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Extraction, purification and anti-cancer activity of curcumin

Pradeep D. Nile, Aditya A. Kulkarni, Ashok A.Shinde *, Archana N. Panche and Sanjay N. Harke

Institute of Biosciences and Technology, MGM University, Aurangabad, Maharashtra, India.

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Abstract

Turmeric has long been used as a strong anti-inflammatory in Indian drug systems. Turmeric is rich in curcuminoids that have different chemical, physico-chemical properties. Current work reports exraction of curcuminoids, curcumin using the Soxhlet extractor. Purification and quantification of curcumin is performed by chromatography. The yield percentage of curcumin reported 4.09% by the Soxhlet extraction method. Different solvent were used for extraction, among them acetone extract was with high yield of curcumin. Individual curcumin extract analysed using UV-VIS Spectroscopy and HPLC. Our results showed that the Salem turmeric contains highest percentage of curcumin. The *in vitro*MTT assay on the U87MG glioma cell line demanstrated anti-cancer activity of isolated curcumin.

Keywords: Turmeric;Curcuminoids; Extraction; Anticancer

1. Introduction

Turmeric (*curcuma longa* L) is a widely used medicinal plant in southern Asia. Turmeric has long been used as a powerful anti-bacterial, anti-oxidant and anti-inflammatory in Indian medical systems. Traditionally turmeric was called "Indian saffron" because of its yellowish colour and is used as a condiment, healing remedy and textile dye [1]. Turmeric is rich in curcuminoids that vary in chemical properties; physico-chemical features. Curcumin (CUR), demethoxycurcumin (DMC) and bisde methoxycurcumin (BDMC) these three compounds are present in turmeric. These three compounds are commonly called curcuminoids. Curcumin is the main compound present in turmeric that gives the yellow colour to the turmeric plant. Curcumin is very expensive and acts as an anti-cancer agent, which is a good supplement to prevent the growth of cancer cells in the body. Indian farmers can sell curcumin as the second most important commodity compared to turmeric in the market [1, 2].

In this current work we focused on extraction purification estimation of curcumin across different turmeric verities. Purification of curcuminis quite challenging as the three curcuminoids are chemically similar only the presence or absence of methoxyfunctional group in each aromatic ring [5]. At laboratory scales chromatography methods are used to separate curcuminoids individually using a separate standing phase, organic solvent as a cellular phase. A combination of crystallization and chromatography is also used to achieve greater curcuminpurit [6]. Various analytical methods have been developed in recent years like HPLC, HPTLC and UV-Visible Spectrophotometry to analyse the quality of curcuminoids in *Curcuma long.* AlthoughUV-spectrophotometric and HPLC methods are the most suitable methods for measuring curcumin in the extract of *Curcuma longa*. Therefore, in the present study, a simple UV and HPLC method was used to measure of the amount of curcumin in *Curcuma longa* [7, 8].

Studies show that curcumin can be used to treat more than 25 diseases In addition to curcuminde methoxycurcumin and bisdemethoxycurcumin has a strong effect on human tumour cells [11]. Curcumin has recently been screened in clinical trials for high-risk cancer and recent literature suggests that curcumin may have many beneficial effects on glioma cells, including blocking pathway and cell growth. Curcumin affects a variety of expression and target cellular processes in several types of cancer [9-19]. [fig 1].

*Corresponding author: Ashok A Shinde
Institute of Biosciences and Technology, MGM University, Aurangabad, Maharashtra, India.

