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Synthesis and Semi-synthesis of Curcumin as a Medical Precursor and Its Derivatives with Desirable Purity and Qualitative and Quantitative Evaluation

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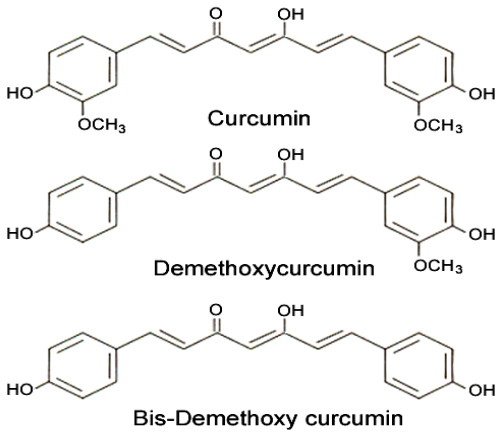
ABSTRACT

Spices and herbs are considered rich sources of powerful antioxidants. Since 2000 years ago, spices and herbs have been utilized as fragrances, colourants, and medicines. Both natural and synthetic antioxidants inhibit or delay oxidation. Due to multiple adverse effects of synthetic antioxidants on human health, there is currently an ever-increasing demand for the natural antioxidants. One of the useful spices, turmeric, with the folk name of *Curcuma longa* belongs to the ginger family. Turmeric usually grows in tropical regions in India, China, Malaysia, and the Philippines. Antioxidant and medicinal substances are derived from turmeric's rhizome. Turmeric has been used to treat many diseases due to its wide range of medicinal applications. Turmeric has been used as herbal medicine. It is traditionally used to treat a variety of diseases such as head cold, cough, sinusitis, anorexia, diabetic ulcers, cramps, and liver obstruction of gastric ulcer. Three different types of curcuminoids exist as bioactive compounds in turmeric including curcumin as the main constituent, and dimethoxy curcumin, and bis-dimethoxy curcumin. These compounds differ in the position of the methoxy group. This study aimed at synthesizing and analyzing the curcuminoid using the HPLC technique and related tests. The results obtained from the analysis of the synthetic curcumin were consistent with those for the standard curcumin. The reaction was carried out at relatively milder conditions than earlier reported methods.

