IJPSR (2011), Vol. 2, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 July, 2011; received in revised form 09 August, 2011; accepted 28 October, 2011

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF STEM OF *CISSUS QUADRANGULARIS* L.

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Keywords:
Cissus quadrangularis L.,
Stem,
Physicochemical,
Phytochemical

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ABSTRACT

The stems of *Cissus quadrangularis* L. (Vitaceae) are reported to have great medicinal value. The present investigation was therefore undertaken to determine the requisite pharmacognostic standards for evaluating the plant material. The macroscopic features of the *Cissus quadrangularis* L. stem were observed under magnifying lens. Microscopic characters and powder analysis were determined under microscope. The physiochemical properties such as loss on drying, total ash value, acid insoluble ash value, water soluble ash value, pH, and extractive values of stem were carried out. The microscopic study showed abundant large cells of mucilage, clusters and bundles of acicular crystals of calcium oxalate scattered throughout the section. The powder microscopy showed the presence of annular, reticulated and boarded pitted thickening xylem vessels, cluster, rosette and acicular crystals of calcium oxalate and starch grains. Phytochemical analysis showed the presence of many important classes of phytoconstituents like alkaloids, flavonoids, cardiac glycosides and triterpenes. The determination of these characteristics will aid future investigators in their pharmacological analyses of this species.

INTRODUCTION: There are lakhs of plants available in nature globally and as per the World Health Organization (WHO) investigations around 85-90% of the world's population consumes traditional herbal medicines. Use of herbal remedies is on the rise in developing and developed countries [1]. Authentication and standardization are prerequisite steps especially for herbal drugs and their formulations in traditional systems of medicine [2].

Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents which may vary depending on various factors, one amongst is the geographical localities which show quantitative variation in their chemical constituents [3, 4]. In most of the cases of herbal medicine, misuse starts with wrong identification. Many of the traditional systems have records where one common vernacular name is given to two or more entirely different species [1].

Cissus quadrangularis Linn. belongs to the family Vitaceae, an edible plant found in India, Sri Lanka, Malaya, Java, West Africa [5] and also found throughout Thailand [6]. It is commonly known as "bone setter"; the plant is referred to as "Asthisamdhani" in Sanskrit and "Hadjod" in Hindi because of its ability to join bones [7]. The plant has been documented in Ayurveda, an alternative system of medicine in India, for its medicinal uses in gout, syphilis, venereal diseases,

piles, tumors, hemorrhoids peptic ulcers and leucorrhoea [8, 9]. The stem juice of the plant is used to treat scurvy and irregular menstruation, otorrhoea and epistaxis [10]. The roots and stem are useful in healing of fractures of the bones [11].

Due to its high profile in medicinal products and less account in morphological, microscopical, physico-chemical and phytochemical aspects, it was thought worthwhile to carry out this work.

MATERIAL AND METHODS:

Collection and extraction of Plant Material: The fresh stem of *Cissus quadrangularis* L. was collected from Jamnagar, Gujarat, in August 2009. The taxonomical identification was done by Dr. P. S. Nagar, former taxonomist, Department of Biosciences, Saurashtra University, Rajkot (voucher specimen No. PSN127). The stem was thoroughly washed by tap water, cut into small pieces and some of them were kept in a jar containing solution of FAA (Formaldehyde : Acetic Acid : Alcohol in the ratio of 90 : 5 : 5) while rest of its parts were air dried and homogenized to fine powder and stored in airtight bottles. For their physicochemical investigation, ten grams of dried powder was extracted with methanol by using Soxhlet apparatus. The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The methanolic extract was used for the solubility study.

Pharmacognostic studies: For morphological observations, fresh stem (approx. 3-4cm in length) was used. The macromorphological features of the plant parts (stem) were observed under magnifying lens [12].

Microscopic characteristics: Free hand section of stem was taken and stained by well known reagent like safranine to confirm its lignification. Powder microscopy was also carried out and their specific diagnostic characters are recorded [13].