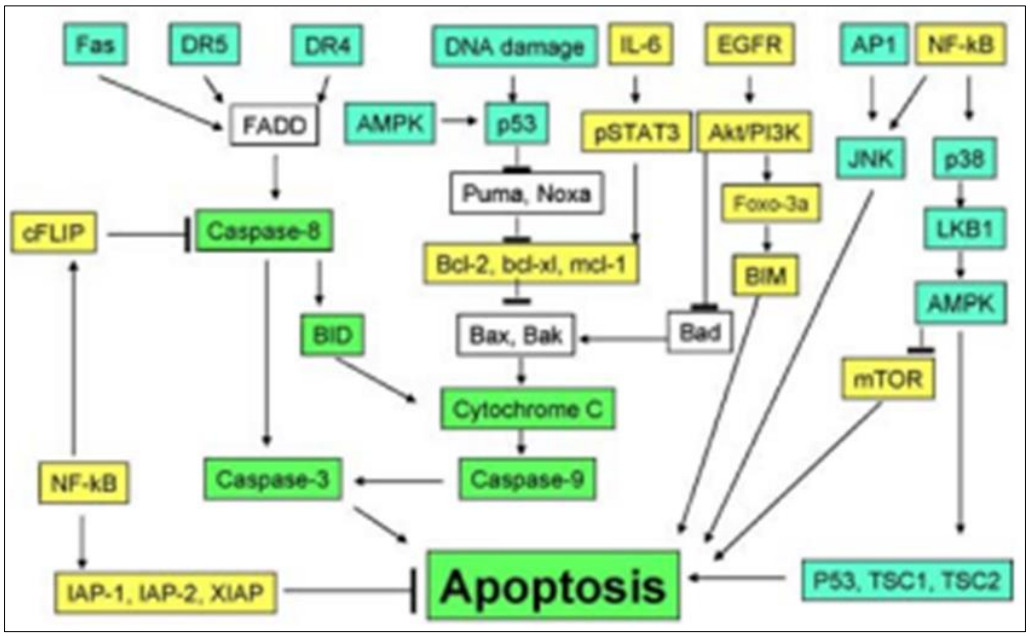

Figure 1 Apoptosis induction by curcumin

Previous research hypothesis that curcumin may induce cytotoxicactivity and overcome natural chemotherapeutic for glioma cell was investigated [19].

Curcumin can be used in dairy products, fats, oils & oil emulsions, confectionary products, grain products, spice mixtures, soups and sauces, meat and meat products. Global curcumin market is expected to reach USD 85 million by 2023, growing at a CAGR (compound annual growth rate) of 12.1% between 2018-2023. The Asia Pacific region is a thriving market place, due to a growing demand from skincare cosmetics manufacturers and processors to consume their fast-growing natural and natural ingredients. The region also has 85% curcumin production worldwide [20]. After the extraction which produce large amount of waste they have also many economic importance [21].

Turmeric is widely cultivated in the Parbani and Nanded region that the turmeric variety varies curcumin content after identifying the best solvent system and the turmeric varieties that provide the highest curcumin content directly to the farmer. Curcumin has many medicinal properties that offer a high market value of turmeric, as well as after identifying anticancer activity curcumin can help in cancer treatment.

2. Material and methods

2.1. Material

Substrate *curcuma longa* (turmeric) local variety collected from Parbhani District. The Salem, china Salem, Rajapuri, these local varieties of turmeric was selected. Pre-coated TLC plate was used. Pure Curcumin (97%) has been approved in Hi-media Pvt Laboratories was used as standerd. All other chemicals and reagents used in the analysis are quality level of the HPLC grade. For column chromatography silica gel (60-120 mesh) from Hi media was used. Human glioma cell line U87MG was purchased from National Centre for cell Science Pune.

2.2. Methodology

2.2.1. Extraction

Curcuminoids were extracted using Soxhlet extraction method. New rhizomes were cleaned, rinsed with water, cut and dried in the sun for one week and then dried at 105°C in a hot oven for three hours. These dried rhizomes were cut into small pieces, powdered with an electronic mill. A 10gmdry sample of each turmeric varieties individually was taken and placed in a thimble of Soxhlet machine; 125 ml of solvent was added and extracted according to their boiling point for seven hours. The solvents used were chloroform (B.P. = 61°C, methanol (B.P. = 65°C and acetone (B.P. = 56.53°C After the extraction the dark brown extract was then cooled and concentrated using a hot plate magnetic stirrer, and this dry

extract that turns a dark orange colour. Each turmeric sample was extracted in the same way and the yield was calculated using formula as below [22].

$$\% \text{ of curcumin } = \frac{\text{Dry weight of extracted curcumin}}{\text{Total weight of turmeric}} \text{ X } 100$$

2.2.2. Thin layer chromatography

A pre-coated TLC plate was used. n-Hexane: Chloroform: Ethanol (41:49:10),Acetone: Methanol (8:2) solvent was used to identify different curcuminoids. After the formation of the chromatogram the plates were removed and dried and the spots were analyzed [23].

