PHARMACOGNOSTICAL EVALUATION OF CALOTROPIS GIGANTEAN (LINN). LEAVES

SWAPNA P* AND ELUMALAI A

Department of Pharmacognosy, SRM College of Pharmacy, SRM University, Kattankulathur-603203, Kancheepuram Dist, Tamil Nadu, India.

Received: 7 May 2011, Revised and Accepted: 18 June 2011

ABSTRACT

Calotrops gigantean Linn is a wasteland weed better known as milkweed, habitat of Asian countries that includes, India, Indonesia, Malaysia, Philippines, Thailand, Sri Lanka and China. Tribal people were using this plant parts to cure several illnesses such as toothache, earache, spin, anxiety, pain, epilepsy, diarrhoea and mental disorders. C.gigantean is scientifically reported for its anti-fertility, anti-inflammatory activity, hepatoprotective activity, anti-myocardial infarction activity and anti-diarrhoeal activity. The main aim of the present investigation is to study the macro, microscopic and some other pharmacognostic characters of leaves of C.gigantean. This could be used to prepare a monograph for the proper identification of the plant.

Keywords: Calotropis gigantean, Pharmacognostic, Macroscopic, Microscopical

INTRODUCTION

The Calotrops gigantean Linn is a perennial shrub belonging to the Asclepiadaceae family found chiefly in wastelands throughout India, in poorly drained and warmer areas, up to an altitude of 1050 meters1. It is called as “Ruvi” in Marathi and “Madar” in Hindi. It is perennial and much more branched handsome shrub or a small tree of 8-10 feet height. It is a genus of about six species, among which C. gigantean is the species that is commonly grown in waste lands throughout India2. Flowers are regular, bisexual, arranged in simple or rarely compound cymose corymb. It has been reported traditionally for anti-fertility, aleyphoric, anthemic, purgative and abortificant activities. The plant has enormous medicinal properties to cure leprosy, leucoderma, ulcers, tumors, and piles, diseases of the spleen, liver and abdomen3.

In folklore medicines C.gigantean roots are reported for their analgesic, anticonvulsant, anxiolytic and sedative properties. The juice is anhelmimtic, laxative; cures piles and “kapha”. The milk is bitter, oleaginous, and purgative; cures leucoderma, tumours, ascites and diseases of the abdomen. The flowers are astringent, bitter and claimed to cure asthma, eczema, leprosy, secondary syphilis, gonorhoea, ascites, helminthiasis, diarrhoea and jaundice in Ayurveda, Siddha and Unani. The flowers of C.gigantean are reported to have hepatoprotective and analgesic activity in mice. Other experiments demonstrated the wound healing property, analgesic, anti-inflammatory, anti-diarrhoeal, hepatoprotective, and anti-pyretic, anti-bacterial, anti-arthritic, skeletal muscle activity4-10.

The major constituents responsible for medicinal properties of C.gigantean are flavonolglycoside, akundarol, uscharidin, calotropin, amyrinmethylbutazone, amyrin, ß-amyrin, ß-amyrin acetate, taraxasterol, ß-sitosterol, ß-amyrin, ß-amyrin acetate, ß-amyrin acetate, taraxasteryl acetate, luepol acetate B, gigantseryl acetate A, gigantseryl acetate B.

MATERIALS AND METHODS

Plant Material Collection And Authentication

The leaves of plant C.gigantean Linn were collected from in and around Anakapthur, Chennai, India in the month of Nov 2010 and were positively identified and confirmed by the botanist, Dr. P.Jayaraman, PhD, Taxonomist, Chennai. A voucher specimen (PARC/2010/598) has been deposited in the herbarium of the Department of Pharmacognosy, SRM College of Pharmacy, SRM University, Chennai. The fresh mature leaves were used for the study of macroscopic and microscopic characters.

Pharmacognostic Studies

Morphological Studies were carried out by using simple determination technique, the shape, size, color, odor, margin and apex. Leaves are elliptic to oblong, 9-15×4-9cm, coriaceous, base auriculate, apex acute, subsessile, in florescence an umbellate panicle, (sub) terminal, 10cm; bracts and bracteolate, l/cm; pedicel 3cm; calyx-lobes ovate valvate, 5mm, puberulous without, ciliate, glandular corolla purpe, 4 cm across; lobes ovate, spreading, valvate, 1.5 cm, thick-fleshy, acute pollinia pendulous; pollinial bags oblong, flattened 2 mm; cajidicle indistinct; receptacle 0.7 mm, corolla single, stamina, laterally compressed, basally incurred, horny and 3-fid at apex, 1.3 cm pubescent at back, ovaries 3mm; styles 1cm, foliacle oblong, inflated, 8×4 cm; seeds oblong to ovate, plano-convex, 9×6cm; coma long, silky, flowers with a peak during December-May, fruits through the year.

The required samples of different leaves were cut and removed from the plant and fixed in FAA (Farnaln-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated withgraded series of (TBA) Tertiary Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks16.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10 - 12µm. Dewaxing of the sections was done by customary procedure (johansen, 1940). The sections were stained with Toludine blue as per the method published by O’Brien et al. (1964). Since Toludine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast green and KI (for starch)17,18.

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine 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 photo 2 microscopic units. 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. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Easu, 1964).

