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Research Article

DEVELOPMENT OF PHARMACOGNOSTICAL PROFILE OF *CRYPTOLEPIS BUCHANANI* ROEM & SCHULT.

DEEPAK SHARMA[1*], ALEKH N. SAHU[1], MOHD. MUJEEB[2], ABINASH BHARTI[1], ANUBHAV SHARMA[1], RATI K. TRIPATHI[1], KAMRAN ASHRAF[2]

[1]Department of Pharmaceutics ITBHU Banaras Hindu University Varanasi, U.P. India, [2]Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Hamdard University, New Delhi, India.

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ABSTRACT

Cryptolepis buchanani Roem & Schult. (Asclepiadaceae), commonly known as jambupatra sariva and Karanta, is a climbing shrub. It is a well known ayurvedic plant found throughout india. The plant is used in Indian folkloric medicine (Ayurveda) for its antidiarrhoeal, antiulcerative, anti-inflammatory, blood purifier, anticough, antibacterial, demulcent, diaphoretic, diuretic properties and in treatment of rickets in children. The roots of this plant are well studied for its medicinal properties but the aerial parts (leaves & stem) are nearly virgin. The aim of present study is to gather information for the systematic identification and authentication of this particular species and pharmacognostic standardization of aerial parts (stem & leaves) of this plant as per WHO guidelines. The result obtained in the present investigation could be useful in the industry for the identification, authentication & quality of the commercial samples supplied by suppliers. The data of the present study will be used for making monograph on this plant for different pharmacopoeias & official books.

Keywords: *Cryptolepis buchanani* Roem. & Schult., Pharmacognostic evaluation, standardization, WHO guidelines.

INTRODUCTION

From the ancient time plants are recognized as a major resource for mankind. Plants are used as food material, in cosmetics and mostly in heath care system. From human civilization up to date plants are primary resources of medicines. Different civilizations used plants in various ways for medicinal purposes. Millions of the people in the third world use herbal medicines because they believe in them and regard them as their own system of medicine[1]. 80% of the world's population has faith in traditional medicine, parti cularly plant drug for their primary health care[2]. The use of herbal medicine, the dominant form of medical treatment in developing countries, has been increasing in developed countries in recent years[3]. According to World Health Organization (WHO) about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated from plants[4].In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patients. But the scenario has changed now; herbal medicines are being manufactured on the large scale in pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, availability of standards, proper standardization methodology of plant etc.

Therefore it has become extremely important to make an effort towards quality control and standardization of the plant material to be used as medicine. The quality control of phytopharmaceuticals may be defined as the status of a drug, which is determined either by identity, purity, content, and other chemical, physical or biological properties, or by the manufacturing process. The process of standardization can be achieved by stepwise pharmacognostic studies. WHO has given certain guidelines for the quality control and standardization of medicinal plants. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of drugs herbal medicines

Cryptolepis buchanani Roem. & Schult. (Asclepiadaceae), commonly known as jambupatra sariva in Sanskrit and as Dudhi or Karanta in Hindi, it is a large evergreen laticiferous, woody climbing, perennial shrub common especially in deciduous forest of sub-himalayan tracts, Bihar, Orisa, East Uttar Pradesh in Varanasi region. The glycosides like sarverogenin and isosarverogenin[5], cardenolides like Cryptosin[6], Buchanin[7], a novel pyridine alkaloid Buchananine[8] and a Serine Protease Cryptolepain[9] are reported in the leaves and roots of this plant. The plant is used in Indian folkloric medicine (Ayurveda) for its anti-diarrhoeal, anti-ulcerative, anti-inflammatory, blood purifier, anti-cough, antibacterial properties[10]. It is widely used as demulcent, diaphoretic, diuretic, cure for paralysis[11], rickets in children[12]. The roots of this plant are well studied for its medicinal properties but the aerial parts (leaves & stem) are nearly virgin. The aim of present study is to gather information for the systematic identification and authentication of this particular species and pharmacognostic standardization of aerial parts (stem & leaves) of this plant as per WHO guidelines. No scientific specifications regarding its pharmacognostical profile has been yet published by any author & agencies.

