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Research Article

DETERMINATION AND COMPARISON OF THE CURCUMINOID PIGMENTS IN TURMERIC GENOTYPES (*CURCUMA DOMESTICA* VAL) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Medicinal plants have the rich source of medicinally and agriculturally useful compounds since they produce raw material for pharmaceutical and phytochemicals for manufacturing drugs. Thus, the germplasm collection, conservation, evaluation and cataloging is necessary. In addition to this, an understanding and exploitation of their existing variability is necessary to find superior genotype having high yield, better quality and high metabolic contents. Considering these facts, the present investigation was carried out on the important medicinal plant: Turmeric. Turmeric (*Curcuma*) is the common name used for the dried rhizome of *Curcuma longa* L., a monocotyledonous plant belonging to the family *Zingiberaceae*. It has been used in traditional medicine as a household remedy for various diseases including anorexia, cough, diabetes, wounds, hepatic disorders, rheumatism and sinusitis. Curcumin (diferuloymethane), the main yellow bioactive component of *Curcuma* has been shown to have a wide spectrum of biological actions. These include its anti-inflammatory, antioxidant, ant carcinogenic, ant mutagenic, anticoagulant, ant fertility, ant diabetic, antibacterial, antifungal activities. Twenty-two genotypes of *Curcuma* were collected from different geographical locations in Chhatisgarh and adjoining areas and High performance liquid chromatography approached to determine curcuminoids in different genotypes of *Curcuma*. Curcuminoids were extracted by Soxhlet Apparatus with organic solvents like Ether, Benzene and Ethanol and purified through column chromatography. These purified fractions were identified by thin layer chromatography and subjected to HPLC. The total curcuminoid percentage ranged from 0.069 to 6.16.The maximum curcuminoid content was found in genotype T22 (Wild local 2) (6.1%) followed by T21 (Wild local 1) (4.04%), T1 (TCP-1) (2.817%), T2 (TCP-2) (2.28%). The variation was recorded for curcumin content and its analogs across all the genotypes. The curcumin content varied from 1.87% to 0.015%. The highest curcumin percentage was recorded in the genotype T22 (Wild local 2) (1.87%) followed by T21 (Wild local 1) (1.76%), T2 (TCP-2') (0.932%), T15 (Maharashtra local) (0.868%).

Keywords: Curcuminoids, HPLC Technique, Turmeric

INTRODUCTION

The success of the drug discovery process is often a function of the diversity of chemo types examined. Natural products screening represents a potential source of organic chemicals of unparallel diversity. The screening of natural products is one of the earliest steps in drug discovery-*Lead identification*.

A *lead compound,* also frequently referred to as a chemical template, is a compound with many of the characteristics of a desired new drug which will be used as a model for chemical modification. Medicinal plants comprise a group of large number of plant species that produce raw material for pharmaceuticals and phyto-chemicals for manufacturing drugs. In the commercial market, medicinal herbs are used as raw drugs, extracts or tinctures. The international medicinal plants market is worth US $60 billion per year, and growing at the rate of 7% per annum[1]. Plants have contributed more than 7,000 different compounds in use as heart drugs, laxatives, diuretics, antibiotics, decongestants, analgesics, anesthetics, ulcer treatments anti-parasitic compounds and so on[2].

For the last few decades, phytochemistry (study of plants) has been making rapid progress and herbal products are becoming popular. Exploitation of already existing variability in available germplasm is very important to identify superior genotype for local condition. Studies on the extent of variation in yield and quality characters of medicinal plants are important for selection of genotype with higher yield and better quality. The present investigation was undertaken to evaluate the performance of different turmeric (*Curcuma*) genotypes with regard to their quality with a view to identify the superior types with high metabolite content.

A medicinal herb can be compared with a chemical factory due to presence of number of chemical constituents like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). With introduction of sophisticated techniques, the scientists started exploring the plant flora for active constituents. The concept of standardization has great impact on quality of herbal products. Standardization helps in adjusting the herbal drug formulation to a defined content of a constituent or constituents with therapeutic activity.

Turmeric is used as traditional medicine in many countries because of the antibiotic and antiseptic effects of curcumin, an important constituent of turmeric. A yellow-pigmented fraction isolated from the rhizomes of *Curcuma* contains curcuminoids belonging to the dicinnamoyl methane group. Curcuminoids are present to the extent of 3 - 5 %[3]. It is an important active ingredient responsible for the biological activity of *Curcuma* . Though the major activity is anti inflammatory, it has also been reported to possess antioxidant, anti allergic, wound healing, anti bacterial, anti fungal and anti tumor activity [4]. In the present study we analyzed and quantified the curcuminoids from turmeric (*Curcuma*) for identification of best quality genotype.