Physicochemical parameters: Various physicochemical parameters such as total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility, pH analysis, petroleum ether, ethyl acetate, acetone, methanol and water soluble extractive values, etc. were determined as per WHO guidelines [14].

Preliminary phytochemical screening: Preliminary phytochemical tests of crude powder and methanolic extract were carried out to identify different phytoconstituents [15, 16].

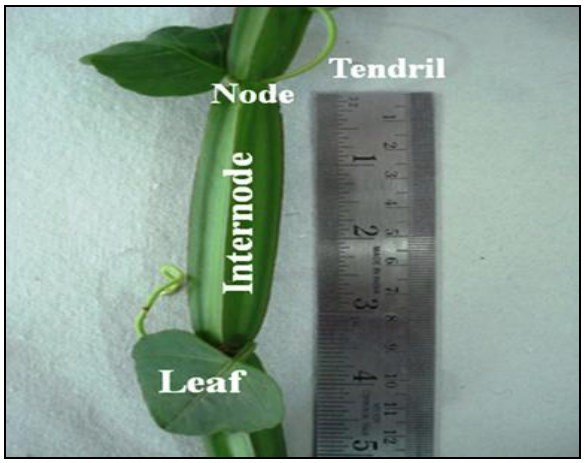
Thin layer chromatographic studies:

Sample preparation: Ten grams of dried powder was extracted with methanol by using Soxhlet apparatus. The solvent was evaporated to dryness and the dried crude extract was used for spotting on the chromatographic plates. Stationary phase: Silica gel G, particle size 10-40 µ, applied as a thin layer on a clean glass plate support and activated just before use. Mobile phase: The mobile phase chosen was toluene: ethyl acetate: glacial acetic acid (8:2:0.1). Development method: One dimensional ascending method was followed [17]. The stem extract prepared by above said method was chromatographed. Visualization: After development visible spots were found. Spots were observed under long UV light (366 nm).

RESULTS:

Macroscopic study: The diagnostic characters of stem of *Cissus quadrangularis* Linn. are the presence of quadrangular stem with winged corners and the internodes on four sides are invaded or depressed deeply in the middle and the corners are exerted with sharp reddish brown to black colored margins, 3-4 cm long (**Fig. 1**).

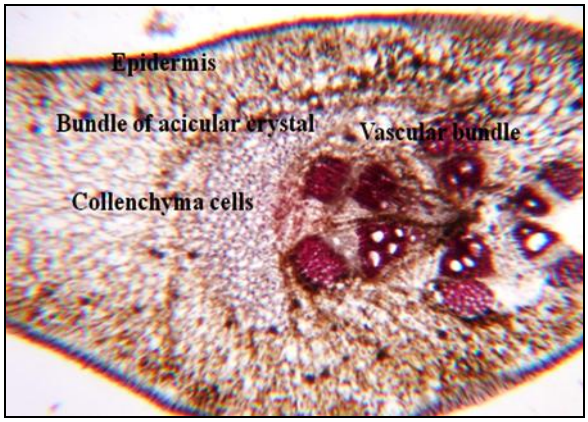
NATURAL VEGETATION OF PLANT



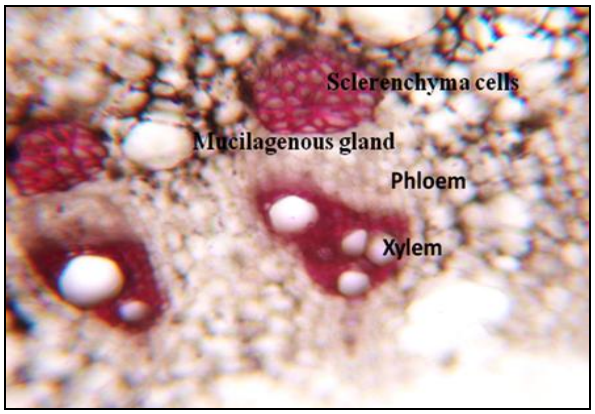
TWIG OF THE PLANT

FIG. 1: MICROSCOPIC CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM

Microscopy: Diagrammatic TS of the stem is four angled; on maturation each goes deep inside forming sharp pointed like projection and shows single layer epidermis followed by hypodermis; narrow cortex and centrally located large pith occupying almost 2/3rd region of the section, surrounded by numerous, small, discontinuous band of vascular bundles (**Fig. 2**).