MATERIALS AND METHODS

Collection of plant material and authentication

The fresh stem and leaves of *Cryptolepis buchanani* plant were collected from the Ayurvedic Garden of Department of Dravyaguna Banaras Hindu University, Varanasi in the month of July and authenticated by Taxonomist N.K. Dubey Department of Botany, Faculty of science, Banaras Hindu University, Varanasi. A voucher specimen No. ITBHU/DP-II is retained in the Department of Pharmaceutics, Institute of Technology Banaras Hindu University, Varanasi.

Drying and size reduction of plant

The collected plant material was dried in shade for about six weeks. The material was then crushed to powder and passed through the mesh 22 and stored in air tight container for further analysis.

Macroscopic and microscopic analysis

To categorize Medicinal plant materials sensory, macroscopic and microscopic characteristics are considered. To determine the identity and the purity of plant materials Examination of macroscopic and microscopic characteristics is the first step.[13] Morphological studies of leaf (such as shape, apex, surface, base, margin, venation, taste and odor) and stem (such as color, odor and taste) were carried out. Free hand transverse section of fresh stem, leaf and petiole were taken, cleaned in chloral hydrate solution with warming, stained with phloroglucinol and concentrated hydrochloric acid. They were mounted on slide in glycerin and studied under microscope. Microphotographs of sections were made using Soni S-708 digital camera.[14,15,16]

Physicochemical studies

Physicochemical studies like ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in WHO guidelines on quality control methods for medicinal plants materials.

Preliminary phytochemical screening

The dried plant material (stem and leaves) were extracted with water, methanol, water & methanol and chloroform. The behavior of powder with various chemical reagent and preliminary chemical tests for various extracts were also carried out.[17]

RESULTS & DISCUSSION

Macroscopic characters

The leaves are green in color, 3-6 cm. in length elliptic oblong, smooth in appearance shining above. The leaves are simple, dorsi-ventral, petiolate, ex-stipulate. The apex of leaves is mucronate and margin is entire. The upper surface is smooth and shining green and lower surface is rough. The venation is reticulate, phyllotaxy opposite and decussate. *Cryptolepis buchanani* is a woody twiner having a weak stem. The stem is lateciferous in nature. Numerous nodes and internodes were present at a distance of about 10-12 cm.

Microscopic features

leaf

The leaf of *Cryptolepsis buchnani* is dorsi-ventral. At the upper and lower surface epidermis is present. There are no intercellular spaces in epidermal cells. Mesophyll tissue, i.e. spongy and palisade cells are present between upper and lower epidermis. There are no intercellular spaces in palisade cells while spongy cells are present with big intercellular spaces. In the midrib region under the upper epidermis collenchymatous and numerous parenchymatous cells are present. In the central portion vascular bundles, i.e. phloem and xylem are present. Phloem is present on both inner and outer sides of xylem (fig.2).

stem

The epidermis is visible in the circular transverse section of the stem. Under the epidermis 5-6 layered parynchymatous cortex is present. Sclerenchyma patches are also visible in this section. Vascular tissues, i.e. xylem and phloem are seen in the section. Along the periphery of the pith, medullary phloem is present. Well developed multiseriate medullary rays are clearly visible. Paryncymatous Pith is present in the centre (fig.1, 1A, 1B).

petiole

A circular section of petiole is shown in the diagram. The outermost layer of petiole is epidermis. Under the epidermis collenchymas cells and a dense mass of parenchyma cells is present. These parenchyma cells are thick walled and have no intercellular spaces. Vascular tissues are present in half circular shape in the middle region. Phloem is clearly visible on both sides of xylem (fig.3).

Surface tissue of leaf

In the surface view of leaf the stomata are clearly visible. Stomata are surrounded with two parallel subsidiary cells hence it is easy to infer that the stomata are paracytic. The guard cells surrounding pore are clearly visible (fig. 4).

