International Journal of Pharmacy and Natural Medicines

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Pharmacognostic Study on *Amaranthus Spinosus* Linn.

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A B S T R A C T

Amaranthus spinosus Linn. (Amaranthaceae) is commonly known as *"Kate Wali Chaulai (Kanatabhajii)"* in "Hindi", also used as vegetable and cultivated throughout in India, Sri Lanka and many other tropical countries. In Ayurveda (Indian traditional system of medicine) the plant is used as digestible, laxative, diuretic, stomachic, antipyretic, improves the appetite, biliousness, blood diseases, burning sensation, leprosy, bronchitis, rat bite, piles, leucorrhoea while the boiled leaves and root are given to children as a laxative, emollient, poultice to abscesses, boils and burn. The leaves are used to treat rheumatic pain, stomeaachache, eczema, gastroenteritis, gall bladder inflammation, boils, abscesses, snakebites, colic menorrhagia and arthritis. The present paper highlights the macroscopic and microscopic characters of leaf, petiole, physico chemical evaluation and preliminary phytochemical studies of the plant (leaves). These observations would be of immense value in the botanical identification and standardization of the drug in crude form. This study would help distinguish the drug from its other species.

Keywords: *Amaranthus spinosus*, microscopy and macroscopy

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Article History: Received 10 October 2016, **Accepted** 25 November 2016, **Available Online** 15 December 2016

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Manuscript ID: IJPNM3192

PAPER-QR CODE

Citation: Hussain Zeashan, *et al.* Pharmacognostic Study on *Amaranthus Spinosus* Linn.*Int. J. Pharm. Natural Med.*, 2016, 4(2): 63-71.

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1. Introduction

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper

control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance.[1,2] Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

Amaranthus spinosus Linn. (Amaranthaceae) is commonly known as *"Kate Wali Chaulai (Kanatabhajii)"* in "Hindi", also used as vegetable and cultivated throughout in India, Sri Lanka and many other tropical countries. In Ayurveda (Indian traditional system of medicine) the plant is used as digestible, laxative, diuretic, stomachic, antipyretic, improves the appetite, biliousness, blood diseases, burning sensation, leprosy, bronchitis, rat bite, piles, leucorrhoea while the boiled leaves and root are given to children as a laxative, emollient, poultice to abscesses, boils and burn.[3,4] The leaves are used to treat rheumatic pain, stomeaachache, eczema, gastroenteritis, gall bladder inflammation, boils, abscesses, snakebites, colic menorrhagia and arthritis.[5]

The leaves of *A. spinosus* are reported to produce inhibition of prostaglandin biosynthesis *in vitro*.[6] In India the root extract is given as a vermicide among the Santhalis and Paharia in eastern Bihar while aqueous decoction of the plant is given to check chronic diarrhea in Southern Orissa.[7] Plant is used in the treatment of diarrhea in traditional medicine system in the tropical countries and it is routinely prescribed as antidiarrheal drugs in Thai traditional medicine.[8]

The juice of *Amaranthus spinosus* is used by tribals of Kerala, India to prevent swelling around stomach while the leaves are boiled without salt and consumed for 2-3 days to cure jaundice.[5] The plant has high concentration of antioxidant components, high nutritive values due to presence of fibre, proteins and high concentration of essential amino acids, especially lysine.[9,10,11,12] *Amaranthus spinosus* is also used as antiinflammatory, antimalarial, antibacterial, antimicrobial, antidiuretic, antiviral and in hepatic disorders.[13,14,15] Water extract of plant showed significant immunostimulating activity[16] and stem extract showed antimalarial activities.[17] *Amaranthus spinosus* has several active constituents like alkaloids, flavonoids, glycosides, phenolic acids, steroids, amino acids, terpenoids, lipids, saponins, anthriquinone derivatives, volatile oils, organic acids, betalains, ß-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins, polyuronides and carotenoids. The betalains in stem bark of *A. spinosus* were identified as amaranthine, isoamaranthine, hydroxycinnamates, quercetin, and kaempferol glycosides.[6,14,17,18] It also contains amaranthoside, a lignan glycoside, amaricin, a coumaroyl adenosine along with stigmasterol glycoside and betaine such as glycinebetaine and trigonelline.[19,20]

