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Research Article

Pharmacognostical and Physicochemical Evaluation of *Coleus spicatus* Benth.

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ABSTRACT
The present study presents a detailed pharmacognostical and physiochemical studies of the root, stem and leaf of the crude drug, *Coleus spicatus* Benth (Labiatae) (*C. spicatus)*. This investigation includes morphological and microscopical evaluation, determination of physicochemical constants, histological study and the preliminary phytochemical screening of different extracts of *C. spicatus*. The parameters presented in this paper may be proposed to establish the authenticity of root, stem and leaf of *C. Spicatus* and may possibly help to differentiate the drug from its other species.

Key Words: *Coleus spicatus*, Labiatae, Pharmacognostical, Physicochemical

INTRODUCTION

Herbal medicine has been practiced worldwide and is now recognized by WHO as an essential building block for primary healthcare[1]. Anatomical characters of powdered drugs have become an important tool for identifying authentic drugs since adulteration of both drugs and food articles has become very common. Quality control of a crude drug and its pharmaceuticals can be attempted by different methods of evaluation depending upon the morphological and microscopical studies of the crude drugs and their physical, chemical and biological behaviour[2].

Coleus spicatus Benth., (synonym: *Plectranthus caninus*) belongs to the Labiatae family and is an important plant in the Indian system of medicine. It is a perennial fleshy herb, which grows in arid places, on rocky ground among bushes[3]. The plant is widely found in Salem and Coimbatore districts of Tamil Nadu, India. It grows up to 50 cm in height with branchlets hispid in nature[4]. Traditionally the plant is used as a stimulant, in the treatment of cough[5]and in the treatment of teeth and gum disorders[6]. The aerial parts of the plant have also been reported to have diuretic, cytotoxic and anti-tumor activities. Diterpenes such as coleon S and coleon T, triterpenes such as α – amyrin, tormentic acid and flavones like kumatakinin, 3,7–dimethyl quercetin and sitosterol[7]are present in the plant.

In spite of the numerous medicinal uses attributed to this plant, there is no pharmacognostical report on the leaf, stem or root of the plant till date. There are also no data available on the anatomical and other physicochemical standards required for the quality control of its crude drug. Hence, the present investigation was undertaken to determine the pharmacognostical standards for authenticating the plant material of *C. spicatus*.

MATERIALS AND METHODS

Collection of plant material: The whole plant of *C. spicatus* was collected from various places of Salem district in Tamil Nadu, India during the month of August 2010. The plant was authenticated by Mr. Chelladurai, Research Officer-Botany, C.C.R.A.S. Government of India, Thirunelveli, Tamil Nadu. A voucher specimen (HS034) has been deposited in the herbarium of the Department of Pharmacognosy, Ezhuthachan College of Pharmaceutical Sciences, Marayamuttom, Thiruvananthapuram, Kerala, India.

Macroscopic and microscopic analysis

The macroscopy and microscopy of the plant were studied according to the method developed by Esau[8]. Cross sections were prepared and stained for the microscopical study as per the standard procedure described[9]. The micro-powder analysis was done according to the established method[10,11].

Physicochemical analysis: Physicochemical values such as the percentage of ash values and extractive values were performed according to the official methods prescribed in Indian pharmacopoeia[12] and the WHO guidelines on quality control methods for medicinal plant materials[13].

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TABLE 1 Preliminary phytochemical screening of the leaf powder of *C. spicatus*

Tests	Pet. ether	Hexane	Chloroform	Acetone	Ethanol	Water
Carbohydrate	-	-	-	-	+	+
Phytosterols	+	+	-	-	+	-
Fixed oils & fat	+	+	-	-	-	-
Volatile oil	-	-	-	-	+	+
Saponins	-	-	-	+	+	+
Phenolic compounds& tannins	+	-	-	-	+	+
Flavonoids	-	-	-	+	+	+

+ denotes the presence of the respective group of compounds

TABLE 2 Preliminary phytochemical screening of the stem powder of *C. spicatus*

Tests	Pet. ether	Hexane	Chloroform	Acetone	Ethanol	Water
Carbohydrate	-	-	-	-	+	+
Phytosterols	+	+	-	-	+	+
Fixed oils & fat	-	-	-	-	-	-
Volatile oil	-	-	-	-	-	-
Saponins	+	-	-	+	+	+
Phenolic compounds& tannins	-	-	-	+	+	+
Flavonoids	+	+	-	-	+	+

+ denotes the presence of the respective group of compounds

Fluorescence analysis was also carried out standard methods[14, 15].

Preliminary phytochemical screening: Preliminary phytochemical analysis was carried out by using standard procedures described by Harborne[16].