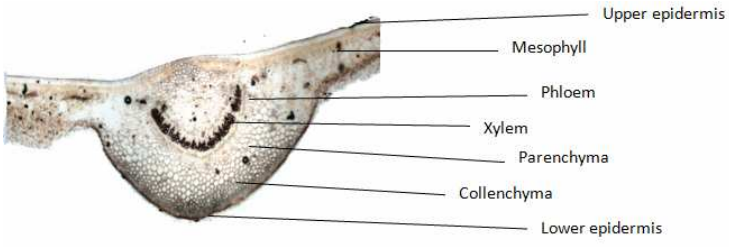

Fig. 2: T.S. of Leaf

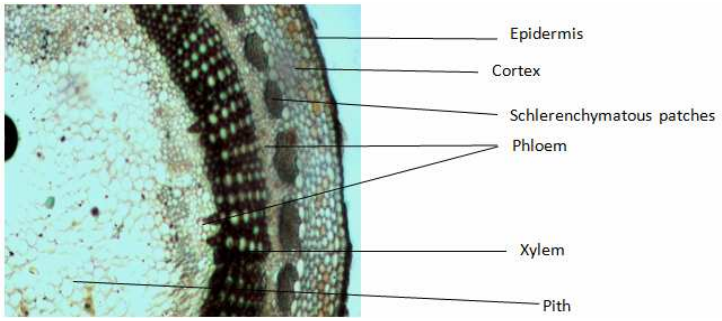

Fig. 1: T.S. of Stem

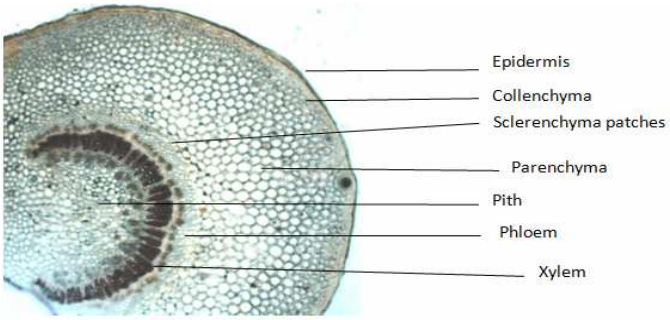

Fig. 3: T.S. of Petiole

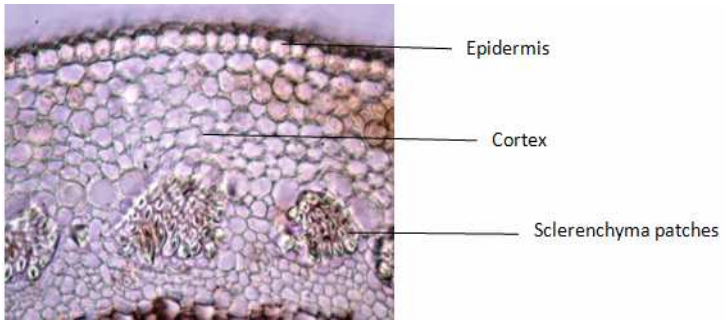

Fig.1A: 40x view of Stem

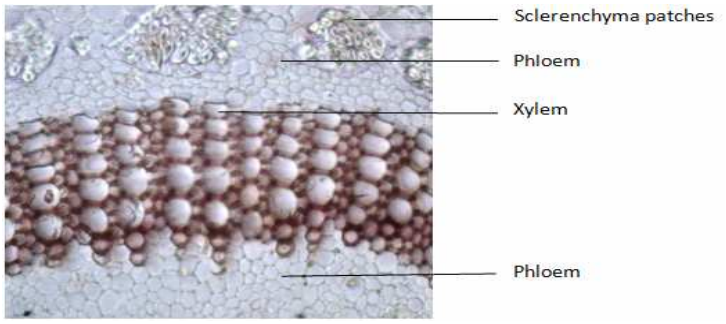

Fig. 1B 40x view of Stem

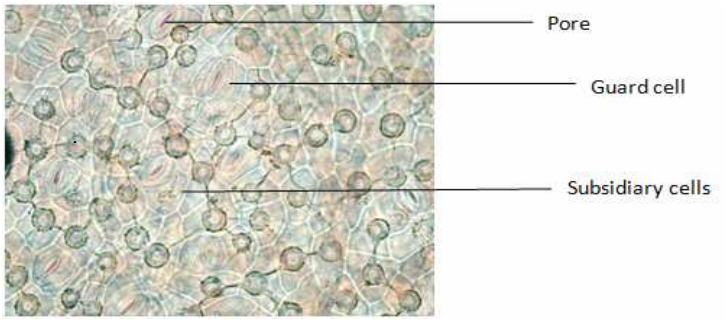

Fig. 4: Stomata

Physicochemical parameters

Physicochemical parameters like foreign matter, loss on drylng, total ash, water soluble ash, acid insoluble ash foaming index, swelling index and volatile oil content are summarized in table 1.

Table 1: Physicochemical parameters

Parameter	Mean value
Foreign matter	Negligible
Loss on drying	10.08%
Total ash	7.32%
Water soluble ash	2.36%
Acid insoluble ash	0.85%
Foaming index	Less than 100
Swelling index	No swelling
Volatile oils	Absent

Extractive values

Extractive values of powder of aerial parts in various solvents are summarized in table 2.

Preliminary phytochemical screening

Various tests were applied on the methanolic extract of the plant material using specific reagents. The results obtained are summarized in the Table 3. The tests showed the presence of alkaloids, carbohydrates, glycosides, flavanoids and tannins.

Table 2: Extractive values of aerial parts of *Cryptolepis buchanani*

Solvent	Extractive value
Acetone	3.2%
Petroleum ether	3.6%
Methanol	8.4%%
Water & Methanol (50:50)	13.6%
Ethyl acetate	2.8%
Chloroform	5.6%

Table 3: Phytochemical screening of methanolic extract of aerial Parts of *Cryptolepis buchanani*

Constituents	Results
