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Research Article

ESTIMATION OF CURCUMINOIDS IN *CURCUMA KARNATAKENSIS* (WHITE TURMERIC) - AN ENDEMIC TAXON

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ABSTRACT

Objective: To estimate the contents of curcuminoids in two samples of *Curcuma karnatakensis* collected from different localities.

Methods: Quantification of curcuminoids was estimated by ultra-high performance liquid chromatography-mass spectrometry/selected reaction monitoring (UHPLC-MS/SRM) method in two samples of *C. karnatakensis* for the first time. Fine powder of rhizomatous rootstocks of two plant samples collected from different habitats were served as Samples A and B. The MS used for the metabolite analysis is a Vantage TSQ triple stage quadrupole MS equipped with heated electrospray ionization. The MS is coupled with an Agilent 1290 infinity UHPLC system. A stock solution of curcuminoid standard was prepared by dissolving 5 mg of standard in 1 mL of methanol. Seven different concentrations of standard (0.15-10 ng on column) were injected for the UHPLC-MS/SRM analysis. Separations were performed using a C-18 column with a flow rate of 0.2 mL/minute.

Results: Contents of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were found to be varied in two samples and lowest than any other species of *Curcuma* studied. Variation in the contents may be due to their different habitats in which they are growing.

Conclusion: The present attempt of analyzing the contents of curcuminoids in this endemic taxon for the first time will provide the basis for further pharmacological analysis to authenticate the efficacy of these active principles as the curcuminoids are known for varied pharmacological activities.

Keywords: *Curcuma karnatakensis*, White turmeric, *Zingiberaceae*, Endemic, Curcuminoids, Ultra-high performance liquid chromatography-mass spectrometry/selected reaction monitoring.

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INTRODUCTION

The taxa categorized under the genus *Curcuma* of the family *Zingiberaceae* are known for their medicinal properties. The rhizome of known species of *Curcuma* yield a yellow - orange and flavor powder when dried and ground except *Curcuma zedoaria*, which is known as white turmeric that yield white powder. *Curcuma longa* commonly referred as turmeric, has a long history of use in food and as medicine. A yellow powder obtained from dried rhizome of *C. longa* has been associated with various pharmacological activities such as anti-inflammatory, antiviral (against human immunodeficiency virus), and anticancerous [1-4]. The three main constituents (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) of turmeric powder have been identified as active ingredients, which are responsible for the various pharmacological activities, attributed to *C. longa* [5,6]. Extensive phytochemical and pharmacological studies were conducted in *C. longa* to estimate the curcuminoids and to study their biological activities [7-10].

Curcuma karnatakensis Amalraj, Velayudhan, and Muralidharan is endemic to Karnataka and reported from only two places. The type locality is near Hirehalli which falls under Western Ghats range of Karnataka region, India [11]. The other locality from where this taxon is reported is Dharwad, Karnataka – plain region with different climatic conditions [12]. It is a perennial as other members of *Zingiberaceae* and grows under shade in red-clay soil (Fig. 1a). The fully grown plant has a short rootstock with stipitate tubers, which are fusiform or conical, white inside (Fig. 1b). During the month from May to September, a spike inflorescence with pale yellow and rosy flowers emerges from the ground followed by broadly ovate dark green leaves (Fig. 1c). Leaves are broadly ovate-elliptic horizontal; petiole shorter than lamina. Inflorescence is spike, which is either lateral or central with pinkish-green bracts. Flowers are rose-to-rosy white with pale yellow corolla tube that has three rose-colored lobes (Fig. 1d). Labellum is three lobed; middle lobe is large, deeply bifid with bright yellow band in the center. Staminoides are multi-colored; terminal part - white, middle part –rose, and basal part - pale yellow. Anther is white with downward pointing rose-colored spur; ovary trilocular with axile placentation.

Except the above taxonomic description and an attempt to raise multiple shoots through *in vitro* culture, the plant is totally under exploited in respect of its chemical constituents-active principles [13]. Unlike the other members of *Curcuma* the dried rhizomatous rootstocks yield white powder as in *C. zedoaria* which is also well known for its medicinal properties and high starch content [14,15]. Hence, the objective of present study is to estimate the contents of curcuminoids in the rhizomatous rootstocks of *C. karnatakensis* as a step forward towards the understanding of its pharmacological importance.

METHODS

Plant source

Two samples were collected from their natural habitat. The specimens were collected during flowering season, i.e., from May to September. The Holotype - Amalraj 807 (MH) is deposited in National Bureau Plant Genetic Resources New Delhi at Thrissur, Kerala state, India [11]. Authenticated herbarium of the specimens of Sample A is deposited in the department of Botany, Karnatak Science College, Dharwad, Karnataka [12]. The herbarium of the two samples studied in the present investigation were authenticated accordingly and deposited in the Department of Botany, Bengaluru University, Bengaluru - (Voucher number: BUB 202 and 203).

