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Pharmacognostic Evaluation of *Curcuma caesia* Roxb. rhizome

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Abstract – *Curcuma caesia* Roxb. (Zingiberaceae) is commonly known as '*Black turmeric*'. In India it grows in West Bengal, Madhya Pradesh, Orissa, Bihar, North-East and Uttar Pradesh and is widely used by ethnic communities for various ailments. Rhizomes of the plant are used for sprains and bruises and are also employed in cosmetics. In West Bengal it is an important place in traditional system of medicine and is also used as a substitute for turmeric in fresh stage. Present communication deals with the detailed pharmacognostical evaluation of the rhizome sample. Inner part of the rhizome is bluish-black in colour and emits a characteristic sweet smell, due to the presence of essential oil. On steam distillation the rhizome yields an essential oil rich in camphor. A detailed HPTLC studies has been carried out for quantitative evaluation of active marker component. HPTLC, physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. caesia* rhizome and may possibly help to differentiate the drug from its other allied species.
Keywords – *Curcuma caesia*, Black turmeric, Pharmacognosy, HPTLC

Introduction

Curcuma caesia (Zingiberaceae), a kind of turmeric with bluish-black rhizome is native to North-East and Central India (Syamkumar and Sasikumar, 2007). Rhizome is claimed to be useful in treating piles, leprosy (Anonymous, 1948-1992), bronchitis, asthma (Kirtikar and Basu, 1933), cancer, epilepsy, fever, wound, sprains, bruises, impotency, fertility, menstrual disorders, toothache, vomiting etc. (Anonymous, 2001). For longevity, impotence, infertility, irregular menstrual flow, a spoonful powder of dried rhizomes is mixed with honey or a cup of milk is taken twice a day, in gastric troubles fresh piece of rhizome is chewed (Rustomjee and Katrak, 1984). In Madhya Pradesh, India plant is regarded as very auspicious and it is stated that a person who possesses it will never experience shortage of cereals and food.

There is no detailed pharmacognostical work reported so far, only some amount of chemical work has been done on this plant, rhizome yields volatile essential oil having about 30 components representing 97.48% of the oil, with camphor (28.3%), *ar*-turmerone (12.3%), (*Z*)-β-ocimene (8.2%), *ar*-curcumene (6.8%), 1,8-cineole (5.3%), β-elemene (4.8%), borneol (4.4%), bornyl acetate (3.3%) and γ-curcumene (2.82%) as the major constituents (Pandey and Ashim, 2003).

In this context, the present study was performed to develop pharmacognostical parameters of rhizome of this species. This will be useful to pharmaceutical industries for authentication, successful commercial exploitation and to maintain batch to batch consistency of the raw material.

Experimental

Plant material was collected from Mariyani Forest Reserve, Assam-Nagaland border. It was authenticated and matched with the voucher specimen and deposited in the institute's herbarium (LWG 262529, 2009). Fresh rhizomes were preserved in 70% ethyl alcohol for histological studies. Microtome sections were cut and stained with safranin and fast green and photographed with Olympus CX 31 camera (Johansen, 1940).

Physico-chemical and phytochemical studies like, total ash, acid insoluble ash, sugar, starch and tannins were calculated from the shade dried and powdered (100 mesh) plant material (Peach and Tracy, 1955; Anonymous, 2007; Anonymous, 1984).

Results

A brief taxonomic description of the plant – A perennial herb. Whole height about 1.2 m. Rootstock

