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EXTRACTION OF BIOACTIVE COMPOUND CURCUMIN FROM TURMERIC (*CURCUMA LONGA L.*) VIA DIFFERENT ROUTES: A COMPARATIVE STUDY

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ABSTRACT

In this study, extraction of curcumin, the bioactive compound of turmeric (*Curcuma longa L.*), through different extraction methods was investigated. For this purpose, microwave-assisted, ultrasound-assisted and enzyme-assisted extractions were used as modern extraction routes to extract curcumin from turmeric powder and some of their essential extraction parameters were optimized; the results were compared to those obtained from Soxhlet extraction as the traditional and reference method. The presence of curcumin in the extracted samples was confirmed by UV-vis spectroscopy and quantification of curcumin was carried out by high performance liquid chromatography (HPLC) analysis. Result showed that the curcumin extraction yield using Soxhlet method (6.9%) was considerably higher than those obtained from microwave-assisted (3.72%), ultrasound-assisted (3.92%) and enzyme-assisted (4.1%) extractions; however, the highlighted features of advance extraction methods including cost-effectiveness (due to much saving in time and energy consumption) and environmentally benign nature make them more favorable extraction methods.

Keywords: Curcumin, Microwave-assisted extraction, Ultrasound-assisted extraction, Enzyme-assisted extraction, Soxhlet extraction

INTRODUCTION

Curcumin, a yellow crystalline polyphenol with low molecular weight, is extracted from rhizome of turmeric. Turmeric belongs to the perennial herb named *Curcuma longa L.* which is prevalent in tropical and subtropical regions, mostly in India, South East Asia and China. India is the first producer, consumer and exporter of *Curcuma longa* in the world (Ching et al., 2014). The biological characteristics of turmeric is known to be attributed to curcuminoids content which exist in dense structure of turmeric. Curcumin has wide range of applications as a dietary food ingredient, dying agent, therapeutic agent and medicament in different diseases. Curcumin has various pharmacological effects including antioxidant, antibacterial, anti-inflammatory, hepatoprotective, anti-tumor and anti-viral activities (Wang et al., 2013, Tajik et al., 2007). It has been proved that curcumin is a potential agent for prevention and treatment of different cancers including: gastro-intestinal, breast, lung, melanoma, head and neck, neurological and sarcoma cancers (Duvoix et al., 2005, Anand et al., 2008, Bar-Sela et al., 2010, Ravindran et al., 2009). *Curcuma longa* contains 2-9 % curcuminoids which are comprised of curcumin, demethoxycurcumin and bisdemethoxycurcumin which all belong to the diarylheptanoids (Salem et al., 2014, Tajik et al., 2008). The percentage composition of curcuminoids depends on geographical conditions; it roughly contains 80 % curcumin, 17 % demethoxycurcumin and 3 % bisdemethoxycurcumin (Esatbeyoglu et al., 2012). The chemical structure of curcumioids is illus-trated in Figure 1 (Huang et al., 2016, Bairwa et al., 2014).

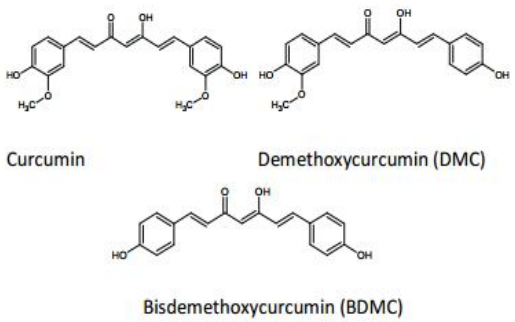


Fig. - 1: Chemical structure of curcumin and its analogs

During the last decades, interests in the field of extraction processes led to new technological achievements to obtain curcumin with admirable properties. The structural characteristics of curcumin and its properties can be significantly affected by the extraction method. The extraction methods can be divided into conventional and modernroutes. Conventional extraction which is based on extracting power of solvents includes Soxhlet extraction, maceration and hydro-distillation. Soxhlet extraction was first designed in 1879 to extract lipid; however, it turned to a common and popular method to extract wide range of bioactive compounds from natural plants with high efficiency (Soxhlet, 1879). This method is considered as a basic extraction technique to evaluate the efficiency of other

conventional and non-conventional extraction methods. Maceration is another traditional method which is inexpensive and popular and is usually performed in a closed vessel with grinded plant material (Azmir et al., 2013). Hydro-distillation is another traditional extraction method used for extraction of bioactive compounds and essential oils. It can be divided into three types; water distillation, water and steam distillation and direct steam distillation. Hot water and steam are used as the main solvents to extract bioactive compounds from plant tissue (Vankar, 2004). This method eliminates separation step of solvent and extract, because oils and organic compounds like curcumin are separated automatically in condenser (Silva et al., 2005). Use of these traditional extraction methods has been limited because of drawbacks such as high temperature, consumption of large amount of solvent, long extraction time, evaporation of huge amount of solvent and low yield (De Castro and Garcıa-Ayuso, 1998).