Whole plants of *Amaranthus spinosus* is used as a food additives and treatment of diarrhea and ulcer in traditional system of medicine however, the plant has already been validated scientifically as antidiarrheal *in vitro* against *Blastocystis hominis*[8] but the effect of plant against diarrhea *in vivo* and antiulcer has not been investigated. However pharmacognosy information about this plant has not been published, particularly the necessary to define quality control procedures of the *A.spinosus.*as raw material. Hence the present investigation deals the pharmacognostical evaluation of the *A.spinosus.* The study includes morphological and anatomical, determination of physico chemical constants and the preliminary phytochemical evaluation of *A. spinosus.*

2. Materials and method

2.1 Plant material collection

Whole plant of *Amaranthus spinosus* Linn. (Family: Amaranthaceae) were freshly collected in the botanical garden of National Botanical Research Institute, India in October 2006. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow. A voucher specimen (NAB 75006) of the collected sample was deposited in the departmental herbarium for future reference.

2.2 Chemical and Instruments

Compound microscope, Camera lucida, stage and eyepiece micrometer, glass slides, cover slips, watch glass and other common glassware were the basic apparatusand instruments used for the study. Photomicrographs in different magnifications of all necessary cells and tissues were taken with Nikon Lab Phot - 2 microscopic Unit.

Some crystals, starch grains and lignified wall photographs were taken under polarized light microscope. Solvents viz. Hexane, chloroform, ethylacetate, methanol and reagents viz. phloroglucinol, glycerin, Hcl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

2.3 Macroscopic study of *A. spinosus*

The external morphological character such as size, shape, colour, odour, taste, texture and odour of *A. spinosus* was evaluated.

2.4 Microscopic study of *A. spinosus*

2.4.1 Preparation of specimens

A. spinosus different parts were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90 ml). After 24 h of fixing, the specimens were dehydrated with graded series of tertiary - Butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 $^{\circ}$C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 /lm. Dewaxing of the sections was by customary procedure.[21] The sections were stained with Toluidine blue as per the method published by[22] wherever necessary sections were

also stained with safranin and Fast-green and IKI. For studying the stomatal morphology, venation pattem 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[23] were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic Unit. Magnifications of the figures are indicated by the scale-bars.

2.5 Physico-chemical evaluation of *A. spinosus*

The physicochemical studies were carried out as mentioned in Indian[24] and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines). The different parameters like determination of foreign matter, loss on drying (LOD), ash values with total ash, acid insoluble ash and water soluble ash, extractive values by successive extraction as well as extractive values in ether, alcohol and water, respectively.

2.6 Preliminary phytochemical tests *for A. Spinosus*

The preliminary phytochemical tests were carried out as mentioned in [25,26] Preliminary qualitative phytochemical screening of 50% ethanolic extract of whole plants of *A. spinosus* (ASE) were performed for alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resin , saponins, steroids and tannins, respectively.

2.7 Total phenolic contents *of A. spinosus*

The total phenolics in the 50% ethanolic extract of whole plant of *A. spinosus* (ASE) was determined using Folin–Ciocalteau reagent. [27]

2.8 HPTLC chemo profiling of *A. spinosus*

10 mg of 50% ethanolic extract of *A. spinosus* (ASE) was dissolved in 1.0 ml of methanol and used for HPTLC analysis. 10 µl of the test solution was applied on precoated silica gel 60 F_{254} TLC plates (E. Merck, 0.20 mm thickness) by linomat V sample applicator. and the plates were then developed in ethyl acetate: methanol: water (9: 3: 0.5) to a distance of 8 cm. Developed plate was then observed under UV light and visible light after derivatisation with anisaldehyde-sulphuric acid reagent. The plate was then scanned densitometrically at various wavelengths and the profile was recorded.

3. Results and Discussion

The macroscopical characters of *A. spinosus* is described below.

Stem

Colour	:	Green
Taste	:	Characteristic
Odour	:	Odourless
Dimension	:	Width – 4 to 5 mm
Appearance	:	Clothed with prickles

Leaf

Colour	:	Green
Odour	:	Characteristic
Taste	:	Characteristic
Apex	:	Obtuse
Margin	:	Entire
Dimension	:	Length – 2.75cm Width – 1.25 mm
Condition	:	Fresh
Surface	:	Smooth (Upper surface), Rough (Lower surface)

Inflorescence is terminal axillary cluster. Bracts and levacteoles membranous, awned, Tepals are five, free, unequal with prominent midvein. Flowers unisexual, male flowers with monocarpellary, one ovuled ovary, ovule on basal placentation. Fruit is utricle, dehisecence is circumscissile.