RESULTS AND DISCUSSION

Macroscopic characteristics : The herb grows up to 50 cm in height. The branchlets are hispid by nature and the leaves are ovate to sub-orbicular in shape with 1-3 x 1.5-3cm in dimensions. The leaves are succulent, pubescent with a cordate-truncate base and a crenate margin. The apex seems to be sub-acute to obtuse. Petioles are up to 0.7cm in length and the Verticils are closely packed. Floral bracts are closely imbricate, ovate and acute. Calyx resemble like tube villows within and tomentus. There are 5 lobes which are unequal in dimension. The upper lip is ovate, flat and 2.5mm, whereas the lower lip is obtuse and 4-toothed. Mid lobes appear to be shorter and are 3mm in length having, acute structure. The corolla is bluish with 5x 2mm across and is a tube which is 5mm in length. There are 5 lobes of which the upper lip is 3mm and the lower lip is 6mm in dimensions. The outer appearance is glabrous and obtuse. There are 4stamens and filaments which are 5.5mm in length, and are connate below. The staminal sheath is 8mm in length. The anthers are 0.8mm in length and the ovary is 0.5mm in length. The style is of around 1.5cm in length. (Fig 1)

Microscopic characteristics: Leaf: The leaf is thick, fleshy and soft. It is isobilateral and apparently less differentiated into midrib from lamina. The lamina of the leaf shows three distinct regions viz., upper epidermis, lower epidermis and mesophyll. The lamina is 1.2mm thick and the epidermal layers are thin comprising small thin walled circular cells covered by a distinct cuticle. The epidermal cells are 30µm thick. Abundant covering and glandular trichomes emerged from the both epidermal layer. The trichomes are multi- cellular

uniseriate and unbranched (2~7 cells) mostly straight and rarely warty with acute tips. Glandular trichomes are small sub sessile as well as wide trichomes, both are peltate type. Stomatas are also seen occasionally in the upper epidermis (Fig 2).

LM- Leaf margin, AdE- Adaxial Epidermis, VS- vascular strands, MT: Mesophyll consists of several (10~12) vertical rows of compact cells. The cells in the upper region are palisade parenchyma is made up of slightly vertically oblong. Spongy parenchyma is shorter towards lower side. The cells are thin walled and their walls are slightly wavy. The vascular strands are found in between the palisade and spongy mesophyll. The lower epidermis is identical to upper epidermis stomata and numerous trichomes. The transverse section of the petiole of *C. spicatus* is horizontally elliptical measuring 2.1mm wide. It has epidermal layer of small squarish cells. The inner epidermis found 2~3 layers of collenchymatous and remaining ground tissue is thin walled parenchyma. Four vascular strands are placed, medium strands are large and lateral strands are small. Vascular strands having thick walled xylem elements and few discrete rests of phloem elements (Fig 3).

Tr-trichomes, AdE-Adaxial Epidermis, PM- palisade mesophyll, VS- vascular strands, Ph-phloem, X- xylem, SM-spongy mesophyll, AbE- Abaxial epidermis

Stem: The transverse section of stem is circular in shape. The margin is even surface. Periderm, cortex, vascular cylinder and pith are seen from periphery to the centre. Periderm is superficial and uniform in thickness all throughout the stem. This comprises about four tabular cells of phloem and four layers of phelloderm. The phloem cells are thin walled and tabular in shape and suberized. The covering and glandular trichomes found in

TABLE 3 Preliminary phytochemical screening of the root powder of *C. spicatus*

Tests	Pet. ether	Hexane	Chloroform	Acetone	Ethanol	Water
Carbohydrate	-	-	-	-	+	+
Phytosterols	+	-	-	-	-	+
Fixed oils & fat	-	-	-	-	-	-
Volatile oil	-	-	-	-	-	+
Saponins	-	-	-	+	+	+
Phenolic compounds & tannins	-	-	-	+	+	+
Flavonoids	-	-	-	-	+	+

+ denotes the presence of the respective group of compounds

Figure 1 Macroscopic characteristics of C. spicatus

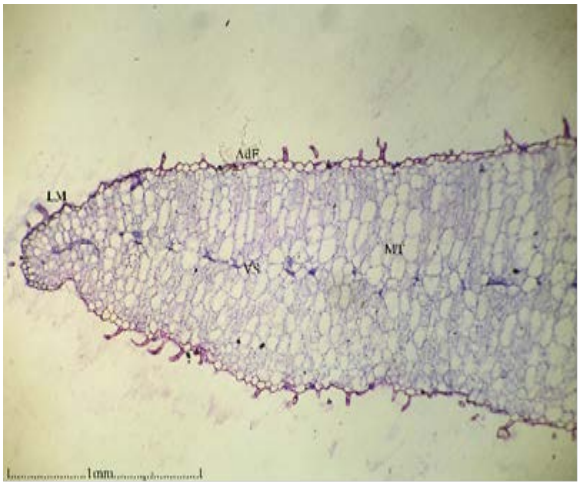

Figure 2 Transverse section of Leaf margin of C. spicatus

TABLE 4 Ash values of the leaf, stem and root powder of *C. spicatus*

Parameters	Value % w/w		
	Leaf powder	Stem powder	Root powder
Total ash	15.43	11.79	14.73
Acid insoluble ash	5.94	1.86	3.15
Water soluble ash	4.30	5.80	5.75
Sulphated ash	18.60	14.14	32.90