The disadvantages associated with traditional extraction methods provokes researchers to look for advance extraction techniques which could incorporate high extraction efficiency and environmentally friendly technology. The goal of modern extraction methods is to use less dangerous volatile organic solvents, applying safer and renewable chemicals, designing methods for energy saving processes, prevention of pollution and producing pure products with fewer derivatives (Azmir et al., 2013). Organic solvents such as hexane, acetone, methanol, isopropanol, ethyl acetate and ethanol have been used for extraction of oleoresins from turmeric (Popuri and Pagala, 2013). The most common advance extraction methods include: microwave-assisted extraction, pressurized liquid extraction, supercritical fluid extraction, enzyme-assisted extraction and ultrasound-assisted extraction(Azmir et al., 2013). Microwave-assisted extraction has been recognized as a useful technique for improving the diffusion of solvent and extraction of bioactive compounds. Microwave is based on electromagnetic energy which can be converted to heat. This conversion depends on the polarity of the utilized solvent. Some advantages of microwave-assisted extraction include faster heating of compounds, reduced thermal gradients, high extraction yield and small equipment size (Cravotto et al., 2008). This technique was used to extract organic and organometallic compounds; it has been recognized as a green technology due to the low consumption of organic solvent (Alupului et al., 2012, Wakte et al., 2011). Ultrasound is a range of sound wave beyond human hearing, between 20 kHz to 100 MHz; the mechanism of ultrasound is divided into thermal and non-thermal. In thermal mechanism, the acoustic energy absorbed is turned to heat, while in non-thermal action, the acoustic streaming leads to cavitation in passing through liquid and liquid containing solid materials. (Azmir et al., 2013). Ultrasound energy cause leaching of organic compounds like curcumin from turmeric powder. Ultrasound-assisted extraction can save energy and time, reduce extraction temperature and the amount of solvent, accelerate energy transfer, provide selective extraction and increase the productivity (Chemat et al., 2008, Li et al., 2014). Generally, extraction methods should have enough selectivity towards targeted compounds and extract the product in suitable form and could recycle the solvents.

The aim of this study was to use several conventional and modern extraction methods for extraction of the bioactive compound, curcumin, from turmeric and compare the extraction efficiency of different methods. For this purpose, microwave, ultrasound and enzyme assisted extraction were implemented to extract curcumin from turmeric and the results were compared to those obtained from Soxhlet extraction as the reference extraction method.

MATERIALS AND METHODS

Materials: Dried rhizomes of turmeric were purchased from Mashhad, Khorasan province, Iran. Standard curcumin was acquired from Merck, Germany. Methanol, acetone, acetonitrile and water (HPLC grade) were provided from Merck for analysis and quantification of curcumin. Enzymes including α-amylase and amyloglocosidase were supplied from SERVA, Germany. All other solvents and chemicals were pure and reagent grade.

Curcumin extraction:

Conventional extraction using Soxhlet: The rhizomes of turmeric were dried in oven at 105 °C for 3 h. Dried rhizomes were triturated using mortar and screened through a sieve with mesh 80 to obtain uniform powder with particle size of 0.18 mm. The turmeric powder was stored in refrigerator to prevent moisture uptake. The Soxhlet extraction, as the reference method, was performed as follows: 15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent. The extraction experiment was carried out at 60 °C within 8 h. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator (Stuart RE300) under vacuum at 35 °C. The residue (oleoresin) was dried and weighed; then dissolved in 10 ml methanol for calculation of curcumin content using HPLC. In all extraction experiments acetone was used as the extraction solvent due to its high solubilization capacity.

Microwave-assisted extraction of curcumin: For microwave-assisted extraction of curcumin, 0.5 g of turmeric powder was weighed and dissolved in 10 ml acetone and put in microwave chamber (domestic Samsung microwave). Acetone which was used as extraction solvent has good dissipation factor (tan δ =

0.5555) which can be heated up to high extent and dissipate the microwave energy. Extraction was carried out at different microwave operating powers varied between 100-450 W and different irradiation times of 0.5-3 min. The samples were subjected to microwave irradiation in an intermittent way of irradiation-cooling-irradiation for extraction time of up to 3 min, because longer irradiation time and higher power caused boiling of solvent. After that, the solvent was separated through 0.45 µm filter and evaporated under vacuum, the residue was weighed and dissolved in 10 ml methanol for HPLC analysis.

