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Quality control standardization of the rhizome of *Curcuma yunnanensis*: A comprehensive standardization process

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Curcuma yunnanensis (CY) (Zingiberaceae) is a plant having a long flowering season (July-October). It is well morphologically characterized by its lance-shaped leaves having a purplish stripe running down the midrib and greenish coloured rhizome when cut transversely. Pharmacognostical standardization of the rhizome has been evaluated as per WHO guidelines. The dried rhizomes are golden-brown colour and vary in size (2 to 7 cm length and 1 to 2 cm diam.). The histological characteristic of the rhizome shows dissimilarity with other existing *Curcuma* species. Physico-chemical standards studied are foreign organic matter (0.16% w/w), loss on drying (9.80% w/w), total ash (7.66% w/w), acid insoluble ash (1.70% w/w), water-soluble ash (3.17% w/w), alcohol soluble extractive (8.77% w/w) and water-soluble extractive (7.70% w/w), foaming index (<100), swelling index (4.3) and volatile oil content (0.8%). Powdered characterization showed the presence of starch grains, unicellular covering trichomes and lignified xylem vessels. Phytochemical screening showed the presence of alkaloids, glycosides, phenolics, tannins and steroidal components. Quantitative estimation of total tannins and phenolics was also determined in the ethanolic extract and was found to contain 21.375 and 22.5 mg/g GAE, respectively. The presence of demethoxycurcumin in the CY ethanolic extract was also documented for the first time using HPTLC by comparing with standard curcumin.

Keywords: Black turmeric, *Curcuma yunnanensis*, Curcumin, Fluorescence analysis, HPTLC, Standardization.

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Introduction

The genus *Curcuma* was established a long time back during 17th century by Linnaeus and belongs to the family Zingiberaceae[1]. Hundreds of species have been so far taxonomically classified and many of them have good economic value because of their volatile oils, showy flowers and are used as spices, medicines, dyes, perfumes, tonics, foods and as tropical ornamentals[2]. Since their utility varies with plant species, choosing the right plant is very important. Many of the *Curcuma* species are well characterized, but others are difficult to distinguish due to their similar morphological nature. During the flowering season, the identification of the *Curcuma* species is easier; however, rhizomes are particularly used in the traditional system of medicine and it is mostly available in the dried form in the market. Hence, pharmacognostical standardization of the crude rhizomes is necessary to select the appropriate species.

Curcuma yunnanensis N. Liu & S. J. Chen is a tall plant which grows on moist shaded places and slopes[3]. The plant is native to China particularly in Yunnan and Guangdong provinces[4]; however, in India, it was first reported in the slopes of Jaintia Hills of the state Meghalaya (North-East India)[3]. The plant can be simply characterized morphologically from its white creamy blossom, lance-shaped leaves having a purplish stripe running throughout the midrib and also from the greenish coloured rhizome when viewed transversely. In India, particularly in the Jaintia Hills district of Meghalaya, it was reported that the plant has a blooming season from July to August and prefers lightly filtered sun[3]. It has blossom featuring many narrow upright bracts that are bright plum-colored[5]. Traditionally, the local tribe particularly the Janitia Tribes of the state Meghalaya (North-East India) uses this plant for the treatment of digestive tract disorder such as stomach pain and the paste of the rhizome is applied locally to the whole body as a

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belief to destroy the evil spirit[3]. The plant is less explored and as per the literature survey, there were no scientific reports published on the pharmacognostical standardization of the rhizome parts of the plant. Hence, the main objective of the present investigation aimed to standardize the *C. yunnanensis* rhizome based on pharmacognostical and phytochemical tests.

Material and Methods

Chemicals and instruments

Standard curcumin was procured from Ranbaxy Laboratory Pvt. Ltd (India). The stationary phase for both TLC and HPTLC was the pre-coated aluminium silica gel plates 60 F_{254} having particle size 2-10 microns (purchased from Merck, Germany). For histological studies, the photomicrographs in different magnifications of all necessary cells and tissues were taken with the trinocular microscope (Nikon microscope E-200). CAMAG (Switzerland) - HPTLC instrumentation equipped with Linomat V sample applicator, CAMAG-TLC scanner 3, and CAMAG-TLC visualizer and CATS 4 software for data interpretation were used for fingerprinting analysis of the samples. All other chemicals, solvents and reagents used were of standard analytical grade.

