DEVELOPMENT OF A RAPID SEPARATION PROCESS FOR CURCUMIN FROM CURCUMA LONGA L. RHIZOMES AND ITS QUANTIFICATION BY HPLC-PDA

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ABSTRACT
In the present work, a shortest and rapid separation process for curcumin from Curcuma longa rhizomes, using microwave-assisted extraction (MAE) technique, has been developed. The obtained extracts were analyzed by using HPLC coupled to photo diode array detector (PDA) to prove the MAE extraction efficiency. The optimal MAE conditions were dichloromethane as extracting solvent, at a temperature equal to 40°C for 3 min. Further, Curcumin was isolated from dichloromethane-MAE extract of C. longa rhizomes and characterized by using FTIR, NMR and EIMS techniques. MAE can be used as an alternative method for isolation of curcumin from C. longa rhizomes due to its efficiency and rapidity as compared to the conventional method with column chromatography. The developed procedure may be useful for researchers, academicians, pharmaceutical and cosmetic industries.

Kew Words: Curcuma longa L., MAE, FTIR, NMR, curcumin.

ABBREVIATIONS
MAE, microwave-assisted extraction; HPLC, high-performance liquid chromatography; PDA, photodiode array detection; ACN, acetonitrile; EtOAc, ethyl acetate; IR, infrared spectroscopy; NMR, nuclear magnetic resonance; LOD, Limit of detection; LOQ, limit of quantification; %RSD, percentage relative standard deviation;
INTRODUCTION
Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Fig. 1], is a major polyphenolic phytoconstituent found in the rhizomes of Curcuma longa L. herb [1].

![Figure 1: Structure of curcumin](image)

The powder of rhizome of C. longa (turmeric) contains mainly three naturally occurring diarylheptanoid curcuminoids, namely, curcumin (60–80%), desmethoxycurcumin (15–30%) and bisdesmethoxycurcumin (2–6%) [2]. Turmeric is commonly used as a spice and coloring agent in food by virtue of its yellowish-orange colour and pleasant aroma. In recent years, curcumin has attracted interest because of its antioxidant, anti-inflammatory, anti-parasitic, anti-mutagenic, anticancer, chemoprotective, hepatoprotective, antimicrobial and antiviral activities [1-4]. In Ayurveda, turmeric has been used internally as a stomachic, tonic, blood purifier and externally in the prevention and treatment of skin diseases [5]. Recently, several studies have been focused on contents of the C. longa and extraction of their high-added value compounds. The traditional solid–liquid extraction technique is based on the correct choice of solvents and the use of heat or/and agitation to improve the extraction efficiency; however, this technique requires longer extraction time and large amounts of solvents. In recent years, much attention has been given to the application of microwave heating in analytical and biological chemistry [6–8]. Major advantages of MAE include short extraction time, low-energy requirement, high extraction efficiency, and minimum degradation of target components [9-17]. Thus, optimizing an appropriate sample preparation technique with significant advantages over conventional methods for the extraction and analysis of medicinal plants is a key factor in the overall effort of ensuring and providing high-quality herbal products. Regarding the great significance of C. longa rhizome in obtaining high added value curcumin, the purpose of this study was to obtain a new rapid and reliable extraction and isolation method based on MAE technique for the rapid isolation and analysis of curcumin.
present in extracts of *C. longa* rhizome by using a HPLC-PDA and details of its characterization and purity check by FTIR, UV, LCMS, HPTLC and HPLC.

**MATERIALS AND METHODS**

**Samples**

Rhizomes of *Curcuma longa* were collected from Village-Jallapur, Post- Salahpur, District-Sitapur, Uttar Pradesh, India in the month of April 2011. After collection, fresh rhizomes were immediately kept in shed, washed with distilled water. Fresh rhizomes were dried under a gentle stream of air in the laboratory till no loss in weight (temperature 40± 2°C and relative humidity 50 ± 5%). Air dried rhizomes were packed in airtight containers and finally, dried samples were stored at −20°C until further use. Rhizomes of *C. longa* L. were identified and characterized by the matching of TLC fingerprints with reference standards of *Curcuma longa* L. rhizome specimen voucher (NISCAIR/RHMD/Consult/2010-2011/1632/230). Dried rhizomes were powdered in an electric grinder for experiments.

