Morphotaxonomic and pharmaco-anatomical studies on Cissampelos pareira L.

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> Manuscript received: 22 August 2012 Accepted for publication: 18 March 2013

ABSTRACT

Singh S. P., Singh V. & Mishra G K. 2013. Morphotaxonomic and pharmaco-anatomical studies on *Cissampelos pareira* L. Geophytology 43(1): 29-40.

In ethnomedicinal practice, the traditional healers use Cissampelos pareira L. (known as 'Laghupatha', 'Madrachi' and several other names) in treatment of various ailments, especially in dysentery, diarrhoea, cough, urinary troubles, skin disorders and snake bite. The crude drug is available in the form of dried and cut pieces in the market where it is difficult to identify the plant material on basis of taxonomic features. Microscopic evaluation is an indispensable tool for identification of medicinal herb and is one of the essential parameters in modern monographic studies. In this regard, the important microscopic characters of the various parts, viz. leaf, petiole, stem and root, have been documented. The leaf architectural studies have been carried out for the correct authentication of leaf drug. Therefore some diagnostic features have been evolved to identify crude drug.

Key-words: Morphotaxonomy, Cissampelos pareira L., Menispermaceae, ethnomedicine.

INTRODUCTION

Herbs have been known for their medicinal effects for a long time, much before the advent of drugs synthesized in the laboratory, in fact, this was the sole source of products for curing every known illness. One of the oldest forms of medicine, known as 'Ayurveda' (Sanskrit: science of longevity), practised in India relies totally on natural sources such as plants (leaf, stem, bark, fruits and roots) and herbs for use in medicinal practice. Medicinal and aromatic plants (MAPs) are local heritage of global importance (Purohit & Vyas 2004). Altogether, 60% of the population of world and 80% of the population in developing countries rely on traditional medicine, mostly plant drugs, for their primary health care needs (Shrestha et al. 2003). About 70% of the population of India (Gadgil & Rao 1998), 80% of Pakistan (Ahmad & Ghafoor 2002) and 80% of Nepal (Kunwar et al. 2006) are dependent on traditional plant based medicines. Medicinal and aromatic plants help in alleviating human suffering and are widely used as additives, beverages, cosmetics, sweeteners, bitters, spices, dying agents and insecticides. For the treatment of malaria, quinine (an alkaloid) is generally prescribed. The synthesis of quinine in laboratory involves many steps and at the end one obtains a product which, in addition to curing, might produce side effects. In case of quinine obtained from natural products, it is pure and has no side effects. The fresh leaves, bark, and roots may be used as such or in the form of tablets, capsules, powders, liquid beverages, extract, tinctures, cream lotion and oils (Agrawal & Ghosh 1985).

The ayurvedic remedies are either crude drugs as traditional medicines or a value added form as in modern

health care systems. Plant materials contribute a major portion of drugs. However, limited availability and genuine identification create a precarious solution at an ever increasing demand of material by collectors, traders and users. There is a need to develop quick assay for the identification of indigenous crude drugs that may be handy and reliable to the first hand users. The study is an effort to fix the identity of true plant material and thereby to ensure its quality.

MATERIAL AND METHOD

The plant material was collected from different localities in Lucknow and Lakhimpur Kheri districts in years 2009-2010. Identification of plants was done on the basis of published literature (Duthie 1973). Pharmaco-anatomical studies on the leaf, petiole, stem and root of *Cissampelos pareira* L. were carried out. Anatomical features were observed on semi-permanent, hand-made, transverse sections. For study of preliminary leaf architecture, the leaves were cut into small pieces and cleared by heating gently in lactic acid.

RESULT AND DISCUSSION

Morphotaxonomy of Cissampelos pareira L. (Plate 1, figures A-F)

Plant sub-erect or climbing twinner, branched, striate, pubescent or sub-glabrous. Leaves peltate, 3.8 to 14 cm in diameter or more orbicular or reniform often slightly broader than long, cordate or sometimes truncate at the base. Leaf apex mucronate, pubescent on both surfaces while young, trichome frequency is