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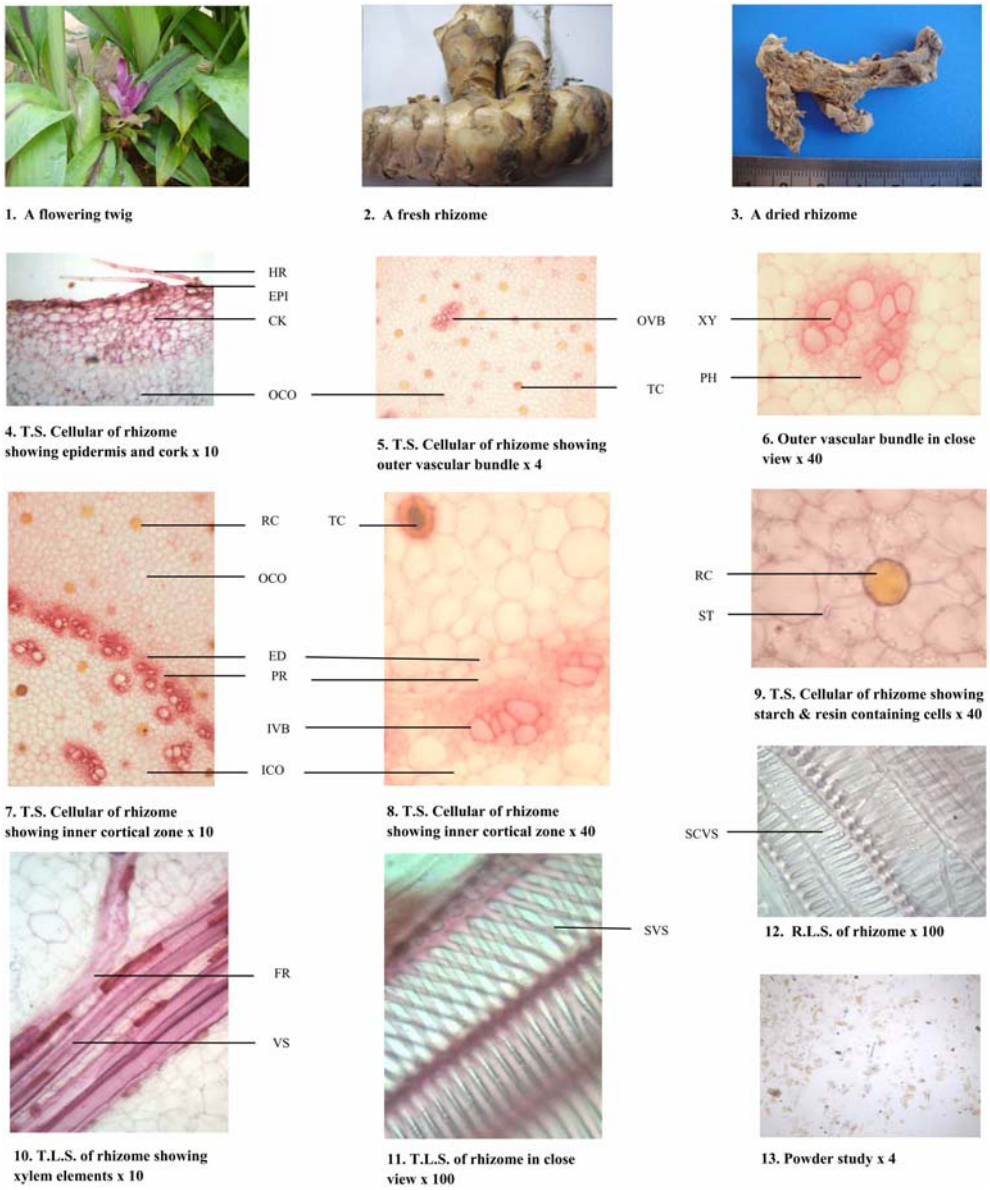

Plate. 1. Macro and Microscopic characters of the rhizome of *Curcuma caesia.*
OCO, Outer cortex; ICO, Inner cortex; EPI, Epidermis; CK, Cork cells; OVB, Outer vascular bundle; IVB, Inner vascular bundle; FR, Fibre; ST, Starch; VS, Vessel; XY, Xylem; PH, Phloem; ED, Endodermis; PR, Pericycle; HR, Hair; TC, Tannin containing cell; RC, Resin containing cell; SVS, Spiral vessel & SCVS, Scalariform vessel.

large, ovoid; tubers sessile, pale grey inside. Leaves 30 - 60 × 12.5 - 15 cm, broadly lanceolate or oblong, glabrous, with a deep ferruginous purple cloud down the middle which penetrates to the lower surface. Petiole and sheath about as long as the blade. Spikes appearing rather before the leaves, about 15 cm long or altogether about 30 cm high with the peduncle. Flowering bracts green with a ferruginous tinge. Coma deep bright red, tending to crimson. Flowers pale yellow, reddish at the outer border, rather shorter than their bracts.

