Pharmacognostical Evaluation of *Amaranthus spinosus* L

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**ABSTRACT**

**Introduction**: In increasing demand in the field of herbal medicines and cosmetics, it has become necessary and pertinent to probe into the area of systematic knowledge about herbal drugs. There is a need for the application of this knowledge in authentication, detailed study and practical utilization of crude drugs. The present paper deals with the taxonomy, anatomy, powder study pertaining to organoleptic, microscopic, and physical constant evaluations of *Amaranthus spinosus* Linn (Amaranthaceae). It is a glabrous herb found in tropical and sub-tropical regions of India. The decoction of the leaves is diuretic and febrifuge. Leaves and stems are also used for treating eye diseases and diabetes.

**Methods**: In the present study macroscopic, microscopic, ash values and extractive values were carried out to develop the diagnostic parameters for quality control of leaf and stem. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol. The specimens were casted into paraffin blocks. **Results**: Powder showed epidermal cell, anamocytic stomata, calcium oxalate crystals and cross section of leaf and stem showed the bundle sheath cells, median bundle, phloem fibers, vessels, periderm, secondary xylem and pith. Phytochemically, the methanol and aqueous extracts of *A. spinosus* showed maximum phytochemicals like alkaloids, glycosides, steroids, flavonoids, saponin, tannin and phenolic compounds, terpenoids, carbohydrates, etc. The average extractive values of leaf and stem can also be used for determine the quality of raw material of *A. spinosus*. **Conclusion**: The results of this study should provide a standard for identification and preparation of monograph of this drug.

**Key words**: *Amaranthus spinosus*, extractive and ash values, microscopical characters.

**INTRODUCTION**

The World Health Organization states that approx 85-90% of the world's population consumes traditional herbal medicines, while the herbal drug industry has been in a high growth in the late 90s due to the growing demand in developing and developed countries.[1] The plant biodiversity in India has served as the foundation for the development of many traditional system of medicine, including Ayurveda, Unani, Siddha and Tibetan.[2] In recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance.[3,4] Standardization is an essential requirement for the whole plant, plant parts or extracts in order to assess the quality of drugs.

*A. spinosus* (Amaranthaceae) is a glabrous herb found in tropical and sub tropical region of India. The root of this plant is used as diuretic and febrifuge.[5] Previous report of this plant showed that its extract was used as an anti-malarial[6], anti-diarrhoeic[7], anti-diabetic, anti-hyperlipidemic and spermatogenic[8], and anti-inflammatory activity[9], stimulates proliferation of β-lymphocytes[10] and haematology.[11] Extract of this plant showed the presence of flavonoid.[12] The present study is focused to Pharmacognostical studies of *A. spinosus*. In folk/tribal medical practice many plants are used to treat many diseases in South India.
MATERIALS AND METHODS

Plant material

The plant parts were collected from the foot hills of Yercaud, Salem, in the month of September 2005. The plant was identified and authenticated by the experts in the department of Botany Govt. Arts College, Salem, Tamil Nadu, and India. A voucher specimen (CHL-03) has been kept in our museum for future reference. The plant material was collected and shade dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No.60.

Preparation of the extract

The powdered material of *A. spinosus* was extracted separately by using soxhlet apparatus with different solvents. After extraction, the extracts were concentrated under reduced pressure.

Instruments used

Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

MATERIALS AND METHODS FOR ANATOMICAL STUDIES

Collection of specimens

The plant specimens were collected from foot hill of Yercaud, Salem Tamil Nadu India, Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + acetic acid – 5ml + 70% Ethyl alcohol – 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol as per the schedule given by Sass, 1940. The specimens were casted into paraffin blocks.

Botanical description

*Amaranthus spinosus* is armed, erect herb. Spines axillary, clustered subtending the inflorescences. Leaves are elliptic-obovate, acute base, apex are acutely emarginated. Fascicle axillary into terminal panicles, bracts and bracteoles narrowly ovate-lanceolate, midribs are green and short. Male flowers: Sepals are in five, unequal with prominent midrib, outer part two lobes which are curved to outwards and inner three lobes which are oblong. Stamens are in five and anthers are oblong. Female flowers: Five sepals which are equal, oblong, flat.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. De washing of the sections were done by customary procedure. The sections were stained with Toluidine blue as per the method published by O’Brien et al. For studying the venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jefferey’s maceration fluid was prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab hot 2 Microscopic Unit. For the study of crystals, starch grains and lignified cells, polarized light was employed. Under polarized light they appear bright against dark background.