more on abaxial surface as compared to adaxial one. underside pale or subglaucous, margin ciliate. Petiole 3.8 to 10 cm long, pubescent inserted 3 to 6 mm in the basal margin. Plant diocious, flowers minute, yellowish in colour. Male flowers, pedicelate in nearly axillary cymose. Peduncle filiform, pubescent; bract small. subulate. Sepals four, hairy, outside obovate, oblong concave. Petals combined to cyathiform corolla filaments longer than corolla anthers, 2-4, sessile. connate, encircling the top of peltate staminal column bursting transversely. Female flowers elongated, solitary or axillary racemose. Pedicels very short, pubescent or villous. Bract foliaceous, more or less stalked or nearly sessile, orbicular or reniform, usually soft, ciliate. persistent. Sepal oblong, ovate, rounded, subglobose. compressed and hairy. Petal 1, 2-nerved, adnate to bract. Carpal 1, hairy, ovule single, parietal placentation. Stigma short, trifid. Stem pieces brown, pale greenish when young. Cut ends whitish in old and greenish in young pieces. Bark thin, wrinkled when dried. Root system seen to run almost vertically downwards, about 40-50 cm deep into soil with lateral branches in all directions. Fresh roots smooth, creamish yellow in colour, develop longitudinal wrinkles, darkens in colour on drying. Periderm easily separable from wood with the help of knife. Fresh material possesses a characteristic odour.

Anatomical characteristics of Cissampelos pareira L.

Leaf: Cissampelos pareira L. leaf epidermal peels have been taken and stained in hematoxylin and mounted in glycerin. The peels have been studied both

Table 1. Preliminar	y leaf architectural	studies (Metcalf	& Cl	nalk 1979).
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Leaf organization	Simple		Consisting of a single lamina
Leaf shape	(A) Lamina	Whole	-
		Symmetrical	
	(B) Apex	Mucronate	Apex terminating in a sharp point which is a
			continuation of the mid vein
	(C) Base	Peltate	Petiole attached within the boundaries of the leaf margin
Form of leaf margin	Entire	Ciliate	-
Leaf texture	Opaque	-	-
Petiole	Normal	-	Without noticeable thickening or other processes
Type of venation	Actinodromous	Perfect	Reticulate, Suprabasal
Ultimate venation pattern	Looped	~	-
Areole development	Well developed	W1	2
Areole shape	Pentagonal, quadrangular	~	*
Veinlets	Branched	Once, twice or	-
		many times	

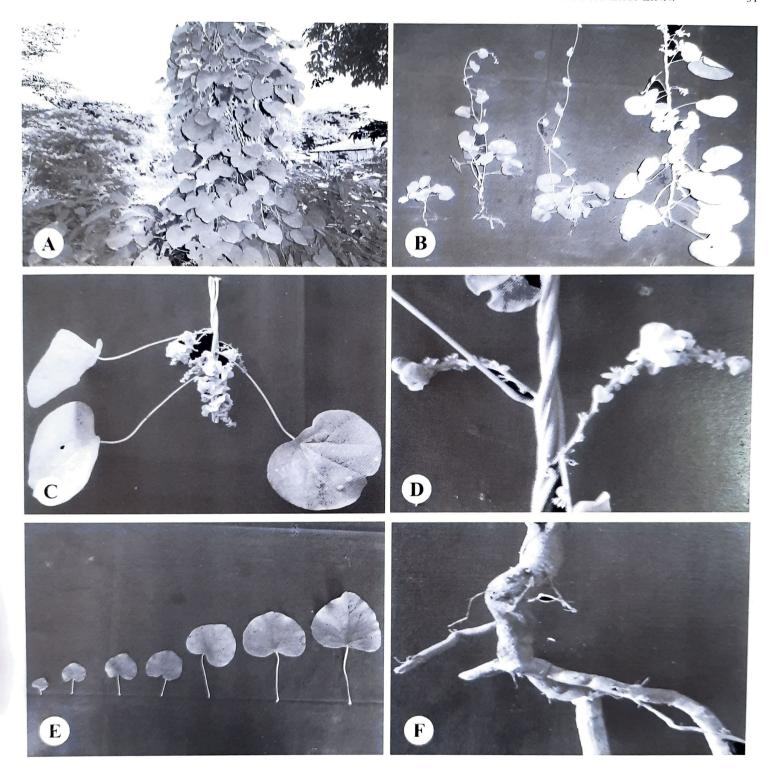


Plate 1

A-B. Cissampelos pareira L. plant, C-D. Inflorescences (female flower in racemose), E. Leaves of variable shape and size, E. External morphology of root showing tap root system.

in low and high power of microscope and following observations have been noted. Surface view of the upper surface has slightly to very wavy anticlinal walls of epidermal cells while lower surface of lamina consists of polygonal to slightly wavy epidermal cells with chloroplast, anomocytic stomata and uniseriate, bicelled trichomes. Stomata are present only on the abaxial surface of leaf, i.e. leaf is hypostomatic.

