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PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES ON *CISSUS VITIGINEA* L.

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It is important to learn the medicinal values of plants through traditional knowledge. The crude drug of *Cissus vitiginea* is used for various ailments by the ethnic people. The present work has been taken up to study the crude drug of *Cissus vitiginea* of the family Vitaceae. The morphological characters of the plant; the anatomical characters of the leaf, petiole, tendril, stem, rhizome and root; microscopic observations of the crude drug; polarized microscopy of the crude drug; qualitative analysis of carbohydrates, alkaloids, tannins, phenols, flavonoids, gums, mucilages, steroids, protein, fixed oils, fat, volatile oils and saponins of the aqueous and methanol extracts of leaves and ash values were studied. It discloses distinctive morphological and anatomical characters for identification. The microscopic observations of the powder and the extraction values help to identify the drug. Qualitative phytochemical observations revealed the presence of many primary and secondary metabolites.

Keywords : *Cissus vitiginea;* Pharmacognosy; Phytochemistry.

Introduction

Since time immemorial people were using plants for curing their ailments. There are about 6000 plants having curative properties and they are used in Indian System of Medicines[1]. The medicinal value of the plants is due to the presence of some chemical substances that produce definite physiological action on the human body. The most important of these substances are the alkaloids, glycosides, essential oils, fatty oils, resins, mucilage, tannins and gums. These are present in the storage organs of the plants particularly in roots and seeds, and in lesser amount in leaves, bark and stem. To promote the proper use of herbal medicine and to determine their potential as source for new drugs, it is essential to study the medicinal plants which have folklore reputations in a more intensified way.

Survey on medicinal plants used by ethnic people of Eastern Ghats of Andhra Pradesh revealed the use of *Cissus vitiginea* L for various ailments[2]. According to them the stem bark powder is used for conjunctivitis, paste of the powder is applied externally for wounds and the decoction is given for anthrax. The dried fruits are also used for asthma. In Kerala the Ayurvedic drug amlavesasah is prepared from four species of Vitaceae (*Ampelocissus latifolia, Cayratia trifolia, Cissus repens and C. vitiginea*) and used for indigestion, liver, spleen and respiratory disorders[3]. The roots of *C. vitiginea* are reported to have antimicrobial activity against Gram positive bacteria[4]. The rhizome is edible and highly nutritive[5]. The plant *C. vitiginea* belongs to the family Vitaceae. The family includes a number of species which have medicinal properties (*Cissus quadrangularis*[6], *Cayratia japonica*[7], *Cyphostemma setosum*[6], *Rhoincissus tridentate*[6], *Vitis hederatia*[8], *Vitis coignetiae*[9]). Though there are reports on the medicinal properties of *C. vitiginea,* organized pharmacognostic work is required. So the present work has been taken up to evalualate the anatomical and phytochemical characters to identify the crude drug of *C. vitiginea.*

Material and Methods

The plant *C. vitiginea* was collected from Auroville, Puducherry and identified with the help of Flora of Madras Presidency[10]. The preserved specimens were deposited in the Post Graduate and research Department of Botany, Kanchi Mamunivar Centre for Post graduate studies, Puducherry. The morphological characters of the plant; the anatomical characters of various parts of the plant; light microscopic observations of powdered plant material; polarized microscopic observations of crystals and starch grains; qualitative analysis of phytochemicals of the aqueous and methanol extracts of leaves were studied. The required samples of different organs were cut and fixed in FAA. The specimens were embedded in paraffin, cut into

blocks and sectioned[11]. The sections were stained with Toluidine blue[12]. Wherever necessary sections were also stained with safranin, fast green and iodine potassium iodide for Starch. For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5 percent sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared[13]. Powdered materials were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and their size measured.

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 bi-refringent property, under polarized light they appear bright against dark back ground. Magnifications of the figures are indicated by the scale bars. The phytochemicals were qualitatively analyzed using standard methods[14].

Observations

Morphological Features-Cissus vitiginea L. is a vine spreading on the thickets with the help of stout unbranched tendrils. The branchlets are densely pubescent. Leaves simple and broadly cordate. The lamina is five angled or deeply lobed, thick and coriaceous, basally three nerved, pubescent, margins dentate. The inflorescence is umbellate cyme and leaf opposed. Flowers small, bisexual; calyx four lobed; petals four, pale yellow; stamens four; ovary 2-celled with two ovules in each cell; fruit pyriform berry; seeds tessellate. Common on the roadsides and in scrub jungles (Fig. 1).

