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PHARMACOGNOSTIC STUDIES ON LEAF AND STEM OF *CRESSA CRETICA* LINN.

P. Sunita*[1], S. Jha [2] and S. P. Pattanayak [2]

Government Pharmacy Institute, Govt. of Jharkhand [1], Ranchi, Jharkhand, India
Department of Pharmaceutical Sciences, Birla Institute of Technology (BIT) [2], Mesra, Ranchi, Jhanrkhand, India

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Correspondence to Author:

P. Sunita

Government Pharmacy Institute, Govt. of Jharkhand, Ranchi, Jharkhand, India

ABSTRACT

Cressa cretica is halophytic plant belongs to family Convolvulaceae, growing extensively all over India. The entire plant is medicinally important and used extensively in traditional system of medicine. The present work attempted to summarize the macroscopic characters of the plant *Cressa cretica*. The microscopic characters, physical constant values, extractive values, fluorescent analysis of the plant were carried out. Preliminary phytochemical screenings of extracts were also performed. The present investigation contributes to establish the Pharmacognostic profile of the medicinally effective plant.

INTRODUCTION: *Cressa cretica* L. belonging to the family Convolvulaceae, is perennial plant with a life cycle that continues in the summer period when the salt marsh area drains. *C. cretica* is a thermo-cosmopolitan halophilous species. *C. cretica* usually grows in sandy or muddy saline habitats along the sea coast along with the species *Suaedamaritima*, *Salicorniaeuropaea*, *Salsola soda*, *Limoniumvulgare* subsp. *Serotinum* and *Crypsisaculeate* [1, 2, 3, 4, 5]. Commonly the plant is known as 'Rudranti' in Hindi, 'Rudravanti' in Bengali and 'Dahna' in Oriya [6, 7, 8, 9]. The entire plant is medicinally important and is used extensively in traditional medicines.

It is reported to be antibilious, antitubercular and expectorant [10, 11]. The plant is used as antihelmintic, stomachic, tonic and aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, asthma, urinary discharges, leprosy and constipation, and as an appetizer [12].The plant is traditionally used in Bahrain as expectorant and antibilious agent [11]. Dry leaves of *C. cretica* crushed with sugar are used as emetic in Sudan [13]. The present investigation deals with studies on some important pharmacognostic profiles of the leaves and stem, which can helpful in authenticating the plant material.

MATERIALS AND METHODS:

Plant Material: *Cressa cretica* was collected from Nalban island of Chilika lake, Orissa and was identified by Dr. H. O. Brahmam, Department of Natural products, Institute of Mineral and Material Technology (formerly Regional Research Laboratory), Bhubaneswar, which was latter on confirmed from Botanical Survey of India, Howrah, West Bengal, India (CNH/I-I/32/2010/Tech. II/237). A voucher specimen has been kept in our laboratory for future reference. One set of the voucher specimen has been preserved in Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra. The leaves were powdered and passed through 40 mesh and stored in an airtight container for further use.

General Morphology of the Plant: *Cressa cretica* is a cushion chemaephyte excretive halophyte, an erect dwarf shrub upto 38 cm in height. Roots horizontal geminate, with lateral branches leading upward to produce above-ground parts. Perennial subshrub or herb, usually much-branched. Stems at first erect and then becoming decumbent, apparently short-lived, grey appressed pilose to sericeous. Young stems herbaceous, more or less cylindrical to oval in shape and slightly swollen at nodes. Stem at young tips more or less rectangular with deep green colour, odour remarkable, taste unpleasant and sour.

Leaves on main branches are often larger than those on branchlets, the blade 1- 12mm long, lanceolate, ovate or elliptic to scale-like, sessile or shortly petiolate. Flowers solitary, axillary, 5- 8mm long, sessile or on short peduncles, bracteate, in spicate to head-like clusters at tips of branchlets, bracteoles unequal in length. Sepals ovate to obovate, imbricate. Corolla salver form, the limb 5-lobed, the lobes mostly ovate, imbricate, spreading to reflexed. Stamens exserted; filaments filiform; styles exserted. Ovary 2-locular, 4-ovulate; styles 2, distinct to the base; stigmas capitate. Fruit capsular, ovoid, unilocular, 2-4-valved, usually 1-seeded. Seeds 3- 4mm long, glabrous and smooth and shining to reticulate, dark brown [6, 7, 8].