Alkaloid	+
Glycoside	+
Tannins	+
Sterols	+
Flavanoids	+
Saponins	+
Carbohydrates	+
Volatile oil	+
Reducing sugars	+

(-): Absent, (+): Present

Fluorescence powder drug analysis

The powdered plant material was treated with different reagents and change in color was studied in both UV and day light. The results obtained are summarized in table 4.

Table 4: Fluorescence powder drug analysis of aerial parts of *Cryptolepis buchanani*

Reagent + Powder	Normal light	Short UV	Long UV
Dry powder	Olive Drab	Green yellow	Yellow green
Powder + 5%NaOH	Dark Goldenrod	Light green	Green yellow
Powder + 5%KOH	Dark Goldenrod	Gray	Yellow green
Powder + Conc. H_2So_4	Black	Gray	Green yellow
Powder + Dil. Ammonia	Dark Goldenrod	Gray	Yellow green
Powder + Conc. HCl	Dark Goldenrod	Gray	Green yellow
Powder + Conc HNo_3	Orange	Gray	Dark gold
Powder + Dil. HCl	Light yellow	Gray	Light yellow
Powder + Dil. HNo_3	Khaki	Dark gray	Yellow green
Powder + Alc.KOH	Olive	Gray	Orange

Pesticide residue determination

The Presence of chlorinated and phosphated pesticides in the powdered plant material was determined as per WHO guidelines and the results obtained are summarized in table 5.

Table 5: Concentration of phosphate & chloride pesticide present in the powdered material of aerial parts of *Cryptolepis buchanani*

Pestisides	TS1	TS2	TS3
Phospahte	NA	NA	0.0278mg/kg
Chloride	0.08mg/kg	0.068mg/kg	-

TS1: First elute containing phosphate pesticides.

TS2: Second elute containing phosphate pesticides.

TS3: Third elute containing phosphate pesticides.

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