color.
RESULTS AND DISCUSSION

TABLE No. 4: Preliminary phytochemical screening of Scleria lithosperma L. (Cyperaceae) extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Chemical Constituents</th>
<th>Chloroform extract</th>
<th>Ethanolic extract</th>
<th>Methanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The phytochemical tests were performed for the identification of different chemical constituents’ present in the plant extract. These tests not only help in identification of the constituents but also help in analyzing the drug for its activity as certain particular chemical constituents’ exhibit particular pharmacological activity. There have been evidences for Flavanoids to exhibit Anti Oxidant activity, Glycosides to exhibit Anti-Hypertensive activity (Slobodan et al., 1994).

CONCLUSION

The identification tests were performed on the extracts of *Scleria lithosperma* L. Methanolic, Ethanolic and Chloroform extracts showed the presence of Glycosides, Flavonoids, Saponins and Sterioids while the aqueous extract showed only positive result for the presence of Saponin glycoside. It was also observed that methanol gave the highest percentage yield and thus methanolic extract of the plant *Scleria lithosperma* L. was used for further studies.

REFERENCES

8. Reviewed by: David C. Dugdale, III, MD, Professor of Medicine, Division of General Medicine, Department of Medicine, University of Washington School of Medicine. Also reviewed by David Zieve, MD, MHA, Medical Director, A.D.A.M., Inc.