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COMPARISON OF CURCUMIN CONTENT OF SOME TURMERIC SAMPLES COLLECTED FROM DIFFERENT PLACES OF NORTHEAST INDIA

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ABSTRACT
Curcumin, derived from the rhizome Curcuma longa, is one of the primary ingredients in Turmeric .Curcumin, the active molecule present in Curcuma longa is known for its antitumour, antioxidant, antiarthritic, anti-inflammatory and antimalarial activities. The pharmacological activity of Curcuma longa depends on quality of curcumin present. Turmeric samples were collected from different places of Northeast India and analyzed for its curcumin content and genetic diversity with RAPD technique. The results of the study disclose that in Hydro-alcoholic extract, turmeric samples collected from Shillong and Arunachal Pradesh have comparatively higher percentage of curcumin than the other samples. In case of Methanolic extract turmeric samples of Shillong and Dibrugarh have higher percentage of curcumin as compared to the other samples. The HPTLC separation was performed on precoated aluminium backed HPTLC plates of 0.2 mm layer thickness with silica gel 60 F254 with Chloroform : Methanol : Acetic acid (10:1:2) combination as mobile phase. The plate was developed up to 80 mm at temperature of 20^0C with 10 min. of chamber saturation. Under this condition the retardation factor (Rf) of curcumin is 0.17, 0.42 and the compound was quantified at its absorbance maxima (λ_{max}) at 366 nm nm.The proposed method is useful as an accurate, simple, cost effective and sensitive for quantitative estimation of curcumin. The findings reveal the curcumin content depends on geographical variation which influences the soil, environment, climatic conditions etc.

KEYWORDS: Curcuma longa, Curcumin, HPTLC, Spectrophotometric, Geographical distribution.

INTRODUCTION
Turmeric is a spice derived from the rhizomes of Curcuma longa, which is a member of the Zingiberaceae family. It is a traditional medicine used in Ayurvedha, Unani and Siddha medicine for various diseases.[1] The bright yellow color of turmeric comes mainly from fat-soluble, polyphenolic pigments known as curcuminoids. Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent. Other curcuminoids found in turmeric include demethoxycurcumin and bisdemethoxycurcumin. In addition to its use as a spice and pigment, turmeric has been used in India for medicinal purposes for centuries. More recently, evidence that curcumin may have anti-inflammatory and anticancer activities has renewed scientific interest in its potential to prevent and treat the disease.[2] Turmeric is known for its anti diabetic, antiseptic, antibacterial, anti-asthmatic, antiulcer drug, insect repellant and wound healing properties.[3] Phytonutrients play a crucial role in many of the chronic diseases due to their pharmacological and biological properties.[4] Curcumin is a free radical scavenger with rich antioxidant activity. Curcumin exhibits great promise as a therapeutic agent and is currently in human clinical trials for diseases like cancer, myelodysplastic syndromes, colon cancer, psoriasis and Alzheimers disease.[5] It is reported to exhibit several pharmacological, microbial and other medicinal properties.[6,7,8] The antidiabetic effect of curcumin and its effects on diabetes-induced ROS generation and lipid peroxidation in type- 1 Diabetes mellitus is reported.[9] Oral intake of curcumin daily for several months is well-tolerated.[10] Curcumin has beneficial effects on various diabetic parameters.[11,12] Formulation of transdermal patch containing turmeric oil collected from North East India is evaluated.[13] Sandhya Rani et al.[14] studied on the development and standardization of turmeric cream by HPTLC. All the activities of turmeric depend on the amount of curcumin. The amount of curcumin in turn depends on various factors. The curcumin content varies from fresh to stored rhizomes.[15] It has been observed that the chemical composition of most of the herbs

changes with geographical region which may be due to climatic conditions and biochemical variations.[16] Previous reports have indicated that the curcumin content varies between the different lines of this species. These results suggest that the difference of curcumin content among the various lines of C. longa was caused by hybridization and introgression with other Curcuma species.[17] The phenotypic and genotypic coefficient of variation, heritability and genetic advance on mean basis were appreciably high for yield and curcumin content.[18] Among the turmeric cultivars of different growing regions in Meghalaya, Lakadong turmeric is reported to have the highest curcumin content (6.8%-7.3%) in a study conducted by Kanjilal et al., (2002)[19] and Chandra et al.,[20] The variations m Curcuma longa cultivars namely Rashmi, Krishna, Roma, CL-315, CEL-6, CL-70, CL-13 and CL-16 collected from various locations in India revealed Rashmi to have highest curcumin content.[21]

Present status of turmeric production in NE region

The turmeric in the NE region is under area of 17.27 thousands ha with a total production of 32.36 thousand tones. The productivity of the crop is much lower (1.87t/ha) compared to the national productivity of 3.47t/ha (Spices Statistics, Spices Board, 2004). The production of turmeric is highest in Meghalaya followed by Assam, Tripura, Nagaland and Mizoram. The most popular cultivated variety in the Mizoram region is Lakadong (7.5 percent) and Megha turmeric-1(6.8 percent) that possesses higher curcumin content and has maximum demand.