Anatomical Features- Leaf : The leaf has a lamina and a prominent midrib. The midrib is quite broad on the abaxial side with thick conical adaxial hump which consists of compact mass of collenchyma cells. The palisade tissue of the lamina extends up to the shoulders of the adaxial hump. The abaxial part has two or three layers of outer collenchyma cells and the remaining portion has wide , compact, thin walled parenchyma cells. Some of the ground cells are wider and are mucilaginous. The vascular system consists of an abaxial row of three bundles. The vascular bundles are collateral, ovate or top shaped in sectional view. The xylem elements are angular, fairly thick walled and 50 - 60 µm wide (Fig. 2).

Lamina : The lamina is about 170 µm thick. The adaxial epidermis is apostomatic. The epidermal cells are polyhedral, randomly oriented, thin walled and the walls are straight. The cells are 50 µm along wider plane and 30 µm along the narrow plane (Fig. 4).The abaxial epidermis is stomatiferous, the stomata are actinocytic and 30x 40 µm in size. The abaxial epidermis cells are also polygonal in outline with thin, slightly wavy anticlinal walls (Fig. 12). The mesophyll tissue is differentiated into a wide adaxial zone of palisade cells which are narrowly cylindrical, compact and 50 - 60 µm in height. The spongy mesophyll has 5 or 6 layers of small, lobed or spherical cells, loosely arranged to form air chambers. The epidermal trichome has a biseriate basal part and a uniseriate, unbranched, tapering terminal part (Fig. 10). The lamina has thin, secondary and tertiary veins. The vein islets are fairly distinct. The islets are wide, polyhedral with thin boundaries. The vein terminations are also distinct. They are simple, unbranched and long. Some of the vein- terminations are branched once forming two dichotomies. More commonly, the terminations are branched many times forming a dendroid appearance. Raphide idioblasts are frequently seen in the vein - islets (Fig. 3).

Petiole : The petiole is eccentric in cross section measuring 2.5 mm in diameter. The surface has dense trichomes arising from thin epidermal layer of small cells. The outer ground tissue is collenchymatous, 5- cell layers thick and 200 µm wide. The inner ground tissue is parenchymatous comprising of three or four layers of wide, compact angular parenchyma cells. The central core has narrow circular canal formed by disintegration of cells. There are about 12 discrete vascular bundles arranged in a ring. The vascular bundles are radially oblong and cylindrical with narrow medullary rays. Five bundles are larger than the others. They have clustered xylem elements which are fairly wide, thick walled and angular, measuring 30 - 40 µm in diameter. Phloem occurs in thick mass on the outer part of the xylem (Fig. 5).

Tendril : It is circular in cross section measuring 2mm thick. The surface is hairy and uneven. The epidermis is thin and consists of small, compact, cubical cells. The ground tissue is heterogeneous and consists of outer 8 to 10 layers of wide compact, angular collenchyma cells and inner, narrow thin walled parenchyma cells. The vascular system consists of 10 to 12 collateral, oblong or elliptical vascular bundles arranged in a ring. Xylem elements occur in radial files and the outer end of the xylem is surrounded by phloem (Fig. 6).

Stem : The stem is circular in cross section. It has continuous cylinder of secondary xylem and secondary phloem. The periderm is superficial and consists of 4 or 5 layers of outer phellem layers and inner 4 layers of phelloderm. The cortex is made of 8 to 10 layers of polygonal, thin walled, compact parenchyma cells. There