Sample preparation

Rhizomatous rootstocks were air-dried for 6 days, cut into small segments before crushed into a fine powder with the help of pestle and

mortar, and stored at 4°C until analysis and quantification. High purity MS grade solvents such as methanol and acetonitrile were obtained from Merck Millipore (Merck Millipore India Pvt., Ltd., Bengaluru). Metabolites were extracted from 10.5 mg of the dried powder of both samples using 1 mL of methanol (vortexed and sonicated for 3 minutes). After centrifugation at 13,000 rpm for 5 minutes the supernatant was dried under speed vacuum. It was then reconstituted in 50 µL methanol and injected 10 µL for the analysis by ultra-high performance liquid chromatography (UHPLC) - MS/selected reaction monitoring (SRM) method.

Standard preparation

Pure mixture of curcuminoids was procured from Sd-fine chemicals, India. A stock solution was prepared by dissolving 5 mg of standard in 1 mL of methanol and stored at 4°C.

UHPLC - MS conditions

UHPLC

An Agilment 1290 infinity UHPLC instrument (Agilent technologies India Pvt., Ltd., India) was coupled to the MS to achieve chromatographic separation. It is equipped with column, oven, auto samplers, and thermocontroller. Separations were performed using a C-18 column (Shim-Pack XR-ODS111, 2.1 × 150 mm, 2 µm) with a flow rate of 0.2 mL/minute. Mobile phase A contains 10 mM of ammonium acetate in water along with 0.1% of formic acid. Whereas mobile Phase B is acetonitrile with 0.1% formic acid. A linear gradient was initiated with a following program: 5% B at 0-2 minutes, 25% B at 3 minutes, 25-100% B at 3-10 minutes, 100% B at 10-12 minutes, and finally 5%B at 12-17 minutes. The temperature in the column oven was maintained at 40°C and in the auto sampler was at 10°C. The sample (10µL) was injected using flow through needle injection mode with a total run time of 17 minutes. To avoid the carry over problem, we have enabled needle wash with acetonitrile (0.1% formic acid) before sample injection.

MS

The MS (thermo scientific - TSQ vantage) was operated in positive ionization mode. The operating conditions were as follows: Spray voltage -3500V; Vaporizer temperature −100°C; Sheath gas flow rate-20 (arbitrary units) and auxiliary gas flow rate -10 (arbitrary units) collision gas: Organ S-lens voltage and collision energy optimized for curcuminoids were incorporated in the method; Scan time is 50 ms/transition. The MS-injector setting of 0-3 minutes for waste, 3-12 minutes for load, and again 12-15 minutes for waste. To check the most intense product ions (MS/MS scans), we have infused 10 µg/mL solution of curcuminoids at a flow rate of 5 µL/minutes through inbuilt syringe pump. We have used collision-induced dissociation and applied collision energy to the precursor ion and monitored the product ions. The third quadrupole scan was set from m/z 50-500 with a cycle time of 1 second to look at the produc tions. The optimized S-lens voltage and collision energy were used to obtain the most intense product ions for curcuminoids. Seven different concentrations of standard (0.15-10 ng on column) were injected for the UHPLC-MS/SRM analysis. For curcumin (369 → 177), demethoxycurcumin (399 → 255), and bisdemethoxycurcumin (309 → 224) transitions were selected. The area under the peak was used to construct the standard curve and to calculate the concentrations of curcuminoids from sample.

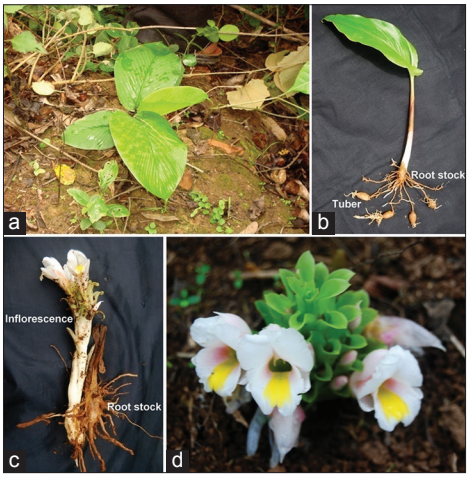

Fig. 1: *Curcuma karnatakensis* - morphology. (a) plant in wild - habitat; (b) plant with rootstock and stipitate tubers; (c) plant with rootstock and spike inflorescence; (d) spike inflorescence with fully bloomed flowers