Plant material and extraction process

The plant specimen was collected from the remote place of Khanduli area situated in the Jaintia Hills district of Meghalaya, Northeast India. Dr R. Kumar (Scientist C in-charge) at the Botanical Survey of India, Shillong (Meghalaya), India identified as *C. yunnanensis* N. Liu & S. J. Chen belonging to Zingiberaceae family. A voucher specimen (COG/CY-09/2009) has been deposited at the Pharmacognosy Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi.

The fresh rhizome was dried under shade and was powdered and the extraction process was done successively for five days using Soxhlet apparatus with organic solvents of varying polarity (*n*-hexane, benzene, chloroform, ethyl acetate, acetone and ethanol). Each successive extracted content was filtered and the filtrate obtained was evaporated under a rotary evaporator (Buchi Pvt. Ltd) and finally stored in a vacuum desiccator to dryness.

Morphological and histological studies

The morphological and histological study of the dried rhizome was done following standard method[6,7]. For the morphological study, dried rhizomes were evaluated for various organoleptic characters such as texture, size, shape, colour, odour and taste. For the histological evaluation, a free-hand section of the rhizome was taken and stained as per the standard method[7]. The sections were dehydrated with varying strength of absolute alcohol and then stained with phloroglucinol and HCl mixture[8]. Finally, the stained sections were permanently mounted with DPX for histological observation.

Physico-chemical evaluation, fluorescence analysis and starch grains quantification

The various physico-chemical parameters of the air-dried plant material were determined as per WHO guidelines on quality control methods for medicinal plants material[9], and as per Indian Herbal Pharmacopoeia[10]. Various physico-chemical constants estimated include foreign matter, loss on drying, ash values, extractive values, swelling index, foaming index, haemolytic index and volatile oil determination (hydrodistillation by Clevenger apparatus). Fluorescence analysis of the powder drug was also carried out following the well known methods[11,12]. The quantitative determination of starch grains was done following the Wallis's Lycopodium spore method[13].

Phytochemical evaluation and quantitative estimation of total polyphenolic content

Extracts which are obtained successively from the Soxhlet extractor was subjected to preliminary phytochemical screening for testing the presence of various active phytoconstituent classes[7,13]. Determination of total phenolic and tannin content was also estimated as per the Folin-ciocalteu method[14,15]. All the results for the quantitative estimation were carried out in triplicates and were expressed as the Mean±S.E.M using the linear regression method.

TLC and HPTLC fingerprinting analysis

Preparation of extracts, thin layer chromatography, and developing of chromatogram was done as per the standard methods[16]. The plates used as the stationary phase are the pre-coated silica gel 60 F_{254} aluminium sheets. The solvent system used for the development of chromatogram contained solvents of different polarity. Spraying/derivatization reagent used for the identification of phytoconstituents were Liebermann-Burchard reagent (for triterpenoids and steroids), vanillin sulphuric acid (for essential oils), 5 % ferric

chloride (for polyphenolics) and benzidine sodium metaperiodate reagent (for glycosides).

For HPTLC analysis, 10 mg/mL ethanolic extract of the rhizome was used. Three different bands of varying concentration (20, 10, and 5 µL in volume) were applied to the plate using HPTLC Linomat applicator. A solution of standard curcumin in ethanol was also prepared (1 mg/mL) and the concentration in terms of volume applied to the plates was 5, 5, and 2 µL. The study was carried out using CAMAG-HPTLC instrumentation equipped with Linomat V sample applicator, CAMAG-TLC scanner 3, and CAMAG-TLC visualizer and CATS 4 software for data interpretation. The chromatogram was developed using chloroform and methanol (9.7:0.3) mobile phase solvent mixture in a Camag twin trough chamber (20 × 10 cm). The R_f value was recorded and the developed plate was screened and photo-documented at 254, 366 nm and white light.