**Chemicals and Reagents**

Solvents and chemicals used were of analytical grade (E. Merck) and those used for HPLC were of HPLC grade. Curcumin (purity ≥ 98%) was isolated from *C. longa* L. rhizomes, identified and characterized by matching of melting point and spectroscopic data (FTIR, ¹H-NMR and ¹³C-NMR) with reported data, respectively. For sample and solvent filtration, 0.45µm membrane filters (Millipore, Germany) were used, and solvents were degassed prior to use.

**Microwave and HPLC operating conditions**

The extraction system comprised of a microwave oven (Domestic) manufactured by KENSTAR (Ahmednagar, Maharashtra, India) equipped with a magnetron of 2450 MHz with a nominal maximum power of 900 W, 10 power levels, time controller, 10 convection temperature sensor and exhaust system was used for microwave extraction.

HPLC analysis of curcumin was carried out on a LiChrospher 100 RP-18 (250 mm x 4.6 mm, 5 µm) column using a Shimadzu High Performance Liquid Chromatographic System LC 2010-CHT with LC solution software, equipped with a degasser, an auto-sampler, a diode array detector (PDA) and 20 µL injector loop. A reverse-phase HPLC was carried out using an isocratic system with a mobile phase of 1% citric acid (\(p^H=3\)) and acetonitrile (45:55, v/v), a column temperature of 28° ± 3°C, a detection wavelength of 427 nm and a flow rate of 1.0
mL/min for 15 min. The injection volume was 20 µL. Curcumin was eluted at retention time of 6.973 min (Fig. 2). HPLC system equilibrated under the starting conditions for 5 min before starting the analysis.

Figure 2: HPLC chromatogram of curcumin at 427 nm wavelength

**Conventional Solvent-Extraction Method**

Rhizome powder (1 g each) was kept in six different flasks and every flask was extracted separately at room temperature with 15 mL of solvents mentioned in Table 1 for 24 hrs. Each extracts were filtered separately. Solvents were removed under vacuum in a rotatory evaporator at 50 °C, and lypholized till each extract was free from solvents. The dried extracts were weighed accurately and percentage yield was calculated and given in Table 1. The concentration of each extract solution was 40 µg/mL prepared for HPLC analysis.

**Table 1: Quantitative determination of curcumin in the extracts of C. longa rhizome**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Powdered rhizome Wt. (g)</th>
<th>Microwave assisted extraction</th>
<th>Conventional extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>1.0255</td>
<td>1.26</td>
<td>5.89</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>1.0457</td>
<td>1.85</td>
<td>78.36</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.0285</td>
<td>2.10</td>
<td>68.84</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.0338</td>
<td>3.58</td>
<td>75.56</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0585</td>
<td>8.25</td>
<td>65.45</td>
</tr>
<tr>
<td>Methanol: water (60:40, v/v)</td>
<td>1.0356</td>
<td>15.75</td>
<td>23.52</td>
</tr>
</tbody>
</table>
Microwave-Assisted Extraction Method

MAE of *C. longa* rhizomes was performed with a variety of solvents ranging from non-polar to polar ones, i.e. *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), acetone, ethanol and methanol: water (60:40, v/v). Dried and powdered rhizomes (1 g each) were kept in six different flasks and every flask was extracted three times (3 x 15 mL) after allowing a preleaching time of 10 min. with each of the above mentioned solvents separately and applied 700W irradiation power for 3 min irradiation time. The sample was treated under microwave irradiation in an intermittent way, i.e. irradiation cooling–irradiation. The irradiation time was kept for 1 min and 1 min was taken to cool the sample solution between two irradiations. Extracts obtained by MAE were filtered and pooled with corresponding extract. Solvents were removed under vacuum rotatory evaporator at 50 °C, separately and lyophilized till each extract was free from solvents. The dried extracts were weighed accurately and percentage yield was calculated and given in Table-1. The extraction efficiency is expressed as the peak area of curcumin. For precision study, repeatability of the optimized method was measured as relative standard deviation (RSD %). Thus, 1 g of sample was extracted under the optimized MAE conditions (n = 3) on the same day (intraday precision) and then analyzed by HPLC-PDA. Each MAE extracts was expressed as percentage of the total peak area of the curcumin. Finally, the *C. longa* rhizome extracts obtained under the optimal MAE conditions were analyzed by using HPLC-PDA method. Peak identification was performed on the basis of their relative retention time values and comparison of UV-Vis spectra with authentic standard solutions when available.