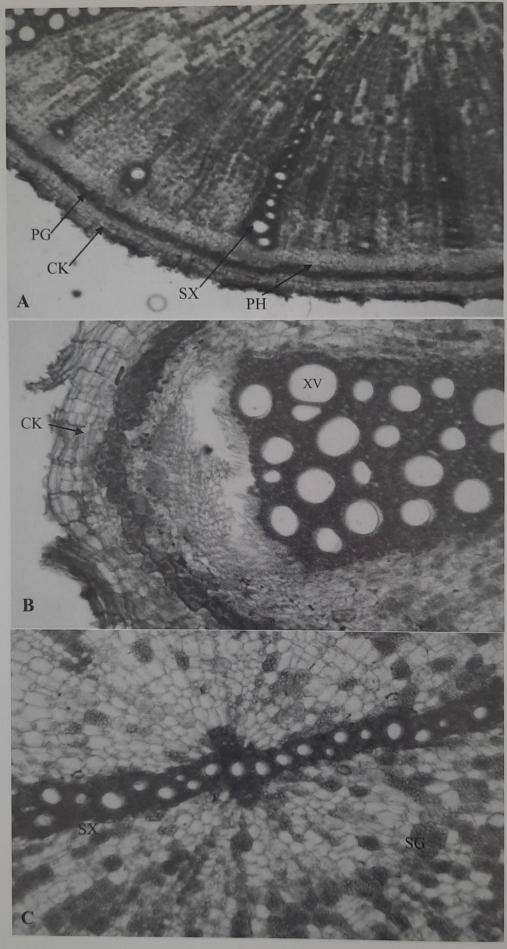


Plate 2

A-C. Transverse section of root of *Cissampelos pareira* L. A. Showing stelar and extra-stelar secondary growth. B. A part of the above, magnified. C. Central portion showing grape-cluster type starch grains. CK: Cork, PG: Phellogen, PH: Pholem, SG: Starch grain, SX: Secondary xylem, XV: Xylem vessel.

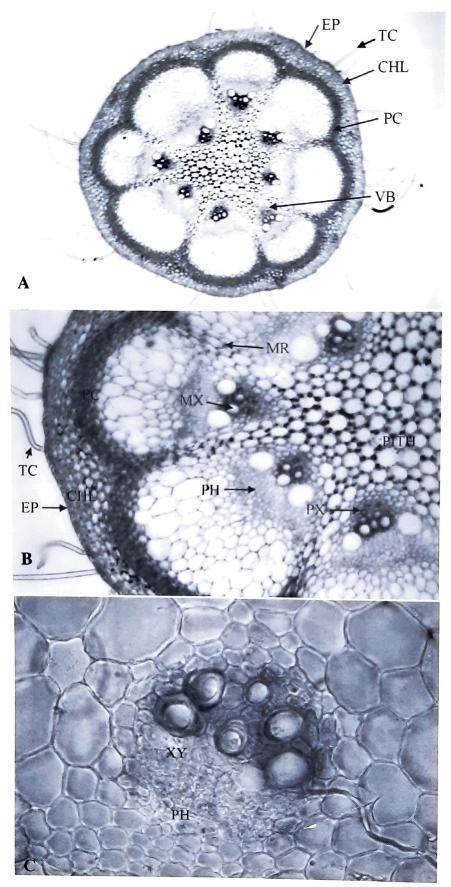


Plate 3

A-C. Transverse section of *Cissampelos pareira* L. stem. A. Showing characteristic sclerenchymatus pericycle. B. A part of the above, magnified. C. Vascular bundle. CHL: Chlorenchyma, EP: Epidermis, MR: Medullary ray, MX: Metaxylem, PC: Pericycle, PH: Pholem, PX: Protoxylem, TC: Terminal cell, VB: Vascular Bundle, XY: Xylem.