Results and Discussion

Pharmacognostical evaluation

Morphological study

Externally, the dried rhizomes are golden-brown to greenish colour, longitudinally wrinkled and vary in size between 2 to 7 cm in length and 1 to 2 cm in diameter. Generally, fresh rhizomes when cut transversely (Fig. 1) shows a greenish colour ring on the outer side with a light yellow colour in the centre which is entirely different from other *Curcuma* species. However, the rhizomes when dried completely showed greenish to yellowish-brown colour when cut transversely, which is more or less similar to that of the fresh ones. The rhizomes are aromatic, pungent in odour and bitter in taste. Morphologically, it differs from other *Curcuma* species, particularly from *C. amada* Robx. in which there is less demarcation of internodal from the nodal region[17]. Scaly leaves are seen to be originating from the nodal region of the rhizomes which give the appearance of growth rings. Numerous long tapering root hairs are also seen to be originating from the rhizomes and are fibrous in nature. A comparison of the different characteristics features of *C. yunannensis* with other *Curcuma* species is shown in Table 1.

Microscopical study

The transverse section (Fig. 2) of the dried rhizomes of *C. yunnanensis* was found to be similar to other species belonging to the Zingiberaceae family and showed to contain five to seven layers of dark brown cork cells which are regular in shape. Cortex is made up of thin-walled round parenchymatous cells (40 to 68 µ in diameter) with intercellular spaces containing abundant of starch grains. Numerous greenish to yellow oily substances were found interspersed with the cortical parenchyma and are believed to be secreted from the lysigenous ducts. This is in concomitant with the literature which reported the presence of lysigenous ducts in other *Curcuma* species[18]. Lying next to the cortex is a single layer of endodermis having radial walls followed by a layer of pericycle. The ground tissue which is the innermost part of the section is made up of large round parenchymatous cells embedded with starch grains and few oleoresins cells. Non-lignified vascular bundle elements are seen lying scattered both in the cortical as well as in the ground tissue region.

Powder characteristics

The powder of the dried rhizome shows a greenish to yellowish-brown colour, aromatic odour and bitter taste. For the study of powder characteristics,

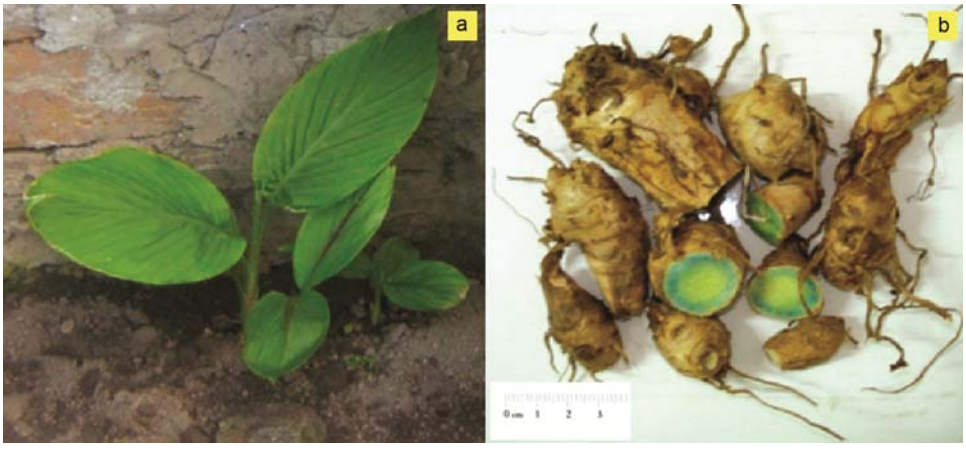

Fig. 1 — Morphology of *Curcuma yunnanensis* plant (a) and rhizome (b).

Table 1 — Comparative characteristics features of *C. yunnanensis* rhizome from other well known *Curcuma* species

