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PHARMACOGNOSTIC STUDIES ON *ASYSTASIA GANGETICA* (ACANTHACEAE)

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Asystasia gangetica (L) T. Anders (Acanthaceae) is known for several therapeutic activities like antipyretic, anti-inflammatory, antipruritic, anthelmintic, antiarthritis, anti-diabetic, analgesic and galactogogue. The present paper highlights the exomorphology and histomorphology of leaf, petiole, internode, root and physicochemical evaluation and preliminary phytochemical study of the whole plant. These observations will enable to standardize the botanical identity of the drug in crude form.

Keywords : Acanthaceae; *Asystasia gangetica* (L) T. Anders; Pharmacognosy.

Introduction

Acanthaceae is an important large family containing biologically active compounds namely, alkaloids, irridoids, terpenoids, polyphenols and phytosterols. Of about 196 species recorded, a meager number of 25 species is considered as economically important. *Asystasia gangetica* (L). T. Anders is also marked as economical plant[1]. The bioactive richness of the raw drug and substantial availability of the plant prompted to select this weed taxon to undertake pharmacognostic analysis. Previous work is lacking with reference to the proposed objectives of the study.

Asystasia gangetica (L) T.Anders (Acanthaceae) known as Methayakeerai in Tamil and Lavanavalli in Hindi, is a straggling herb used for variety of ailments [2,3]. Leaves and flowers are used as intestinal astringent. One tablespoon of leaf juice mixed with equal quantity of milk given in the morning and evening in empty stomach for diabetes. Irula tribals of North Arcot District (Tamil Nadu) used this plant along with black gram pepper, garlic, palmyrrah and seasamine oil for healing fractures. The tender leaves are good source of Thiamine. It is also used as anthelmintic, given in swelling and rheumatism. Earlier workers reported the valuable compounds namely Leutolin, Leutolin 7-0 glycoside, Apigenin, Apigenin –0- glycoside and Isosalipurposide[4, 5]. The plant yielded during the present investigation â–amyrin, η-octocosanol, β–sitosterol and its glycoside[6].

Materials and Methods

The plant study involves macroscopical, microscopical, preliminary phytochemical standardization including fluorescence analysis of *Asystasia gangetica* (L) T. Anders. For microscopical investigation customary techniques of microtomy were followed [7]. Paraffin sections of 10μm thickness were stained with safranin fast green. Photomicrographs were prepared with NIKON Labphot-2 microscope. Physical constant, behavior of powder with chemical reagents, preliminary phytochemical tests with extracts and fluorescence analysis were carried out [8].