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Contiguous stomata were also observed. Uniseriate covering trichomes are present on both the surfaces of leaf lamina but particularly along veins on the lower surface and on the entire margin of leaf lamina. Trichomes are non-glandular, simple, short and thick, bi-celled, basal highly thickened stalk cell and tapering terminal cell. Shape and size of trichome show variations in different age group of leaves. Occasionally twisted or sickle shaped tapering terminal cells are also observed (Plate 6, figures A-C, Plate 7, figures A-B). Transverse section shows typical characters of bifacial leaf. In transverse section, the lamina consists of upper and lower epidermis with stomata and trichomes. Following are the important features in lamina and midrib region (Plate 4, figures A-B). Epidermis is single layered. It consists of tabular cells and is covered by cuticle externally. Uniseriate, bi-celled trichomes are present on both adaxial and abaxial epidermis. A distinct differentiation of mesophyll into palisade and spongy parenchyma is present. Palisade constitutes one layer towards the adaxial epidermis with rod shaped compactly arranged cells filled with chloroplast. Spongy parenchyma consists of up to five layers of loosely arranged parenchyma cells. Palisade tissue extends up to midrib region except the bulging. The mesophyll tissue does not extend up to midrib region. A single collateral vascular bundle is present in the central portion of the midrib with phloem facing towards the abaxial epidermis. The vascular bundle is caped with collenchymatous tissue on either side. Surrounding the vascular bundle is rounded thin walled parenchyma.

Petiole (Plate 5, figures A-B): Epidermis is single layered with barrel shaped cells cuticularised exteriorly. Uniseriate trichomes are present on the epidermal surface. Following the epidermis, 3-4 layered chlorenchymatous ground tissue with intercellular spaces is present. A sheath separates the vascular bundles and cortical region. Vascular bundles are seven in number with protoxylem at the centre and metaxylem towards the periphery. Pith constitutes central portion with polyhedral parenchymatous cells.

Stem (Plate 3, figures A-C): Transverse section of *C. pareira* L. stem is circular in outline and shows following characteristics. Epidermis is single layered with

thick cuticle followed by 7-8 layers of chlorenchymatous cortex. The cells of this region are packed with chloroplast. Uniseriate, bicelled trichomes are present on the surface of epidermis. Endodermis is not clear and following the cortex pericycle is present. Pericycle constitutes an undulated composite and continuous sclerenchymatous ring, which is composed of arc of primary hard bast belonging to separate vascular bundle. There are 8 ridges in the pericycle and each ridge follows a vascular bundle with protoxylem element situated towards the centre and metaxylem towards the periphery. The xylem is characteristic with wide lumina and simple perforation. 6-7 layer of phloem are present just above the metaxylem element with sieve tube, sieve plate, companion cells and phloem parenchyma. Broad primary medullary rays separate the vascular bundle of axis from one another. No inclusions are seen. Pith is large made up of rounded to hexagonal cells.

Root (Plate 2, figures A-C): Transverse section of C. pareira L. root is circular in outline and shows following characteristics. Periderm consists of 12-15 layers of tangentially elongated cells. Phellogen constitutes 3-4 layers, immediately below the cork. Cells are tangentially elongated and slightly compressed due to formation of secondary tissue. Cortex is composed of 6-8 layers of thin walled parenchyma cells with small intercellular spaces. The primary xylem bundles have tapering arms of protoxylem and centrally located large metaxylem. The secondary vasculature appears in the form of arcs and does not lead to formation of complete cylinder. The xylem rays form ribbon like sheaths of tissue traversing radially. Large amount of parenchymatous cells are produced which lead to the development of discrete vascular bundles with very wide parenchymatous medullary rays. The parenchyma cells are laden with large grape cluster type starch grains. Few layers of cambium separate xylem and crushed phloem.

ETHNOMEDICINAL USES OF CISSAMPELOS PAREIRA L.

Cissampelos pareira L. Sp. pl. (1753) (Family Menispermaceae) is widely used by different tribes in several countries in both traditional and modern