Rhizome features	*Curcuma yunnanensis*	*Curcuma longa*[10]	*Curcuma amada*[17]
Morphological characters			
Colour: Externally	Light brown	Yellowish	Buffed coloured
Internally	Yellowish green to blue	Orange-yellow to yellow	Yellow
Size	4-10 cm in length and 1-3 cm in diameter	3-8 cm in length and 2-3 cm in diameter	2-6 cm in length and 0.5-2 cm in diameter
Shape	Cylindrical to round and elongated. Almost sessile	Ovate, oblong, round or cylindrical to elongate. Lateral branches are sessile.	Laterally flattened and longitudinally wrinkled. Sympodial branching.
Odour	Aromatic	Aromatic	Raw-mango like odour
Taste	Pungent and bitter	Warmly aromatic and bitter	Pungent
Microscopical characters			
Cork	Compressed, rectangular to oval elongated cells.	Polygonal thin-walled brown cells.	Suberized cells, rectangular to oval, thick cuticle.
Trichomes	Unicellular long covering trichomes.	Unicellular, elongated and bluntly pointed covering trichomes.	Unicellular hairs present.
Cortical vascular bundle	Vascular bundles are arranged scattered both in cortex as well as in the ground tissue. Xylem vessel contains spiral and reticulate thickenings.	Vascular bundle are scattered and are of collateral type. vessels with spiral, reticulate and annular thickenings.	Irregularly arranged scattered vascular bundle without bundle sheath. Xylem vessels with reticulate thickenings.
Oil cells	Oil cells distributed in the cortex and ground tissue.	Not mentioned	Oil cells with suberized walls found in cortex and central region.
Starch grain	Starch grain is simple and round. Hilum circular with concentric lamellae.	Gelatinized starch grain present in the ground tissue.	Starch grain is oval-ellipsoidal sometimes polygonal shaped. Hilum circular and lamellae concentric.
Quantitative standards			
Foreign matter	Not more than 1.0 %	Not more than 2.0%	Not more than 1.0%
Total ash	Not more than 8.0 %	Not more than 9.0%	Not more than 12.0%
Acid-insoluble ash	Not more than 2.0 %	Not more than 1.0%	Not more than 2.0%
Water-soluble ash	Not more than 4.0 %	Not mentioned	Not mentioned
Alcohol-soluble extractive	Not less than 8.0 %	Not less than 8.0%	Not less than 9.0%
Water-soluble extractive	Not less than 7.0 %	Not less than 12.0%	Not less than 14.0%
Loss on drying	Not more than 9.0 %	Not mentioned	Not more than 5.0%
Essential oil	Not less than 0.8 %	Not less than 4.0%	Not less than 1.0%
Starch grain quantity	1,32,187 per mg crude powder drug	Not mentioned	Not mentioned
Phytochemical constituents			
Essential oil	0.8%	2-7%	1.0%
Curcuminoids	Des-methoxycurcumin	Curcumin, bis-desmethoxy curcumin and demethoxy curcumin	Curcumene

visualization of fine powder was done under a pale background using a simple microscope having the eyepiece 10× and 45× magnifying power (Fig. 3). The powdered rhizome shows the presence of simple starch grains which are oval to round shaped (11 to 18 µm). Xylem vessels with spiral thickenings ranging from 270 to 1080 µm were observed. Spiral thickenings of xylem vessels were also observed in other *Curcuma* species as reported in literature[18]. Round to oval parenchymatous cells (41 to 60 µm) and elongated unicellular covering trichomes which are believed to be epidermal hairs were also found in the rhizome. The presence of epidermal hairs in *C. yunnanensis* is in particulars with other *Curcuma* species (*C. longa*, *C. aromatic*, *C. amada* and *C. zedoaria*) as reported in the literature[18].

Physico-chemical evaluation, fluorescence analysis and starch grains quantification

The results for the quantitative physico-chemical constants of *C. yunnanensis* are shown in Table 2. Hydrodistillation of the essential oil matter content was found to be 0.8 % v/w. Other parameters like swelling, foaming index, haemolytic index and loss on drying were also evaluated and shown in Table 1. The powder of the rhizomes swells in the presence of water with a swelling index value of 4.3 mL. This