Macroscopic characters of the rhizome – Rhizome cylindrical, 4 to 9 cm long, 2.5 to 8 cm in diameter, branched, aromatic, pale grey or bluish grey inside; sessile tubers fleshy, finger shaped, branched; branching sympodial, horizontal; roots long, unbranched, tapering, thread like, yellowish brown (Plate-1).

Microscopic characters of the rhizome – Transverse section (TS) of rhizome is almost circular in outline. Epidermal cells are rectangular-oval in shape and covered with thick cuticle. Long unicellular trichomes are present

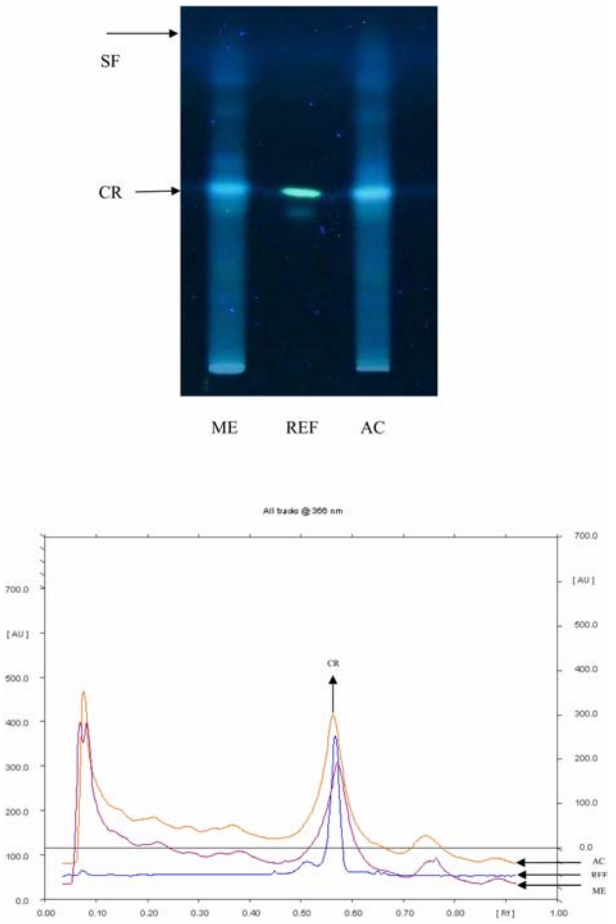

Plate. 2. HPTLC profile of *Curcuma caesia* rhizome and reference sample (Under UV- 366 nm).
SF, Solvent front; CR, Curcumin; REF, Reference sample; AC, Acetone extract and ME, Methanol extract.

on the epidermis. Followed to these, 6 to 8 layered storied suberized cork cells are present. A wide cortex having irregularly scattered vascular bundles are present. Each vascular bundle is enclosed within a prominent fibrous sheath, inner limit of cortex marked by endodermis, and pericycle followed by vascular bundles which are devoid of bundle sheath, arranged in a ring; numerous resin cells with their dusky orange colored content are present in cortical region. Most of the parenchymatous cells are filled with starch grains, which are oval-ellipsoidal in shape. Vascular bundles in the central cylinder are similar to those found in the cortex and are scattered, closed, collateral, surrounded by thick walled bundle sheath. Secondary wall thickening reticulate, fibres thin walled with lignified narrow central lumen (Plate-1).