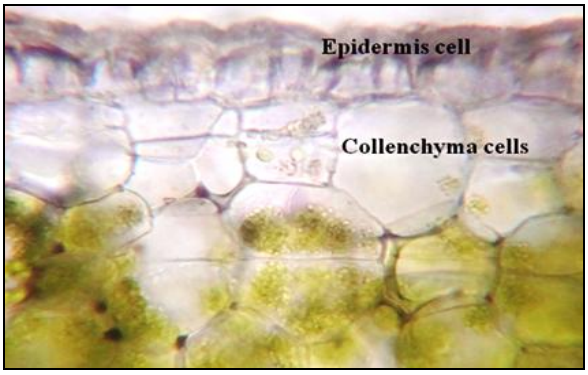
TS OF STEM PASSING THROUGH ANGLE



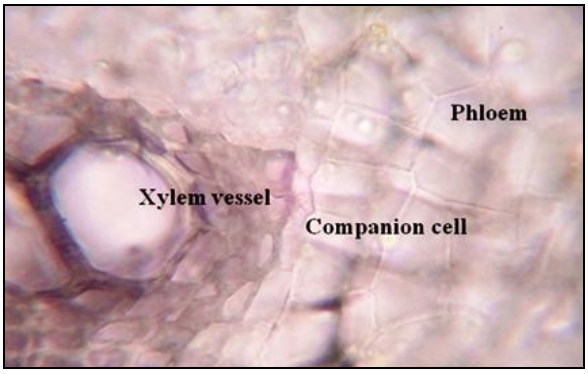
STELE REGION IN ENLARGE VIEW

FIG. 2: MICROSCOPIC CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM

Detailed section shows rectangular - pentagonal, 1-2 layered, epidermis covered by thin cuticle, followed by 3-4 layered, circular-polygonal, chlorenchymatous hypodermis deposited more near the angle; cortex very narrow, cortical parenchymatous, 5-7 layered; pith very large, parenchymatous similar to that of region surrounded by discontinuous band of numerous, small, conjoint, collateral vascular bundles, each shielded with sclerenchymatous sheath, stele near the angle formed into strip, capped with collenchymatous band; few starch grains and rosette crystals and abundant large cells of mucilage, clusters and bundles of acicular crystals of calcium oxalate scattered throughout the section (**Fig. 3-5**).

