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Original Article

Curcuminoid and essential oil components of turmeric at different stages of growth cultivated in Iran

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Abstract

Turmeric is an important herb used as medicine, condiment and cosmetic. Its main active compounds are curcuminoids and essential oils. The biomass production, essential oil composition and curcuminoid content of *Curcuma longa* are known to be dependent on the growth and development conditions of rhizome. Iran is a major importer of turmeric in the Middle East. The aim of this work was, therefore, to evaluate the curcuminoids and essential oil composition as well as curcumin content at different stages of growth of *C. longa* rhizome cultivated in Isfahan. Rhizomes of *C. longa* were obtained from Thailand and cultivated in University of Isfahan greenhouse. The growth media was 10 cm diameter pots containing perlite and cocoperlite watered using Hoagland nutrient solution. Plants were harvested at different stages of growth and rhizomes' fresh and dry weights were measured. The curcuminoids and the essential oils were analyzed by TLC and GC-MS, respectively. α-Phellanderen, terpinolen, α-zingiberene, β-sequiphellanderen, r-turmerone, and α-turmerone were the main components of essential oils. The curcuminoids were detected as curcumin, desmethoxy curcumin, and bisdesmethoxy curcumin. The turmerone in harvested rhizome accounted for almost 90% of the oil composition. It has been reported that the major compounds found in turmeric oil, up to 50-60%, are the sesquiterpene ketones, ß-, and r-turmerone. The rhizomes of the *C. longa* cultivated in Iran have similar flavor and color to those of the turmeric rhizome imported to Iran. It seems that Iranian turmeric production can be cured and dried for condiment use.

Keywords: *Curcuma longa*; Rhizome; Cultivation; Curcuminoid; Essential oil

INTRODUCTION

Curcuma longa L. (Turmeric) is a perennial rhizomatous herb which belongs to Zingiber family. It is originated in India and now is cultivated in tropical and subtropical parts of the world (1). Turmeric is an important herb and is widely used worldwide as medicine, condiment, dye and cosmetic (2). The main active compounds of turmeric are curcuminoids and essential oils. Curcumin, the most active curcuminoids found in turmeric, has been shown to possess a multitude of beneficial effects in the treatment of cancers (3-5), cardiovascular disease (6), inflammation (7,8) and alzheimer's disease (9). All these features of turmeric render it a good candidate for development of pharmaceutical usage. The biomass production, essential oil composition and curcuminoid contents of *C. longa* of the rhizome are affected by its cultivation conditions, including factors such as nutrient availability, growth media and climatic conditions (10-13). Most commercial turmeric productions contain 2-8% active curcumin (14). Iran is a major importer of turmeric in Middle East (15) and at the same time, the consumption of turmeric is increasing in Iran. Food and Agriculture Organization of the United Nations estimated that approximately 5000 tons of turmeric is imported annually to Iran for consumer use (15). In concordance with increasing use of turmeric as well as the high amount of imported turmeric to Iran, we decided to evaluate the possibility of cultivation of *C. longa* in Isfahan and also to

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assay the curcuminoids and essential oils composition of its rhizome as well as the curcumin production at different stages of growth.

MATERIALS AND METHODS

Plant materials

Rhizomes of *C. longa* used in this study were obtained from Chulalankarana University of Thailand on 5th of February 2002 and cultivated in University of Isfahan greenhouse. The rhizomes were sterilized for 30 min in Banomyl fungicide and simultaneously the peat-cocoperlite and the pots were inoculated for sterilization. The uniform rhizomes in size (5-7 cm in length and 1-1.5 cm in diameter) were selected and then were planted on 15th of February 2006. The growth media consisted of perlite and cocoperlite in 10 cm diameter pots. The pots were placed in a greenhouse with temperatures set at 30/25 ºC day/night and then a preventive drench of Banrot 40% WP (Scotts-Sierra, Marysville, OH) at 62.5 ml/100 l was applied immediately after planting. Once shoots had emerged, the budded rhizomes were moved to a 25 cm diameter pots and kept in greenhouse in a completely randomized design in 3 replicates. Plants were irrigated using Hoagland solution (16) daily to keep the soil moisture almost at field capacity. Plants were harvested at different stages of growth (2, 4, 6, 8, 10 months after budding). Harvested plants were separated into leaf, root and rhizomes and then wet and dry weights of each plant parts were measured.