Microscopic Characters: The species was collected from the marshy saline soil of Nalban Island of Chilika Lake of Orissa in the month of March. During collection care was taken to select healthy plants with normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (formalin-5ml + Acetic acid-5ml + 70 percent ethyl alcohol 90ml). After 24hrs of fixation, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) as per schedule given by Sass [14]. Infiltration of the

specimen was carried out by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. Mid rib portion of leaf was taken and cut about 10-12 µm and Stained with Safranin for 2-3 minutes. Then, mounted in a micro slide with Glycerin water and visualized under compound microscope. Color reactions to chemical reagents and their location in which they are present in the plant are determined [15, 16, 17].

Determination of Leaf Constants: As a part of quantitative microscopy stomatal number and stomatal index of the leaves was determined by usual techniques [18], the observations were recorded, result was calculated and reported.

Physico-chemical Evaluations: Physico-chemical parameters such as the total ash, acid insoluble ash, acid soluble ash, water insoluble ash, water soluble ash were determined as per reported methods[19]. Considering the diversity of chemical nature and properties of contents of drugs, seven different solvents were used for determination of extractive values as per reported methods [19]. All determinations were performed in triplicate and the results are presented as mean ± standard error of mean (SEM).

Preparation of Extracts: The fresh aerial parts were dried under shade, powdered and pass through 40 mesh sieve and stored in closed containers for further use. The powder was extracted with different solvents ranging from non-polar to polar solvents. About 500g of the crude drug powder was subjected for extraction (Soxhlet extraction) in round bottom flask, first with hexane for 18-20 hours. The extract was concentrated under reduced pressure at 50-60^{o}C. The dried marc of *C.cretica were* once again subjected to successive extraction with different solvents viz, ethyl acetate, and methanol respectively.

Phytochemical screening: The dried and powdered leaf was subjected to preliminary phytochemical screening for qualitative detection of phytoconstituents. The concentrated extracts were evaporated about to dryness and the extracts obtained with each solvent were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material [20, 21].

Fluorescence analysis: The colour changes of the powdered seeds with respect to different chemical reagents on the basis of different chemical constituents was observed in day and ultraviolet light (UV 254nm and 366nm) as per methods described by Chase and Pratt 1949 and Kokoski *et al*., 1958 [22, 23, 24].

RESULTS AND DISCUSSION: The following characters are found in plant:

Trichomes are of two types;

A. Non-glandular Types:

a) Long unicellular with highly thickened pointed ends.
b) Multicellular- with a unicellular roundish base. Hairs short cell somewhat larger and elliptical with a little thickening. Some cells at the middle collapsed (with contracted cytoplasmic content). Base multicellular, non-bulbous.

B. Glandular Types: Stalk small, 2-3 cells long - with a round topped 2-seriate apex. The leaf has undulate epidermal layers, thin lamina and less distinct midrib. The mesophyll tissue is differentiated into slightly wider adaxial zone of palisade cells, narrow abaxial palisade zone and spongy parenchyma in between.

Stomata: Large numbers of paracytic as well as anomocytic types of stomatas are present on both the surfaces. The physical constants of leaf are represented in **Table 1**.