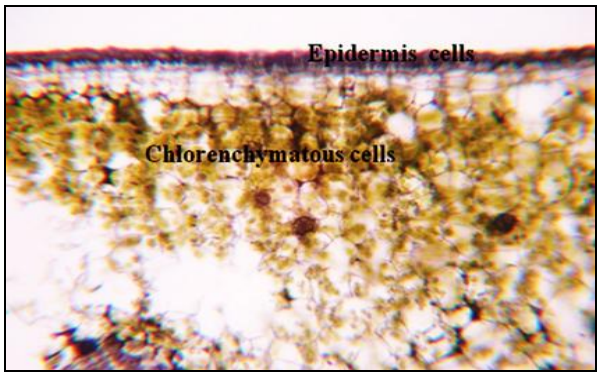


EPIDERMIS AND HYPODERMIS

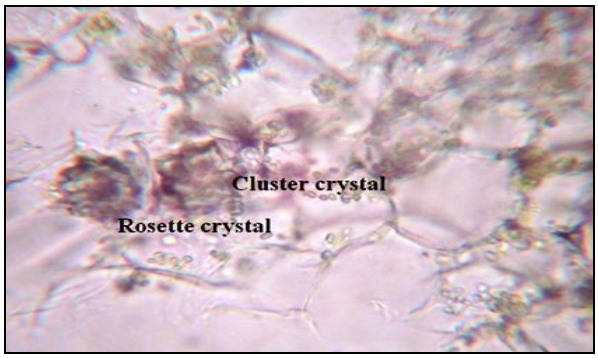


CORTICAL REGION WITH VASCULAR BUNDLE

FIG. 3: MICROSCOPIC CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM

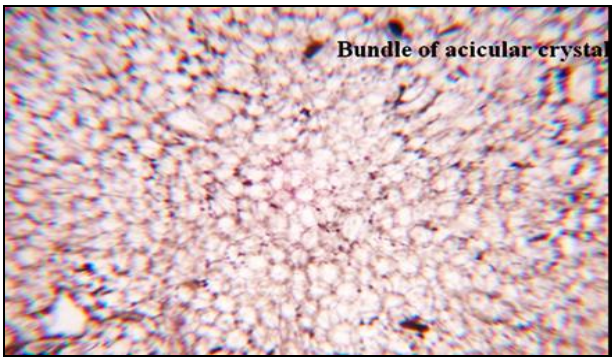


TS WITH OVERALL ARRANGEMENT OF TISSUES

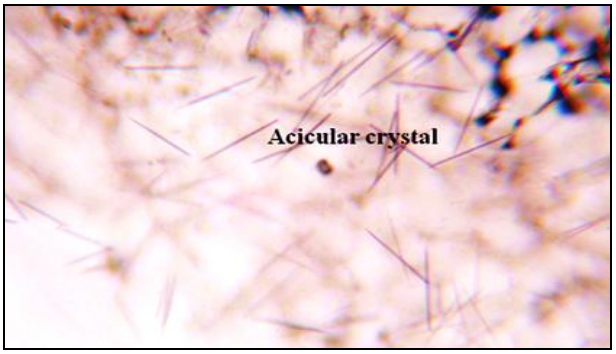


CORTEX IN ENLARGE VIEW SHOWS CLUSTER AND ROSETTE CRYSTALS OF CALCIUM OXALATE

FIG. 4: MICROSCOPIC CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM



PITH CONSISTING BUNDLES OF ACICULAR CRYSTALS OF CALCIUM OXALATE

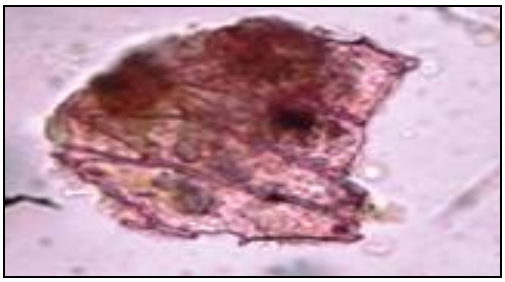


CRYSTALS IN SCATTERED FORM

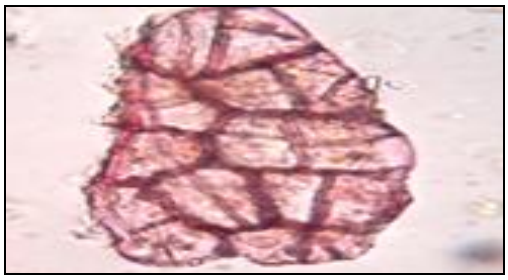
FIG. 5: MICROSCOPIC CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM

Powder study: The fine powder is green colored with faint odor. The diagnostic features of powder are plenty of cluster, rosette and acicular crystals of calcium oxalate scattered as such throughout or embedded with parenchymatous cells. Simple and compound with 2-celled starch grains scattered as such or embedded in parenchyma. Fragments of epidermis in surface view embedded with anisocytic stomata. The fibers isolated or in groups, thin walled, occasionally exhibiting dentate margin, vessels with annular, reticulate and boarded pitted thickening. Cells of the medullary rays with pitted thickening (**Fig. 6, 7**).