RESULTS AND DISCUSSIONS

Quantification of curcuminoids in two samples of *C. karnatakensis* was carried out for the first time following UHPLC-MS/SRM method. MS-based target quantification of biological extracts is considered as quite precise and reliable because of its specificity, sensitivity, and selectivity [16-18]. Goren *et al.* [5] have compared NMR with LC-MS/MS method for quantification of curcumin in commercial turmeric samples. They are of the opinion that LC-MS/MS method is more suitable for trace analysis of curcumin, while NMR technique for rapid analysis. While Paramasivam *et al.* [19] have quantified the curcuminoids by HPTLC method citing its advantages such as shorter analysis time and minimal sample preparation. Jayaprakasha *et al.* [10] employed improved HPLC method for the determination of curcuminoids. Contents of curcumin, demethoxycurcumin, and bisdemethoxycurcumin were found to be 1.06±0.061 to 5.65±0.040%, 0.83±0.047 to 3.36±0.040%, and 0.42±0.036 to 2.16±0.06%, respectively, in four different samples of commercially available curcumin. *C. zedoaria*, known as white turmeric is a well-known ethnomedicinal plant that is also used in Ayruveda. Paramapojn and Gritsanapan [20] have analyzed curcuminoids in ethanolic extracts of *C. zedoaria* and found an average of 2.73±1.24% w/w of curcumin, average of 7.37±2.71% w/w of demethoxycurcumin and average of 1.40±0.82% w/w of bisdemethoxycurcumin. We have used the highly sensitive UHPLC-MS/SRM method to quantify the curcuminoids. When the stock solution was injected (10 ng on column), curcumin is the most abundant compared to the other two curcuminoids. We have checked the MS/MS spectrum of curcuminoids to select the specific product ion for the quantification. Both curcumin and demethoxycurcumin showed 177 m/z as one of the major product ions and in the bisdemethoxycurcumin, 149 m/z as a major product ion. We have selected these product ions for the quantification of both standards and samples. All three showed clear sharp eluting peaks in the gradient elution. Bisdemethoxycurcumin eluted slightly before at 9.47 minutes, demethoxycurcumin was at 9.59 minutes and curcumin at 9.74 minutes (Fig. 2). Since curcumin is abundant (90%) in the curcuminoid standard; we have calculated the concentration of demethoxycurcumin and bisdemethoxycurcumin derivatives based on the curcumin amount. We have constructed seven-point standard curve (0.15-10 ng on column) from the stock solution to calculate the concentration of curcuminoids in the samples. It looks like there is around 10% (1.19 ng) of demethoxycurcumin and 0.1% (0.14 ng) of bisdemethoxycurcumin present in 10 ng of stock. In the same way, we have also calculated the curcuminoids from both samples. As shown in Table 1, the contents of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in Sample A were found to be 0.96 ng (0.0453%), 0.09 ng (0.0042%), and 0.02 ng (0.0009%) in 2.12 mg of dried powder as against 1.53 ng (0.0722%), 0.14 ng (0.0066%), and 0.01 ng (0.0005%) the Sample B, respectively (Fig. 2).

It is well established that biosynthesis of active principles in medicinal plants is largely depends on the environmental conditions [21,22]. Variations found in the contents of curcuminoids in two samples may be due to their two different habitats - Sample A growing in plains, whereas Sample B growing in Western Ghats region. Akbar *et al.* have found variations in the curcumin content in 131 genotypes collected from different agro climatic zones of India [23]. According to Liu *et al.* [24] the annual average precipitation and soil organic matter are two important limiting factors that have caused 98.13%