TABLE 1: PRELIMINARY EVALUATION OF LEAF CONSTANTS OF *C. CRETICA.*

Parameters	Leaf constants
Stomatal index of lower surface	8.3±2
Stomatal index of upper surface	6.69±2
Mean palisade ratio (upper surface)	4.4±1
Mean palisade ratio (lower surface)	7.2±1
Vein islet number(upper surface)	16±2
Vein islet number(lower surface)	19±2

One or two roundish somewhat thickened cells are situated at the apex of the tracheids. Thickness of the lamina is 4.6-8.8µm**.** Both the surfaces of the lamina are entire, without any outgrowth except the epidermal hairs, mostly provided with cystoliths having calcium carbonate. Internally, ground tissue forming the mesophyll is differentiated into palisade and airenchyma cells. Palisade cells are columnar, chlorophyllous, arranged in a single row with many intercellular spaces. Airenchyma cells are roundish, loosely arranged in 3-5 rows, round to sub-elliptical chloroplast grains of both the types of cells.

Single layered starch sheath consisting of irregular shaped cells occurs at the innermost cortical layer, only at the abaxial side of the vascular bundle. The phloem consists of sieve tube, companion cells and some narrower cells with a brownish content. Phloem-ray cells are iso-diametric, somewhat roundish in appearance, having tangential breadth. The cambial zone is irregular and not well defined.

Stem: Transverse and longitudinal sections of young stem revealing compact cork cells, ranging from 3-5 layers, more or less rectangular, interrupted frequently by large oval cells with dense gummy contents, glands containing some coloured substance in the epidermis also; alternate patches of smaller loose chlorenchymatous and larger collenchymatous tissues, the latter being in the form of large bands, radially 6-12 layers, cells. These layers followed by 8-12 layers of cortical parenchyma, smaller near the vascular bundles, oval to round, and thin walled, endodermis in conspicuous. Vascular bundles are large. Pith large containing parenchymatous cells.

The phloem consists of sieve tube, companion cells and some narrower cells with a brownish content. Phloem-ray cells are isodiametric, somewhat roundish in appearance, having tangential breadth of 2.1-6.3µmand radial breadth of 3.5-8.3µm**,** respectively. The cambial zone is irregular and not well defined.

The xylem arch consists of about 18-23.5 radial rows of tracheary elements. Pores are circular in outline; mean number 2-7 in each radial row. Chloroplast grains are distributed all through the lamina. The microhistochemical investigations obtained by treatment with chemical reagents and the location in which the chemical constituents are present mentioned in **Table 2**.

Microscopical Examination of Powder: Powder of the crude material was pale green colored, coarse, and free flowing it was unpleasant in taste.The dried leaf powder, boiled in saturated solution of chloral hydrate reveals;

1. Some isolated epidermal cells in surface view, irregular in outline,
2. Stomata of paracytic type and anomocytic type;
3. Parenchyma cells with crystals;
4. Spirally thickened vessel members and tracheids; members short with horizontal end wall and simple perforation plate, length
5. Round to ellipsoidal chloroplasts in parenchyma cells;
6. Long, narrow, pitted fibres with pointed ends;
7. Glandular as well as nonglandular trichomes;
8. Columnar palisade cells.

TABLE 2: MICROHISTOCHEMICAL TESTS OF *C. CRETICA*

Test for	Reagent	Result	Location
Lignin	Phloroglucinol– HCl(Saturated	+	Xylem portion
	Solution in 18% HCl, Aniline sulphate with H2SO4	+	Xylem and outer periphery
	Chlor-Zinc-Iodine solution	+	Xylem and periphery
Cellulose	Chlor-Zinc-Iodine solution	+	All parenchyma cells
Suberin	Sudan III	+	Some of the trichomes
Gummy deposits	Disappeared by hot water	+	Epidermal cells.
Fixed oils and Fats	Sudan III	+	Parenchyma cells and epidermal cells.
Resin	Saturated aqueous solution of copper acetate	–	
Tannin	Ferric chloride	+	Here and there in midrib.
Starch	Weak Iodine Solution	+	Cortex
Protein	Lugols solution	+	Vascular cells, guard cells.
	Millons reagent	+	Xylem parenchyma and hypodermal cells.
Calcium oxalate	Acetic acid and caustic alkali HCl.	+	Parenchyma cells.
Alkaloids	Mayers reagent	-	Leaf cells.
	Wagner's reagent	-	Leaf cells, epidermal cells.
	Dragondroffs reagent	-	Leaf cells, epidermal cells.
Sugar	Saturated solution of CuSO4.aqueous solution KOH solution (1: 1)	+	Subepidermal cells, especially the intercellular spaces, positive cortical cells
	Fehling's solution	Weakly positive	Peripheral and cortical cells