3.1 Microscopic features

3.1.1 Leaf: The leaf has a prominent midrib and there isobilateral lamina (Fig 1, A). The lamina has thin delicate epidermal layers and narrow abaxial and adaxial zones palisade cells. Two or three layers of wide darkly stained spongy mesophyll tissue. The lamina is 150 µm thick (Fig. 1, B).

3.1.2 Midrib

The midrib has wide deep adaxial concavity and prominently projecting abaxial side. It is 400 µm thick. It has thin epidermal layer of small, less prominent elliptical cells. The ground tissue has wide, circular, thin walled compact parenchyma cells (Fig 2, A). The vascular strand is single, large and arc shaped. It is collateral with adaxial band of wide, circular, thick walled xylem elements and abaxial arc of fairly wide sieve elements of the phloem (Fig. 2, B). The metaxylem elements are 30 µm wide and the protoxylem elements are 8 µm wide.

3.1.3 Crystals

Calcium oxalate crystals are abundant in the midrib and lamina. The crystals are sand crystal type or micro crystals. Minute sand like bodies of the crystals are seen aggregated within the cells. In the midrib the sand crystal masses are seen within the ground parenchyma cells as well as within the lumen of the xylem elements (Fig. 3, A). In the leaf the sand crystals occur in the wide mesophyll cells (Fig 3, B and C). The crystal masses are about 50 µm wide.

3.1.4 Venation pattern

In surface view the venation appears to the loose reticulate type. The vein islets are distinct but wide and much elongated into rectangular area (Fig. 4, A). The vein terminations are mostly simple, long slender and curved. The sand crystal masses are seen scattered within the vein-islets. On either side of the veins occur, radially oblong, bundle sheath cells which possess dense chloroplast (Fig. 4). The bundle sheath cells occur in single row an either side, in transactional view, the bundle sheath cells appear as circular rosette of cells.

3.1.5 Epidermal cells of the lamina

In surface view, the epidermal cells of the lamina appear large, thin walled with highly lobed anticlinal walls, so that the epidermal cells appear amoeboid in outline. The stomata are anomocytic type, without specific subsidiary cells. The guard cells are elliptic with distinct stomatal pores. (Fig.5). Thus stomata are 20-25 X 10-12 µm in size. No cuticular markings are seen on the surface (Fig. 6, B).

3.1.6 Adaxial (upper) epidermis

The upper epidermis has no stomata. There are wide, circular, subsessile glandular trichomes seen occasionally on the adaxial epidermis. They are darkly stained and are 40 µm in diameter (Fig. 6, A).

3.1.7 Stem: The stem is roughly circular in cross sectional outline with smooth surface (Fig. 7, A). It has epidermis, cortex and anomalous type of vascular system. The epidermal layer is thin comprising of narrow rectangular, less prominent cells (Fig. 8). The Cortex is 200-250µm wide; it is heterogeneous comprising of outer about 8 layers of angular collenchymas and inner portion of three layers of wide, thin walled angular parenchyma cells. The vascular system in unusual or anomatous type. It consists of numerous central scattered collateral vascular bundles which are known as pith bundles or medullary bundles (Fig. 7, A, B, 8). The medullary bundles are elliptical and have wide circular, or angular thick walled cluster of xylem elements and outer shallow arc of phloem with small cluster of scalerenchyma cap (Fign 7, B). The meta xylem elements are up to 30 µm wide. The outer portion of the vascular system has a continuous, narrow cylinder of secondary xylem where xylem elements are in radial files; some of the vascular bundles are seen abutting the secondary xylem cylinder (Fig. 9). These peripheral bundles also have wide, circular thick walled xylem elements and outer cluster of phloem elements are the outer part of the secondary xylem cylinder, occur small nests of phloem which are discrete and discontinuous.

3.1.8 Determination of leaf constant

A number of leaf measurements are used to distinguish between some closely related species not easily characterized by general microscopy. Leaf constant of *A. spinosus* was tabulated in table 1.