Ultrasound-assisted extraction of curcumin: An ultrasonic bath (Elmasonic S 10 H, Elma Schmibauer GmbH, Germany) with tank capacity of 0.8 liter was used for extraction of curcumin. The bath power was 90 W with 37 kHz frequency. For extraction experiment, 0.5 g of turmeric powder was dissolved in 10 ml acetone and immersed in ultrasonic bath. The extraction was performed at different extraction times from 10 to 40 min and temperatures of 25-40 °C. During the experiments, the Erlenmeyer containing the sample was covered with parafilm in order to prevent solvent loss. The extract was filtered through 0.45µm filter and the solvent was evaporated under vacuum. The residue was weighed as oleoresin and dissolved in 10 ml methanol for HPLC analysis to determine its curcumin content.

Enzyme-assisted extraction of curcumin: In this set of experiments, 1 g of turmeric powder was weighed in 250 ml Erlenmeyer flask and mixed with 100 ml water followed by addition of 50 ml McIlvaine's buffer pH 5. α-Amylase and amyloglocosidase enzymes were used for turmeric hydrolysis because more than 80% of turmeric is composed of carbohydrate which can be hydrolyzed by these type of enzymes (Kurmudle et al., 2013). Different concentrations of α-amylase and amyloglocosidase varying between 1-5% w/w of turmeric for each enzyme was added to the described mixture. Water is poor extraction solvent but necessary for enzyme activity. The flask was sealed with cotton and aluminum foil and shaken in shaker incubator (IKA IC4000) at 130 rpm and incubation temperature of 65 °C which was the suitable temperature for high activity of α-amylase and amyloglocosidase. The turmeric powder was hydrolyzed at different incubation times of 2-8 h. After enzyme pretreatment, the suspension was settled down and water was separated from turmeric powder; the precipitated turmeric was dried at 60 °C for 6 h and then subjected to curcumin extraction using 10 ml acetone for different extraction times of 1-5 h. The solvent was then separated from turmeric and evaporated by rotary evaporator. The residue was weighed as oleoresin and dissolved in 10 ml methanol for analysis of its curcumin content.

Analytical methods: High Performance Liquid Chromatography **(**HPLC, Knauer, Germany) with UV detector was used for calculation of curcumin concentration. The column was C18 with dimension of 250×4.6 mm; the mobile phase was composed of acetonitrile and water at ratio of 90/10; the flow rate was 1.0 ml/min at room temperature. For HPLC analysis of extracted powder, standard curcumin with different concentrations of 1, 2.5, 8, 10 ppm was prepared and dissolved in HPLC grade methanol and then injected into HPLC. The calibration curve of standard curcumin was developed. To determine the curcumin content of unknown samples, a certain concentration of sample (5 ppm) was made, passed through 0.45 µm filter and injected into the system. Quantification of extracted curcumin was performed using the peak area at specific retention times and standard curve. The amount of extracted curcumin (% w/w) from turmeric was calculated as follows:

$$\text{Curcumin yield (\%)} = \frac{M_{\text{extracted Curcumin}}}{M_{\text{pristine turmeric}}} \times 100$$

The extracted solutions were also analyzed for presence of curcumin by UV-vis spectrophotometer (Analytik Jena AG, Germany).

RESULTS AND DISCUSSION

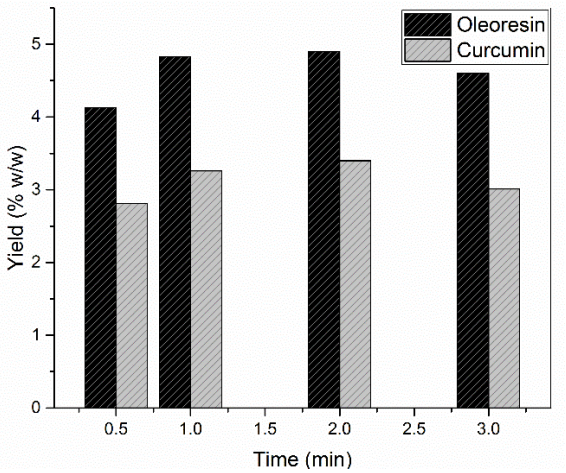
The extraction of curcumin from turmeric through different extraction methods was investigated. To evaluate the efficiency of the examined methods for curcumin extraction, Soxhlet extraction was considered as the base method and the extraction yields were compared. Soxhlet extraction is one of the foremost and most common extraction techniques wherein long extraction time at high temperature facilitate the extraction of target compound; moreover, the repeated contact of solvent with turmeric can enhance the extraction yield. In Soxhlet extraction, the total yield of extracted curcumin and oleoresin was determined by HPLC analysis which was 6.9 and 8.29%, respectively.