Fig. 1 *Cissus vitiginea*

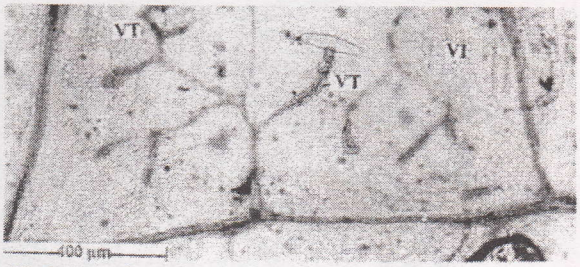

Fig.3. Venation

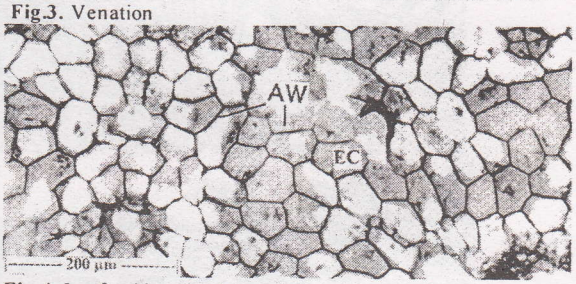

Fig.4. Leaf epidermis- Adaxial

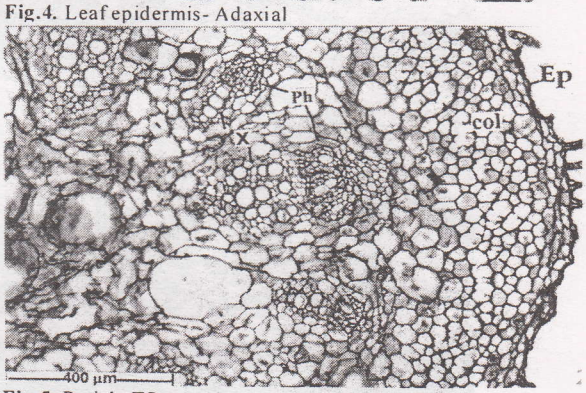

Fig.5. Petiole TS

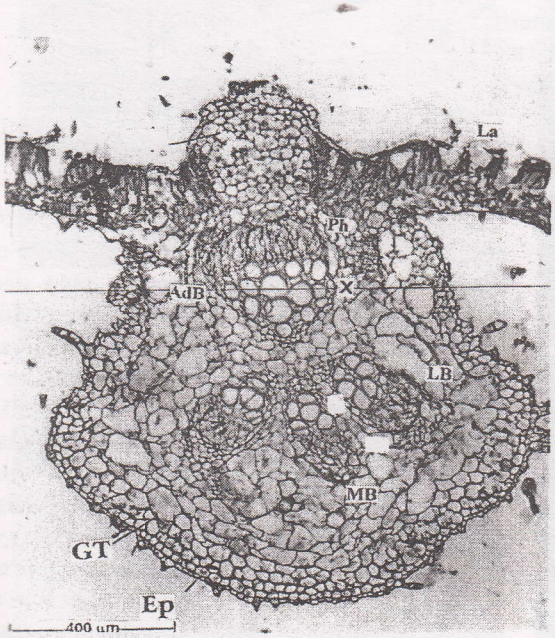

Fig.2. Leaf TS

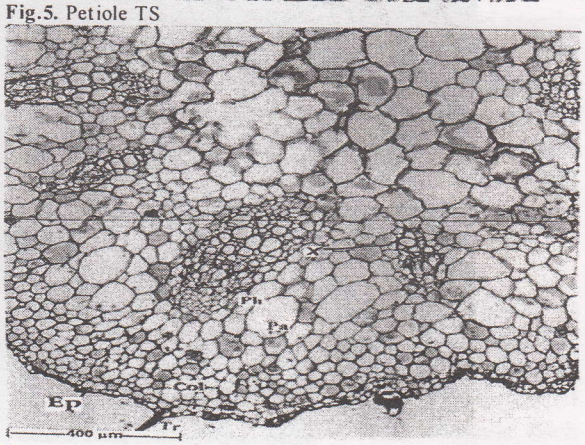

Fig.6. Tendril TS

AdB- Adaxial bundle; AdE- Adaxial epidermis; AW- Anticlinal wall; Co- Cortex ; Col- Collenchyma; Dr- Druses; EC- Epidermal cell; Ep- Epidermis; ETr- Epidermal trichome; Fi- Fibre; GT- Ground tissue; La-Lamina; LB- Lateral bundle; MB- Median bundle; MT- Mesophyll tissue; Pa- Parenchyma cells; Pe- Periderm; Pa- Parenchyma; Ph- Phloem; Pi- Pith; PM- Palisade mesophyll; PX- Primary xylem; Ra- Raphide; RaC - Ray cells; SC- Subsidiary cell; SG- Starch grain; SM- Spongy mesophyll; St- Stomata; SX- Secondary xylem; Tr- Trichome; V- Vessel; VB- Vascular bundle; VE- Vessel element ; VI- Vein islets; VT- Vein termination; X- Xylem.