4.1 Physico-chemical parameters

In order to standardize the raw materials, the various physicochemical parameters such as loss on drying, foreign matter, ash values and extractive values were carried out. The moisture content plays important role in storage of the drugs and as it varies a lot the extractive values will vary which will affect the dose of the drugs. Foreign organic matter and loss on drying were found in a range of 6.8 and 0.2 respectively (Table 2). Ash values are helpful in determining the quality and purity of crude drugs in powder form. The total ash usually consist of carbonates, phosphates, silicates and silica which includes both physiological ash derived from drug itself and non-physiological ash which is residue of adhering material such as sand and soil. The acid insoluble ash value particularly indicates contamination with silicious material like earth or sand. The total ash very high temperature may result in the conversion of carbonate to oxides. The treatment with dilute sulphuric acid results in sulphated ash where the oxides are converted to sulphates. The percentage of total ash and acid insoluble ash were found to be 2.42 and 0.22% w/w (Table 2). The extractive values are useful for evaluation of crude drugs and give an idea about the nature chemical constituents present in them. The ethanol and water soluble extractive values were 20% and 15.12% respectively (Table 2). The fluorescence character of powder of whole plants of *A. spinosus* was studied both in daylight and UV light[28,29] In the near UV region of the spectrum some of the phytoconstituents shown more or less brilliant coloration when exposed to radiation. These fluorescence techniques are useful for the detection of adulterants and qualitative examination of herbal drugs. The fluorescence analysis of powder of whole plants of *A. spinosus* are recorded in table 3.

5.1 Preliminary phytochemical tests

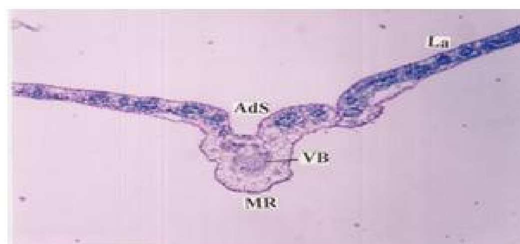
The pharmacological action of crude drug is determined by the action of its constituents. Thus the plant species may be considered as the biosynthetic laboratory not only for the chemical compounds like carbohydrates, protein and fats, but also for a multitude of compounds including alkaloids, terpenoids, flavonoids and glycosides etc., which exerts definite physiological effects. These chemical compounds are responsible for the desired therapeutic properties. To obtain this pharmacological effects, the plant materials are used as such in their crude form or they may be extracted with suitable solvent to take out the desired components and the resulting principle being employed as therapeutic agents. Hence, the plant extracts were subjected to phytochemical screening for detecting of various plant constituents present.[30] In the preliminary phytochemical screenings of the 50% ethanolic extract of whole plant of *A. spinosus* (ASE) found to contain alkaloids, carbohydrates, flavonoids, glycosides, triperpinoids, steroids and tannins (Table 4).

5.1 Total Phenolics contents

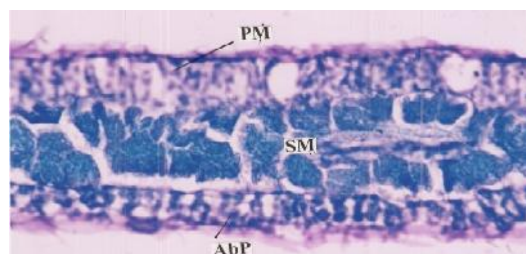
The 50% ethanolic extract of whole plant of *A. spinosus* (ASE) was found to contain 336 ± 14.3 mg/g total polyphenolics expressed as gallic acid equivalent (GAE, mg/g of GAE).

6.1 HPTLC chemoprofiling

Satisfactory separation of ethanolic extract of *A. spinosus* (ASE) was obtained in solvent system acetate: methanol: water (9: 3: 0.5) respectively. Developed plate was then observed under UV-Visible light after derivatisation with anisaldehyde-sulphuric acid reagent. The plate was then scanned densitometrically at various wavelengths and the profile was recorded (Figure 10). Also the Rt values and the relative percentage of area in each peak in the extract applied were also calculated and are presented in table 5 at 290 nm.



(A) Transverse section of leaf through midrib with lamina



(B) Transverse section of lamina

Figure 1: Anatomy of leaf of *A. Spinosus*

(Abp: Abaxial part, Ads: Adaxial side, La: Lamina, MR: Midrib, PM: Palisade mesophyll, SM; Spongy mesophyll, VB: Vascular bundle)

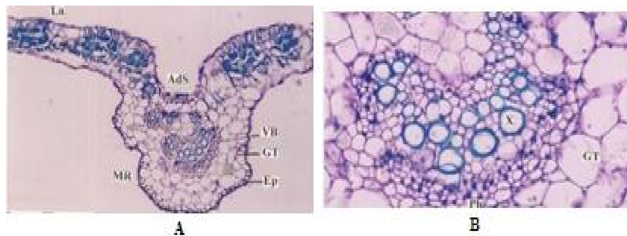


Figure 2: Anatomy of midrib of *A. spinosus*

A and B: Transverse section of midrib with lamina magnified and midrib vascular bundle enlarged.