GC analysis

The dried rhizome was grounded to a fine powder using a pestle and mortar. The essential oils from powdered rhizome were obtained by hydrodistillation methods immediately after collection (17). The essential oils were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectroscopy (GC-MS). GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with FID detector and a BP-1 capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The carrier gas was nitrogen with a flow rate of 2 ml/min, the oven temperature was initially set at 60 °C and then increased at a rate of 4 °C/min until reached to the temperature of 150 °C, then increased to 280 °C at a rate of 15 °C/min. Injector and detector temperatures were set at 280 °C.

GC-MS analysis

The mass spectra were recorded using a Hewlett Packard (HP) 6890 MS detector with EI source, coupled with Hewlett Packard 6890 gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The gas chromatographic conditions were set as described above for GC analysis. Mass spectrometer conditions were as follows: ionization potential 70 eV, source temperature 200 °C. Identification was based on retention data and computer matching with the WILEY275.L library as well as by comparison of electron-impact-mass spectra with those of relevant reference materials and the literature (18).

TLC analysis

The finely powdered rhizomes at various stages of collection (0.1 g) of *C. longa*, grown in Iran and also the control sample (original rhizome) were extracted by methanol (1 ml) at room temperature. The mixture was shaken for 30 min, filtered and the filtrate was chromatographed on a pre-coated TLC silica gel plate (Silica gel G 60 F_{254} plates). Chloroform:benzene:ethanol (45:45:10) was used as developing solvent system. Visualization of the separated bands was carried out under U.V. light (365 nm) after spraying with boric acid:methanol reagent. The individual zones were detected and identified. Comparison was made with the relevant literature and pure curcumin as standard (15,19).

Spectrophotometric analysis

A known amount of powder prepared from rhizomes collected at various stages of growth (2, 4, 6, 8 and 10 months after budding) was quantitatively extracted with sufficient amount of redistilled methanol. The intensity of their absorbance was measured using a spectrophotometer (Secomam 1000, France). The methanolic extract of samples were

quantitatively diluted in order that their absorbance lay in the linear part of the calibration curve and the absorbance of each sample was then measured at 530 nm against the acetic acid as blank. Curcuminoids content of each sample was then calculated using the published pharmacopoeia equation (20) and their total contents were reported as curcumin.

RESULTS

The GC-MS analysis of *C. longa* rhizome (Fig. 1) showed the presence of 6 major peaks eluted at 4.93, 6.97, 19.34, 20.14, 24.79, and 25.43 min which were identified as α-phellanderen, terpinolen, α-zingiberene, β-sequiphellanderen, r-turmerone, and α-turmerone, respectively. Simultaneously, 6 minor compounds were also identified. The retention times and Kovat indices of major and minor compounds are presented in Table 1. Turmerone was one of the main compounds found in the rhizome and is also believed to be an intermediate for formation of zingiberene, and sesquiphellanderen, which are two major sesquitrpene of the plant rhizome (21,22).