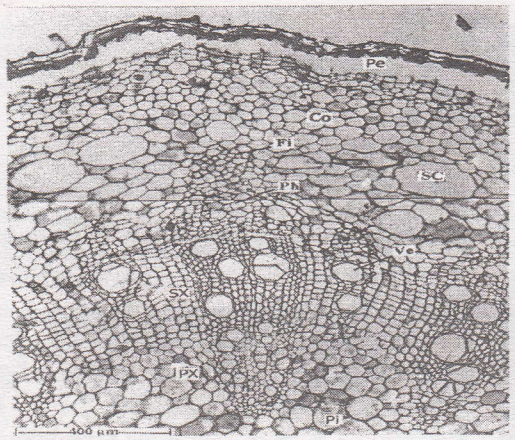

Fig.7. Stem TS

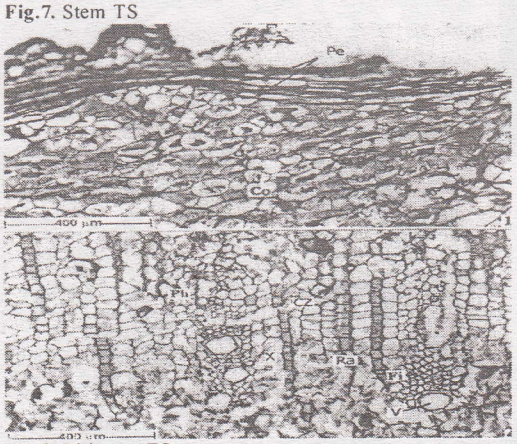

Fig.8. Rhizome TS

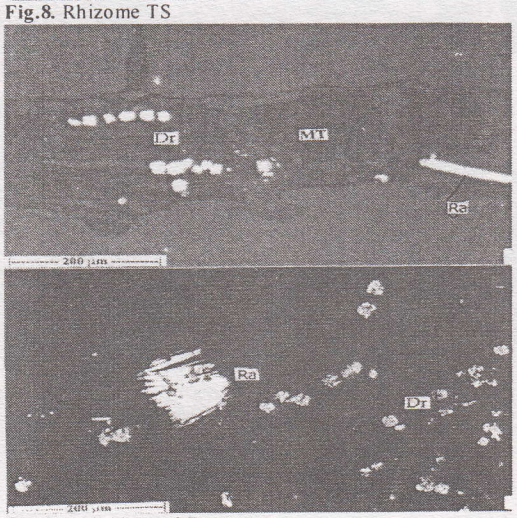

Fig.9. Raphides and Druses

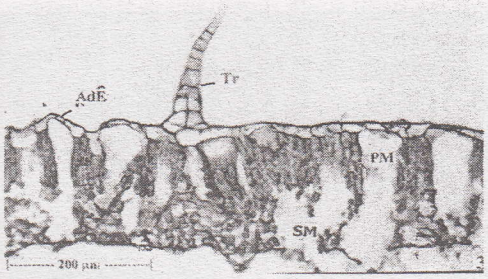

Fig.10. Lamina T S

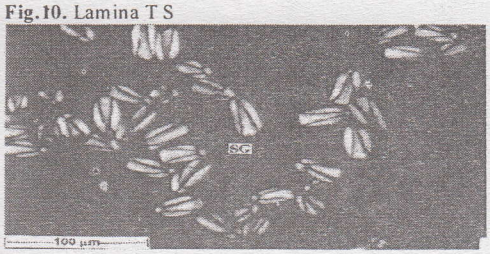

Fig.11. Starch grains

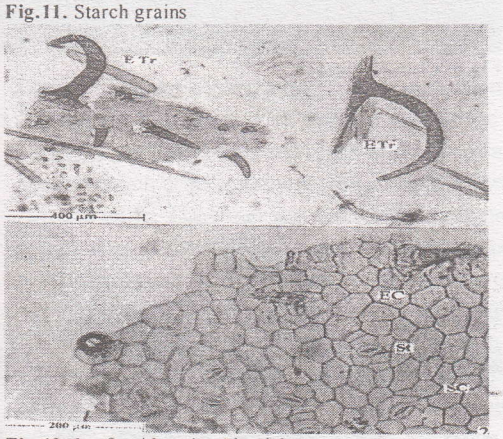

Fig.12. Leaf epidermis- Abaxial

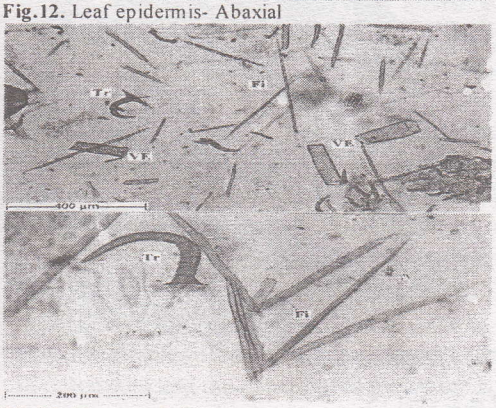

Fig.13. Macerated elements

AdB- Adaxial bundle; AdE- Adaxial epidermis; AW- Anticlinal wall; Co- Cortex ; Col- Collenchyma; Dr- Druses; EC- Epidermal cell; Ep- Epidermis; ETr- Epidermal trichome; Fi- Fibre; GT- Ground tissue; La-Lamina; LB- Lateral bundle; MB- Median bundle; MT- Mesophyll tissue; Pa- Parenchyma cells; Pe- Periderm; Pa- Parenchyma; Ph- Phloem; Pi- Pith; PM- Palisade mesophyll; PX- Primary xylem; Ra- Raphide; RaC - Ray cells; SC- Subsidiary cell; SG- Starch grain; SM- Spongy mesophyll; St- Stomata; SX- Secondary xylem; Tr- Trichome; V- Vessel; VB- Vascular bundle; VE- Vessel element ; VI- Vein islets; VT- Vein termination; X- Xylem.

Table 1. Qualitative phytochemical screening of aqueous extract of leaf of *Cissus vitiginea.*

Sl. No.	Name of the compound	Name of the test	Status of the substance
1.	Carbohydrates	Fehling's	++
		Benedict's	++
2.	Alkaloids	Mayer's	+
		Hager's	+
		Wagner's	++
		Dragen Dorfff's	+
3.	Steroids	Chloroform + acetic acid + H_2SO_4	–
4.	Tannins & Phenols	10% Lead acetate	+
		5% Ferric chloride	+++
		1% gelatin	+
5.	Saponins	Foam test	+
6.	Fixed oils & Fats	Spot test	++
7.	Mucilage	Alcoholic precipitation	-
8.	Proteins	Biuret test	++
9.	Flavonoids	Na OH / HCl	+
10.	Volatile oils	Hydro distillation method	-

Note: +++ - Rich amount; ++ - Moderate amount; + - Minimum amount; - - Absent