(Ads: Adaxial side, EP: Epidermis,GT: Ground tissue, MR: Midrib, La: Lamina, Ph: Pholem, VB: Vascular bundle, X: Xylem)

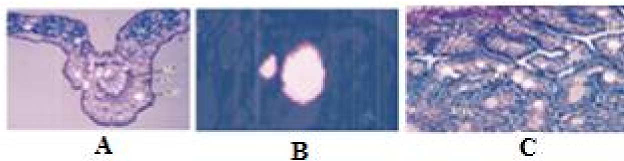
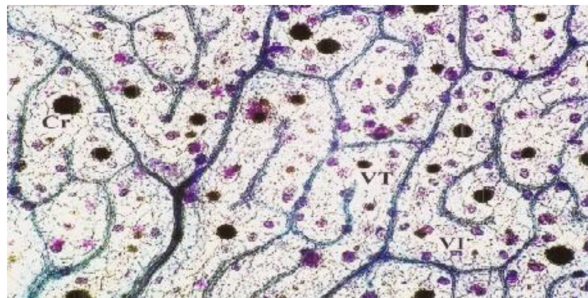


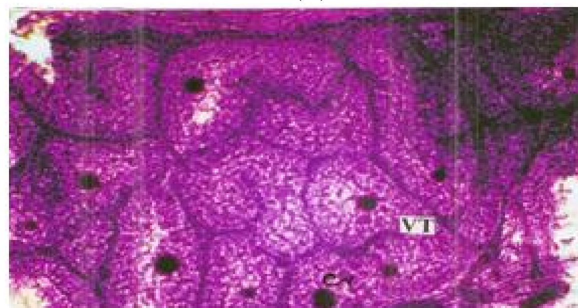
Figure 3: Crystal distribution in the leaf of *A. Spinosus*

A, B and C: Transverse section of leaf showing sand crystal in the ground tissue and mesophyll tissue, a crystal magnified and paradermal section showing crystal in the mesophyll tissue. (MT: Mesophyll tissue, SCr: Sand crystal, Ve: Vein,, VB: Vascular bundle, X: Xylem)



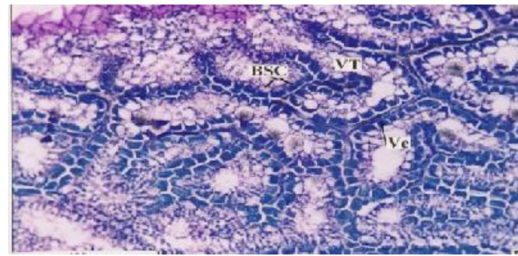
(A) Cleared leaf showing vein-islets and vein termination with crystal distribution

(B)

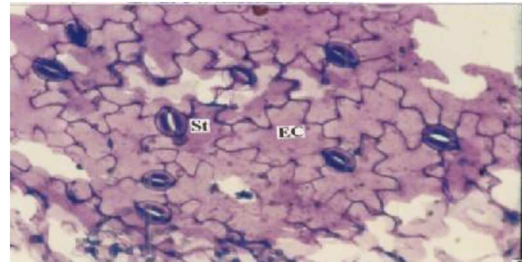


(B) Same as enlarged (Cr: Crystal, VI: Vein islets, VT: Vein termination)

Figure 4: Venation pattern in *A. spinosus*

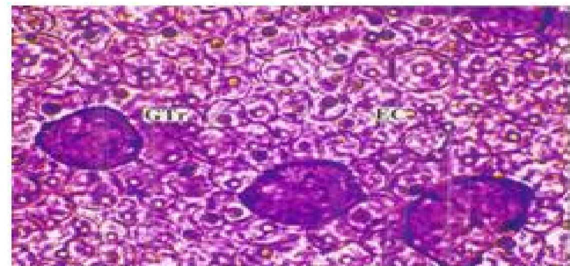


(A) Paradermal section showing vein-islets and vein-termination

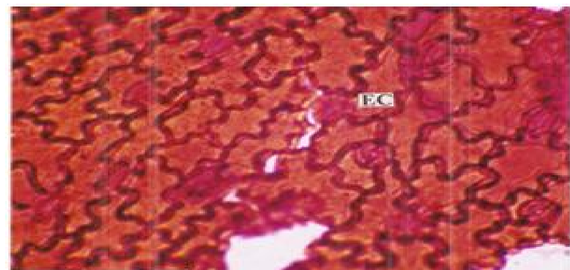


(B) Abaxial epidermis with stomata *(Bsc: Bundle sheath cell, EC: Epidermal cells, St: stoma, Ve: Vein, VT: Vein termination)*