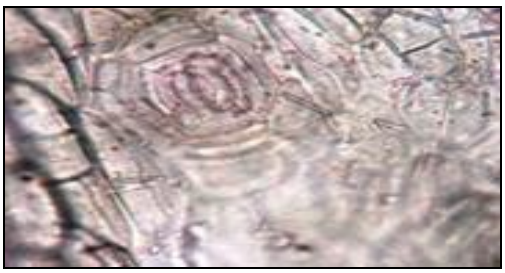
EPIDERMAL CELL IN SURFACE VIEW



EPIDERMAL CELLS FIXED WITH CLUSTERS OF CALCIUM OXALATE

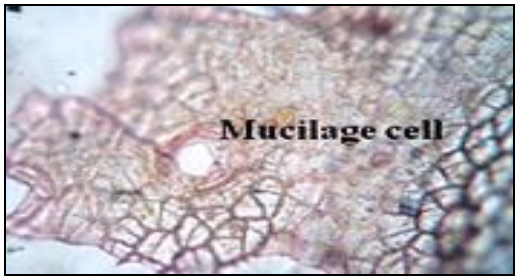


EPIDERMIS OVERLAPPED WITH HYPODERMIS



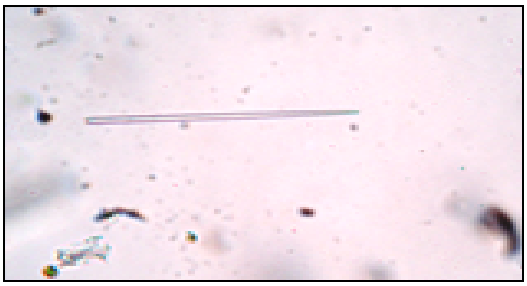
EPIDERMAL CELLS WITH ANISOCYTIC STOMATA

XYLEM VESSELS WITH ANNULAR THICKENING



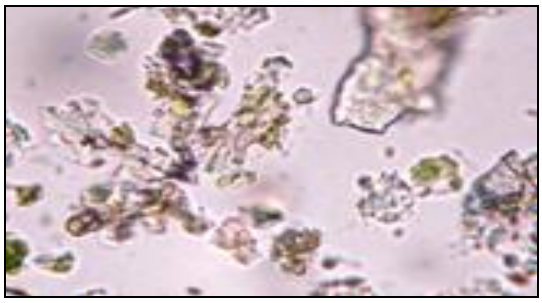
CELLS OF MUCILAGE IN PARENCHYMA

FIG. 6: POWDER CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM

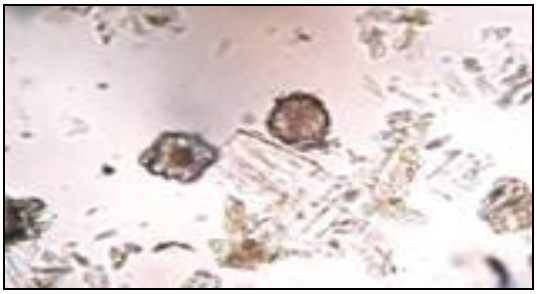


ACICULAR CRYSTALS

BUNDLE OF ACICULAR CRYSTALS



STARCH GRAIN



CLUSTERS AND ROSSETTES OF CALCIUM OXALATE

IDIOBLASTS- VASCULAR STRANDS SURROUNDED BY CLUSTERS AND ROSSETTES OF CALCIUM OXALATE

FIG. 7: POWDER CHARACTERISTIC OF *CISSUS QUADRANGULARIS* L. STEM

Physicochemical investigations: The result of proximate analysis of crude powder of *Cissus quadrangularis* stem is shown in **Table 1**. The average values are expressed as percentage of air-dried material. The loss on drying was 9.5 %. Total ash was 19.41 %, acid insoluble ash was 17.0 % and water soluble ash was 14.16 %. The extractive value of crude powder was maximum in water (19.18%), followed by methanol (7.81 %) and minimum was in petroleum ether (1.11%). pH of methanol extract was 4.25.