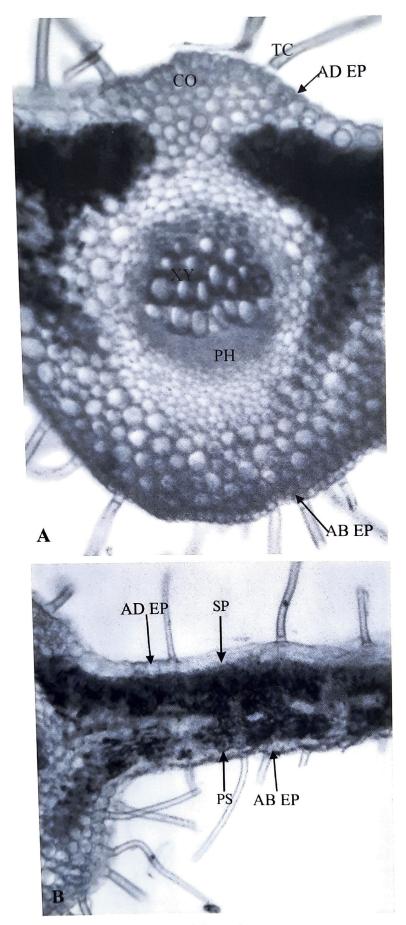


Plate 4

A. Transverse section of leaf- midrib region of *Cissampelos pareira* L., B. T.S. of leaf- lamina region. AB EP: Abaxial epidermis, AD EP: Adaxial epidermis, CO: Collenchyma, PH: Pholem, PS: Palisade, SP: Spongy paranchyma, TC: Terminal cell, XY: Xylem.

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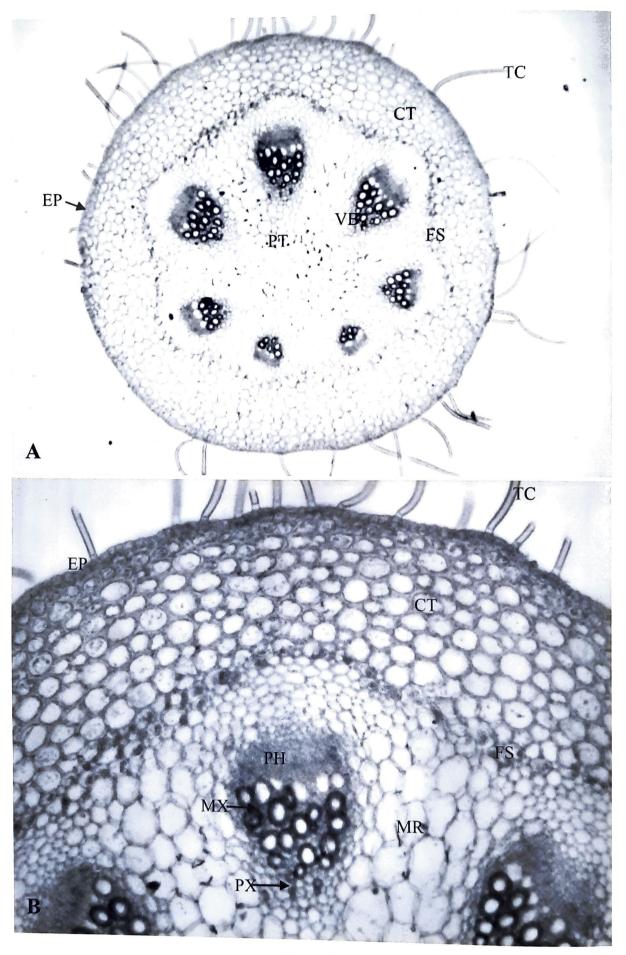


Plate 5

A-B. Cissampelos pareira L. Transverse section of petiole. CT: Cortex, EP: Epidermis, FS: Fibrous sheath, MR: Medullary ray, MX: Metaxylem, PH: Pholem, PT: Pith, PX: Protoxylem, TC: Terminal cell, VB: Vascular Bundle.