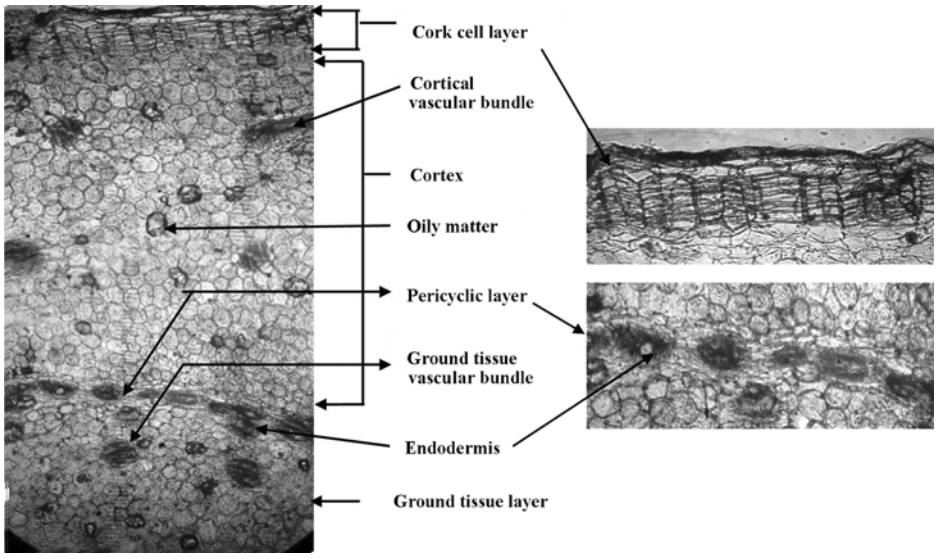

Fig. 2 — Transverse section of *Curcuma yunnanensis* rhizome.

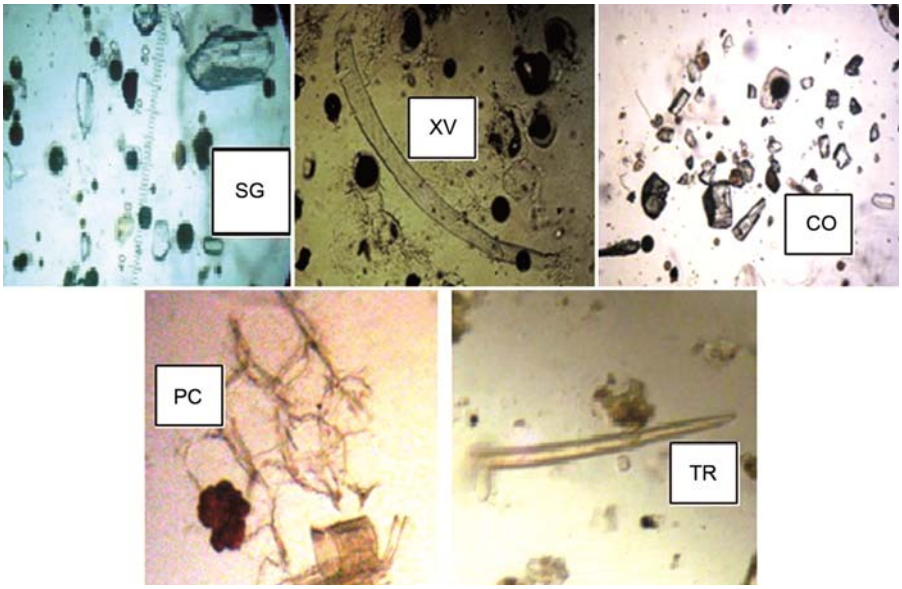

Fig. 3 — Powder characteristics of *Curcuma yunnanensis* rhizome (SG- Starch grain; XV- Xylem vessel; CO- Calcium oxalate crystal; PC- Parenchymatous cell; TR- Trichome).

Table 2 — Physico-chemical values of *C. yunnanensis* rhizome

S. No.	Physico-chemical parameters	Obtained values in percentage
1	Foreign matter	0.16% w/w
2	Loss on drying	9.80% w/w
3	Total ash	7.66% w/w
	Acid-insoluble ash	1.70% w/w
	Water-soluble ash	3.17% w/w
4	Alcohol extractable matter	8.77% w/w
	Water extractable matter	7.70% w/w
5	Foaming index	Less than 100
6	Swelling index	4.3
7	Haemolytic index	110
8	Volatile oil determination	0.80% v/w

might be attributed to the presence of any adhering mucilaginous and gummy substance in the plant. As per the literature, a report on the presence of mucilage and gums was also found in the rhizome of other *Curcuma* species especially *C. aromatic*[19]. Swelling index is mainly attributed to components of medicinal plants which are mucilage and gums and they are playing an important role as a swelling agent in pharmaceutical excipients. Crude drugs having the capability to swell in aqueous phase might possess viscous matter like mucilage, pectin and hemicellulose which are of pharmaceutical

importance[9]. It was observed that there are few similarities and differences between the rhizome of *C. yunnanensis* and other well known *Curcuma* species with regards to the physicochemical values[10,17].