GRAPHICAL ABSTRACT



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Introduction

Over the last few decades, extensive research in the field of medicine and various sciences has led to significant growth in the discovery and investigation of bioactive pharmaceutical compounds. Among these compounds, curcumin was first extracted by Vogel in 1842 from the root of the *Curcuma longa* [1, 2]. Turmeric, the root of the *Kuma Longa*, is traditionally used as a spice and a food colorant [3]. Turmeric plays an important role in traditional medicine among Southeast Asian countries and has been used as a soothing agent for wounds and inflation for thousands of years in China and India [4]. This characteristic can be attributed to the presence of curcumin as a bioactive compound in the turmeric structure [5]. The root of Curcuma longa contains 2–9% curcumin on average. Curcumin with the chemical name di-Ferro ethyl methane is a yellow crystalline polyphenolic compound. Curcumin is naturally present in combination with two other curcuminoids, namely dimethoxy curcumin and bismetoxic curcumin [6]. The curcumin structure consists of two ferric acid rings linked together by an alpha-beta de ketone chain. As two stable isomers of curcumin, enol and keto are hydrogen-bonded and change with changes in the ambient pH (Figure 1) [7, 8]. The literature on the biological activity of curcumin indicates antioxidant and anticarcinogenic properties of this compound. The curative properties of curcumin are related to the phenolic, beta-keto, and methoxy groups. Curcumin can act as a radical donor by shifting the carbonyl oxygen electrons of the enol group in its structure and plays an important role in scavenging free radicals to prevent Alzheimer's and Parkinson's diseases. Curcumin also affects the signaling pathways of the nuclear factor-kB (the transcription factor in many eukaryotes for gene regulation, whose activity induces swelling, thereby proliferating and producing tumor cells). Swelling prevents the development and growth of cancer cells [9]. Curcumin can interact with many biological molecules such as tumor cytokines, transcription factors, kinases, enzymes, receptors, growth factors, proteins, and other ligands [10]. Biological availability is considered the main prerequisite for the biological activity of curcumin in the human body. However, over 90% of curcumin is excreted unchanged by the gallbladder with the stool due to low intestinal absorption and rapid metabolism in the liver. Therefore, there has been recently great interest in finding ways to utilize this active plant compound and increase its bioavailability [11]. The interaction mechanism of the Cur have very well been recognized based on the obtained quantitative and qualitative results by both of DFT calculations and MD simulations, in which the results indicated that the investigated Cur structures are supposed to be considered in further investigations for antidepressant activity evaluations [12]. Curcumin (a constituent of turmeric). New treatment option against COVID-19 [13]. Both quantitative and qualitative studies on curcumin are dependent on the selection of appropriate extraction methods. Liquid extraction of curcumin using organic solvents is a time-consuming process with low efficiency and requires a large volume of solvent at high operating temperatures leading to environmental pollution [14]. Due to these problems, the use of novel methods consuming lower amounts of organic and synthetic chemicals with a shorter extraction time and higher quality and high yield of the product is recommended [15]. Over the past decades, carbohydrate ionic liquids have been particularly employed in the extraction processes due to their prominent physiochemical properties such as low vapor pressure, low toxicity and flammability, and biological activities [16]. Protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), moisture content (13.1%), essential oil (5.8%), Felandren (1%), Sabinene (0.6%), Cineol (1%), Borneol (0.5%), Zingiberene (25%), and Curcuminoid (3-6%) are responsible for yellowish color [17]. Turmeric is used as an herbal remedy. Turmeric antioxidants are extracted from its rhizome. Traditionally, it is used to treat various types of diseases such as cold, cough, sinusitis, inappetence, diabetic

ulcers, stomach obstruction [18], and liver obstruction [19].

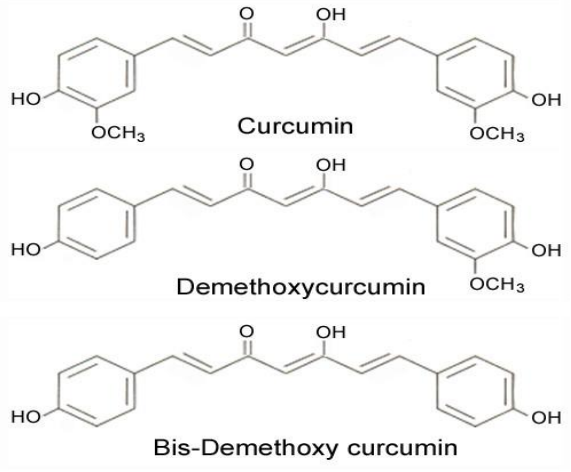

Figure 1: Different types of curcuminoid [18]

Material and methods

Chemicals and Method

The turmeric plant stem Standard curcumin (No. 1 Code 820345) depropylamine, diethylamine, and methanol were purchased from the Merck (Germany, Darmstadt). Solid carbon dioxide was obtained from Zarrin Branch (Karaj, Tehran).

Synthesis Method

First, 5 g of boric acid was added to a reflux balloon. Then, 2 g of N-butanol and 1 mL of toluene was added to the reflux balloon. The reflux balloon was then placed in the melted paraffin bath and the heater was set a constant temperature in the range of 118 °C to 130 °C. The reflux lasted for 6 -7 h to extract tri-butyl borate. The solution was then filtered and the filtrate was placed in an oven for 1 h to be dried. Then, it was placed in a refrigerator for 3 h to be crystallized. A total of 4.346 g crystal was obtained. Of this, 2.173 g crystal was poured into a watch glass and then placed in an oven for 2 h. The product removed from the oven weighed 1.872 g. The melting points of the synthesized solids were measured using the Electrothermal Model 9100. The infrared spectra of the samples were recorded on an FTIR spectrophotometer (Bruker) via KBr tablets in cm^{-1}. The magnetic resonance spectra of the proton nuclei were recorded using the Avance Bruker DRX-500 spectrometer (500 MHz hydrogen frequencies) with TMS internal standard and in DMSO as the solvent.