The physicochemical characters are like ash values showed the inorganic silicates, carbonates, phosphates present in the aerial parts of *Cressa cretica* and acid insoluble ash values, and water soluble ash shows the inorganic elements that were soluble in acid and water respectively. Sulphated ash was found out and this is useful to find out free metals present in the drug with sulphate form. The high percentage of water insoluble residue in the ash of *C. cretica* was evaluated and the results are depicted in **table 3**.

TABLE 3: ASH VALUES

Ash values	Values (in % w/w)
Total ash	5.23
Water soluble ash	0.87
Acid soluble ash	1.24
Sulphated ash	3.12

The extractive values were assessed and represented in **table 4**.

TABLE 4: EXTRACTIVE VALUES

Solvent	Extractive Values (in %w/w)
Hexane	3.390
Ethyl acetate	8.621
Methanol	4.440

The Preliminary phytochemical screening shows the presence the phytoconstituents like carbohydrates, flavonoids, phytosterols, terpenes, tannins, glycosides, fixed oil and sugars. The fluorescence analysis also represented the behavioral changes of the powder leaf, extracts with different chemical reagents in different wavelengths in UV, which can be the identifying character of the plant *C. cretica.* The fluorescence characteristics of the powder when treated with

various chemical reagents have been extensively studied in different wavelengths (265nm and 354nm), which sets a standard parameter for authentication. The results are shown in **Tables 5(a) and 5(b)**.

TABLE 5(A): FLUORESCENT STUDIES OF POWDER OF *C. CRETICA*

S. no.-		Day light	UV 254	UV 366
1	Powder (P) as such	Green	Fluorescent green	Fluorescent green
2	P+ nitrocellulose in amyl acetate	Green	Fluorescent green	Fluorescent yellow
3	P+1N NaOH in water	Fluorescent green	Dark Fluorescent green	Fluorescent green
4	P+ 1N NaOH in nitrocellulose in amyl acetate	Fluorescent green	Fluorescent green	Fluorescent yellow
5	P + 1N HCl	Light green	Light fluorescent	Brown
6.	P+1NHCl+nitrocellulose in amyl acetate	Reddish green	Cremy fluorescent	Fluorescent green
7	P+ 1N NaOH in methanol	Fluorescent green	Fluorescent green	Fluorescent yellow
8	P+ 50% KOH	Light green	Dark Fluorescent green	Fluorescent yellow
9	P+ 50% H_2SO_4	Fluorescent green	Light Fluorescent green	Fluorescent yellow
10	P + 50% HNO_3	Light green	Fluorescent green	Dark brown
11	P + concentration HNO_3	Reddish green	Fluorescent green	Fluorescent yellow
12	P + acetic acid	Light green	Fluorescent yellow	Green
13	P + I_2 water	Light green	Fluorescent yellow	Yellow

TABLE 5 (B): BEHAVIOR OF *CRESSA CRETICA* POWDER WITH DIFFERENT CHEMICAL REAGENTS

Treatment	Observations (Color)
Powder as such	Dull green
Powder + Acetic acid	Green
Powder + Conc H_2SO_4	Yellow
Powder + Conc HNO_3	Brown
Powder + $Fecl_3$	Green
Powder + Aq. NaOH	Yellowish green
Powder + Conc HCL	Dark green
Powder + Picric acid	Yellowish green

CONCLUSION: In conclusion, the present study on pharmacognostical characters of *Cressa cretica* may be useful to supplement information in regard to its identification and can be an authenticate parameter of standardization.

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