To see the effectiveness of microwave for extraction of curcumin from turmeric, experiments were conducted in microwave and the effect of irradiation time and power on extraction yield of curcumin was evaluated. The results of this investigation are presented in Fig. 2. The effect of extraction time (0.5 to 3 min) at constant microwave power of 100 W is depicted in Fig. 2 (a). Results show that increase of extraction time from 0.5 to 2 min enhanced the extraction yield of oleoresin and curcumin from 4.13 and 2.81% to 4.9 and 3.4%, respectively; however, prolonging the extraction time to 3 min adversely affected the extraction yields of oleoresin and curcumin. This reduced yield could be attributed to the disruption of the curcumin structure and evaporation of solvent at lengthy extraction time. The effect of microwave irradiation power on extraction yields at constant irradiation time of 2 min is depicted in Fig. 2 (b). As

indicated in this figure, increase of microwave power from 100 to 300 W increased the extraction yield of oleoresin and curcumin, while further increase of power to 450 W reduced the extraction yield. At high microwave power, heat build-up inside the material exposed to microwave irradiation possibly led to the cell disruption and lowered the extraction yield. The microwave power of 300 W and irradiation time of 2 min wherein extraction yields of 5.19 and 3.72% were obtained for oleoresin and curcumin and considered as the suitable operation conditions.

Extraction of curcumin from turmeric was carried out using ultrasound waves and the effect of sonication time (10-40 min) and temperature (25-40 °C) on curcumin extraction yield was examined. Fig. 3 (a) shows the effect of extraction temperature on extraction yields at fixed sonication time of 20 min. The extraction yields of oleoresin and curcumin improved from 4.51 and 3.37% to 5.28 and 3.65%, respectively, as ultrasonic temperature increased from 25 to 35 °C. Increase of temperature to 40 °C decreased the yields considerably because of evaporation of acetone which was used as extraction solvent or partial destruction of curcumin during sonication. Fig. 3 (b) exhibits the effect of sonication time on extraction yields at constant temperature of 35 °C. As shown in the figure the highest extraction yields were obtained at sonication time of 30 min which were 5.72 and 3.92% for oleoresin and curcumin, respectively. Prolonging the sonication time to 40 min was not effective to enhance the extraction yield and even reduced the amount of oleoresin and curcumin being extracted which could be due to the dissociation of curcumin structure at long exposure to ultrasound wave.

Curcumin is captured in polysaccharide-lignin network in turmeric (Azmir et al., 2013). Enzymatic pretreatment of turmeric powder prior to extraction can be very effective to disrupt the turmeric wall and facilitate the diffusion of solvent, where it can easily pass through the structure of turmeric and extract the curcumin. In this study, hydrolytic enzymes including α-amylase and amyloglocosidase were used for pretreatment of turmeric which was then subjected to extraction using acetone. Fig. 4 (a) and (b) represent the effect of enzyme concentration (1-5 w/w of turmeric %) and incubation time (2-8 h) on extraction yields, respectively; in these experiments enzyme-pretreated turmeric was subjected to extraction using acetone at room temperature for 4 h and incubation time of 2 h. Studies on enzyme concentration (Fig. 4 (a)) showed that as enzyme loading increased from 1 to 5%, the oleoresin and curcumin extraction yield enhanced; however, the increase in extraction yields was not significant and there was only a slight improvement in yields at enzyme loadings above 3%. At this concentration, the extraction yields for oleoresin and curcumin were 5.46 and 3.69%, respectively.