are wide circular secretary cells in the cortex. Xylem vessels are wide, polygonal, thick walled and solitary. Xylem fibres are narrow, lignified and thick walled. Secondary phloem occurs outside the xylem segments and consists of sieve tubes, phloem parenchyma and small nests of fibres at the periphery. The xylem elements are 40 - 70 µm in diameter, primary xylem is thick, triangular and occurs along the inner periphery of the secondary xylem cylinder (Fig. 7).

Rhizome : It has deeply fissured periderm and consists of outer narrow cortical tissue and inner periderm cells. The cortex is broken forming the fissures. The periderm has narrow, tangentially stretched, thick walled tabular phellem cells. The phellem zone is nearly 100 µm wide. Inner to the phellem is wide parenchymatous cortex comprising of large, circular to polygonal cells. Tannin and starch grains are abundant in the cortical cells. The cortex gradually merges in to a wide hollow vascular cylinder with several radial segments of collateral vascular bundles which are separated from each other by wide inter fascicular parenchymatous rays. The vascular segments have inner conical xylem with wide, thin walled angular vessels and xylem fibres. The vessels are 80 m wide. Outside the xylem segments are radial clusters of phloem (Fig. 8). Starch grains of various shape and size are abundant in the cortex, rays and pith. Most of the starch grains when viewed under the polarized light microscope are elongated and cylindrical with Y-shaped dark markings; other starch grains are circular and concentric with X - shaped dark lines (Fig. 11).

Root : The root has a thin superficial periderm which has shallow wide fissures.The periderm is 200 µm wide. The cortex is wide and parenchymatous comprising of small, compact cells of varying shape and size. The inner part of the root has small nests of isolated vascular strands. Each has a wide, thin walled xylem element, ensheathed by a thin layer of sclerenchyma cells. The central core of the root has larger, segments of xylem radiating from the center. The xylem segments have narrow thin walled vessels and fibres. Tannin is abundant in the central ground tissue.