Rapid isolation procedure for curcumin

The powdered rhizomes of *C. longa* (100 g) were extracted three times with DCM (3 x 300 mL) after preleaching time 10 min., under microwave conditions (applied 700 W irradiation power, at 40°C temperature for 3 min), applying microwave irradiation in an intermittent way, i.e. irradiation cooling–irradiation. The irradiation time was kept for 1 min. and 1 min. was taken to cool the sample solution between two irradiations. The extract was filtered and solvent was removed under vacuum at 50°C up to dryness. Total extract 1.89 g was obtained which was dissolved in mixture of chloroform: acetone (60:40, v/v) and kept overnight at -4°C temperature for precipitation. Yellow-orange crystals i.e. **compound 1** (1.42 g) solid was obtained with purity of ≥ 98% by HPLC (Figure 2), TLC of obtained solid was performed on TLC plate (silica 60F254) developed in chloroform: methanol (95:5, v/v) and plate was
visualized at UV-366 nm, showed single major spot at $R_f$ 0.48. Which was gave red brown colour with ferric chloride and reddish pink colour with alcoholic sodium hydroxide.

Spectral data analysis of yellow-orange crystals (compound 1)
FTIR v max (KBr) cm$^{-1}$: 3507 (OH), 2918, 2849, 1627 (C=O), 1602, 1509, 1458 (aromatic), 1429, 1282, 1206, 1154, 1027, 962, 856, 814 and 714 cm$^{-1}$. Spectra of $^1$H and $^{13}$C NMR of compound 1 were determined and TMS was used as an internal standard [18]. NMR data is presented in Table 2. Compound 1 showed molecular ion peak on EIMS: m/z 368, [M]$^+$ C$_{21}$H$_{20}$O$_6$.

Table 2: NMR data of compound 1 in DMSO-d$_6$

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>$^1$H NMR (300 MHz), δ ppm</th>
<th>$^{13}$C NMR (75 MHz), δ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.02 (s, 2H)</td>
<td>100.9</td>
</tr>
<tr>
<td>2, 2’</td>
<td>-</td>
<td>183.3</td>
</tr>
<tr>
<td>3, 3’</td>
<td>6.74 (d, $J$ = 15.8 Hz, 2H)</td>
<td>121.2</td>
</tr>
<tr>
<td>4, 4’</td>
<td>7.52 (d, $J$ = 15.8 Hz, 2H)</td>
<td>140.8</td>
</tr>
<tr>
<td>5, 5’</td>
<td>-</td>
<td>126.4</td>
</tr>
<tr>
<td>6, 6’</td>
<td>7.28 (brs, 2H)</td>
<td>111.5</td>
</tr>
<tr>
<td>7, 7’</td>
<td>-</td>
<td>148.1</td>
</tr>
<tr>
<td>8, 8'-OH</td>
<td>9.63 (brs, 2H)</td>
<td>149.5</td>
</tr>
<tr>
<td>9, 9’</td>
<td>6.80 (d, $J$ = 8.0 Hz, 2H)</td>
<td>115.8</td>
</tr>
<tr>
<td>10, 10’</td>
<td>7.11 (dd, $J_{9,10}$ = 8.0 Hz, $J_{6,10}$ = 1.2 Hz, 2H)</td>
<td>123.2</td>
</tr>
<tr>
<td>-OCH$_3$× 2</td>
<td>3.70 (s, 6H)</td>
<td>55.8</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Optimization of MAE conditions
Solvent optimization is of primary importance in MAE [19] for this reason, different polarity solvents such as n-hexane, DCM, EtOAc, acetone, ethanol and methanol: water (60:40, v/v) were investigated to determine the effective extraction of curcumin. By comparing the extraction efficiency of all solvents (Table 1), it can be seen that at fixed microwave power of 700 W, temperature (40°C) and time (3 min) increasing polarity solvents have a benefit in terms of increasing the extraction efficiency for the majority of the curcuminoids. The highest recovery of curcumin was obtained with DCM. Nevertheless, EtOAc, acetone, ethanol and methanol: water did not provide as much yield as DCM extraction. Thus, DCM was selected as the most efficient solvent for maximum extraction of curcumin. It has been reported that acetone is often the solvent of choice for recovery of a wide range of curcuminoids from C. longa rhizome [20]. DCM can penetrate easily into the cells of the plant matrix and facilitate
better heating with traces of water present in plant matrix. This in turn increases the mass transfer of the active phytoconstituents into the extracting solvent [19]. For optimization of microwave irradiation power, the obtained results are shown in Table 1, from these results, microwave power of 700 W, was chosen for further MAE of rhizomes of C. longa rhizomes.