Physicochemical Analysis

The dried powdered material of root was subjected to physicochemical analysis including fluorescence analysis, moisture content, total ash, water soluble, ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive to determine the quality and purity of the plant material.

Preliminary phytochemical screening

The leaves were dried under shade, powdered with a mechanical grinder and pass through sieve no 45. The sieved powder was stored in airtight container and kept in room temperature until further study. The dried powdered material (250 g) was extracted with methanol by hot extraction method by using soxhlet apparatus and aqueous extraction by cold maceration. The solvents were completely removed under reduced pressure by using vacuum evaporator.
RESULTS

Determination of physicochemical parameters of selected plants

Fresh materials of *A. spinosus* (stem and leaf) were collected and subjected to various physicochemical parameters such as moisture content were observed and recorded.

Ash values are helpful in determining the quality and purity of crude drug, especially in the powder form. Total ash reflects the care taken in its preparation as all traces of organic matters were removed during ash formation and usually consists of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. A higher limit of acid insoluble ash reflects the cases where silica may be present or when the calcium oxalate content of the drug is very high (Table 1).

Extractive values of selected medicinal plants like *A. spinosus* (leaf and stem) were observed and tabulated no 1.

The total percentage of ash values, acid insoluble ash, water soluble ash and percentage yield of extractives in different solvents are constant features of a part of the plant which may constitute individual drug. These reports would be of much significance in finding out the genuineness of the drug sample. Medicinal plants are valuable natural sources and regarded as potential and safe drugs. They have been playing an important role as natural drugs to alleviate human sufferings by contribution herbal medicines to the primary health care systems of rural and remote areas where more than 70% of population in India depend on folklore and traditional systems of medicines.

<table>
<thead>
<tr>
<th>Parameters (w/w%)</th>
<th><em>A. spinosus</em> Stem</th>
<th><em>A. spinosus</em> Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>17.8-14.2</td>
<td>16.26-17.8</td>
</tr>
<tr>
<td>Foreign matters</td>
<td>0.2-0.4</td>
<td>0.6-0.8</td>
</tr>
<tr>
<td>Total ash</td>
<td>18.4-20.42</td>
<td>14.0-18.62</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>12.4-14.64</td>
<td>11.5-13.22</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>11.2-13.64</td>
<td>9.6-10.32</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>16.2-18.84</td>
<td>12.2-14.65</td>
</tr>
<tr>
<td>Extractive values</td>
<td>Pet. Ether</td>
<td>6.10-12.26</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>5.21-10.24</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>4.43-8.56</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>9.45-16.8</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td>9.52-10.24</td>
</tr>
</tbody>
</table>

Preliminary phytochemical studies

The qualitative chemical investigation of all the extracts of selected plant was carried out to check the presence of various phytoconstituents. It revealed the presence of alkaloids, flavonoids, phenolic compounds, phytosterol, tannins, glycosides and carbohydrates.

Botanical description

*A. spinosus* is armed, erect herb, spines axillary, clustered subtending the inflorescences. Leaves are elliptic-obovate, acute base, apex are acutely emarginated. Fascicle axillary into terminal panicels, bracts and bracteoles narrowly ovate-lanceolate, midribs are green and short. Male flowers: Sepals are in five, unequal with prominent midrib, outer part two lobes which are curved to outwards and inner three lobes which are oblong. Stamens are in five and anthers are oblong. Female flowers: Five sepals which are equal, oblong, flat.

Anatomy of *A. spinosus* (Fig 1a, b)

**Leaf**

The leaf has prominent abaxial midrib and thin lamina. The midrib is deeply hallowed on the adaxial side and prominently hemi spherical on the abaxial side. The midrib is 400 µm in vertical plane and 450 µm in horizontal plane. The abaxial part of the midrib is wavy in outline. It has thin epidermal layers of small cells. The ground tissues are homogeneous, parenchymatous, thin walled and compact. There is a wide prominent bowl shaped vascular bundles. The vascular bundles are collateral with wide circular thick walled vessels and parenchymatous xylem elements. Phloem fibers have a thick abaxial sheath. The vessel elements are 30-40 µm in diameters.