Leaf powder observations - Powder of all organs of the

Table 2. Qualitative phytochemical screening of methanol extract of leaf of *Cissus vitiginea.*

Sl. No.	Name of the compound	Name of the test	Status of the substance
1.	Carbohydrates	Fehling's	+
		Benedict's	+
2.	Alkaloids	Mayer's	+
		Hager's	+++
		Wagner's	++
		Dragen Dorffff's	+++
3.	Steroids	Chloroform + acetic acid + H_2SO_4	–
4.	Tannins & Phenols	10% Lead acetate	+++
		5% Ferric chloride	++
		1% gelatin	++
5.	Saponins	Foam test	—
6.	Fixed oils & Fats	Spot test	++
7.	Mucilage	Alcoholic precipitation	—
8.	Proteins	Biuret test	++
9.	Flavonoids	Na OH / HCl	++
10.	Volatile oils	Hydro distillation method	—

Note: +++ - Rich amount; ++ - Moderate amount;
+ - Minimum amount; - - Absent

Table 3. Extractive values and Behavior of various solvent extracts of *Cissus vitiginea.*

S.N.	Solvent	Extractive value % (w/w)	Behaviour of the extract		
			Colour	Consistency	Odour
1	Aqueous	5.4	Brown	Pasty	Pungent
	Methanol	5.9	Dark brown	Pasty	Pungent

Table 4. Analytical ash values of *Cissus vitiginea.*

Sl. No.	Parameters	Percentage
1.	Total ash value	28
2.	Acid insoluble ash value	17
3.	Acid soluble value	83
4.	Solubility in methanol	18
5.	Solubility in water	16

plant showed epidermal trichomes, stomata, vessels, fibres, druses, raphides and starch grains. Epidermal trichomes are more frequent and are of two types, the short and long ones. Short trichomes are 1 - 4 celled, unbranched and straight or slightly curved tubercled, 30 - 150 µm long and 30 µm thick. Most of the trichomes are long and curved, 8 - 10 celled, uniseriate, unbranched, thick walled, finely echinate, up to500 µm long and 50 µm thick(Fig. 12). Vessel elements are cylindrical, elongated, have simple, wide, horizontal perforation plate and dense scalariform or reticulate lateral wall pits. The vessel elements are 130 - 170 µm long. Xylem fibres are abundant and they may be narrow and thick walled or wide and thin walled. The thick walled narrow fibres are 250 - 400 µm long and the wide and thin walled fibres are 300 µm long (Fig. 13). Calcium oxalate crystals (druses and raphides) and starch grains are abundant in the leaf, stem and rhizome. These two types of crystals occur mixed in leaf (Fig. 9). Druses are 40 µm in diameter and mostly seen along the veins of the leaf. The raphides are 50 µm

long and 20 µm thick. Starch grains are abundant in the pith ray cells of the rhizome and stem. They are either cylindrical, measuring 20 - 40 µm long and 15 µm thick, some of the grains are circular measuring 20 µm in diameter.

Phytochemical Studies- Rich amounts of tannins and phenols; moderate amounts of carbohydrates, alkaloid, fixed oils and protein and low amounts of saponin and flavonoids were observed from the aqueous extract of the leaf (Table 1). The methanol extracts yielded rich amounts of alkaloids, tannins and phenols; moderate amounts of fixed oils, fats, proteins and flavonoids and low amount of carbohydrate (Table 2). The extraction values were 5.4 and 5.9 percent in aqueous and methanol extracts respectively. The extracts were brown, pasty and pungent (Table 3). The values of total ash, acid insoluble ash and acid soluble ash were 28 percent, 17 percent and 83 percent, respectively. The solubility was 18 percent in alcohol and 16 percent in water (Table 4).

Discussion

The qualitative microscopic characters such as trichomes, stomatal type, tracheids, vessel members, druses, raphides and starch grains are some of the diagnostic characters that are useful in the identification of the crude drug sample[15]. These features are believed to be constant for a given species[16]. The plant *C. vitiginea* was subjected to pharmacognostical studies to identify the plant materials and to differentiate them from the spurious crude drugs. It showed many important features useful for the identication of the drug of the plant. Thick midrib on the abaxial side of the leaf, discrete vascular bundles with wide vessels, occurrence of short straight and long curved echinate trichomes, hypostomatic leaves, actinocytic stomata, cylindrical elongated vessel members with simple perforation plate, narrow thick walled fibres, abundant druses and raphides, long cylindrical starch grains with Y-shaped dark lines are the diagnostic characters of the species studied. 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 a plant which may constitute individual drug.

This report would be of much significance in establishing the identity of the drug plant. The morphological, anatomical, powder microscopic and polarized microscopic observations and the extraction values help to ascertain the identity of the drug. Qualitative phytochemical observations revealed the presence of many primary and secondary metabolites which may be useful in the studies of antimicrobial activities of *C. vitiginea*.

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