The main objective of the present study is to compare curcumin content and genetic diversity with RAPD technique on some Turmeric samples collected from different places of Northeast India. There are different turmeric species available in Northeast regions. There are vast differences in the climatic as well as in soil conditions in different areas of Northeast regions. So there is a chance of genetic diversity as well as differences in curcumin content in the turmeric species grown in different environmental conditions. Therefore in the present study, we have etimated the curcumin content of samples collected from different places of Northeast India.

MATERIALS AND METHODS

The turmeric samples were collected from Karbi Anlong, Nagaland, Arunachal, Shillong, Dibrugarh and Ledo of Northeast India.

Chemicals and reagents

Standard curcumin was obtained from Sigma Chemicals, Germany. All other chemicals and reagents used were of analytical grade.

Preparation of Standard stock solution

Standard stock solution (A) of curcuminoids was prepared by dissolving 10mg of each drug in 100 ml volumetric flask separately by using methanol and ethanol. From the stock solution final concentration (100µg/ml) of the individual working standards were prepared with methanol and 60% ethanol.

Simultaneous equation methods

Working standard solution was scanned in the range of 200 to 600 nm to determine the λmax ₌427 of drugs using methanol and 60 % ethanol as blank. The max of curcuminoids was found to be 427 nm. From the stock solution (i) 10 ml was taken and diluted to 100ml with methanol and 60% ethanol. From this solution (ii) 1ml, 2ml, 3ml, 4ml and 5ml were taken and volume was made up to 10ml in volumetric flask to get a concentration of 10,20,30,40 and 50µg/ml. The absorbance of the resulting solution was measured at 427 nm and a calibration curve were plotted at these wavelength as shown in Table 1 , Table 2, Table 3 and Table 4.

High performance thin layer chromatography (HPTLC) of the test samples

Sample and Standard Preparation

Solvent used was methanol. Plates were dried and store in dust free atmosphere.

Activation of pre-coated plates.

Aluminum sheets were kept in between two glass plates and was placed in oven at 110-120ºc for 15 minutes.

Application of sample and standard

The concentration range was between 2.0µl -6µl. Nitrogen gas was sprayed in the samples and standard from syringe on TLC plates as bands by CMAG Automatic TLC Sampler 4.

Selection of mobile phase

Mobile Phase used was chloroform: methanol: acetic acid (10:1:2 drops).

Chromatographic development and drying

After development of the spots, the plate and mobile phase was removed from the plate to avoid contamination of lab atmosphere and was dried in oven at 60ºC.

Detection and visualization

Detection was done under UV light and the spots of fluorescent compounds were seen at 254 nm (short wave length) or at 366 nm (long wave length).

Quantification

Samples and standard was chromatographed on same plate-after the development chromatogram was scanned by CMAG TLC scanner 3 at the speed of 20mm/s and grannular Size-60. Statistics such as RSD report concentration of analyte in the sample was automatically calculated by considering the sample initially taken and dilution factors.

Scanning densitometry

The absorption derivative substances were measured at the wavelengths of 254nm. At this wavelength the evaluated and spectra of the peaks were recorded. (winCATS Planar Chromatography Manager).