The respective Table 3, specify the results in which the powder drug of the *C. yunnanensis* rhizomes produces fluorescence in day light as well as under long UV light (365 nm). Fluorescence analysis of crude drug is very important to predict the presence of phyto components which are UV active. In addition to the quantitative physico-chemical parameters, the number of starch grains present in the powdered drug of *C. yunnanensis* was also determined and it was found to contain 1, 32, 187 starch grains per mg of the powdered drug.

Phytochemical evaluation

Phytochemical screening

The result for the preliminary phytochemical screening of the various solvents extracts of *C. yunnanensis* was shown in Table 4. Results revealed that alkaloids and glyocosides are presents in chloroform, ethyl acetate, acetone and ethanolic extract; whereas, flavonoids were found to be present in chloroform, ethyl acetate and ethanolic extract. Steroidal/triterpenoids components were found to be dominated in all the extracts except water extract. Phenolics and tannins were found to be distributed in benzene, chloroform, ethyl acetate and ethanolic extract. Mucilage, proteins, amino acids and sugars were found to be distributed only in the polar water extract and absent in all the extract (exception is for ethanolic extract which bears the sugar components).

Quantitative estimation of total phenolic and tannin content

The polyphenolic class was also spectrophotometrically estimated using Folin-Ciocalteu methods and the extract of *C. yunannensis* was found to contain 21.375±2.1 mg/g (tannic acid equivalent) of total tannin and 22.500±3.4 mg/g (gallic acid equivalent) of total phenolic content.

Table 3 — Fluorescence powder drug analysis of *C. yunnanensis* rhizome

S. No.	Powder + Reagent	Fluorescence in daylight	Fluorescence under UV light (365 nm)
1	Powder as such	Brown	NF
2	Powder + 1N NaOH in methanol	Golden rod	Spring green
3	Powder + 1N NaoH in water	Gold	Spring green
4	Powder + 1N HCl in methanol	Dark green	Light green
5	Powder + 1N HCl in water	Corn silk	Pale green
6	Powder + 1N HNO_3 in methanol	Dark Red	Yellow green
7	Powder + 1N HNO_3 in water	Tan	Aquamarine
8	Powder + Iodine (5%)	Crimson	No fluorescence
9	Powder + $FeCl_3$ (5%)	Green yellow	No fluorescence
10	Powder + KOH (50%)	Golden rod	Light green
11	Powder + Ammonia (25%)	Yellow	Green yellow
12	Powder + Picric acid (saturated)	Yellow	No fluorescence
13	Powder + Acetic acid	Orange	Yellow green

Table 4 — Preliminary phytochemical screening of various extracts of *C. yunnanensis* rhizome

Phytoconstituents classes	Hexane extract	Benzene extract	$CHCl_3$ extract	Ethyl acetate extract	Acetone extract	Ethanolic extract	Water extract
Alkaloids	-	-	+	+	+	+	-
Glycosides	-	-	+	+	+	+	+
Flavonoids	-	-	+	+	-	+	-
Steroidal/ triterpenes	+	+	+	+	+	+	-
Phenolic & tannins	-	+	+	+	-	+	-
Saponins	-	-	-	-	-	-	-
Mucilages	-	-	-	-	-	-	+
Proteins	-	-	-	-	-	-	+
Amino acids	-	-	-	-	-	-	+
Sugars	-	-	-	-	-	+	+