Figure 5: Venation system and stomatal morphology in *A. Spinosus*

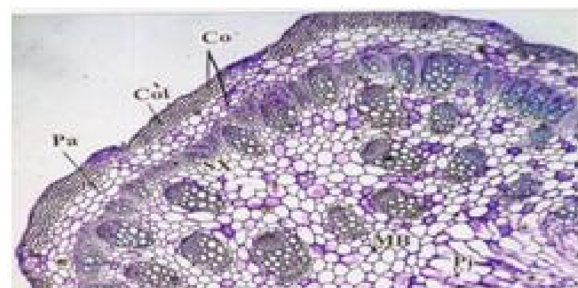


(A) Glandular trichome on the surface view

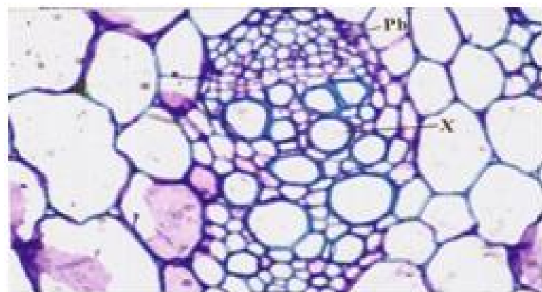


(B) Adaxial epidermis cell (Non stomatiferous)
(EC: Epidermal cells, GTr: Glandular trichome)

Figure 6: Epidermal morphology in *A. Spinosus*



(A) Transverse section of stem half portion



(B) One medullary vascular bundle enlarged

Figure 7: Anatomy of the stem of *A. spinosus*

(Co: cortex, Col: Collencyma, MB: Medullary bundle, Ph: Pholem, Pi: Pith, Pa: parenchyma cortex, X: Xylem)

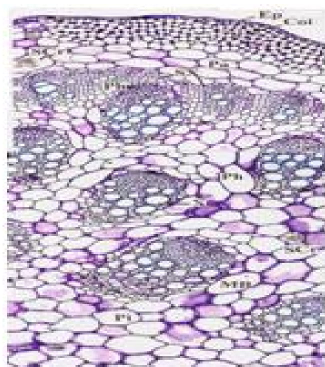


Figure 8: Transverse section of stem of A. spinosus: a sector enlarged

(Col: Collencyma, Ep: Epidermis, MB: Medullary bundle, Pa: Parncyma cells, Ph: Phloem, Pi: Pith, SCr: Sand crystal,SX: Secondary xylem, X: Xylem)

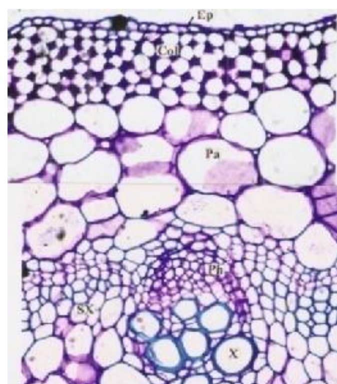
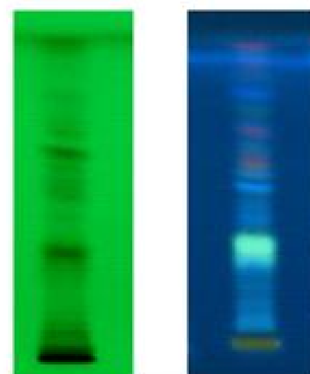


Figure 9: Transverse section of stem of *A. spinosus* showing one outer vascular bundle magnified

Col: Collencyma, Ep: Epidermis, pa: Parencyma cells, Ph: Phloem, SX: Secondary xylem, X: Xylem)



Under UV 254 nm Under UV 366 nm

TLC profile of 50% ethanolic extract of whole plant of *A. spinosus* (Solvent system: Ethyl acetate: Methanol: Water 9:3:0.5) (Spray reagent: anisaldehyde -sulfuric acid).