The extraction time must be optimized to ensure maximum recovery in the minimum analysis time. For optimization of extraction time, obtained results showed that after 3 min there was no remarkable increase of curcumin extraction with the increase in extraction time; therefore, 3 min was selected as an appropriate extraction time. For optimization of temperature, obtained results are shown in Table 1. The extraction efficiency of the curcumin increased with the rise of temperature. Nevertheless, up to 40 °C, the extraction efficiency began to decrease for most of the curcuminoids under study. It can be explained by the thermal degradation of some of the curcumin. Thus, the optimal temperature was chosen at 40°C.

**Precision Study:** The RSD % values for the yield and the major and well-known curcumin of the extract are represented in Table 1. Intraday repeatability of the developed method was between 1.30% and 2.49%, whereas the interday repeatability was from 3.01% to 8.47%. Intraday precision was higher than the interday precision, and the method showed a good overall repeatability.

**Characterization of compound 1 (yellow-orange crystals)**

The purity of the compound 1 was demonstrated by HPLC analysis and melting point determination. HPLC analysis of the isolated compound 1 showed single peak, as indicated in Figure 2 with the retention time 6.96 min with percentage area of more than 98% [18]. The melting point of compound 1 was 184–186°C. UV–vis spectra indicated that the wavelengths of maximum absorption in ethanol were 240 nm and 427 nm for compound 1 indicated the presence of α, β-unsaturated carbonyl group (Figure 1). The IR spectrum indicated the presence of a hydroxyl group (3507 cm⁻¹), a carbonyl group at 1627 cm⁻¹ and a band at 1509 cm⁻¹ for double bonds as evident in Figure 1. A singlet at δ 1.02 (2H) in ¹H NMR was assigned for C-1 proton. Two broad singlets at δ 6.74 and 7.52 were assigned for magnetically equivalent H-3/H-3’ and H-4/H-4’ protons, respectively. The ortho-coupled protons of the aromatic ring appeared as doublets at δ 6.80 and 7.11 (J =8.0 Hz each) assigned for H-9/H-9’ and H-10/H-10’, respectively, present in identical environment. A singlet corresponding to the two methoxy groups (OCH₃) in compound 1 was observed at δ ppm 3.70 in the ¹H NMR spectrum (Table 1 and Figure 1). The ¹³C NMR signal for the
methoxy groups of **compound 1** was observed at δ ppm 55.8 (Table 1 and Figure 1). Accurate EIMS of **compound 1** was found m/z 368 corresponding to molecular formula \( \text{C}_{21}\text{H}_{20}\text{O}_{6} \) (Figure 1). Therefore, **compound 1** was characterized as 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (**curcumin**, Fig.1), which was further compared and confirmed with the reported data [18, 20].

**Efficiency of the MAE Method**
To improve the efficiency of the optimized MAE method, it was compared to the conventional extraction method. With respect to the extraction time, MAE was the fastest, requiring just 3 min giving the highest yield (15.75% of dry weight), whereas in the extract obtained by the conventional extraction the yield did not exceed 11% (Table 1). Quantitatively, the statistical analysis showed significant differences between both methods (p < 0.05). The main significant observation was that the major detected curcumin in the extracts showed better recoveries with MAE. Being the major compound identified in the extract under study, curcumin is represented in Table 1 and expressed as percentage of the total peak areas.

**CONCLUSION**
In conclusion, in this study, a cost-effective, precise and timesaving extraction method, based on the use of microwave energy, has been optimized for the analysis of curcumin from Indian *C. longa* rhizomes. Moreover, dichloromethane is proposed as most favorable solvent for MAE of curcumin from *C. longa* to get the maximum yield. The characterization of the extracts obtained under the optimized MAE conditions, by using a combination of HPLC-PDA, revealed the existence of curcumin in *C. longa* rhizomes. The proposed MAE method allows the extraction of these compounds in a shorter time (3 min) with higher efficiency when compared to the conventional solvent method. Therefore, MAE proved to be an attractive alternative to conventional extraction methods for the extraction of curcumin from *C. longa* rhizomes. Consequently, we suggested that proposed method can be helpful for rapid isolation of curcumin and routine assay of *C. longa* containing extracts, formulations and commercial products.

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*Author has no conflict of interest*
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