Experimental work

Experimental materials

Twenty-two genotypes of *Curcuma* were collected from the farmers fields, forests of Chhattisgarh and were grown in the field are shown in Table 1.

A known quantity of turmeric (*Curcuma*) powder (5.0 g) was used for extraction of curcuminoids. The extraction was done by soxhlet apparatus with petroleum ether, benzene and 95% ethanol. The ethanol extract was concentrated to small volume and passed through silica gel (60-120 mash) column (2 cm x 25 cm) saturated with benzene. Fractions were collected and examined by thin layer chromatography. The spots were developed in acetone and ethanol (50:50) mixture with the standard and iodine used as a spraying agent. The active fraction eluted by ethanol and dried though vacuum dryer. The dried alcoholic extract dissolved in HPLC solvents and subjected to HPLC for separation and estimation of curcuminoids.

Table 1: Collection of *Curcuma* genotypes from different regions

Genotype no.	Common name	Collection from
T 1	TCP-1	Raigarh
T 2	TCP-2	Raigarh
T 3	RH-5	Raigarh
T 4	IT-1	Raigarh
T 5	IT-2	Raigarh
T 6	IT-3	Raigarh
T 7	IT-4	Raigarh
T 8	IT-5	Raigarh
T 9	IT-6	Raigarh
T 10	IT-7	Raigarh
T 11	IT-8	Raigarh
T 12	IT-9	Raigarh
T 13	Shyama Haldi	Bilaspur
T 14	Local Bilaspur	Bilaspur
T 15	Maharashtra local	Raipur
T 16	Ama haldi	Raipur
T 17	Ambikapur Local	Ambikapur
T 18	Bastar Collection	Raipur
T 19	Pratibha	Raigarh
T 20	Local Raipur	Raipur
T 21	Wild local 1	Bastar
T 22	Wild local 2	Bastar

HPLC conditions

The HPLC method (http//www.curcuminoid.com)[3] was used to separate and estimate the curcuminoids. The area under the major peaks was measured for quantitative analysis of the samples.

Chromatographic system (Model No. 1100 series)

HPLC Pump: LC 10 AD – Agilent make
HPLC Detector SPD 10 A – Agilent make
Syringe: Hamilton 100 µl Syringe
Column: 250 x 4.8 mm SS (C-18) column containing amino packing 5 micron particle size.

Instrument conditions

Mobile Phase: Ethanol and methanol were mixed in the ratio 60: 40 and the mixture was degassed and filtered.
Injection size: 20 µl
Flow rate: 1 ml per minute
Detector: UV
Wavelength: 254 nm

Preparation of standards

25 mg of standard curcumin was accurately weighed into a 25 ml volumetric flask and dissolved in the mobile phase with warming and the volume was made up with solvent mixture.

Preparation of sample

25 mg of the sample was accurately weighed into a 25 ml volumetric flask, dissolved and diluted to volume with methanol and further diluted to inject in HPLC machine.

Procedure

Standard preparation and sample preparation were injected, separately into the chromatograph The response of the sample preparation in terms of areas under the three major peaks corresponding to two analogs and curcumin were measured.

RESULT AND DISCUSSION

A mixture of curcuminoid in the ethanol extract of powdered samples of *Curcuma* were analyzed by an Agilent make C18 column at a flow rate of 1.0 ml/min and detected at a wavelength of 254 nm. Well-resolved chromatograms of curcuminoids were obtained with a gradient elution of ethanol–methanol 60:40 (v/v). The total time required for a single analysis was approximately 20 min. The curcuminoid content varied in the *Curcuma* genotypes. HPLC analysis profile was obtained for standard curcumin at 2.445 min in 20 min run time as shown in fig 1. Purified samples were injected in a fixed loop of 20 ul. The chromatogram from the HPLC analysis showed three major peaks corresponding to curcumin, analog 1 and analog 2shwon in fig 2. The maximum curcuminoid content was found in genotype T22 (Wild local 2) (6.1%) followed by T21 (Wild local 1) (4.04%), T1 (TCP-1) (2.817%), T2 (TCP-2) (2.28%). Tonnesen used the HPLC method for separation and estimation of curcumin and its structural isomers[5].