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF POWDER OF *CISSUS QUADRANGULARIS* L. STEM

Physicochemical Parameters	Average value %W/W
Total Ash	19.41 %
Acid insoluble Ash	17.0 %
Water soluble Ash	14.16 %
Loss on Drying	09.5 %
Petroleum ether soluble extractive	01.11 %
Ethyl acetate soluble extractive	02.08 %
Acetone soluble extractive	01.94 %
Methanol soluble extractive	07.81 %
Water soluble extractive	19.18 %

Solubility test: The methanolic extract of stem was made to evaluate its solubility in 11 solvents with varied polarities. The extract was highly soluble in dimethylformamide, distilled water and methanol but less soluble in ethyl acetate, 1-4 dioxan and petroleum ether (**Table 2**).

TABLE 2: SOLUBILITY OF METHANOLIC EXTRACT OF *CISSUS QUADRANGULARIS* L. STEM IN DIFFERENT SOLVENTS

Solvents	Soluble in mg/ml
Petroleum ether	10.5
Toluene	13.3
Ethyl acetate	07.1
Acetone	10.8
Methanol	79.1
Chloroform	24.5
DMSO	52.0
DMF	115.6
1-4 Dioxan	07.2
Glacial acetic acid	53.8
Distilled water	107.8

pH: 4.23

Phytochemical analysis: Preliminary phytochemical analysis revealed the presence of medicinally important secondary metabolites like alkaloids, flavonoids, cardiac glycosides and triterpenes (Table 3). However, the methanolic extract was rich in alkaloids (Wagner test) while crude powder was rich in cardiac glycosides.

TABLE 3: PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *CISSUS QUADRANGULARIS* L. STEM

Phytochemical	Test	Powder	Methanolic extract
Alkaloids	Dragendroff test	+	+
	Mayer test	+	-
	Wagner test	+	+++
Flavonoids	Alcoholic extract	-	-
	Alkaline reagent	+	-
Tannins	$FeCl_3$ test	-	-
Phlobatanins	HCl test	-	-
Triterpenes	H_2SO_4 test	+	+
Steroids	Liebermann-Burchard reaction	+	+
Saponins	Frothing test	-	+
Cardiac glycosides	Keller-kilianni test	+++	++

Thin Layer Chromatographic Studies: The methanol extract yielded five light yellow spots of different intensity. Rf values are 0.56, 0.63, 0.71, 0.77. Two test spots were found to exhibit bright blue fluorescence under long UV radiation (365 nm).

DISCUSSION: The main diagnostic microscopic features are 4-angled shape of stem, presence of three types of crystals such as rosettes, clusters and bundles of acicular. These three types of calcium oxalate crystals are found both in intact stem and powder form. The physico chemical constants are important parameters for detecting adulteration or improper handling of drugs. The percentage of active chemical constituents in crude drugs is mentioned on air-dried basis.[18] Therefore, the loss on drying of plant materials should be determined and the water content should also be controlled. The moisture content of dry powder of stem was 9.5 % which is not very high, hence it would discourage bacteria fungi or yeast growth.

The important microscopic features of the plant that have been documented are transverse section of stem showing four angled stem; single layered epidermis followed by hypodermis and narrow cortex; midrib showing both upper and lower continuous epidermis; arc shaped vascular bundles; all three types of calcium oxalate crystals scattered throughout.

The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods *viz*. total ash, acid-insoluble ash and water-soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the water soluble portion of the total ash [19, 20, 21]. These ash values are important quantitative standards. All these three parameters were determined in *Cissus quadrangularis* stem.

Phytochemical analysis shows the presence of many medicinally important secondary metabolite types of phytoconstituents like alkaloids, cardiac glycosides, saponins, triterpenes, which indicates that the plant possesses high profile values and can be used to treat various kinds of diseases.