TABLE 5 Extractive values of the leaf, stem and root powder of *C. spicatus*

Parameters	Value % w/w		
	Leaf powder	Stem powder	Root powder
Water soluble extractive	5.74	4.61	3.70
Ethanol soluble extractive	5.24	4.33	3.04
Ether soluble extractive	1.12	0.78	0.56

periderm. In cortex, the outer zone, 3~5 layers of cortical parenchyma appear like collenchyma cells contain no inclusions and the inner zone of 5~7 layers of circular less compacts parenchyma. The vascular cylinder is four angled with thicker xylem tissue along the four corners and the zones in between the thick corners being thin. The xylem fibers that are in the outer zone of the cylinder are thick walled lignified and radially oblong. Phloem elements are more in frequency in the thicker zones of vascular cylinder than in narrow zones. In both zones, the sieve elements occur in small groups 2~6 elements. Pith occupies in major parts of transverse section and is made up of thin walled, compact, circular parenchymatous cells. Starch grains are fairly abundant in pith parenchyma. They are circular and have central hilum (Fig 4).

Col- collenchyma, Pa- parenchyma, SG, Pe-periderm, Co-cortex, Ph- phloem, XR- xylem ray, XF- xylem fibers, Ve- vessel, Pi- pith, SX- secondary xylem