Results

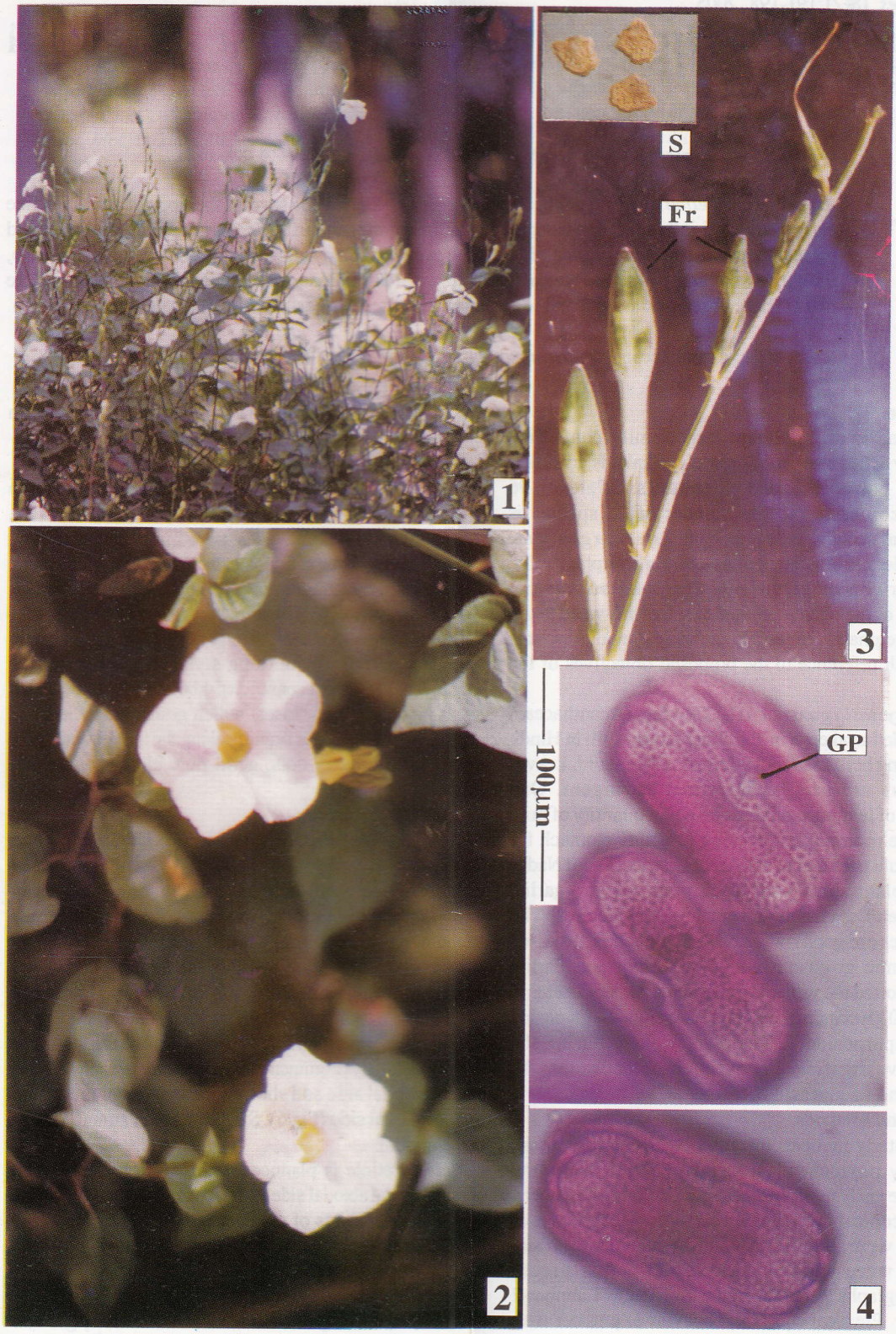
Exomorphology : The plant is a straggling perrenial herb (fig 1). The leaves are opposite, elliptic ovate to ovate, grey pubescent on both sides, base truncate to rounded , apex acute to shortly acuminate, lateral nerves up to 5 pairs, petiole 2cm long, inflorescence spike like receme with flowers on one side of the peduncle, bracts lanceolate, calyx lobes 5, lanceolated, corolla white (Fig 2), stamens in two pairs ovary 3.5mm long style 2cm long, capsule 1.5x0.5cm pubescent constricted about the middle (Fig 3), seeds angular (Fig 3, Inset) pollen grains are cylindrical with two parallel ridges running along the longitudinal axis, exine with minutes dots, germ pore circular and wide (Fig 4).