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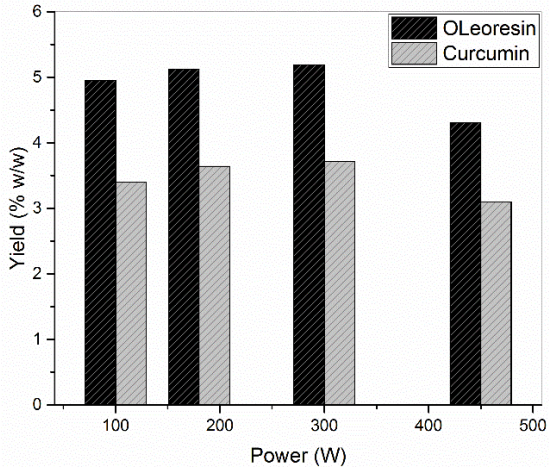
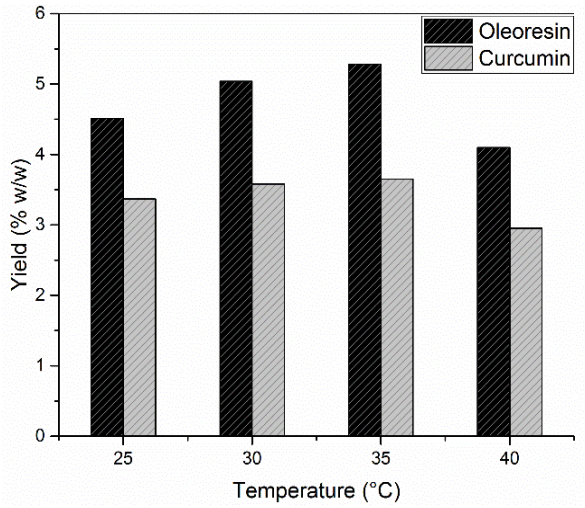


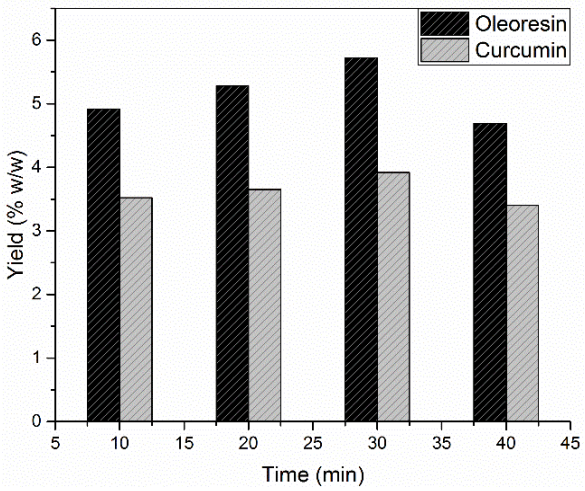
(b)

Fig. - 2: The effect of (a) irradiation time at constant power of 100 W and (b) microwave power at constant time of 2 min on oleoresin and curcumin extraction yields

Studies on incubation time (Fig. 4 (b)) revealed that increase of time from 2 to 6 h improved the curcumin yield from 3.69 to 4.1%, but after 6 h, the extraction yield remained almost constant (4.12%) as the extraction time was extended to 8 h. After determination of suitable conditions for enzyme pretreatment (enzyme loading 3% and incubation time of 6 h), the effect of extraction time using acetone was investigated; the results are reflected in Fig. 5. Results showed that the extraction time of 4 h was suitable to obtain the extraction yields of 6.27 and 4.1% for oleoresin and curcumin, respectively; as the extraction time was prolonged to 5 h the enhancement in extraction yields was very insignificant.

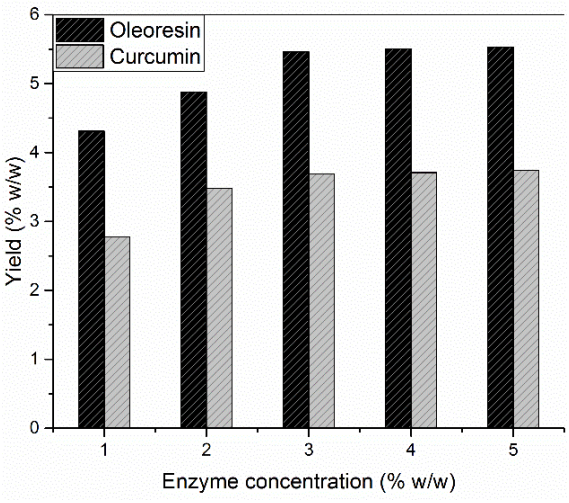


(a)

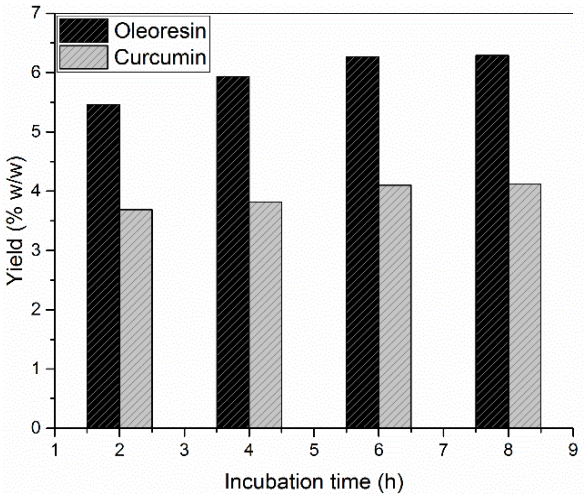


(b)