CONCLUSION: The quantitative phytochemical investigation gave valuable information about the different phytoconstituents present in the powder extract, which helps the future investigators regarding the selection of the particular extract for further research in isolation of new active compounds. This study serves important criteria in standardization of the stem material, isolation of medicinally important phytoconstituents, performing pharmacological investigations and ensuring quality formulations.

REFERECECS:

1. Dineshkumar C: Pharmacognosy can help minimize accidental misuse of herbal medicine. Current Science 2007; 93:1356-1358.
2. Ahmad M, Khan MA, Rashid U, Zafar M, Arshad M and Sultana S: Quality assurance of herbal drug valerian by chemotaxonomic markers. African Journal of Biotechnology 2009; 8:1148-1154.

3. Mallavarapu GR, Ramesh S, Chandrasekaran RS, Rao RBR, Kaul, PN and Bhattacharya, AK: Investigation of the essential oil of Cinnamomum leaf grown at Bangalore and Hyderabad. Flavour and Fragrance Journal 1995; 10:231-249.
4. Chanda S, Nagani K and Parekh J: Assessment of quality of *Manilkara hexandra* (Roxb.) Dubard leaf (Sapotaceae): Pharmacognostical and physicochemical profile. Pharmacognosy Journal 2010; 2:520-524.
5. Udupa KN, Chaturvedi GN and Tripathi SN: Advances in research in Indian medicine. Banaras Hindu University, Varanasi, India Vol. 12, 1970:165-196.
6. Pongboonrod S: Mai Thed Mung Muang Thai. Bangkok: Kaeseam-bunnakit Printing, Thailand, 1995: 428-429.
7. Shirwaikar A, Khan S and Malini S: Antiosteoporotic effect of ethanol extracts of *Cissus quadrangularis* Linn. on ovariectomized rat. Journal of Ethnopharmacology 2003; 89:245-250.
8. Yoganarisimhan SN: Medicinal Plants of India. Cyber Media Bangalore 2000; 2: 136-137.
9. Warrier PK, Nambiar VPK and Raman CK: Indian Medicinal plants, Orient Longman, India, 1994:112-113.
10. Deka DK, Lahon LC, Saikia J and Mukit A: Effect of *Cissus quadrangularis* in accelerating healing process of experimentally fractured Radius-Ulna of dog: A preliminary study. Indian Journal of Pharmacology 1994; 26:44-45.
11. Kumbhojkar MS, Kulkarni DK and Upadhye AS: Ethnobotany of *Cissus quadrangularis* L. from India. Ethnobotany 1991; 3:21-25.
12. Tyler V, Brady L and Robbers J: Pharmacognosy, K. M. Varghese Company, India, 1977:103-141.
13. Khandelwal KR: Practical Pharmacognosy, Nirali Prakashan, Pune, India, Edition 19, 2008:49-70.
14. WHO: Quality Control Methods for Medicinal Plant Materials. (An authorized publication of World health organization, Geneva). A.I.T.B.S. Publishers & Distributors, New Delhi. 2002.
15. Harborne JB: Phytochemical Methods. London: Chapman & Hall. Edition 2, 1973.
16. Parekh J and Chanda S: Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research 2007; 10:175-181.
17. Indian Pharmacopoeia: New Delhi: Controller of Publications, Government of India; Vol. 4. 2008: 48-50.
18. Bhattacharya S and Zaman MK: Pharmacognostical Evaluation of *Zanthoxylum nitidum* Root. Pharmacognosy Journal 2009; 1:15-21.
19. Dave R, Nagani K and Chanda S: Pharmacognostic studies and physicochemical properties of the *Polyalthia longifolia* var. pendula leaf. Pharmacognosy Journal 2010; 2:572-576.
20. Evans WC: Trease and Evans Pharmacognosy (Ed Saunders WB) Edinburgh, UK. Edition 15, 2002.
21. Kokate CK, Purohit AP and Gokhale SB: Pharmacognosy, Nirali Prakashan, Pune, India, Edition 34, 2006.

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