Microscopic Features

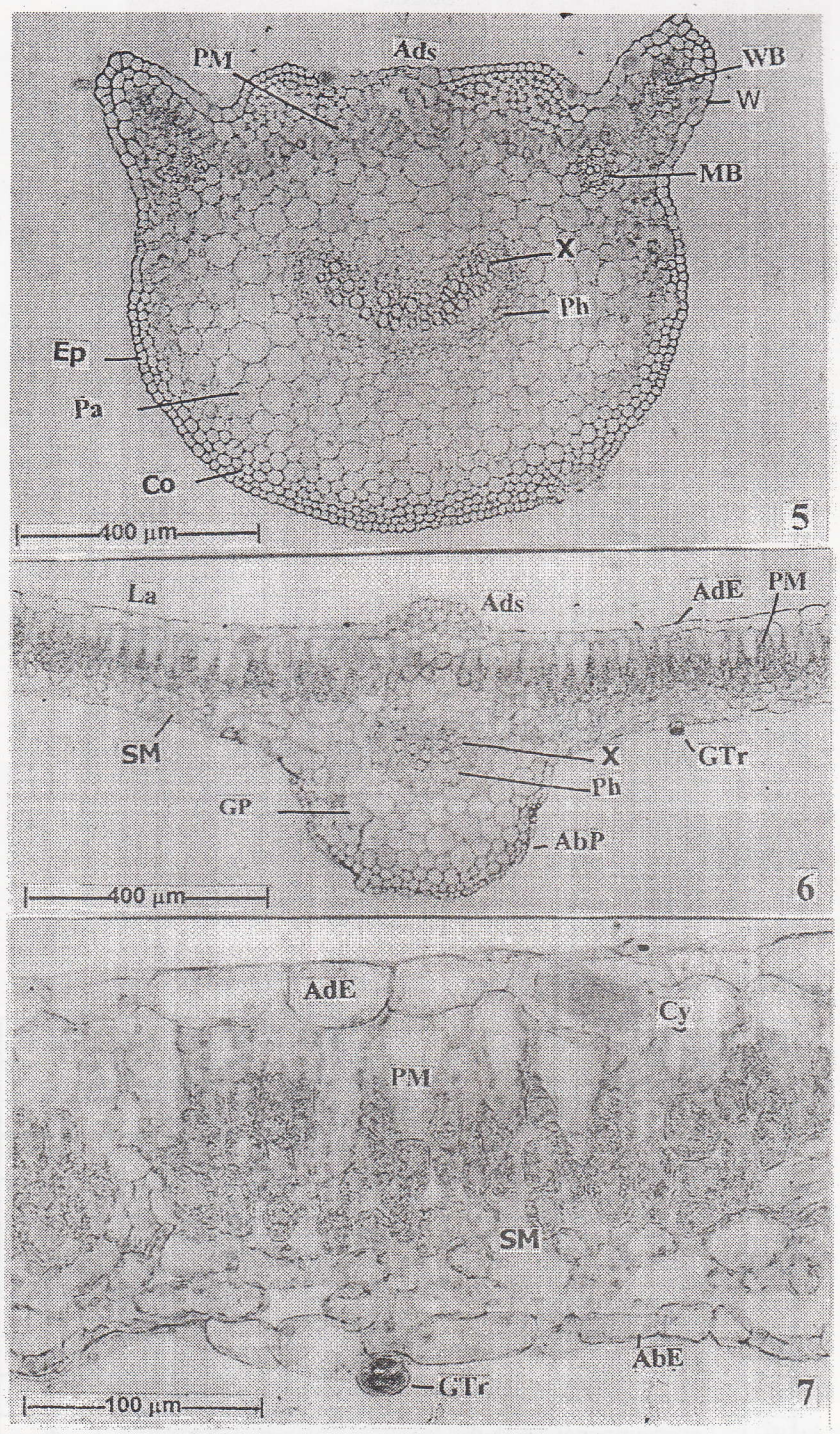
Leaf : The leaf exhibits mesomorphic features and dorsiventral organization (Fig 7). The lamina is amphistomatic with diacytic stomata and thick wavy anticlinal walls of the epidermal cells (Fig 10). Epidermal trichomes are occasional, glandular, sessile, spherical and multicellular (Fig. 7). Cystolith are frequent in adaxial epidermis (Fig 7, 8). Mesophyll is differentiated into single layer of chlorenchymatous palisade cells and six layers of lobed spongy mesophyll cells (Fig 6, 7). The midrib of the lamina distinctly prominent hemispherical parenchymatous on the abaxial side and short broad pyramidal projection on the adaxial side (Fig 6). Single vascular strand present at the middle.

Petiole : Petiole is planoconvex in sectional view with hemispherical abaxial side and flat adaxial side (Fig 5). The vasculature consists of single arc shaped bundles in the middle and two traces, one within the wing and another at the marginal part of the wing (Fig 5).

Internode : The young internode is angular tending to assume circular (Fig 11), outline due to secondary growth.



Figs. 1-4. *Asystasia gangetica* (L) T. Anders. 1. Bushy herbaceous habit of the plant, 2. Flowers enlarged, 3. Young capsules and seeds (inset), 4. Pollen grains in polar and equatorial views (GP-Germ-pore).