Fig. - 3: The effect of sonication (a) temperature at constant time of 20 min and (b) time at fixed temperature of 35 ^{o}C on extraction yields of oleoresin and curcumin



(a)



(b)

Fig. - 4: The effect of (a) enzyme concentration and (b) incubation time on extraction yields of oleoresin and curcumin

To confirm the presence of curcumin in the extracted sample, UV-vis spectroscopy analysis was conducted. The absorbance spectra of standard and extracted curcumins using different methods were collected in the range of 350 to 600 nm as shown in Fig. 6. The standard curcumin represented an absorption peak at around 420 nm. This characteristic peak which corresponds to the diarylheptanoid chromophore group of curcumin is observed in the spectrum of different samples obtained from Soxhlet, different samples obtained from Soxhlet, microwave-assisted, ultrasound-assisted and enzyme-assisted extraction. Also, the intensity of the peaks corresponds to the efficiency of the implemented extraction methods where Soxhlet extraction was the most effective one followed by enzyme-assisted extraction; microwave-assisted and ultrasound-assisted extractions showed almost same performance.

The HPLC analysis was performed for quantification of extracted curcumin from turmeric powder. Fig. 7 represents the results of HPLC analysis for curcumin extracted using Soxhlet, enzyme-assisted, ultrasound-assisted and microwave-assisted extractions as compared to standard curcumin. As could be inferred from the HPLC chromatograms, the highest peak was obtained from Soxhlet extraction, followed by enzyme-assisted, ultrasound-assisted and microwave-assisted extractions from which the curcumin extraction yields were obtained as 6.9, 4.1, 3.9 and 3.7%, respectively.

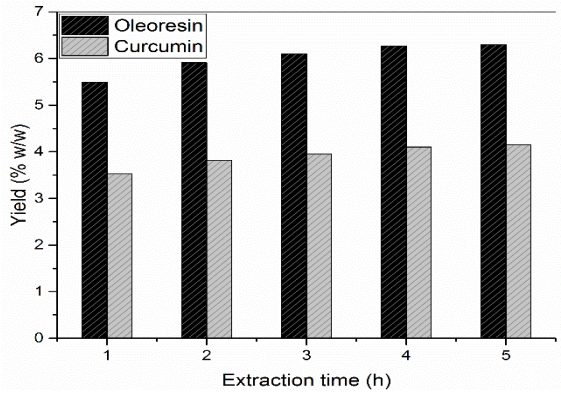


Fig. - 5: The effect of time on extraction of curcumin from enzyme-treated turmeric using acetone

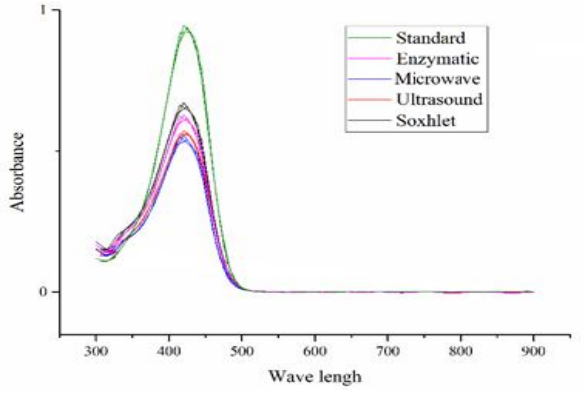
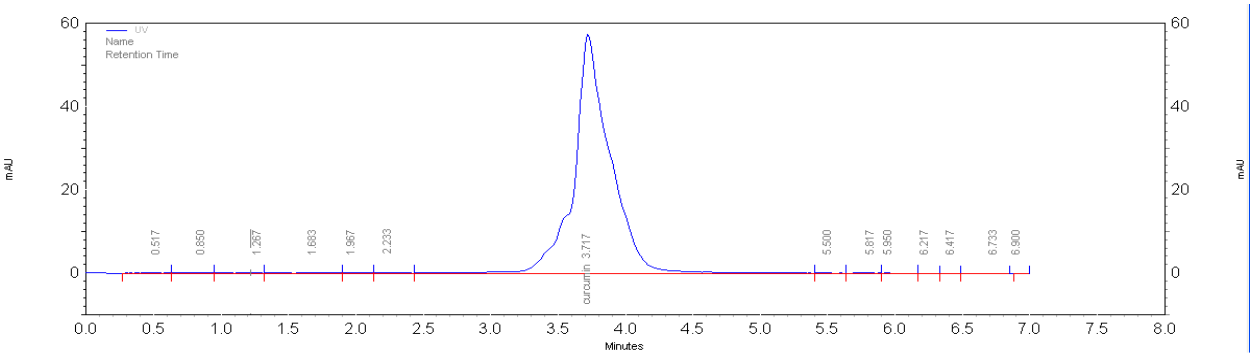