RESULTS AND DISCUSSION

The samples collected in triplicate from six places of Northeast India were analyzed for its curcumin content by UV-Visible spectrophotometry. The UV-Visible absorbance was recorded for all the samples were compared with that of UV-Visible absorbance of standard curcuminoids. The absorption band at 427 nm is characteristic of Curcumin. The calibration curve constructed for standard curcumin is represented in Figure 1 and Fig 2. Calibration curve was plotted using different concentration values of curcumin versus their respective absorbances. The results of Curcumin content for the turmeric rhizomes obtained from different regions of Northeast India are summarized in Table 2 and Table 4. It has been observed that the climatic conditions have significant impact on curcumin content of turmeric rhizome. Because of diverse weather conditions and soil type the curumin content was found to be varied for samples collected from different geographical source. The results of the study disclose that in hydroalcoholic extract, turmeric samples collected from Shillong and Arunachal Pradesh have comparatively higher percentage of curcumin than the other samples. In case of methanolic extract turmeric samples of Shillong and Dibrugarh have higher percentage of curcumin as compared to the other samples. The HPTLC separation was performed on precoated aluminium backed HPTLC plates of 0.2 mm layer thickness with silica gel 60 F_{254} with chloroform : methanol : acetic acid (10:1:2)combination as mobile phase. The plate was developed up to 80 mm at temperature of 20 ± 4^{O}C with 10 min. of chamber saturation. Under this condition the retardation factor (Rf) of curcumin is 0.17, 0.42 and the compound was quantified at its absorbance maxima (λ_{max}) at 366 nm nm.The proposed method is useful as an accurate, simple, cost effective and sensitive for quantitative estimation of curcumin. The findings reveal the curcumin content depends on geographical variation which influences the soil, environment, climatic conditions etc.

Table-1: Spectrophotometric absorbance (λmax $_{=}$427) of the standard curcuminoids in 60% Ethanol.

Concentration(µg/ml)	Absorbance
10	0.405
20	0.457
30	0.543
40	0.657
50	0.758

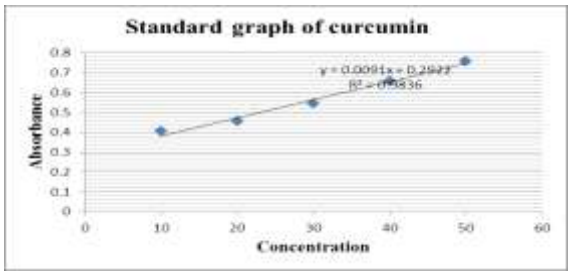

Fig.1: Standard curve for Curcuminoid

Table 2: Spectrophotometric absorbance (λmax $_{=}$427) of the Test samples (Hydro-alcoholic extract - 50µg/ml) in 60% Ethanol.

Test samples	Absorbance	Concentration (µg/ml)
Karbi Anlong	0.439	12.19
Nagaland	0.657	18.25
Arunachal	1.252	34.78
Shillong	0.873	24.25
Dibrugarh	0.567	15.75
Ledo	0.672	18.67

Table 3: Spectrophotometric absorbance (λ max $_{=427}$) of the standard curcuminoids in Methanol

Curcuminoid Conc. (µg/ml)	Absorbance
10	0.445
20	0.557
30	0.643
40	0.753
50	0.857

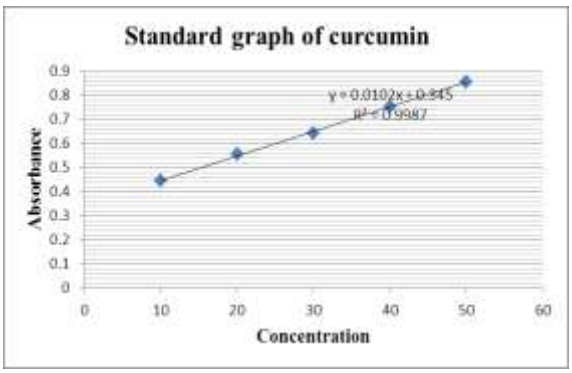

Fig.2: Standard curve for Curcuminoid

Table: 4 Spectrophotometric absorbance of the Test samples (Methanolic extract-50µg/ml) in Methanol

Test samples	Absorbance	Concentration (µg/ml)
Karbi Anlong	0.539	234.63
Nagaland	0.457	266.72
Arunachal	1.252	282.72
Shillong	0.473	333.45
Dibrugarh	0.767	287.90
Ledo	0.872	269.45

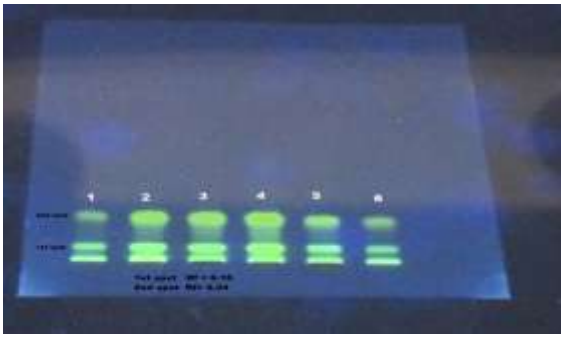

Fig 1: HPTLC fingerprints of different turmeric samples (1-Karbi Anlong, 2-Shillong, 3-Ledo, 4-Nagaland, 5-Arunachal and 6-Dibrugarh)