(+) indicates present, (-) indicates absent

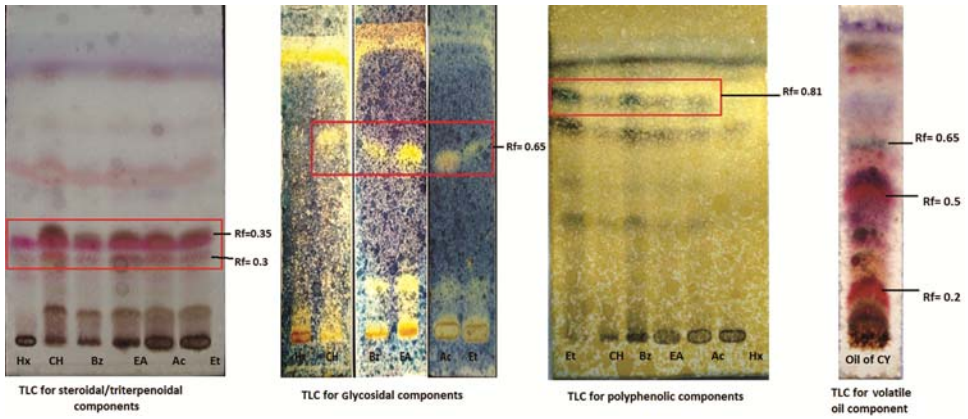

Fig. 4 — TLC fingerprinting of various solvent fractions and volatile oil of *Curcuma yunnanensis* rhizome [Hx- Hexane fraction; CH- Chloroform fraction; Bz- Benzene fraction; EA- Ethyl acetate fraction; Ac- Acetone fraction; Et- Ethanolic extract]

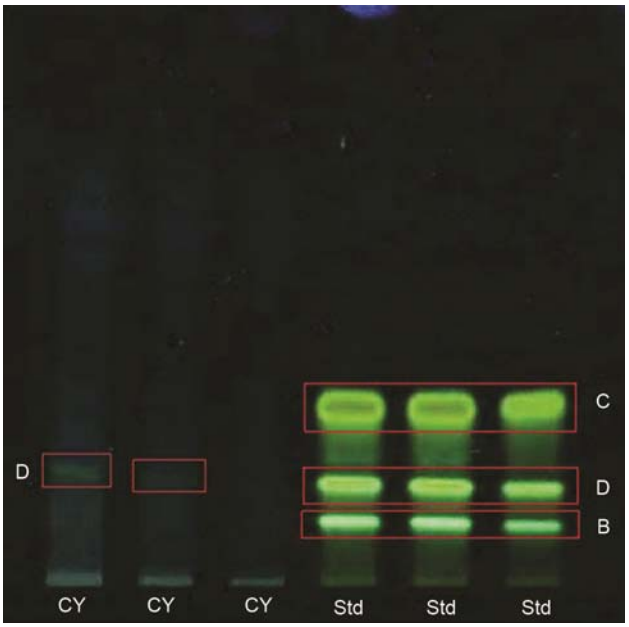

Fig. 5 — HPTLC fingerprinting of ethanolic extract of *Curcuma yunnanensis* and standard curcumin containing derivative (at 366 nm) [CY- *Curcuma yunnanensis* extract; Std- Standard Curcumin; B- Bis-demethoxycurcumin; D- Demethoxycurcumin; C- Curcumin]

TLC and HPTLC analysis

The thin layer chromatographic fingerprinting of the various extracts (*n*-hexane, benzene, chloroform, ethyl acetate, acetone and ethanolic) obtained from Soxhlet and volatile oil from Clevenger apparatus was screened for the identification and confirmation of the phytoconstituents in *C. yunnanensis* rhizome and is shown in the Fig. 4. Solvent system for the screening of volatile oil was *n*-hexane and ethyl acetate in the ratio of 4:1; for steroidal component (*n*-hexane and ethyl acetate 9:1); for polyphenolic components (chloroform and methanol 4:1) and glycosidal components (chloroform and methanol 7:3). The respective R_f value of all the chromatograms in various phytoconstituents is labelled in Fig. 4. TLC still showed up to be one of the reproducible technique required for the confirmation of the active constituent classes in medicinal plants.

Fig. 5 represents the HPTLC fingerprinting of standard curcumin and *C. yunnanensis* extract at 366 nm. In the present investigation, HPTLC result showed that the ethanolic extract of *C. yunnanensis* showed the presence of demethoxycurcumin (at 366 nm) only whereas curcumin and bis-demethoxycurcumin were found to be absent. It is well documented from the literature that standard curcumin always bears two derivatives which are demethoxycurcumin and bis-demethoxycurcumin[20]. This had led to the conclusion, that the rhizome of *C. yunannensis* confirms the absence of the yellowish-orange compound curcumin. Hence, this signifies the fact that the plant is truly black turmeric which is devoid of curcumin.