The variation was recorded for curcumin content and its analogs across all the genotypes. The curcumin content varied from 1.87% to 0.015%. The highest curcumin percentage was recorded in the genotype T22 (Wild local 2) (1.87%) followed by T21 (Wild local 1) (1.76%), T2 (TCP-2') (0.932%), T15 (Maharashtra local) (0.868%). The curcumin content was found comparatively low in T16 (Ama haldi) (0.01%) followed by T10 (IT-7) (0.131%), T9 (IT-6) (0.168%). The percentage of analog-1 varied from 1.08 to 0.02 across the genotypes. The maximum percentage of analog 1 was found in genotype T22 (Wild local 2) (1.08%) followed by T2 (TCP-2) (0.932%). The minimum percentage of analog 1 was found in T16 (Ama haldi) (0.02%) followed by T9 (IT-6) (0.06%). The percentage of analog-2 varied from 3.21 to 0.02% across the genotypes.

The highest content of analog 2 was found in T22 (Wild local 2) (3.21%) followed by T21 (Wild local 1) (1.56%). Comparatively low percentage of analog 2 was found in T16 (Ama haldi) (0.02%) followed by T10 (IT-7) (0.103%). The turmeric (*Curcuma*) is an important spice valued for the characteristics yellow color and flavor. Curcuminoids constitutes the major coloring matter in turmeric. The three major constituents were identified as diferuloyl methane (curcumin) and its two analogs, viz. p-hydroxy-cinnamoyl-feruloyl methane and p,p- dihydroxy-dicinnamoyl-methane. The total curcuminoid percentage ranged from 0.069 to 6.16. The variation was observed in curcumin and its analogs. The highest curcuminoid content was recorded in T22 (Wild local 2) and T21 (Wild local 1), which were collected from forest area of Chhattisgarh. Study suggested that the Indian forests have pure and quality secondary metabolites as compared to cultivated crops.

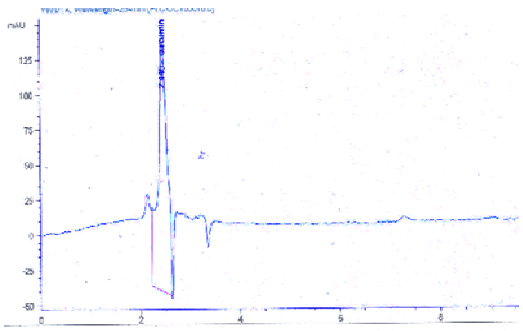

Fig. 1: Standard peak of curcumin at 2.445 retention time monitored at 254 nm wavelength

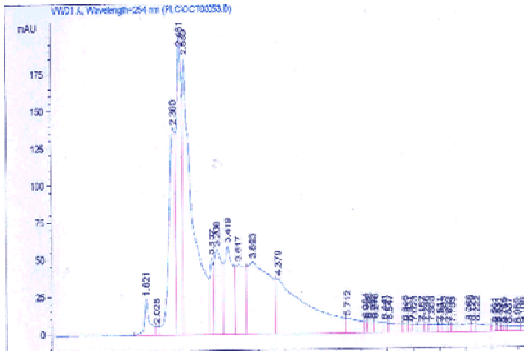

Fig. 2: Peaks of curcuminoid extract from genotype T4

Table 2: Variation in Curcuminoid contents of collected *Curcuma* genotypes

Genotypes	Curcumin (%)	Analog 1 (%)	Analog 2 (%)	Total Curcuminoid (%)
T 1	0.725	0.652	1.44	2.817
T 2	0.921	0.932	0.435	2.288
T 3	0.34	0.091	0.375	0.806
T 4	0.421	0.245	0.513	1.179
T 5	0.725	0	0	0.725
T 6	0.391	0.222	0.522	1.135
T 7	0.802	0.177	0	0.979
T 8	0.497	0.208	0.716	1.421
T 9	0.168	0.061	0.351	0.58
T 10	0.131	0.288	0.103	0.522
T 11	0.313	0.402	0	0.715
T 12	0.3	0.133	0.302	0.735
T 13	0	0	0	0
T 14	0.408	0.18	0.516	1.104
T 15	0.868	0.28	0.26	1.408
T 16	0.02	0.022	0.023	0.065
T 17	0.578	0.226	0.505	1.309
T 18	0.591	0.242	0.57	1.403
T 19	0.433	0	0.34	0.773
T 20	0.293	0.116	0.306	0.715
T 21	1.76	0.725	1.56	4.045
T 22	1.87	1.08	3.21	6.16

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