Table 5: R_f values of standard and Test samples

Sl NO.	Sample 1	Concentration	Rf value	% Area	Absorbance (nm)
1.	Standard (Curcuminoids)	2µl	0.17 0.42	28.50 71.50	350 350
2.	Sample 1	2µl	0.12 0.21 0.47	28.01 7.83 5.86	250 350 249
3.	Sample 2	2µl	0.10 0.21 0.48	30.81 14.16 21.32	250 350 350
4.	Sample 3	2µl	0.10 0.23 0.46	27.65 13.20 11.11	250 350 350
5.	Sample 4	2µl	0.11 0.20 0.47	30.52 13.01 18.50	250 350 350
6.	Sample 5	2µl	0.11 0.20 0.49	23.64 12.06 21.84	250 350 350
7.	Sample 6	2µl	0.11 0.19 0.45	41.35 9.94 13.73	250 350 350

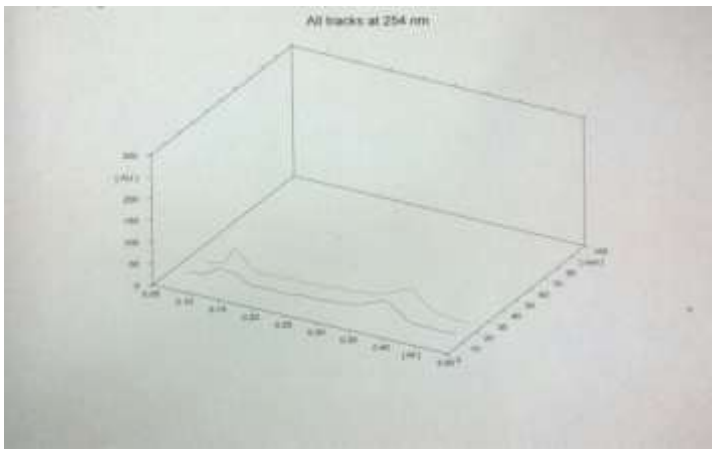

Fig 2: HPTLC Peaks of Standard (curcuminoid)

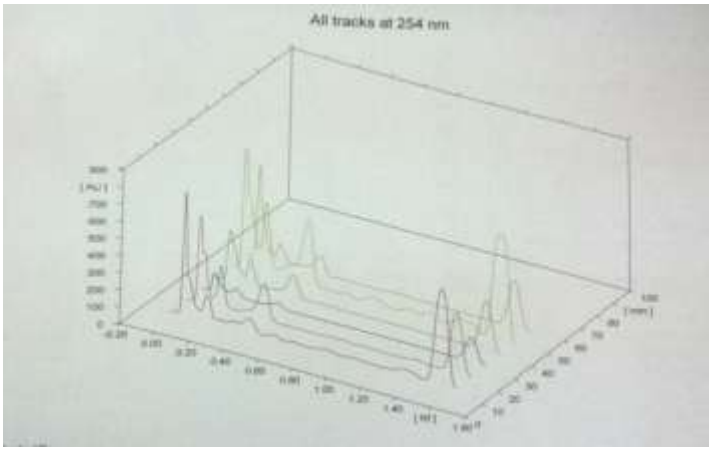

Fig 3: HPTLC peaks of test samples of turmeric.

CONCLUSION

The proposed method was found to be simple, sensitive, accurate, precise, economical and rapid for the routine simultaneous estimation of phytoconstituents. The HPTLC fingerprint is also suitable for rapid and simple authentication and comparison of the suitable difference among samples with identical plant resource but different geographic locations. Curcumin, the main bioactive component of turmeric has been shown to have a wide spectrum of biological actions viz. anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic and antimicrobial activities. The content of curcumin in turmeric determines its colour, quality, therapeutic utility and hence its cost The quantity and quality of curcumin vary due to the changes in ecological factors in different places of Northeast India. Hence, in order to increase the quality of turmeric, it is essential to domesticate and systematically cultivate these plants on a large scale. The curcumin percentage present in turmeric can be correlated to the inhibitory potential of many diseases and further analysis can be carried out to find the need for planting turmeric with higher quality of curcumin, which possesses tremendous medicinal properties. This data will be useful to ayurvedic and Pharmaceutical industries for deciding the source /supply of turmeric. Further research work need to be carried out to study the impact of different factors such as geographic localization, harvest time and climate conditions etc. on cultivation in terms of chemical composition of turmeric rhizome.

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