Curcumin Synthesis

First, 0.5 mL of acetylacetone was removed with petroleum and added to a 10 mL balloon, and 0.25 g of oxide was weighed. The mixture was added to a 10 mL closed balloon containing 5 mL ethyl acetate and mixed with a magnet and cooled to temperature 0 ℃. At this point, a complex of acetone (borane) was formed. $B(OBu)_3$ is a very strong acid that catalyzes the reaction by reacting with the carboxyl group of vanillin to produce more active carboxyl carbon.

Description of Experiment

Ethyl acetate was evaporated by a rotary evaporator at 65 °C after mixing the first and second solutions. It was the mixed 18 h on the heater, cooled to 50 °C, and 5 mL HCl 10% was added to the solution at the same temperature. Mixing lasted for 60 min. The resulting solution was then washed with ethyl acetate, and ethyl acetate was evaporated with the rotary evaporator. After this step, a certain amount of magnesium sulfate ($MgSO_4$) was poured on one side of the watch glass to accelerate the drying of curcumin. The evaporated ethyl acetate was then poured into a watch glass. The upper and lower phases were dried at 70 °C and 80 °C, respectively. These phases were dried on separate watch glasses for 5 days. The dried phases were then weighed, and curcumin content in the samples was measured by NMR, UV, FTIR, and HPLC techniques. The polymers were dried and powdered to prepare KBr tablets for the FTIR test.

Uv-Vis Test

First, a 100 ppm solution was prepared from 87% standard curcumin. Then, 50, 25, and 10 ppm solutions were prepared from the mother solution. The solution was diluted from the synthetic curcumin and then, the absorbance was recorded on the spectrophotometer in a

wavelength compatible with the solvent. Different concentrations were used to plot the calibration curve. In the resulting linear equation, instead of Y, the recorded figures were plotted against the blank sample to obtain X and the total curcumin content (mg of curcumin per 1 g turmeric).

HPLC Analyses

HPLC Agilent 1200 Series**,** Mobile Phase: methanol and acetic acid 1% (50:50), Detector Type: UV, Wavelength: 425 nm, Column: C18 (4.6 mm, 150 mm), Particle Size: 5 µm

HNMR Analysis

Nuclear magnetic resonance (NMR) is a physical observation in which nuclei in a strong constant magnetic field are perturbed by a weak oscillating magnetic field (in the near field and therefore not involving electromagnetic waves [20, 21]). This process occurs near resonance when the oscillation frequency matches the intrinsic frequency of the nuclei, which depends on the strength of the static magnetic field, the chemical environment, and the magnetic properties of the isotope involved. In practical applications with static magnetic fields up to ca. 20 tesla, the frequency is similar to VHF and UHF television broadcasts (60–1000 MHz). (**VHF**) is the ITU designation for the range of radio frequency electromagnetic waves (radio waves) from 30 to 300 megahertz (MHz), with corresponding wavelengths of ten meters to one meter. Frequencies immediately below VHF are denoted high frequency (HF), and the next higher frequencies are known as ultra-high frequency (UHF). (**UHF**) is the ITU designation for radio frequencies in the range between 300 megahertz (MHz) and 3 gigahertz (GHz), also known as the decimetre band as the wavelengths range from one meter to one tenth of a meter (one decimeter). NMR is the result of specific magnetic properties of certain atomic nuclei. NMR spectroscopy is widely used to determine the structure of organic molecules in a solution and also to study the molecular physics of crystals as well as non-crystalline materials. NMR is also routinely used in advanced medical imaging techniques such as in magnetic resonance imaging (MRI). The overall spin of the nucleus is determined by the spin quantum number S. If the numbers of both the protons and neutrons in a given nuclide are even then S = 0, i.e. there is no overall spins. Then, just as electrons pair up in nondegenerate atomic orbitals, so do even numbers of protons or even numbers of neutrons (both of which are also spin ½ particles and hence fermions), giving zero overall spin.