TLC densitometric chromatogram scan at 290 nm of 50% ethanolic extract of whole plant of *A. spinosus*

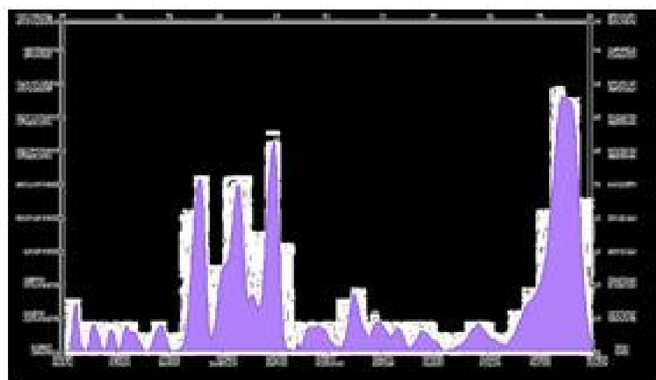


Figure 10: HPTLC finger print profiling of 50% ethanolic extract of *A. spinosus* (ASE)

4. Conclusion

The present paper highlights the macroscopic and microscopic characters of leaf, petiole, physico chemical evaluation and preliminary phytochemical studies of the plant (leaves). These observations would be of immense value in the botanical identification and standardization of the drug in crude form. This study would help distinguish the drug from its other species.

Table 1: Leaf constants evaluation of *A. Spinosus*

Parameters	Range	Mean
Stomatal number		
Upper epidermis	10-16	13
lower epidermis	-	-
Stomatal index	17-19	18
Upper epidermis	-	-
Lower epidermis		
Vein islet number	20-24	22
Vein termination number	39-43	41

Table 2: Physico-chemical evaluation of whole plant of *A. spinosus*

Parameters	Value
Foreign matters (% w/w)	0.2
Loss on drying (% w/w)	6.8
Total ash (% w/w)	2.42
Acid insoluble ash (% w/w)	0.22
Ethanol soluble extractive value (% w/w)	20.00
Water soluble extractive value (% w/w)	15.12

Table 3: Powder florescence test of crude *A. spinosus* powder

S.N.	Material (P)	*A. spinosus*		
		Day light	UV 254 nm	UV366 nm
1.	Powder as such	Light green	Light green	Light green
2.	Powder + water	Light green	Light green	Light green
3.	Powder + 1N HCl	Light brownish	Light green	Light brown
4.	P + in NaOH	Dark green	Dark green	Dark green floresce
5.	P + in NaOH in Methanol	Greenish brown	Light green	Light green
6.	P+ 50% HNO_3	Dark yellow	Dark yellow	Dark yellow
7.	P+ Conc. HNO_3	Brown	Green	Green
8.	P+ 50% H_2SO_4	Brownish orange	Green	Light green
9.	P+ Conc. H_2SO_4	Brownish black	Green florescent	Brown
10.	P+ Acetic acid	Light green	Green florescent	Green
11.	P+ Iodine in H_2O	Reddish brown	Green florescent	Green

Table 4: Preliminary phytochemical screening of 50% ethanolic extract of *A. spinosus* (ASE)

Phytochmicals	Tests	Inference
Alkaloids	a) Dragendorff's test	+ve
	b) Hager's test	+ve
	c) Wagner's test	+ve
	d) Mayer's test	+ve
Carbohydrates	a) Benedict's test	+ve
	b) Fehling's test	+ve
	c) Molisch's test	+ve
Flavanoids	Shinoda's test	+ve
Glycosides	a) Legal's test	+ve
	b) Baljet's test	+ve
	c) Brontagar's test	+ve
	d) Killar Killani 's test	+ve
Triterpenoids		-ve
Resins		+ve
Steroids	a) Liebermann -Burchard's test	+ve
	b) Salkowski reaction	+ve
Saponins		+ve
Tannins	Lead acetate	+ve
	Ferric chloride	+ve

Table 5: HPTLC chemo profiling of 50% ethanolic extract of *A. spinosus* (ASE)

No. Peaks	Retention time (RT)	% amount in each spot
1	1.273	18.37
2	1.775	3.51
3	2.047	4.83
4	2.335	4.76
5	2.761	10.80
6	6.229	17.11
7	8.196	22.04
8	9.767	5.64
9	15.67	13.33

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