Conclusion

Standardization method of medicinal plants is one of the foremost steps in achieving the proper authenticity of the crude drug which depicts its genuine nature. In our present investigation, an attempt was successfully made to evaluate the various standardization parameters for *C. yunnanensis* and this will be helpful to all the researchers working on different field related to *Curcuma* species. The presence of demethoxycurucmin is also one of the main characteristics features which can be considered as an essential chemical marker for this plant. Hence, by developing the standardization methods for *C. yunnanensis* it is possible to

differentiate its rhizomes from rhizomes of other *Curcuma* species which can be understood by comparing their various pharmacognostical and phytochemical parameters. This will mainly facilitate in maintaining the genuine nature of the drug and also in preventing the process of one drug being adulterated with another.

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Conflict of interest

The authors report no conflict of interest.

References

1 Chaveerach A, Sudmoon R, Tanee T, Mokkamul P, Sattayasai N, *et al.*, Two new species of *Curcuma* (Zingiberaceae) use1d as cobra-bite antidotes, *J Syst Evol*, 2008, **46**(1), 80-88.

2 Sirirugsa P, *The genus Curcuma (Zingiberaceae) in Thailand, Songkla*. Ph D Thesis, Prince of Songkla University, Thailand, 1966.

3 Bhaumik M and Samati H, *Curcuma yunnanensis* N. Liu & S. J. Chen (Zingiberaceae)- A new record for India, *J Bombay Nat Hist Soc*, 2008, **105**(1), 113-114.

4 Zhang L, Wei J, Yang Z, Chen F, Xian Q, *et al.*, Distribution and diversity of twelve *Curcuma* species in China, *Nat Prod Res*, 2018, **32**(3), 327-330.

5 Anonymous, Curcuma Linnaeus: Flora of China, 2000, **24**, 359-362.

6 Brain K R and Turner T D, *The Practical Evaluation of Phytopharmaceuticals*, vol 1, (Bristol: Wright-Scientechnica, United Kingdom), 1975, 36-45.

7 Khandelwal K R, *Practical Pharmacognosy: Techniques and Experiments*, 17th edn, (Nirali Prakashan Publisher, New Delhi), 2007, 9-22.

8 Johansen D A, *Plant Microtechnique* (McGraw Hill, New York), 1940, 182.

9 Anonymous, *World Health Organization (WHO), Quality control methods for medicinal plant materials*, *Geneva*, (A.I.T.B.S Publishers, New Delhi, India), 2002, 8-60.

10 Anonymous, *Indian herbal Pharmacoepeia* (Indian Drug Manufacturers' Association, Mumbai), 2002, 1-521.

11 Chase C R and Pratt R, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J Am Pharm Assoc*, 1949, **38**(6), 324-331.

12 Laloo D, Prasad S K, Kumar M and Hemalatha S, Pharmacognostical and phytochemical standardization of the roots of *Potentilla mooniana* Wight, *Pharmacog J*, 2014, **6**(1), 70-79.

13 Kokate C K, *Practical Pharmacognosy*, 1st edn, (Vallabh Prakashan Publisher, New Delhi), 1986, 15-30.

14 Kumaran A and Karunakaran R J, *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India, *LWT Food Sci Technol*, 2007, **40**(2), 344-352.

15 Grubesic R J, Vukovic J, Kremer D and Vladimir-Knezevic S, Spectrophotometric method for polyphenols analysis: Prevalidation and application on *Plantago* L. Species, *J Pharm Biomed Anal*, 2005, **39**(3-4), 837-842.

16 Wagner H, Bladt S and Zgainski E M, Plant Drug Analysis: A thin layer chromatography atlas, 2nd edn (Springer-Verlag Berlin Heidelberg, New York), 1984.

17 Anonymous, *Curcuma amada* Roxb (Karpura haridra) in *Quality control standards of Indian Medicinal plants*, Vol I, (Indian Council of Medical Research, New Delhi, India), 2003, 82-88.

18 Sherlija K K, Remashree A B, Unnikrishnan K and Ravindran P N, Comparative rhizome anatomy of four species of Curcuma, *J Spices Aromat Crops*, 1998, **7**(2), 103-109.

19 Jain S D, Pathak R, Koka S S and Nema R K, Evaluation of quality control parameters of *Curcuma aromatica* Salisb, *J Pharmacog Phytochem*, 2016, **5**(5), 51-54.

20 Paramasivam M, Aktar M W, Poi R, Banerjee H and Bandyopadhyay A, Occurrence of curcuminoids in Curcuma longa: A quality standardization by HPTLC, *Bangladesh J Pharmacol*, 2008, **3**(2), 55-58.