However, a proton and neutron will have lower energy when their spins are parallel, not anti-parallel. This parallel spin alignment of distinguishable particles does not violate the Pauli Exclusion Principle. The lowering of energy for parallel spins has to do with the quark structure of these two nucleons. As a result, the spin ground state for the deuteron (the nucleus of deuterium, the 2H isotope of hydrogen), which has only a proton and a neutron, corresponds to a spin value of 1, not of zero.

Result and Dissection

Curcumin Synthesis by Common Methods

Tri-butyl borate synthesis: After preparing the solution and storing it in a refrigerator for 3 days and crystallization, the obtained crystal was dried and weighed (4.346 g). Of this, half of the crystals (2.346 g) was dried. The total weight of 1.872 g was obtained after drying. Figure 2 and Figure 3 demonstrate the UV spectra of the standard and the synthetic curcumin upper phase at 70 and 80 °C. The peak observed at 390 nm at 70 °C such is assigned to curcumin. There is an impurity between 290 min to 340 nm indicating the lack of synthesis of a 100% pure curcumin. However, the traditional curcumin is close to the standard curcumin at 70 °C.

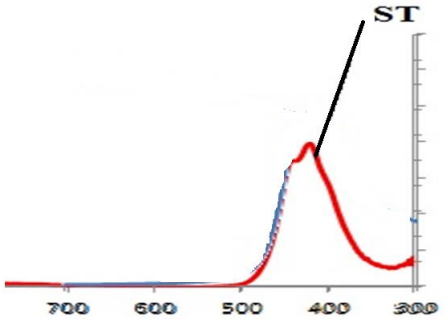

Figure 2: UV-Vis spectra of the synthetic and standard curcumin