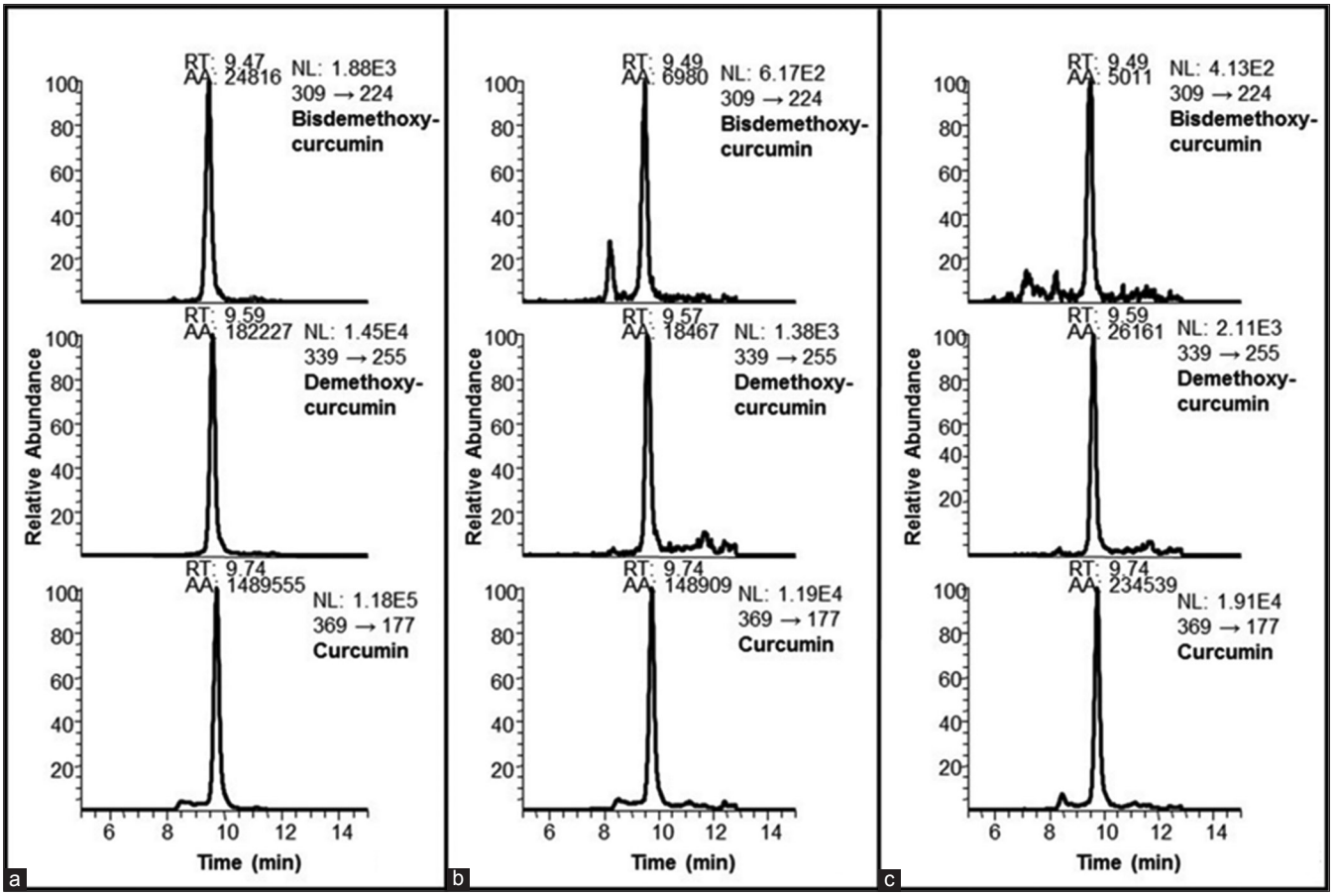

Fig. 2: Ultra-high performance liquid chromatography-mass spectrometry/selected reaction monitoring chromatogram - (a) curcuminoid standard, (b) Sample A and (c) Sample B

Table 1: Contents of curcuminoids in sample A and B of *C. karnatakensis* analyzed by UHPLC-MS/SRM method

Samples	Area	Slope	Intercept	Concentrations (ng) (%)
Sample A				
Curcumin	137510	139039	3664	0.96 (0.0453)
Demethoxycurcumin	16216	139039	3664	0.09 (0.0042)
Bismethoxycurcumin	6305	139039	3664	0.02 (0.0009)
Sample B				
Curcumin	216142	139039	3664	1.53 (0.0722)
Demethoxycurcumin	23642	139039	3664	0.14 (0.0066)
Bismethoxycurcumin	4372	139039	3664	0.01 (0.0005)

C. karnatakensis: Curcuma karnatakensis, UHPLC: Ultra-high performance liquid chromatography, MS: Mass spectrometer, SRM: Selected reaction monitoring

of the total geographical variation of the active ingredient contents in *Sinopodophyllum hexandrum.* As Sample B, growing in Western Ghats receives more annual average precipitation and exposed to significantly more organic matter compared to Sample A which grows in plains. However, in *S. hexandrum*, the annual average precipitation and altitude are negatively correlated and organic matter is positively correlated with the contents of active ingredients. Whereas the contents of active principles in *Sinapsis alba* was reported to have enhanced with increased precipitation as in the present study [25]. Liu *et al.* [22] have however reported that the effect of various environmental factors on the contents of active ingredients varies with the type of the secondary metabolite and on the taxon. Altitude has negative correlation with the contents of tannins and shown positive correlation with the contents of flavonoids and rutin in populations of *Potentilla fruticosa* collected from different localities in China [22]. Arya *et al.* have correlated the antioxidant potential of *C. longa* to geographical and climatic conditions and concluded that the occurrence of bioactive compounds can be affected by these conditions [26].

CONCLUSION

The content of curcuminoids in *C. karnatakensis* was quantified using UHPLC-MS/SRM method that is considered as highly sensitive and can analyze small quantities in the sample. Perusal of literature reveals that the amount of curcuminoids detected in the present samples is the lowest reported so far in any other species of *Curcuma* including *C. zedoaria* - white turmeric. Further phytochemical and pharmacological studies are needed to ascertain the efficacy of these active principles in *C. karnatakensis*.

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