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. Pine 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, Curcuminoinds, Ultra-high performance liquid chromatography-mass spectrometry/selected reaction monitoring.

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, antirival (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-rose 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 axial placenta.

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 rootstock 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 Sample A is deposited in the department of Botany, Karnataka 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...
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/MSE method for quantification of curcumin in commercial turmeric samples. They are of the opinion that LC-MS/MSE 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%, 0.93±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 Ayurveda. Paramapojin 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 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-climate 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%
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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**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
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