Calcium oxalate crystals are abundant in the midrib. Some of them are quite large measuring up to 50 µm in diameter and some of them are smaller measuring up to 20 µm in diameter. They occur in the ground parenchyma in the midrib and they are also localized within the vessel wall (Fig 1a, b).

**Lamina**

The lamina is thin glabrous and has thin delegate epidermal layers. The mesophyll tissue has narrow zone of adaxial palisade cells and three or four layers of small lobed spongy parenchyma cells. The median part of the lamina, there are several circular small vascular bundles surrounded by bundle sheath cells called “Kranz-tissues” (Fig 1c).

**Venation Pattern**

The lamina shows very characteristic venation pattern. The veins are thin and reticulate with distinct or indistinct vein-islet. The vein-terminations are distinct, shortly and thick.
The veins are surrounded by dilated bundle sheath cells (Kranz-tissue) which contain dark prominent chloroplast.

**Stem**

The young stem measuring about 4 mm diameter. The stem is circular in outer line and smooth, the places where leuticle are present. The epidermis is thin and has started dividing transversely giving rise to initial periderm (Fig 1 h). The periderm is fairly 300 µm wide, it is homogeneous and parenchymatous. The sclernchyma zone has thick walled and lignified in outer part and thin walled in inner part. Cellulose is present in the inner zone.

The leuticle has several layers of filling tissue. The sieve tubes are wide, angular thick walled having the companion cells along the corners. Phloem rays are thin and narrow. Secondary xylem is a dense hollow cylinder comprising of their rays and vessels. The vessels are sparse, wide, thick walled and mostly solitary. The widest vessel is 80µm in diameter and

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**Figure 1:** Microscopical Characters of *Amaranthus spinosus*

narrow vessels are less than 50µm (Fig 1 a, b). The xylem fibers are thick walled, lignified and random in arrangement. The pith is wide, homogenous and parenchymatous. When the section is viewed under the polarized microscope, phloem parenchyma tissues are seen. The lignified tissues appear bright against dark background.

![T.S of old stem entire view](image1)

Co- Cortex, Ep- Epidermis, Pe- periderm, Sclerenchymatous, Sph- Secondary phloem, Lenticells, Cr-Crystals.

![T. S of Young Stem- ground and round plane](image2)

Co- cortex, Ep- Epidermis, Fi- Fibers, Ph- phloem, Pi- Pith.

**Figure 1: continued**

**Table 2: Phytochemical screening of extracts of *Amaranthus spinosus***

<table>
<thead>
<tr>
<th>Name of the constituents</th>
<th>Pet. ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>methanol</th>
<th>aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tanin &amp; Phenolic compound</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terphenoid</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Fixed oils</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gums &amp; mucilage</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**When the section is viewed under the polarized microscope, phloem parenchyma tissues are seen. The lignified tissues appear bright against dark background.**
Powder microscopy of *A. spinosus* (Fig 1 d)

The powder of the leaf shows small fragments of lamina. These fragments show reticulate venation with Kronz-tissue.

Some of the fragments show epidermal morphology. The epidermal cells are large and amoeboid in shape. There walls are thin and highly lobed. The stomata are large and elliptical. They are mostly anomocytic type, because no distinct subsidiary cells are seen around the stomata. Crystal distribution is also seen in the powders. Calcium oxalate crystals are abundant in the mesophyll tissue as well as along the vein. The crystals are either very large or very small and these two type of crystals are seen inter mixed.

DISCUSSIONS

Bioprospecting of medicinal plants entails the search for pharmacologically and economically valuable biochemical resources. India has great potential to utilize these resources and knowledge base in traditional medicines as population as well as for economical gain. There is an urgent need for the documentation of folk knowledge, systematic, phytochemical and pharmacognostical studies of medicinal plants and their natural products.

REFERENCES