Figs. 5-7. *Asystasia gangetica* (L). T. Anders. 5. Cross sectional of the petiole, 6. T.S. of the leaf through midrib, 7. T.S. of lamina (AbE- Abaxial epidermis; AbP- Abaxial part of the midribs; AdE- Adaxial epidermis; AdS- Adaxial side; Co- Collenchyma; Cy- Cystolith; Ep- Epidermis; GP- Ground Parenchyma; GTr- Glandular trichome; La- Lamina; MB- Marginal bundle; Pa- Parenchyma; Ph-Phloem; PM- Palisade mesophyll; SM- Sponge mesophyll; W- Wing; WB-Wing bundle; X- Xylem).

Table 1. Quantitative microscopic data.

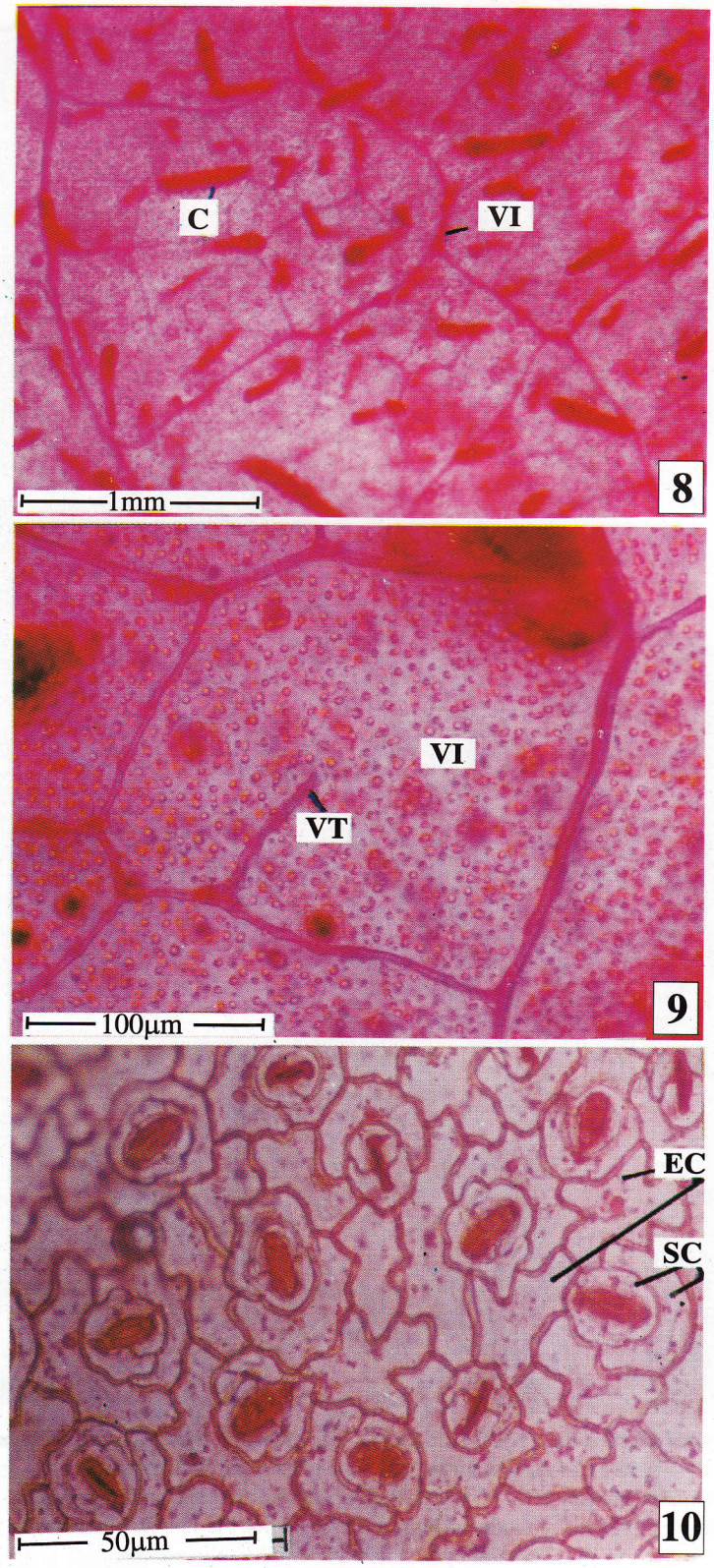
Parameters	Value
Stomatal number	
Adaxial epidermis	22-27/mm²
Abaxial epidermis	33-37/mm²
Stomatal Index	
Adaxial epidermis	11.25-12.76/mm²
Abaxial epidermis	14.5-16.92/mm²
Palisade ratio	4.0-6.0/mm²
Vein islet number	11.25-14.28/mm²
Vein termination number	10.46-12.5/mm²

Table 2. Physico chemical constants.