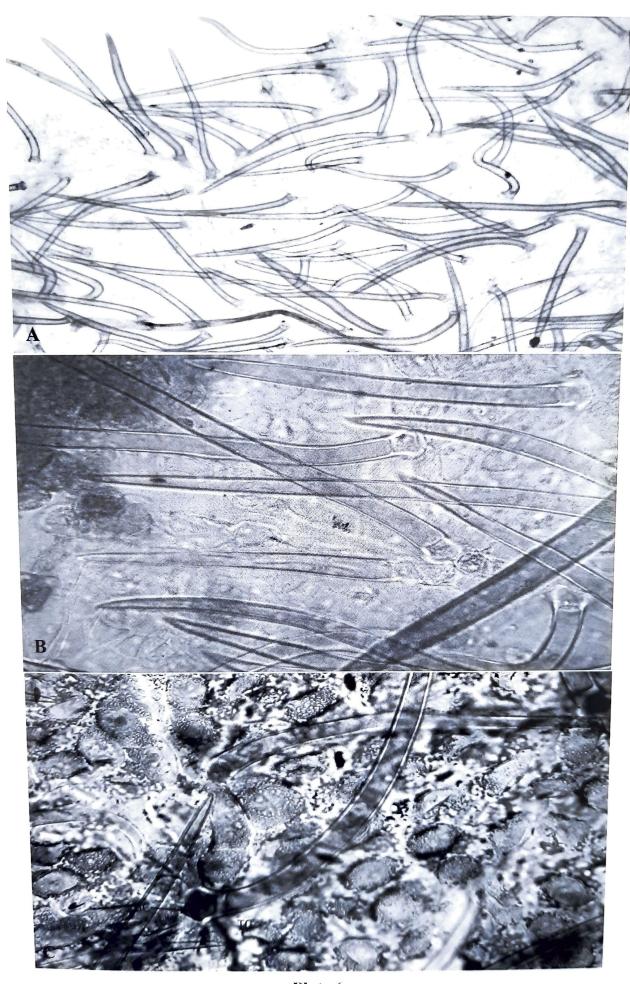


Plate 6

A-C. Epidermal peelings showing uniscriate, bi-celled, nonglandular trichomes in Cissampelos pareira L. BC: Basal cell, TC: Terminal cell.

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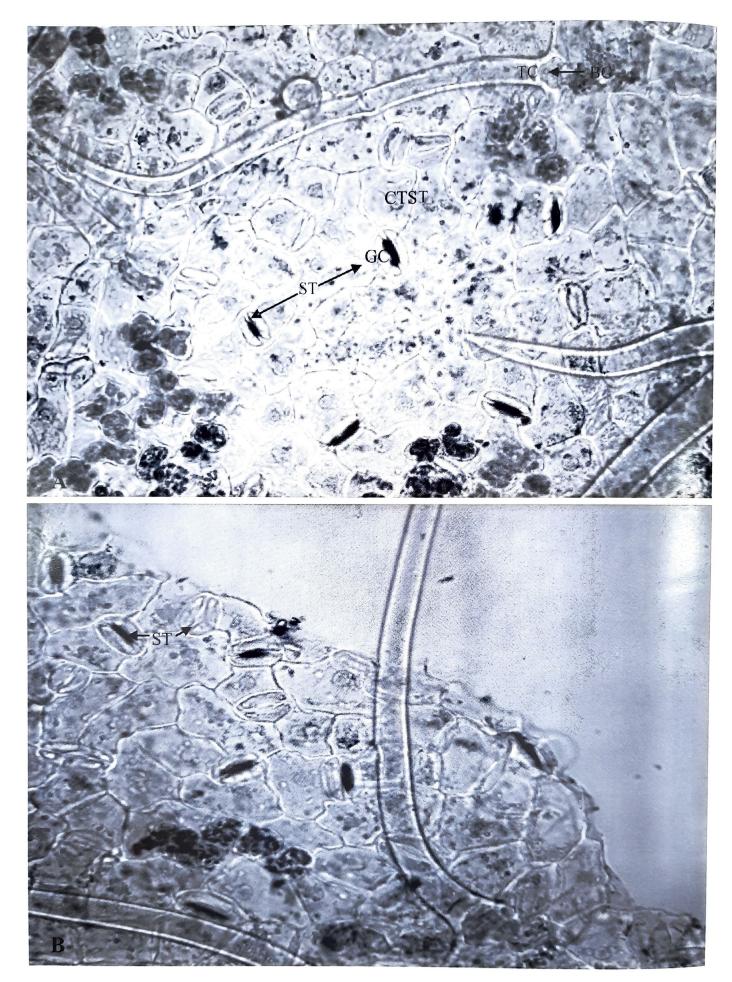


Plate 7

A-B. Epidermal peeling showing stomata and trichome in *Cissampelos pareira* L. BC: Basal cell, CTST: Contiguous stomata, GC: Guard cells, ST: Stomata, TC: Terminal cell.

medicines. The trade name is 'Laghupatha' but plant is also known as 'Madrachi' (Tharus) (Singh & Maheshwari 1994). The leaves and seeds are given in worm cases and in certain bowel affections of children in India. In North India, they are considered stimulant and laxative. In Konkan, juice is used to cure ringworm. The Santhals use the root as a remedy for amenorrhoea. Among the Mundas of Chota Nagpur, it is used to stop the diarrhoea, half a handful of whole plant being powdered and drunk in a mixture of water with sugar candy. In La Reunion, the herb is used as an astringent in diarrhoea and dysentery. The expressed juice of powdered plant is administrated internally with wine as remedy for snake bite and it is applicable to the part bitten; but whether given internally or applied externally the plant is equally useless in the treatment of snake bite (Mhaskar & Caius 1931).