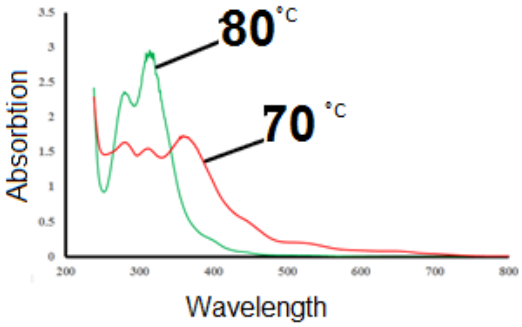

Figure 3: UV-Vis spectra for the upper phase at 70 and 80 °C

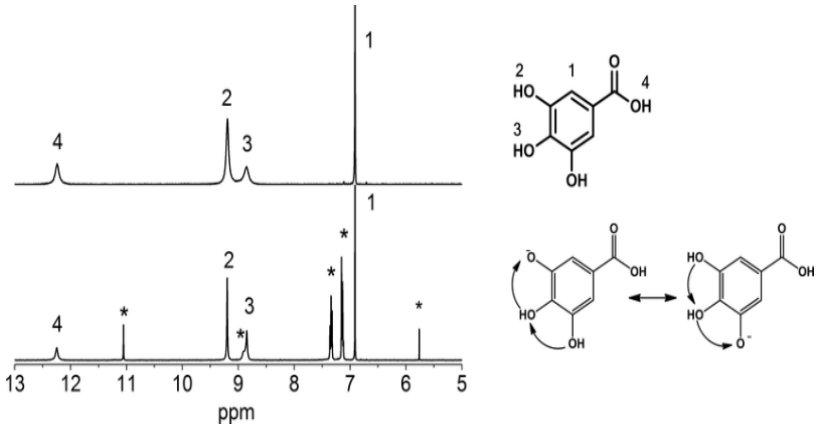

Figure 4: The HNMR spectrum for the standard curcumin

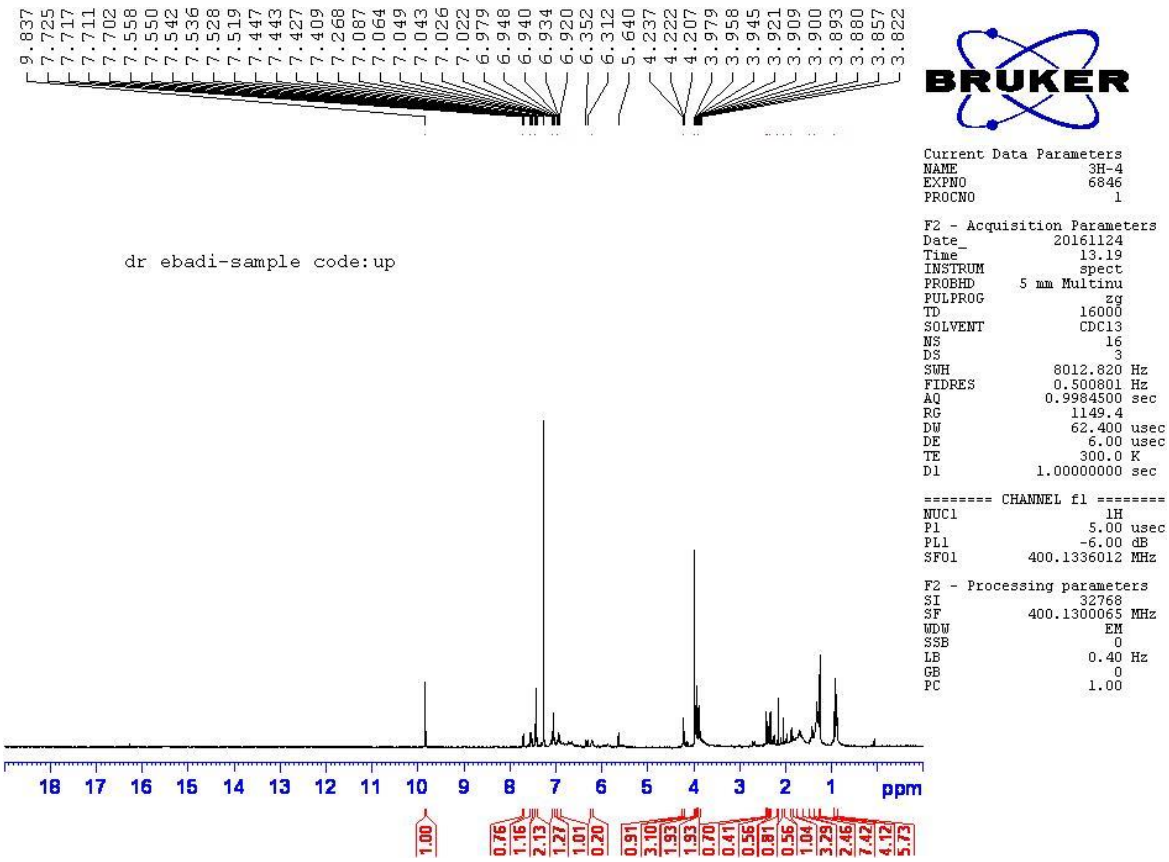

Figure 5: The HNMR spectrum for the synthesized curcumin

[1]HMMR Analysis

The powdered curcumin contained both phases I and II at 70 °C. After decentering, the powder was washed in ethyl acetate to confirm the synthesis of curcumin. Figure 4 and Figure 5 illustrate the NMR spectrum of the standard and the synthesized curcumin. As can be seen, the synthetic curcumin is closely matched to the standard curcumin. There is also a low level of impurities in the synthesized curcumin. The H-NMR spectrum of the synthesized curcumine indicated a sharp singlet at 10 ppm and it was assigned to the hydroxyl proton in the chain. The NMR signal of the methoxy proton in the ring at 3.9 ppm revealed that one methoxy group (with integral 3) located at C-2 of the ring. The chemical shift of two aromatic ring protons and methine groups in the chain are at 6-8 ppm which show the total integral equal to 11. Therefore the H-NMR data is in agreement with dimethoxy curcumine structure.

Table 1. Tri-butyl borate as the suitable Lewis acids.