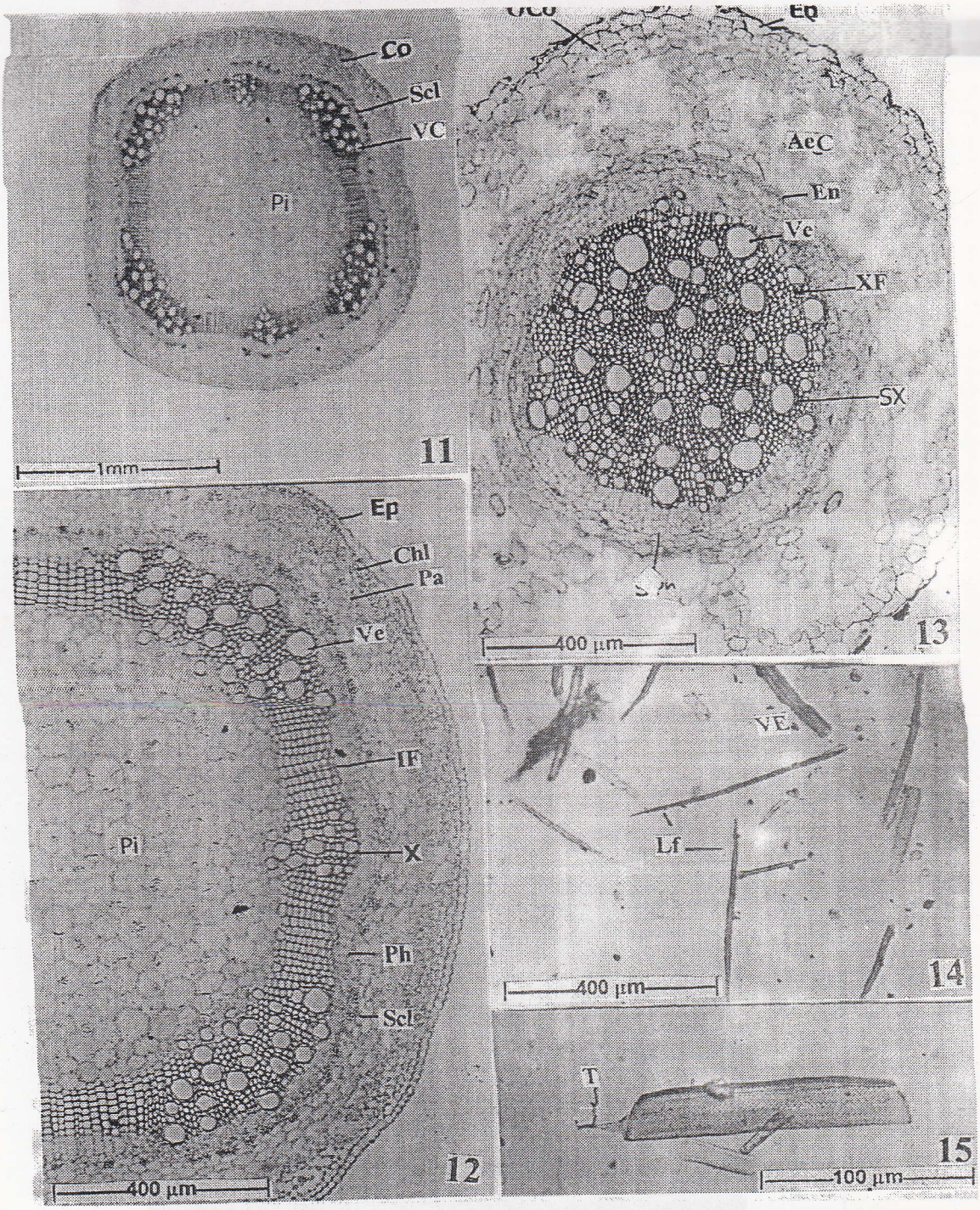
Parameters	Value W/W
Total Ash content %	10.996
Water soluble ash %	9.516
Alkalinity of water soluble ash (0.IN HCl)	2.10 ml/g
Acid soluble ash%	2.78
Loss on drying %	8.8
Successive Extractive Values%	
Hexane	0.5666
Benzene	1.0
Chloroform	0.48
Solubility %	
Alcohol	6.5878
Water	11.076

Table 3. Behaviour of drug powder with different chemical reagents.