Each spots Rf value was calculated by following formula [24]

$$\text{Rf } = \frac{\text{Distance moved by the compound}}{\text{Distance moved by the compound}}$$

2.2.3. Column chromatography

Sample of soxhlet extract from acetone solvent 1gm curcuminoid used in silica gel column chromatography. The extract was filtered by muslin cloth and concentrated in using hot plate magnetic stirrer this raw curcuminoid mixture contains curcumin, demethoxycurcumin, bisdemethoxycurcumin. Extracted curcuminoid from acetone was inserted into the silica glass column (60-120 mesh). Approximately 1gm of raw Curcuminoids was mixed with 8gm of silica gel and loaded into a 46 × 2 cm column and extracted with chloroform followed by chloroform: methanol (95:5) with increasing polarity. All the collected fractions were analysed using a TLC plate using chloroform: methanol (95:5). Mobile phase of Column chromatography fractions removed has content pure curcumin. The total curcuminoid content of each curcuminoid collected was analysed by UV spectrophotometry at 425 nm [25, 26].

2.2.4. UV Visible Spectroscopy

Standard CurcuminSolutionPreparation

Curcumin 10 mg was accurately measured and transferred to a 25 ml volumetric flask. Methanol was added up to a mark of 25 ml volumetric flask to obtain a concentration of 400µg / ml of stock solution. Solutions were diluted 10ml with methanol to obtain a concentration of 1, 2, 3, 4, 5, 6, 7µg / ml dilutions respectively. The wavelength associated with the high absorption of curcumin in methanol was detected at 425 nm.

Standard Curcumin Curve

The standard curcumin curve was obtained by measuring the absorption of curcumin solution at concentration (1-7µg / ml) prepared from stock solutions in methanol at 425 nm. The curcumin balancing curve was then arranged by section on the y-axis and the curcumin concentration on the x-axis [7, 8].

Test Solution Preparation

1 mg of extracted *Curcuma longa* was accurately measured and transferred to 10 mL of volumetric flask. Methanol was added to the mark and the resulting solution was used for analysis.

2.2.5. HPLC

High Performance Liquid Chromatography (Dionex, Ultimate 3000) with UV detector was used to calculate curcumin concentration. The standard curcumin purchased from HI-MEDIA laboratory. The column was C18 used (C18, 5mm, 250 x 4.6mm); the mobile phase is composed of acetonitrile and water at a rate of (90:10); the flow rate was 1.0 ml / min at 33°C. Cycle time for each sample was 8min. For the analysis of curcumin wavelength used 425nm. [12] The standard solution (400µ/ml) to prepare by dissolving them in solvent and solution were passes through filter 0.45µm, To analyse with the HPLC, standard curcumin containing 20, 24, 40, 48, 60 mixtures was prepared and dissolved in HPLC grade methanol and then injected into HPLC and standard curcumin calibration curve was developed. To determine the curcumin content of unknown samples, a specific concentration of the sample (1 mg/ml) was developed, Obtained from extraction process were collected and there solvent were evaporated using hot plate magnetic stirrer, The solution were prepared by dissolving them in methanol, Passed through a filter of 0.45 µm and injected into the system. The measurement of the extracted curcumin was done using compared with standard calibration cure [4].

2.2.6. Starch Extraction and Validation

8gm of turmeric residue (both types of Curcuma) after extraction was used. The remains were immersed in water for 12 hours. Then remove the soaked residue from beaker and passed through double layer muslin cloth. The high-strength layer was reduced and the weight was allowed to sit for about an hour. To eliminate impurities, turmeric starch was washed twice with water in a centrifuge. The collected starch is dried in an oven at 400°C [21].

2.2.7. Iodine test for starch identification:

1 ml of sample provided in a clean, dry test tube. 1 ml distilled water at one tube for control. About 2-3 drops of Iodine solution in both tubes and mixed them in a vortex. Observe colour visibility in test tubes. Heat the test tubes in the bath water until the colour disappears. Test tubes are out to cool. Visiblecolour appeared in test tubes [26].

2.2.8. Determination of cytotoxicity by MTT assay

Human glioma cell line (U87MG) were purchased from National Centre for Cell Science, Pune was used. Cells were seeded 10k per well at 96 well plates incubated for 24 hr. various concentration of curcumin (25µM/L and 50µM/L)were added in wells and incubated 48hrs for the interaction of curcumin with cells. After the incubation MTT was added in each well had 10 µl of MTT concentration (5 mg/ml) and incubate for 6 hours. After the incubation 100µl of DMSO was added and incubated for 20 minutes and showing the formation of a purple colour. And absorbance observed on ELISA plate reader [27].