	Lewis acids	Temperature (°C)	Time (h)	Product yield (%)
1	ETOAC	25	24	44.5

FTIR analysis

As seen in Figure 6, the peal appeared at the wavenumber 3509.81 cm^{-1} is assigned to the OH bond in the phenolic ring, and that at 1627.63 cm^{-1} is attributed to the C=C bond and the carbonyl group in the alkene chain of curcumin. The peak observed at 1428.99 cm^{-1} is related to the C=C bond in the aromatic ring of curcumin, and that at 856.24 cm^{-1} is assigned to the CH bond in the phenolic curcumin ring. The resulting FTIR spectrum confirms the chemical structure of curcumin (Mohan *et al.*, 2012). According to the FTIR spectra recorded at different temperatures (Figs. 6(b), 6(c), and 6(d), the FTIR of curcumin synthesized at 70 °C well matches that of standard curcumin. In Fig. 6(c), a slight shift is observed at a wavenumber of 3415 cm^{-1} correspondings to a carbon bonded with oxygen bound to hydrogen in the phenolic ring. The peak appeared at 1616 cm^{-1} is assigned to the C=C bond and the carbonyl group in the alkene chain, and that observed at a wavenumber of 1514 cm^{-1} is related to carbon-oxygen bonding to the methyl group in the phenolic ring. The peak appeared at 631 cm^{-1} is assigned to the carbon-hydrogen bond in the phenolic ring of curcumin.

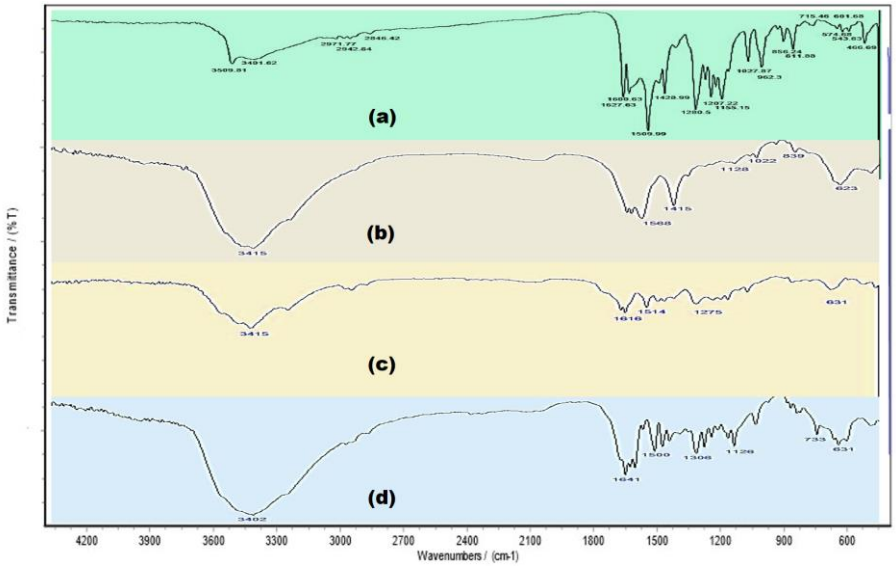

Figure 6: The FTIR spectrum of the standard curcumin(a), curcumin synthesized at 65 °C(b), curcumin synthesized at 70 °C**(c)** and curcumin synthesized at 80 °C(d)

Confirming Purity of Synthesized Curcumin by HPLC

Figures 7 and 8 display the HPLC spectra of standard and synthesized curcumin, respectively. As seen, synthesized curcumin (Figure 8) depicts the lowest extra peak compared to the standard curcumin (87%, Sigma), as seen in (Figure 7). The sharp peak of synthesized curcumin (B) reduces the likelihood of interference, indicating almost the identical purities of the synthesized and standard curcumin.

