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Pharmacognostic Evaluation of Aerial Parts of *Cleome rutidosperma*

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ABSTRACT

Different parts of *Cleome rutidosperma* are used in many ways, as an antiplasmodial, analgesic, locomotor, antimicrobial, diuretic, or laxative. In the present investigation, a detailed pharmacognostic study of *C. rutidosperma* leaves was carried out to establish standards that could be useful in future experimental studies. The study includes macroscopy, microscopy, powder microscopy, physical analysis and physicochemical evaluation.

Keywords: macroscopy, microscopy, extract

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started giving emphasis on the ancient healing systems like Ayurveda, Siddha and Unnani, this is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all 'plant parts to be potential sources of medicinal substances (Khandelwal *et al.* 1998). However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is a need for documentation of research work carried out on traditional medicines (Dahanukar *et al.* 2004). With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The process of standardization can be achieved by stepwise pharmacognostic studies (Ozarkar *et al.* 2011). These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Newbould *et al.* 1963). *Cleome rutidosperma* (Capparidaceae) is a low growing herb, up to 70 cm tall, found in waste herb, grounds and grassy places with trifoliate leaves and small, violet-blue flowers, which turn pink as to West Africa, although it has become naturalized in various parts of tropical America as well as Southeast Asia (Widespread *et al.* 1972; Waterhouse *et al.* 1998). According to traditional use, the different parts of the plants of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, vesicant, rubifacient and carminative (Kirtikar *et al.* 1991). The antiplasmodial, analgesic, locomotor antimicrobial, diuretic, laxative (Bose *et al.* 2006) activities of *Cleome rutidosperma* were reported earlier. *C. rutidosperma* is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, pain and skin disease. The popular use of the roots, however, refers mainly to its analgesic, anti-inflammatory and anthelmintic activity (Saha *et al.* 1961; Bose *et al.* 2007). Since the plant, *C. rutidosperma*, is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug. As no work has been reported on the diagnostic features of *C. rutidosperma*, the present study reports the macroscopic and microscopic and some other pharmacognostic characters of the aerial parts of *C. rutidosperma*, which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Collection of plant part

The plant material (whole plant) was collected from North 24-Pargana district of West Bengal, India during September 2004 and was authenticated at Botanical Survey of India, Shibpur, Howrah, West Bengal, India. A voucher specimen (C.R.-1) has been kept in our research laboratory for future reference. The fresh aerial parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

Macroscopy

The macroscopic characters such as size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture were noted for the aerial parts of the fresh plant (Wallis *et al.* 1985; Trease *et al.* 2002).

Microscopy

Thinnest possible transverse section of plant parts (leaves and stem) was cut, colouring material was cleared with chloral hydrate, mounted with glycerin and observed under a compound microscope (Olympus, Hicksville, New York).

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Research Note

Powder microscopy

Examination of the powder (aerial parts) were carried out using standard techniques for presence of various cells, starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin (Abere *et al.* 2007).

Quantitative microscopy of leaf constants

The vital quantitative microscopic leaf constants like vein islet, vein termination number, palisade ratio and stomata index were carried out according to the method (Iyenger *et al.* 1987).

Histochemical color reactions

Histochemical color reactions were carried out on the transverse sections of leaf and stem by the reported methods (Wallis *et al.* 1985; Ansari *et al.* 2006).

Behavior of powder with chemical reagents

Behavior of powder (aerial parts) with different chemical reagents was studied to detect the presence of phytoconstituents with color changes under daylight (Venkatesh *et al.* 2008).

Fluorescence characteristic of the powder

Fluorescence characteristic of the powder with different reagents were studied by standard methods (Pratt *et al.* 1949).

Ash values

Total ash, acid-insoluble ash, water-soluble ash, and sulphated ash values of the powder (aerial parts) were done (Kokashi *et al.* 1958) as described below.