Fig. - 6: UV-vis spectra of extracted curcumin using Soxhlet enzyme-assisted, ultrasound-assisted and microwave-assisted extractions



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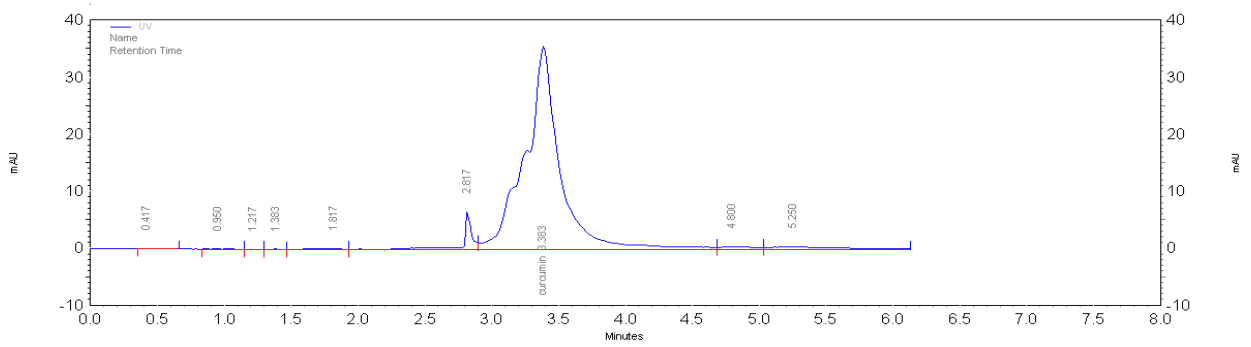

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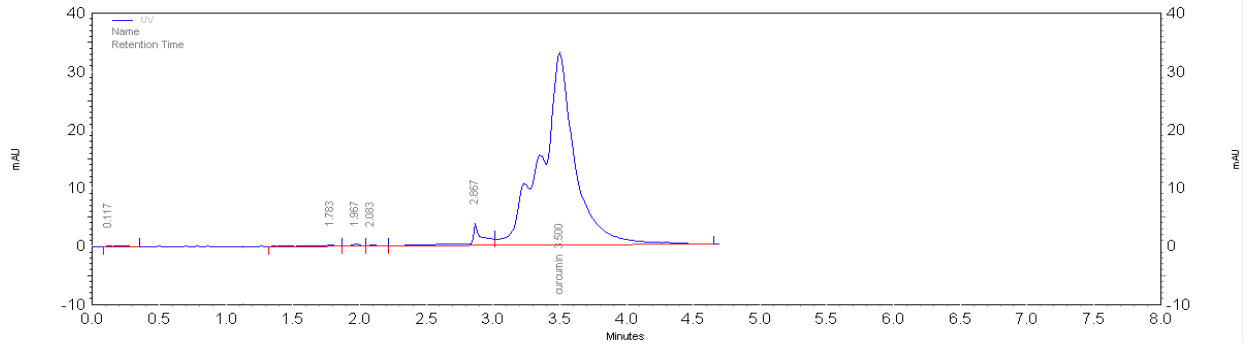

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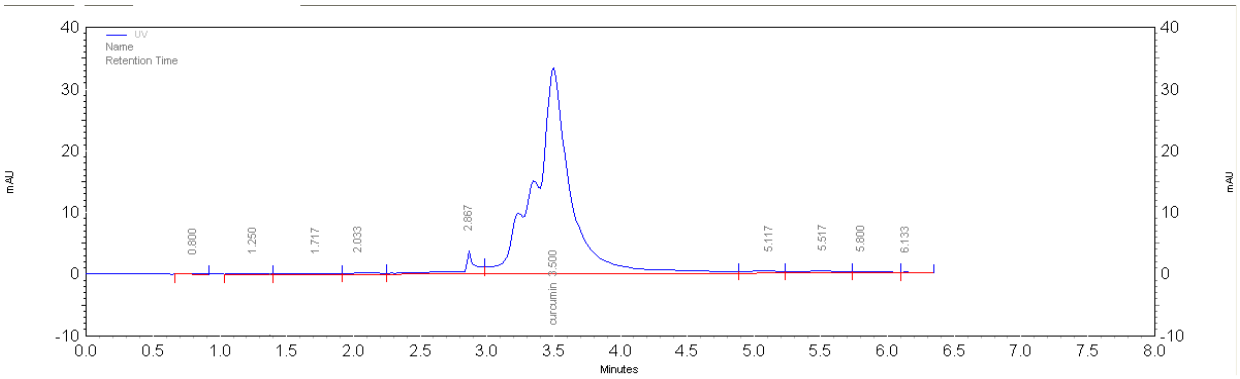