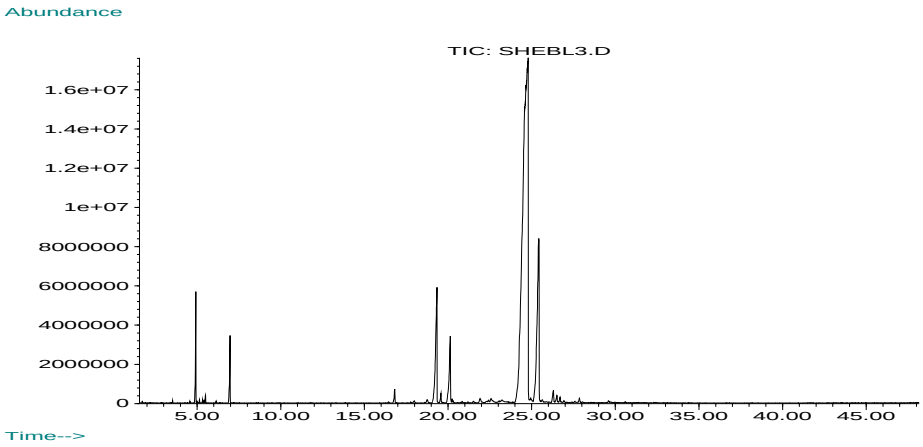

Fig. 1. Gas chromatogram of Turmeric rhizomes essential oil

Table 1. Composition of essential oil of *Curcuma longa*

Compound	%	RI
α -phellanderene	2.2	1005
α-terpinene	0.2	1018
p-cymene	0.4	1026
1& 8-cineol	0.4	1033
terpinolene	1.5	1088
β-Caryophyllene	0.6	1418
r-curcumin	0.8	1483
α -zingiberene	1.5	1495
β -bisabolene	0.4	1509
β -sesquiphellanderene	1.3	1524
r-turmerone	68.9	1591
α -turmerone	20.9	1664

Determination of curcuminoids was carried out using TLC analysis. Three curcuminoids were detected in *C. longa* rhizome when the 2, 4, 6, 8, and 10 months old rhizomes were extracted by organic solvent. As illustrated in Fig. 2 the curcuminoids were detected as three red fluorescent individual bands and identified by comparing with their corresponding reference standards as following; R_f = 0.4 curcumin, R_f = 0.35 desmethoxy curcumin, and R_f = 0.25 bisdesmethoxy curcumin (20). Quantitative analysis of curcuminoids was carried out at all stages of growth using their spectrophotometer visible absorbance (20). The production of 0.24 g rhizome and 0.6 mg curcumin from each plant respectively, at the second month (Table 2 and 3) indicated that rhizome initiation and curcumin synthesis is started before plant approaches this stage of growth and then reaches to its maximum values when the plants are 10 months old.

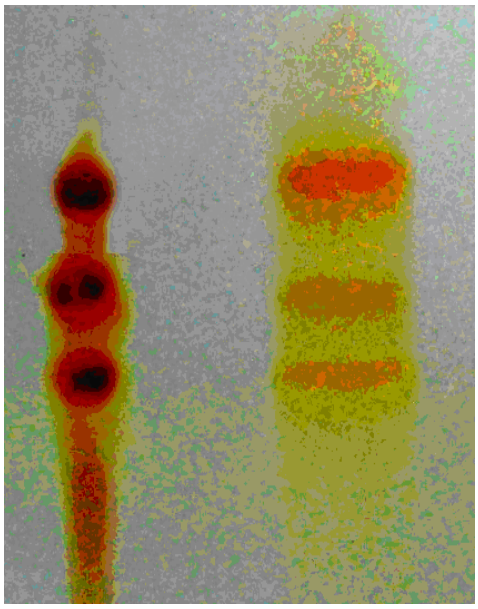

Fig. 2. TLC chromatogram of Turmeric rhizomes extract (right), with curcuminoids standard (left), R_f = 0.4 curcumin, R_f = 0.35 desmethoxy curcumin, and R_f = 0.25 bisdesmethoxy curcumin

DISCUSSION

The main sesquiterpene components of the essential oil of *C. longa* rhizome were determined as r-turmerone, α-turmerone, zingiberene, sesquiphellanderene and bisabolene. While the main monoterpene constituents were phellanderene, terpinolene, 1,8-cineol, para-cymene and terpinene (Table 1). There were no remarkable differences between the *C. longa* cultivated in Iran and those cultivated in tropical countries in their mono and sesquiterpenoids pattern of the essential oils (23,24). In contrast, the contents of two sesquiterpenes of zingeberene and r-curcumene have been reported to be up to 25% and 35% respectively (25). The interesting point to note is that in the present study, turmerone was determined to be about 90% of the total oil composition. Similar to our findings, the sesquiterpene ketones, ß-, and r-turmerone were found to be the major constituents of the turmeric oil comprising up to 60% of the oil (24).