No.	Test for	Reagent	Reaction	Result
1.	Gum	Powder+drop of water	No. reaction	-
2.	Saponin	Water soluble	Froth	+
3.	Protein	Picric acid	Yellow	+
4.	Tannin	Lead acetate soluble	White PPT	+
5.	Sterol	Acetic anhydride + H_2SO_4	Red	+
6.	Terpenes	Tin and Thionyl chloride	Pink	+
7.	Sugar	Anthrone+conc. H_2SO_4	Green	+
8.	Phenol	5% $FeCl_3$	Green	+
9.	Flavonoid	10% NaOH	Magenta	+
10.	Anthraquinone	5% KOH	Red	+
11.	Furan	Alcohal+Ehrlich's reagent	No reaction	-
12.	Alkaloid	Dragendrffs reagent	No reaction	-



Figs. 8-10. ***Asystasia gangetica*** (L) T. Anders. 8. Lamina cleared to show the veins and the cystolith, 9. one islet with a vein termination enlarged , 10. Abaxial epidermis showing the stomatal morphology (C- Cystolith; EC-Epidermis Cells; Sc- Subsidiary Cell; VI- Vein islet; VT-Vein termination).



Figs. 11-15. *Asystasia gangetica* (L). T. Anders. 11. T.S. of young stem, 12. T.S. of young stem; a sector enlarged, 13. T.S. of tap root, 14. Fibres and vessel element, 15. A single tailed vessel element.
(AeC- Aerenchymatous cortex; Chl- Chlorenchyma; Co- Cortex; En-Endodermis; Ep- Epidermis; IF- Interfacicular Fibres; LF- Libriform Fibres; OCo- Outer cortex; Pa- Parenchyma; Ph- Phloem; Pi- Pith; Scl- Sclereids; SPh- Secondary phloem; T-Tail; Ve- Vessel; VC- Vascular cylinder; X- Xylem; XF- Xylem fibre).

Table 4. Preliminry phytochemical test of extracts.

Test	Hexane	Benzene	Chloroform	Alcohol
Steroid	+	+	+	+
Terpenes	+	+	+	+
Sugar	-	-	+	+
Alkaloid	-	-	-	-
Phenol	-	-	+	+
Flavonoid	-	-	+	+
Furan	-	-	-	-
Acid	-	-	+	+
Tannin	-	-	+	+
Saponin	+	+	+	+
Quinone	-	-	-	-

Table 5a. Fluorescence analysis of drug powder.

Treatment	Day light	UV light
Drug powder	Green	Green
Drug powder +aq. 1N NaOH	Green	Dark green
Drug powder + alc. 1N NaOH	Yellowiish green	Dark green
Drug powder + 1N HCl	Light brown	Light green
Drug powder + 50% H_2SO_4	Dark green	Dark green

Table 5b. Fluorescence analysis of extracts.

Name of extract	Day light	UV light
Hexane	Light green	Light green
Benzene	Light green	green
Chloroform	Dark green	Dark green
Alcohol	Dark green	Dark green
Water	Light green	Green
Acetone	Dark green	Dark green

Epidermal cells are thin, barel shaped and single layered (Fig 12). Cortex is heterogeneous, outer zone of three layers of collenchyma, middle zone of chlorenchyma and inner three to four layers of thin walled parenchyma. Stomata are frequently seen in the epidermis with stomatal chamber and chlorenchymatous cells.

Cortex is bounded internally by a thin discontinuous layer of fibres which are just one or two cells thickness. The pith is wide, homogeneous and parenchymatous. The vascular cylinder consists of a solid four angled zone; four corners of the cylinder consist of vessel grouping and two smaller vessel groups occurring juxtaposed to each other (Fig 11). The intervening portion of the cylinder consists of radial rows of fibres where vessels are lacking phloem occurs in a continuous zone all around the xylem cylinder. (Fig 12).