Extractive values

For preparation of ethanolic extract, the powdered plant material was extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black coloured sticky residue. A portion of dried ethanolic extract was suspended in water and fractionated successively with petroleum ether (40-60°C), diethyl ether, ethyl acetate and *n*-butanol. For preparation of water extract, the powdered plan material was boiled with distilled water for 10 min with frequent shaking. It was then filtered rapidly and the process residual powder was again boiled twice again using the same procedure. The total filtrated accumulated was accumulated. All the extracts were evaporated and dried at 100°C to constant weight. Percentages of the extractive values were calculated with reference to air-dried powder. Color and consistency of extracts were also observed (Dymock *et al.* 1976).

Colour and fluorescence analysis of extracts

All the extracts were examined in daylight, short and long UV to detect the fluorescent compounds by the reported method (Trease *et al.* 2002).

RESULTS AND DISCUSSION

Macroscopy

The plant is a procumbent, branching 30-100 cm tall perennial herb. Stems are twisted, angular, and sparely pubescent. Leaves are 2-3 cm long, trifoliolate, petiolate, green when young, purplish green when mature. Leaflets are 3 in number, glabrous, chartaceous, rhomboidal-elliptic. Petiolules are 1 cm long with both ends acute, margin is minutely serrulate and revolute, veins are prominently depressed above, elevated below. Terminal leaflets are larger than lateral, 2 cm long, and 1.1 cm wide with 7-10 pairs of lateral veins. Lateral leaflets are 1.5 cm long, 0.8 cm wide, with 8-9 pairs of lateral veins. Flowers are axillary and solitary. Pedicels are slender, 1.5-1.7 cm long. Sepals are pinkish from outside. Petals are 4 in number, free, linear-lanceolate, membranaceous with 1 midrib, blue coloured with caudate apex, entire margin. Stamens are 6 in number with elongated anthers, sagittate at base. Pistil is glandular hairy. Ovary is subsessile. Style is elongated with disc beaked at apex. Carpels are 2 in number, 1 celled. Placentation is parietal. Fruit are linear capsules with apical beaked disk, 2.5 cm long, 1 mm thick. Peduncles and stock are 3 cm and 1-4 mm long, respectively. Seeds are 4-25 per capsule, reddish brown to black with white funicular aril, 1-1.5 mm, slender, transversely ridged. The morphology is done for first time so the botanical information is not copied but only observed.

Microscopy

T.S. Transverse section of the leaf have the following characteristics:

1. Epidermis: Single layered tightly packed, rectangular cells with straight or slightly wavy walls and anomocytic stomata. Lower epidermis consisted of single layer, tightly packed, rectangular cells with wavy and with few anomocytic stomata. Numerous unicellular covering trichomes were present on both upper and lower epidermis. The upper and lower epidermis was covered by thin layer of cuticle.
2. Mesophyll: It consisted of two layers of elongated upper palisade parenchyma arranged just below epidermis without any intercellular space. Just below the palisade parenchyma two to three layers of parenchyma were present. Spongy parenchyma contained isodiametric cells which had intercellular space in between them.
3. Midrib: It showed thick walled two layers of collenchymatous cells just above the lower epidermis. The vascular bundle consisted of xylem vessels and phloem fibres. The phloem fibres surrounded the xylem vessels or centroxylic condition.

T.S. of the stem consisted of following three regions:

1. Epidermis: It consisted of a single layer of covering cells which were closely packed. The walls were thickened and covered with thin layer of cuticle. Unicellular hair like or trichomes appeared in the epidermis.
2. Cortex: This region consisted of collenchymas, parenchyma and endodermis.
3. Vascular cylinder/stele: It consists of pericycle, vascular bundle and pith.

Various cells observed under microscope like epidermal cells, trichomes, stomata, palisade cells, fibres.

Other parameters

Results of evaluation of other parameters, i.e., quantitative microscopy of leaf constants (**Table 1**), histochemical color reactions (**Tables 2, 3**), behavior of powder with chemical reagents (**Table 4**), fluorescence characteristic of the powder (**Table 5**), ash values (**Table 6**), extractive values (**Table 7**), colour and fluorescence analysis of extracts (**Table 8**) are also described in different tables.

CONCLUSION

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The vein islet, and vein termination numbers and the other parameters determined in the quantitative microscopy, are relatively constant for plants and can be used to differentiate closely related species. The physical constant evalua-

Table 1 Quantitative microscopy of *C. rutidosperma* leaf.