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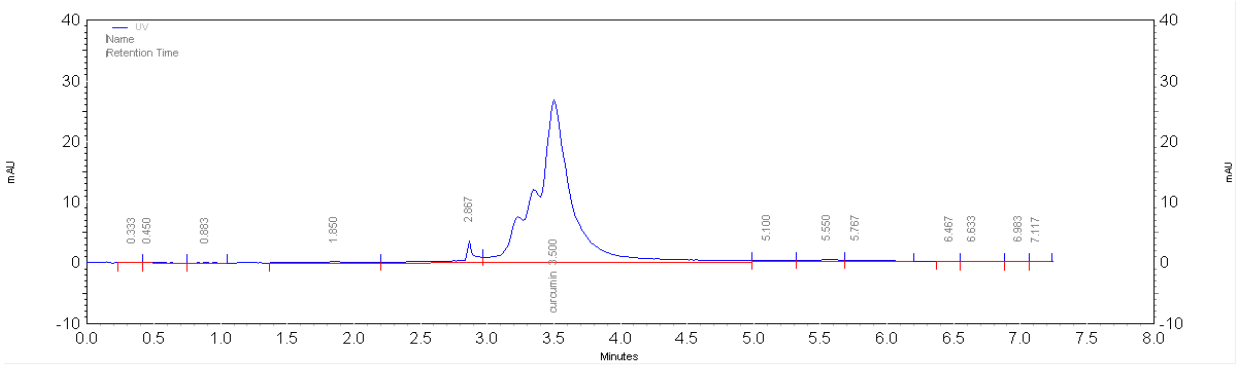

(e)

Fig. - 7: HPLC chromatograms of (a) standard curcumin, (b) curcumin extracted using Soxhlet extraction, (c) curcumin extracted using enzyme-assisted extraction , (d) curcumin extracted using ultrasound-assisted extraction and (e) curcumin extracted using microwave-assisted extraction

The suitable extraction condition for different implemented extraction routes; i.e., microwave-assisted, ultrasound-assisted and enzyme-assisted extraction as well as maximum extraction yields are summarized in Table 1. The objective of this work was to evaluate the efficiency of different extraction methods for extraction of curcumin from turmeric as compared to the Soxhlet extraction as the most common and traditional extraction method. As indicated by results in Table 1, the extraction yields obtained by Soxhlet extraction were considerably higher than those obtained from other methods; however, the harsh operation condition of this method including long extraction time (8 h), high extraction temperature (60 °C) and use of huge amount of solvent (350 ml as compared to only 10 ml for same amount of turmeric in other methods) makes this method unattractive. Although the other extra-ction methods used in this study resulted in lower yields but seem to be more promising from both economic (much less time and energy consumption compared to Soxhlet) and environmental (consumption of much less solvent) views.

Table - 1: Curcumin and oleoresin extraction yields obtained from different extraction methods

Method	**Extraction condition**	**$Y_{oleoresin}$ (%)**	**$Y_{curcumin}$ (%)**
Soxhlet	Extraction temperature= 60°C Extraction time=8 h	8.29	6.90
Microwave-assisted extraction	Microwave power= 300 W Irradiation (extraction) time = 2 min	5.19	3.72
Ultrasound-assisted extraction	Sonication temperature= 35 °C Sonication (extrac-tion) time= 30 min	5.72	3.92
Enzyme-assisted extraction	Enzyme concentration=3 w/w% Incubation time= 6 h Extraction time= 4 h	6.27	4.1

CONCLUSION

Extraction of curcumin from turmeric using several advance methods was investigated and the results were compared to those obtained from Soxhlet as the most common and reference method. Although the modern

extraction methods including microwave-assisted, ultrasound-assisted and enzyme-assisted extractions did not show high extraction yields as high as Soxhlet method, but their highlighted advantages such as low extraction temperature, short extraction time and use of very few amount of solvent makes them more favorable extraction methods.

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