Cell viability index was calculated according to the following equation [19]

$$\text{Cytotoxicity \%} = 1\frac{\text{Mean absorbance of toxicant}}{\text{Mean absorbance of negative control}}\text{X } 100$$

Viability % =100-Cytotoxicity %

3. Results and discussion

3.1. Extraction

Different varieties of turmeric from the Nanded region, Maharashtra were used to study how to extract Curcuminoids by soxhlet from turmeric. After extraction bySoxhlet method crude was measured and the percentage of weight of curcuminoids was calculated. In this study we used three local varieties of turmeric, Selam, Rajapuri, Kocha, dry powder was made and used to extract Soxhlet using various solvents; this is extracted in the form of a dark orange colour obtained when dried [25]. The raw curcuminoid is calculated and achieved from Selam, Rajapori, kocha RF value as 4.2, 3.8, and 5.9 respectively in acetone solvent system [1].

3.2. Qualitative analysis

TLC separation of curcuminoid pigments in silica gel using different solvent systems resulted in the Rf values indicated. All of the spots showed fluorescence under UV light. Some of the developing solvents employed did not promote separation of DMC and BDMC. The use of n-Hexane: Chloroform: Ethanol in the proportion (41:49:10) achieved the best results, compare to Acetone: Methanol (8:2) CUR, DMC, BDMS compound Show RF values that is 0.7, 0.61, 0.59 and 0.83, 0.79, 0.58 respectively [23, 27].

3.3. Estimation of starch

8.5gm of turmeric residue soaked and starch were collected after washing twice with distilled water approximately 0.51gm starch received that is 6% of total weight of turmeric residue.After collection of starch and drying presence of starch identify using iodine test it gives dark blue black colour represent the positive test, indicating presence of starch [21].

3.4. Purification

Silica gel column chromatography carried out using Chloroform: Methanol (95:5) mobile phase and analysed by thin layer chromatography.Using a constant flow rate of 5 ml / min, a non-isocratic elution profile was used with gradually increase the methanol concentration in the mobile phase chloroform-methanol. From the pure chloroform solvent, pure CUR was extracted from the column. Then increase the methanol content to 5%, first extracting the remaining CUR

mixture and DMC, after which the pure DMC fraction is obtained. In the third step the concentration of methanol rises to 25%, which initially provides CUR after mixture of the remaining DMC and BDMC, then the pure BDMC fraction is obtained. Total 10 fraction took and indidual fraction analyazed using TLC in this observation shows fraction 1-2 CUR, fraction 3 CUR-DMC, fraction 4-5 dmc, fraction 6 DMC, fraction 7-8 DMC-BDMC and last fraction was BDMC [6,25].

3.5. Quantification

3.5.1. UV-VIS Spectroscopy

The linearity graph obtained from absorbance of standard. CUR shows compatibility at a concentration range of 1-7µg / ml at 425 nm [fig 2] of UV Visible spectroscopy. Measurement curves are designed as a focus against UV absorption.The coefficient was determined by the level of CUR absorbance [7, 8].