Leaf constants	Value range	Mean*
Palisade ratio	6 – 10	8.0
Stomata index	15 – 18	16.66
Vein-islet number	6 – 9	7.2
Vein termination number	4 – 6	5.2

*Mean value of five counts

Table 2 Histochemical colour reactions of *C. rutidosperma* leaf.

Reagent	Colour	Inference
Weak iodine solution	No dark bluish purple colour	Starch absent
A drop of H_2SO_4	Yellowish red colour	Saponins present
Millons reagent	No red colour	Proteins absent
Phloroglucinol + HCl	Reddish brown colour	Lignin present
Dragendorff's reagent	No orange colour	Alkaloids absent
$SbCl_3$	Reddish pink	Steroids/Triterpenoids present
$FeCl_3 + Na_2CO_3$	Bluish colour	Tannins present
5% Aq. KOH	Deep yellow colour	Flavonoids present

Table 3 Histochemical colour reactions of *C. rutidosperma* stem.

Reagent	Colour	Inference
Weak I_2 solution	No dark bluish purple colour	Starch absent
A drop of H_2SO_4	Yellowish red colour	Saponins present
Millon's reagent	No red colour	Proteins absent
Phloroglucinol + HCl	Reddish brown colour	Lignin present
Dragendorff's reagent	Very faint orange colour	Very less amount of alkaloids present
$SbCl_3$	Reddish pink	Steroids/Triterpenoids present
$FeCl_3 + Na_2CO_3$	No Blue colour	Tannins absent
5% Aq. KOH	No deep yellow colour	Flavonoids absent

Table 4 Behavior of the powder of the aerial parts of *C. rutidosperma* different chemical reagents.

Treatment	Colour/precipitate	Constituent
Powder as such.	Green	–
Powder + Conc. H_2SO_4	Reddish brown	Steroids/Triterpenes present
Powder + Aq. $FeCl_3$	Greenish black	Tannins/Flavonoids present
Powder + I_2 solution	No blue colour	Starch absent
Powder + Picric acid	No precipitation	Alkaloids absent
Powder + 5% Aq. KOH	No change	Anthraquinone glycosides absent
Powder + Aq. $AgNO_3$	No precipitation	Proteins absent

Table 5 Fluorescence characteristic of the powder of the aerial parts of *C. rutidosperma*.

Treatment	Colour in ordinary light	Colour under UV light	
		Short UV (254 nm)	Long UV (365 nm)
Powder as such	Green	Green	Dark green
Powder + 1N NaOH in methanol	Green	Green	Bluish green
Powder + 1N NaOH in water	Greenish yellow	Green	Greenish black
Powder + 1N HCl	Blackish brown	Greenish black	Black
Powder + 50% HNO_3	Yellow	Greenish yellow	Black
Powder + 50% H_2SO_4	Reddish brown	Greenish black	Blackish brown

Table 6 Ash values *C. rutidosperma* aerial parts.

Type of the ash value	% w/w
Total ash	5.74
Acid insoluble ash	2.85
Water soluble ash	3.10
Sulphated ash	6.81

Table 7 Extractive values of *C. rutidosperma* aerial parts.

Mother extract	Ethanolic extract fraction	% w /w
Water		19.61
90% Ethanol		12.15
	Petroleum ether 40-60°	3.24
	Diethyl ether	1.09
	Ethyl acetate	0.78
	n-Butanol	1.98

Table 8 Colour and fluorescence character of ethanolic extracts and its fractions *C. rutidosperma* aerial parts.

Parameter	Ethanolic extract	Ethanolic extract fractions			
		Petroleum ether	Diethyl ether	Ethyl acetate	*n*-Butanol
Color (day light)	Brownish green	Yellow	Green	Pale yellow	Yellowish red
Short UV (254 nm)	Green	Pale green	Pale green	Pale yellow	Pale green
Long UV (365 nm)	Red rose (F)	Deep orange (F)	Red (F)	Red rose (F)	White (F)

* F indicates fluorescence.

tion of the drugs is an important parameter in detecting adulteration or improper handling of drugs. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Thus the present study on pharmacognostical characters of *C. rutidosperma* Linn. DC aerial parts will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species of *Cleome*.

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