Hot, sharp and bitter taste destroy 'Vata' and 'Kapha', remove pain, fever, dysentery, skin eruptions, heart troubles, burning, itching, alleviate vomiting, asthma, remove intestinal worms, cure enlarged spleen and ulcers, in hemicranias, help parturition, useful in piles and uterine complaints. The variety laghupatha has same properties. In the traditional folk medicine, the roots are used against a lot of ailments. They have a bitter taste and possess diuretic, purgative and antiperiodic properties. Furthermore, they are judged to be good against dyspepsia, diarrhoea, cough, asthma, dysentery and heart diseases (Mukerji & Bhandari 1959). The root is also prescribed in combination with other drugs for the treatment of snake bite. (Charaka, Shushruta, Vagbhat, Yogratnakar, Vrindamadhava, Baishajayaratnavali, Chakradatta, Asthangsangraha-Kritikar & Basu 1935).

The leaf paste is used in boils and juice taken with common salt for dysentery. In the simplest cases, leaves are good as an antiseptic against inflammation and can be put on wounds in order to heal sores (Neuwinger 1994). The aqueous extract of the root is given thrice in a day in fever. (Singh & Maheshwari 1994). The root is the part most esteemed. It has an agreeable bitterish taste and is considered valuable stomachic. It is frequently prescribed in the later stage of bowel complaints in conjunction with aromatics. It is given for

pain in stomach and for dysentery, diarrhoea, dropsy and cough also for prolapus uteri. Among the Mundas of Chota Nagpur, the roots ground and mixed with water is used against stomach-ache and diarrhoea, especially against infantile diarrhoea (Encyclopedia Mundarica). The Chuanas, Subias and Kobas of S. Africa drink an infusion of powdered roots for stomach-ache. The Filabusi natives and Xosas drink a similar preparation for snake bite. The Pedias use a decoction of root as a wash for children who have pimples on their body. In Madagascar, the root is considered as diuretic emmenogoguge and antipyretic. It is given for urinary gravels and bladder troubles. An infusion is expectorant and is considered a laxative. The root acts as an antiseptic of the bladder and is used in chronic inflammation of urinary passage (Kritikar & Basu 1935). Traditionally, C. pareira L. is reported for its blood purifier and anti-inflammatory properties in India (Gogte 2000).

Leaves: The leaf juice is given in eye troubles. Plaster of crushed leaves applied on various skin ailments, burns and wounds. Leaves are chewed in fever, crushed and applied on forehead in cold and fever. A decoction is given in colic and dysentery, cholera and bronchitis (Asolkar et al. 1965). The leaves are said to have a peculiarly cooling quality and are used locally in case of unhealthy sores and sinuses. In Gold Coast, they are applied to abscesses. The Xosas apply a paste to wounds (Mhaskar & Caius 1931).

Root: Employed in leucorrhea, gonorrhea and also in chronic inflammation of bladder; decoction is given in enlarged spleen; in pregnancy for abortion. Lower blood pressure, extreme hypoglycemic in animals (Bhatnagar et al. 1967). Powdered root is given internally in indigestion and headache. Internally, roots are useful in anorexia, indigestion, abdominal pain, gastric disorder, diarrhoea and dysentery (Amresh et al. 2004). The roots show significant antibacterial activity against gram-positive organisms than against gram-negative (Adesina 1982). Its root is common bazaar drug sold under name 'pareira brava' and was sometimes confused with true pareira brava derived from *Clarodendron tomentosum* Reci. & Pav. and *C. platyphyllum* Miers., a native of Peru and Brazil.

Since there is no record on the pharmacoanatomical standardization of this drug plant, an attempt has been made to standardize this herb using botanical parameters. The study has revealed some diagnostic features which facilitate detection of authentic and adulteries materials.

ACKNOWLEDGEMENT

The authors are thankful to the Head, Department of Botany, Lucknow University, Lucknow for providing necessary laboratory facilities. One of the authors (S.P.S.) is grateful to the Council of Scientific & Industrial Research, New Delhi for providing CSIR-JRF and is also thankful to Prof. Seshu Lavania, Department of Botany, Lucknow University, Lucknow for co-operation and encouragement.

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