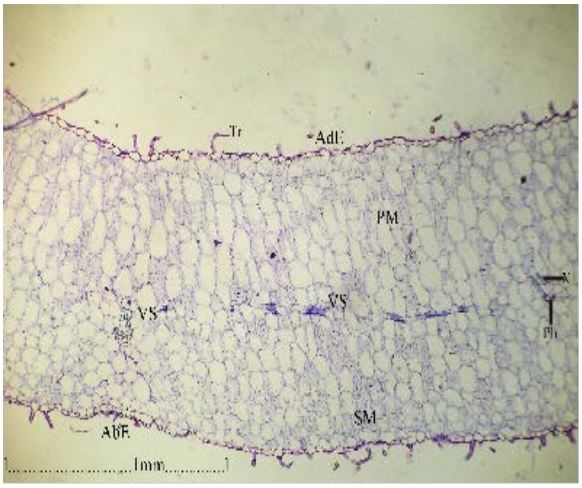

Figure 3 Transverse section of middle lamina of C. spicatus

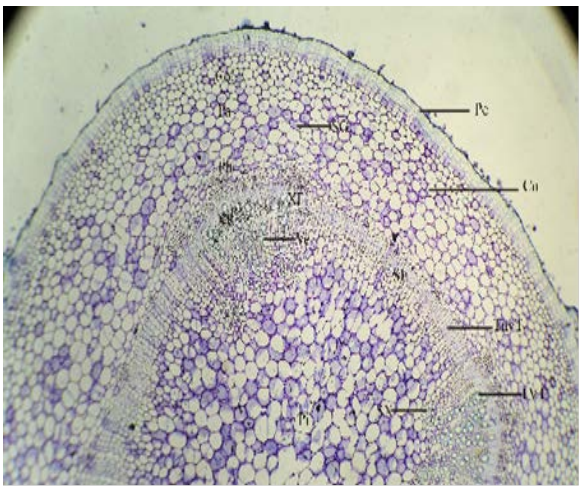

Figure 4 Transverse section of stem of C. spicatus

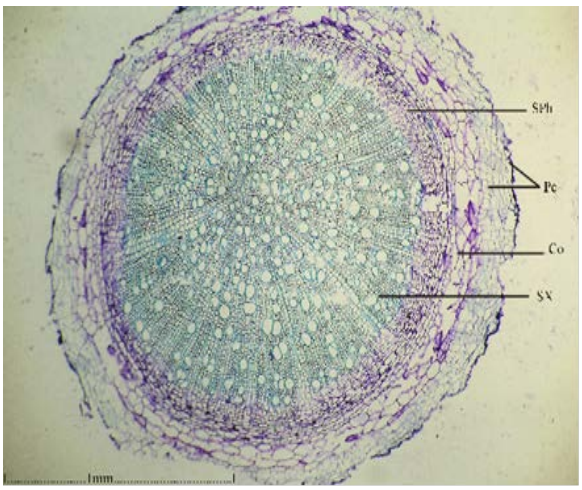

Figure 5 Transverse section of thick root of C. spicatus

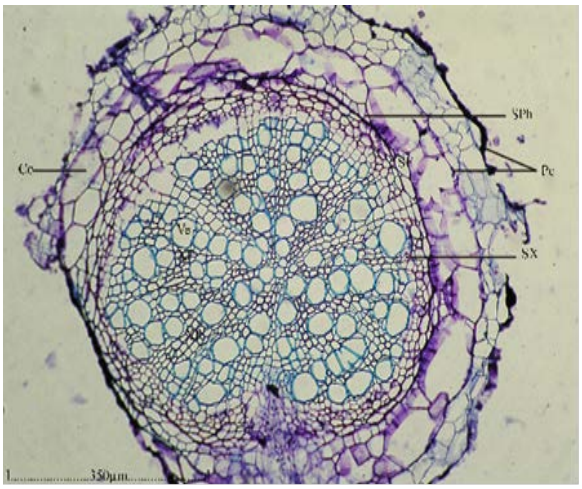

Figure 6 Transverse section of thin root of C. spicatus

Root: The transverse section of the root is more or less circular. The margin is permanently wavy. Cork, cork cambium and cortex are the tissues present from the periphery to the centre. The cork is thin, brown and made up of 2 layers of irregular parenchymatous cells. The cortex consists of 3~4 layers of wide, rectangular, compact parenchymatous cells. The secondary xylem occupied about three fourth of the transverse section and transversed regularly by rows of medullary rays. The xylem fibers are fairly wide, thick walled, lignified

TABLE 6 Fluorescence analysis of the leaf, stem and root powder of *C. spicatus*

Treatment	Leaf powder		Stem powder		Root powder	
	Day Light	UV light (254nm)	Day Light	UV light (254nm)	Day Light	UV light (254nm)
Powder as such	pale green	brownish green	pale yellow	brown	yellowish white	brown
Powder +1N NaOH	green	brown	yellowish brown	violet	yellow	pale violet
Powder +1N NaOH (alcoholic)	light green	violet	yellow	pale violet	brown	brownish violet
Powder +1N HCl	yellow	violet	yellowish brown	violet	yellowish brown	brown
Powder + 50% H_2SO_4	green	brown	yellowish brown	brownish violet	brown	brownish violet

occurring compact radial rows. The fibers are either single or in pairs and shows scalariform. The xylem fibers appear as rounded or polygonal structure with thick lignified wall. Secondary phloem is fairly wide and encircles the xylem cylinder. Phloem elements are thick radial segments (Fig 5 and Fig 6).

Sph- secondary phloem, Pe- periderm, Co- cortex, SX- secondary xylem

Co-cortex, SPh- secondary phloem, Pe-periderm, SX- secondary xylem, Ve-vessel

Powder characteristics

Leaf: The organoleptic evaluation of leaf powder revealed the following characteristics. The leaf powder is

coarse and bulky and pale green in color, with characteristic odor and bitter taste. Both covering and glandular trichomes are seen. The covering trichomes are multicellular 2~7 celled unbranched, lignified with short tips. The glandular trichomes are numerous of both stalked and sessile types with multicellular heads and unicellular stalks. Diacytic and cyclocytic (3 or 4 subsidiary cell) stomatas are seen.

Stem: The powder is pale yellow in color with characteristic odor and mucilaginous taste. Narrow and rectangular, compact layer of ray parenchyma is common in stem. Scalariform xylem fibers are thin or thick walled and narrow or wider. Some of the fibers and parenchyma are septate.

Root: The root powder is pale yellowish in color with characteristic odor and mucilaginous taste. The cork cells are suberized and are narrow in appearance. The cortex is of 3~4 layers with wide, rectangular parenchyma cells. The secondary phloem is fairly wide.

Preliminary phytochemical screening: Preliminary phytochemical screening on the leaf, stem and root powder of *C. spicatus* revealed that the presence of carbohydrate, phytosterols, fixed oils, volatile oils, saponins, phenolic compounds and tannins and flavonoids (Table 1 to 3).

Physicochemical constants: Ash values of drug given an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The Sulphated ash value of the leaf, stem and root powder of *C. spicatus* showed higher content followed by total ash (Table 4).

The extractive values are preliminary useful for the determination of exhausted or adulterated drug. The water soluble extractive was high in both leaf and stem and root powder of *C. spicatus* (Table 5).

Fluorescence Analysis: Fluorescence analysis of crude drug powder was carried out using various reagents. The results are presented in Table 6.

CONCLUSION

The leaves, stem and root of *C. spicatus* were ethno-medically used by local people to treat various ailments without standardization. The standardization of a crude drug is an essential part to establish the correct identity and authenticity of this medicinally useful plant. The results of these investigations could inform about the proper identification, collection and investigation of the plant. The pharmacognostic features investigated on *C. spicatus* in the present study may serve as tool for validation of the raw material and its standardization.

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