In transverse longitudinal section (TLS), cortical cells irregular in shape with variable size. Vessels elongated with spiral and scalariform thickenings and with no clear

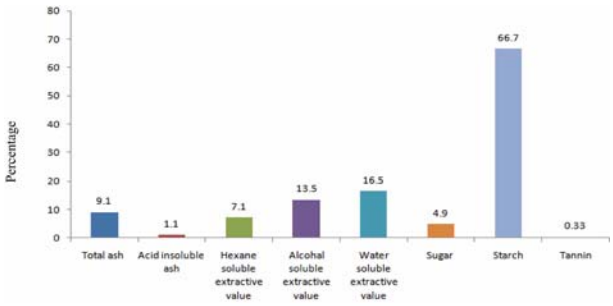

Fig. 1. Physico-chemical values of *Curcuma caesia* rhizome.

end-walls. Tracheids with bordered pits are observed.

Powder pale yellowish-grey with sweet, aromatic and camphoraceous odour, shows fragments of storied cork, xylem vessels with spiral and scalariform thickenings, lignified xylem fibres, resin cells, and patches of parenchymatous cells filled with starch grains which are oval-ellipsoidal, sometimes polygonal in shape.

Physico-Chemical Studies – On physico-chemical studies, air dried rhizome sample exhibits- total ash 9.1%, acid insoluble ash 1.1%, hexane soluble extractive 7.1%, alcohol soluble extractive 13.5%, water soluble extractive 16.5%, sugar 4.9%, starch 66.7% and tannins 0.33% (Fig. 1).

HPTLC Studies – Reagents used were from Merk (Germany) and standard curcumin was procured from Sigma-Aldrich (Steinheim, Germany). Air dried (45 -55 °C) powdered rhizome of *C. caesia* (1.0 g) in triplicate were extracted separately with 3 × 10 ml methanol. Extracts were concentrated under vacuum, redissolved in methanol, filtered and finally made up to 100 ml with methanol prior to HPTLC analysis.

Chromatographic conditions – Chromatography was performed on Merk HPTLC percoated silica gel 60 GF254 (20 × 20 cm) plates. Methanolic solutions of samples and standard compound curcumin of known concentrations were applied to the layers as 6 mm- wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl/s from application syringe. These conditions were kept constant throughout analysis of samples.

Detection and Quantification of curcumin – Following sample application, layers were developed in a Camag twin trough glass chamber (20 cm × 10 cm) that had been presaturated with mobile phase of chloroform: ethanol: acetic acid, (95: 4: 5, v/v) till proper separation of bands up to 8 cm height. After development, layers were dried at room temperature (27 °C) and curcumin was simultaneously quantified using Camag TLC scanner

model 3 equipped with Camag Wincats 321 software. Following scan conditions were applied: slit width, 6 mm × 0.45 mm; wavelength, 366 mm; and absorption-reflection mode. In order to prepare calibration curves, stock solution of curcumin (1 mg/ml each) was prepared and various volumes of these solutions were analyzed through HPTLC, calibration curves of peak area vs. concentration were also prepared. Calibration curve (range, 1 - 20 μg) of curcumin was linear. Different values for determination of curcumin were as follows: R_f 0.59; regression equation, $y = 1185.456 + 3602.145x$; and r^2, 0.943.

Discussion

From the above studies rhizome can easily be differentiated on the basis of its organoleptic characters for example odour and taste of rhizome is quite characteristic and is aromatic with sweet camphoraceous taste. On microscopical examination oval-ellipsoidal starch grains are observed in the rhizome. Similarly number of curcumin containing cells is abundant in the rhizome.

Physicochemical values *viz.* total ash, acid insoluble ash, alcohol and water-soluble extractives were observed. The total ash (9.1%) and acid insoluble ash (1.1%) are considered to be an important and useful parameter for detecting the presence of inorganic substances like silicate ion. Similarly the hexane (9.1%), alcohol (13.5%) and water-soluble extractives (16.5%) are indicators of the total solvent soluble components.

On quantitative HPTLC analysis acetone and methanol extract yield 0.179% and 0.067% of curcumin respectively.

Conclusion

Above parameters of this species may be useful to pharmaceutical industries for the authentication and batch to batch consistency of the commercial samples.

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