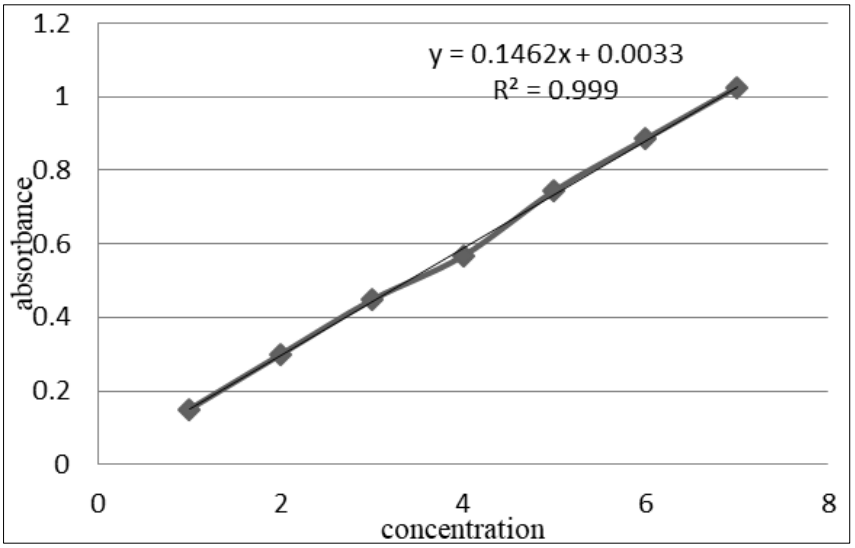

Figure 2 Linearity graph of curcumin by UV

Curcumin concentration present in the test calculated and Concentration of curcumin in turmeric variety that is China selum, Rajapuri, Kocha is respectively 6.82, 5.2, 12.41 µg/ml.

3.5.2. HPLC (high pressure liquid chromatography)

The median accuracy was analysed using three curcumin concentrations and that is 20, 24, 40, 48, 60µg/mL. The absorption of the test solution was measured at 425 nm. The peak area, peak height and retention time of each standard reference and sample compound were evaluated from HPLC chromatogram. The percentage of Curcumin present in the extract is calculated as curve measurement method and result was reported. [fig 3].

The peak area of standard concentration 20, 24, 40, 48, 60µg/mL shows 58.6666, 68.8763, 99.3666, 117.333, 135.1819, and sample1 277.9832, sample2 543.832, sample3 1883.9135 at 425nm. Calibration standard curves were obtained using the HPLC plotted concentration against peak area the calibration curves of the showed good linearity within the test range (R2 =0.9957). [Fig:4] After the calculation of test value with standard cure. Based calibration cure concentration of the three samples that is three different varieties selam, rajapuri, kocha respectively 132.87, 523.37, 666.29µg/ml per curcumin present in crude curcuminoids was obtained. As per these values kocha and selam contain high value of curcumin [4].