Root : Mature lateral root was studied. The root showed well developed secondary growth and initiation of periderm development. Epidermal layer is broken and remains as small fragments. The cortex is broad and aerenchymatous. There are wide air chambers formed by radial anastomosing cell filaments (Fig 13). The centre is occupied by a solid cylinder of secondary xylem ensheathed by secondary phloem. The primary xylem is pentarch. Secondary xylem

consists of narrow and wide vessels, circular in cross sectional view mostly solitary and thick walled (Fig 13). Xylem fibers are libriform type and thick walled. Powder microscopy revealed narrow long vessel elements with or without tails (Fig 15) and simple oblique perforation plate. The fibres are thin walled lignified with wide lumen (Fig 14).

Quantitative Microscopy : Quantitative microscopical data pertaining to stomatal frequency, palisade ratios and veination features are presented in Table 1.

Physicochemical Constants : The whole plant powder was studied for their physicochemical constants, which include ash values, successive extractive values and solubility. The higher hexane extractive value reflexes the significant amount of waxy materials (Table 2).

Behavior of powder with different chemical reagents : The drug powder reacted positively for saponins, proteins, tannins, steroids, terpenes, sugars and phenols, quinones and gums, and no reaction for furans and alkaloids (Table 3).

Preliminary Phytochemical Tests : Preliminary phytochemical tests for hexane, benzene chloroform and alcohol extracts of drugs were carried out. Steroids, terpenes and saponins were present in all the extracts. Sugars, phenols, flavonoids, acids, tannins were present in both chloroform and alcohol extracts (Table 4).

Fluorescence analysis of extract and drug powder : Fluorescence analysis of drug powder and its various extracts treated with acids, alkali was studied (Table 5 a and b).

Discussion

Pharmacognostic studies on *Asystasia gangetica* (L) T. Anders has brought to light certain microscopic features as well as preliminary phytochemical datas of diagnostic values. Anatomy of the plant sometimes proves helpful for individual identification of fragmentary samples. The presence of cymose or racemose inflorescence and loculicidal, elastically dehiscent capsule and superior ovary characterize the Acanthaceae family. Collective microscopic data of all organs have proved to be simple technique of identification. Bilateral symmetry of the lamina, diacytic stomata with equal subsidiaries, the epidermal cells bearing the cystolith; occasional sessile, spherical, multicellular glandular trichomes are characteristic of leaf. Midrib of the lamina is prominent with less distinct adaxial projection and wide, projecting abaxial part, single vascular strand present at the middle, plano convex transectial outline of the petiole with single arc shaped vascular bundle in the middle and two strands within the wings. The young internode is four angled in transectional profile with intact epidermal layers. The cortex consists of collenchyma and forms discontinuous patches followed by chlorenchyma and parenchyma. The vascular cylinder is four angled and the stele also follows the same outline, the vessels occur in four to six clusters within the stele, the intervening space being filled with xylem fibers. In the mature lateral root, the cortical zone is aerenchymatous; air chambers are formed by radially running anastomosing filaments, so that the aerenchyma is multiseriate. Xylem tissue is typical of the root having wide abundant vessels and dense xylem fibres.

Quantitative microscopic data such as stomatal number, stomatal index palisade ratio, vein islet number and vein termination number have been highly relied upon by pioneer pharmacognocists [8]. It is believed that these features are constant for given species and can be employed for inter specific identity of drugs. Physicochemical constants such as solubility, successive extractive values and other parameters of the drug are corroborative evidences in drug standardizations. The drug powder exhibits specific color reactions when mixed with different reagents, thereby indicating the presence or absence of different compounds in the drug. As showed in the Tables 1- 5, the powder drug of *Asystasia gangetica* (L) T. Anders was found to contain sterols, terpenes, saponins, phenols, tannins and acids. Fluorescence analysis of the drug powder as well as drug extract is the other test for standardizing the drug for the presence of chromophores (Table 4, 5). Thus, the anatomical characters coupled with preliminary phytochemical results are specific for the weed drug *Asystasia gangetica* (L) T. Anders.

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