RESULTS AND DISCUSSION

Microscopic Features of the Leaf

T.S of Petiole (Fig 1, 2)

The leaf consists of a thick plano convex petiole and thick lamina. The petiole is flat on the adaxial side and broadly convex on the abaxial side. It is 1.1 mm thick and 2.8 mm wide. The epidermal layer of the midrib is thin made up of small rectangular cells. The cells in the outer zone are small compact and collenchymatous; the cells in the inner portion are circular, thick walled and parenchymatous, fairly wider and comparatively thick walled circular laticifers are common in the ground tissue.

Central Section Of Petiole (Fig 2)

The vascular strand is shallow, wide and bicollateral. The strand consists of several parallel rows of xylem elements; the rows consists of four or five xylem elements in each row, they are angular, thick walled and are in radial multiples. Phloem occurs in small clusters all along the lower and upper parts of the xylem arc; the xylem elements are 20 µm wide.

---

Fig. 1: T.s of petiole (40 X); AdS – Adaxial side; GT – Ground Tissue; Ph – Phloem; X – Xylem

Fig. 2: central section of petiole (10 X); AdP – Adaxial side; X – Xylem
T.S of Leaf Margin (Fig 3)
The marginal part of the leaf is conical measuring 300 µm thick. The epidermal cells are smaller and thick walled. Compact parenchyma cells without differentiation of palisade and spongy parenchyma.

T.S of Central part of the Lamina (Fig 4)
The lamina is smooth and even on both surfaces. It is 420 µm thick. Both epidermal layers are thin with squarish cells and prominent cuticle; epidermis 20 µm thick. The lamina is amphistomatic (stomata occur on both surfaces).

The mesophyll tissue is differentiated into adaxial zone of three layers of short cylindrical compact palisade cells and abaxial zone of lobed spongy mesophyll cells which form loosely reticulate aerenchyma. The lateral vein is large circular and collateral; it consists of a few xylem elements and phloem elements surrounded by parenchymatous bundle sheath.

Fig. 3: T.s of leaf margin (10X); AdE – Adaxial side; LV – Lateral vein; LM – Leaf margin; PM – Palisade mesophyll; SM – Spongy mesophyll

Fig. 4: T.s of central part of the lamina (10X); AbE – Abaxial epidermis; AdE – Adaxial epidermis; BS – Bundle sheath; SM – Spongy mesophyll; X – Xylem

Stomata
Stomata occur on both adaxial and abaxial surfaces.

Adaxial Epidermal Layer (Fig 5)
The adaxial stomata are paraectytic or cycloctytic, the epidermal cells have thin smooth walls. No cuticular markings are seen.

Abaxial Epidermal Layer (Fig 6)
The abaxial epidermal cells are fairly thick walled with thick cuticular lamellae; the stomata are mostly paraectytic type. The guard cells are elliptic, circular and small cells which are the cells from the trichomes originate. A radiating whorl of rectangular cells is seen around the trichome bearing epidermal cells.
Venation Type (Fig 7, 8)
The veins are thick and straight, they form dense reticulate venation with distinct vein-islets. The vein terminations are thick and short, either simple or lobed ones, the lobes being unequal. Laticifers are seen running along with the veins, they appear darkly stained.

Powder Microscopy
The leaf powder when examined under the microscope exhibits the following elements:

1. Epidermal Trichomes (Fig 9, 10)
Epidermal trichomes of curious type are abundant in the powder. They are unicellular, unbranched; thin walled, dilated and club shaped. They are either straight or curved. They have short, unicellular stalk cell with which they are attached with epidermis). No specific inclusions are seen in the cells.

2. Laticifers (Fig 11, 12)
Long tubular bodies which are the latex containing structures are seen in the powder. The laticifers are unicellular and unbranched; the walls are thin.

3. Epidermal Fragments
Small fragments of adaxial epidermis are commonly seen in the powder. The adaxial epidermal feelings exhibit small polygonal epidermal cells with fairly thick, straight walls.

4. Stomata (Fig 13, 14)
A stoma is encircled by five or more subsidiary cells. Some of the stomata are paracytic type. The abaxial epidermal peeling shows epidermal cells with thin straight walls. The stomata are cycloctic type or paractic type. These are small circular thick walled cells in the epidermal layer which are the basal cells of the epidermal trichomes. The epidermal trichomes are surrounded by a ring of triangular rosette cells.
Fig. 7, 8: Paradermal sections of the lamina (10X); LF - Laticifier in vein; VI - Vein islet; VT - Vein termination
Fig. 9, 10: Epidermal trichomes in the leaf powder (10X); B - Body cell, ST - Stalk cell

Fig. 11: Trichomes enlarged, laticifier (40X); SC - Stalk cell

Fig. 12: Laticifers Enlarged (40X)
CONCLUSION

Now a day the standardization of crude drugs has become very important for identification and authentication of a drug. But due to certain problems the importance was not up to the mark. Thus, the lack of standardization technique fails to identify the drug from its originality which there by exploits the usage of drug from its Traditional System of medicine. The plant Calotropis gigantean is used widely for curing various diseases and gives a helping hand to the Humans. Here a perfect protocol was designed for its Authentication and identification on the basis of Macroscopy and Microscopy. Thus the present investigation was aimed and the results were found to be significant and encouraging towards the goal for Standardization.

ACKNOWLEDGEMENT

The authors are Grateful to Mrs. Suseela Janarthan and Mrs. Chandramathi Arunachalam for providing facility to carry out research work.

REFERENCES


11. Sorimuthu Pillai Subramanian Venkatesan Saratha. Evaluation of Antibacterial Activity of Calotropis gigantea Latex Extract on...