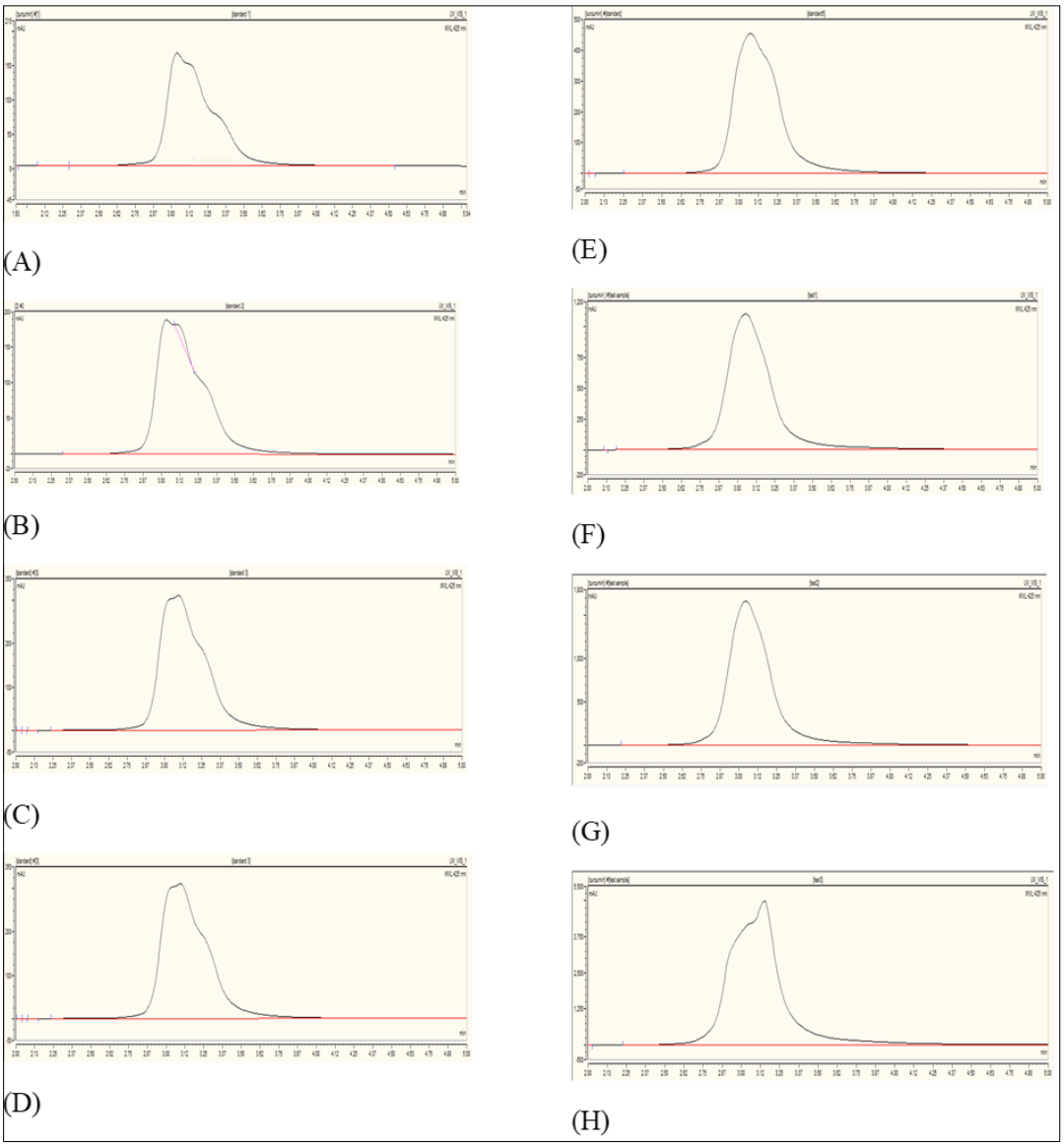

Figure 3 HPLC chromatograms of standard curcumin (A, B, C, D, E) Test samples (F, G, H)

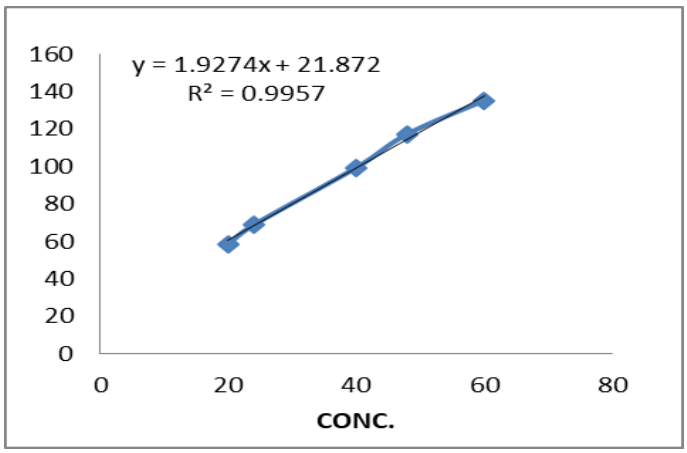

Figure 4 Linearity graph of curcumin by HPLC

3.6. Determination of cytotoxicity by MTT assay

Curcumin has reduced the viability in glioma cell line. At 25 and 50mol/L one time concentration was added in cell seeded plate. This effect was observed within 48 hours after treatment and lasted 4 days. Curcumin exerts cytotoxicity in glioma cells and viability observed for 25μM/L and 50μM/L concentrations at 490nm in ELISA plate reader was 68% and 27% respectively. In this observation 50μM/L concentration exerts high amount of cytotoxicity in glioma cell [19]. Anticancer activity of curcumin in U87MG glioma cell was observed and that is possibility curcumin modulate the cellular signalling pathway responsible for toxicity in GBM cells [11].

4. Conclusion

In this study, we extracted natural curcumin and compared there concentration in different sample. Among the acetone, methanol and ethanol solvent for extractionacetone gave high amount of crude curcuminoids. We found among all types of turmeric china selam showing the highest 4.2% curcuminoids using acetone solvent. Silica gel chromatography simple method for Separation of each curcuminoid. UV Spectroscopy and HPLC methods were appropriate for the detection of curcumin. The analysis method built into the UV-Visible spectrophotometer was simple, reliable, accurate and reproducible.

As curcumin is a single drug and an important product compared to turmeric in the market, therefore farmers can be extract crude curcuminoid and sold in market. In the future more work should be done to explore the therapeutic properties of curcumin. The use of curcumin work as natural anti-cancer medicine. With the help of targeted drug delivery a brain tumour can be curative.

Compliance with ethical standards

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Disclosure of conflict of interest

Disclosure of conflict of interest Author declared no conflict of interest.

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