Results obtained from this experiment showed that the dry weight and curcumin content of *C. longa* rhizome increased over maturation of the plant. Statistical analysis indicated a linear significant relationship between the concentration of curcumin and biomass of the rhizome (r^2 = 0.91). According to Table 2, the smallest monthly variation ranges were observed within February, while the largest one in November and May. The mean values of the curcumin are different between 4 and 6 months of harvest that is different for the weight of rhizomes at these stages. With regards to the amount of harvested curcumin at different stages of growth, no significant difference between 8 and 10 months of harvest was observed. This means that harvesting the plant when it is 8 to 10 months old does not make a significant difference in terms of curcumin contents. Therefore plant can be harvested two months earlier and there would be no curcumin gain for this 2-month delay. There are a number of reports that the concentration of phytochemicals such as alkaloids (26), essential oils (27), sesquiterpenes (28), phenolics (29), taxanes (30), and monoterpenoids (31) vary during seasons. Although Isfahan has a temperate climate, it seems that temperature, rainfall, and hours of day light are climatic factors of great importance for the development of *C. longa*.

Table 2. Curcumin content and rhizome dry weight of *C. longa* at different stages of growth. The different letter on the mean values indicated the significant difference within the means of each column at 5% on the basis of Duncan's multiple test range

Growth stage (month)	Rhizome (g/plant)	Curcumin (%)	Weight of curcumin (mg/plant)
2	0.24^{d}	0.25^{c}	0.6^{e}
4	0.55^{c}	1.2^{b}	6.6^{d}
6	2.40^{b}	1.5^{b}	36.0^{c}
8	5.80^{a}	2.6^{a}	150.8^{b}
10	6.30^{a}	2.7^{a}	170.1^{a}

Table 3. The rate of rhizome and curcumin production at different stage of growth. The different letter on the mean values indicated the significant difference within the means of each column at 5% on the basis of Duncan's multiple test range.

Harvest time (month)	Rhizome production rate (mg/plant/day)	Curcumin production rate (mg/plant/day)
2	4.0^{c}	0.01^{d}
4	4.6^{c}	0.06^{c}
6	10.0^{b}	0.20^{b}
8	24.2^{a}	0.63^{a}
10	21.0^{a}	0.57^{a}

According to the data presented in Table 2, the differences in the weight of rhizome at different stages of growth were statistically significant and also their weights correlated significantly to their curcumin content. However, the rise in curcumin content was not parallel to the rise in the rhizome weight. The results in Table 3 indicate that curcuma exhibited higher growth rate between month 4 and 8, however, higher amount of curcumin was produced between month 2 to 4, month 4 to 6, and then month 6 to month 8. This behavior might be due to the allocation of synthesis to different locations or due to selection of different synthesis pathways for different components production. These results show the time that is most appropriate to harvest *C. longa* rhizome in order to obtain an optimum yield of curcumin. It is recommended that the rhizomes in most plant be collected at the end of vegetation period (32).

The maximum curcumin content of the rhizome was 2.7% which is lower than curcumin content of plant cultivated in most tropical countries. Previous literature reported curcumin concentrations ranging from 2.9 to 5.1% (33), and 0.61 to 1.45% (8). Our values for curcumin contents ranged from 0.25 to 2.7% depending on the phenological stages. The yield of curcumin from commercial dried turmeric root is about 5% (34).

Although the curcuminoid content of the rhizomes showed a lower accumulation, the curcuminoid pattern was rather similar to previous reports. The lowest and highest curcumin content for all samples tested in the current study are somewhat lower than those found in some preceding works (13,33,35). These results suggest that the rhizome of the *C. longa* cultivated in Iran has similar flavour and color to those of turmeric rhizome imported to Iran. It seems that although there are considerable differences between Iran and Thailand climatic conditions, Iranian turmeric can be cured and dried for condiment use. The present findings also demonstrated the importance of performing detailed cultivation experiments, including seasonal variations, on the content of active compounds when introducing new crop plants for the production of known chemicals.

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