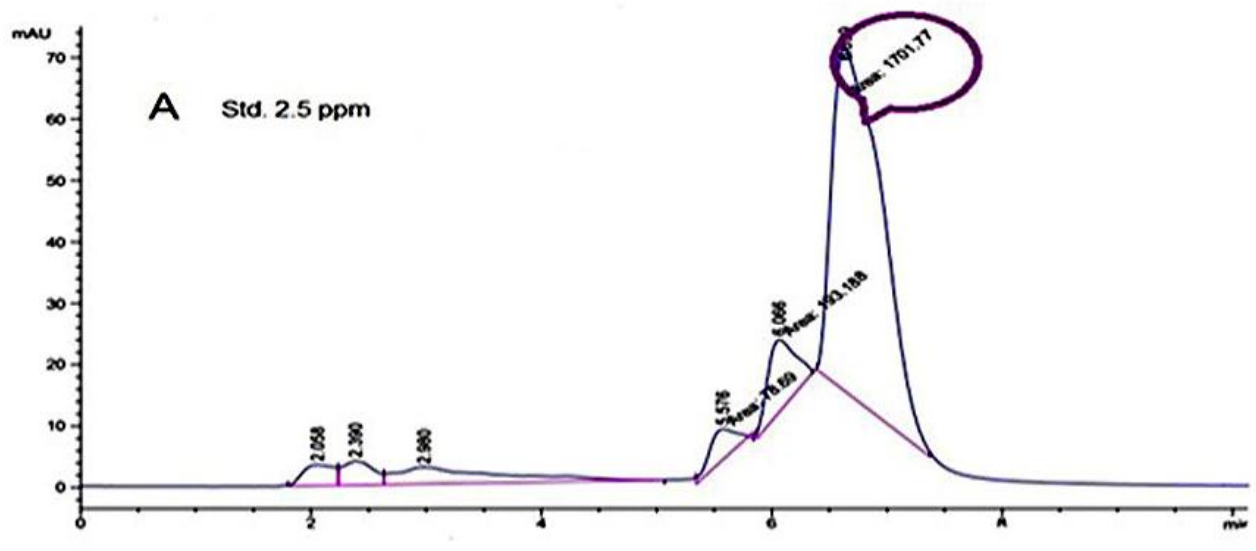

Figure 7: The HPLC spectrum of standard curcumin

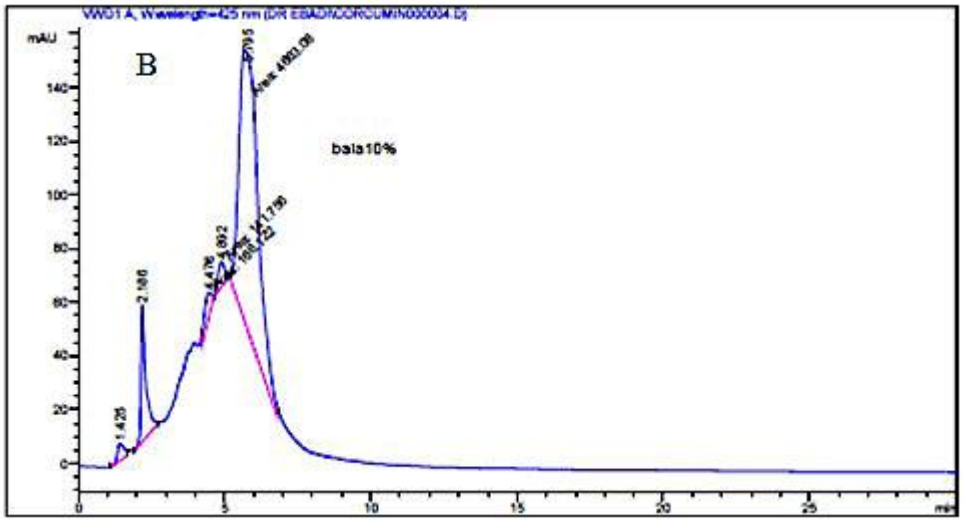

Figure 8: The HPLC spectrum of the upper phase of curcumin synthesized above 70 °C (HPLC interpretation)

Best-Synthesized Curcumin

Curcumin samples synthesized at 65, 70 and 80 °C were dried and weighed. In order to measure the curcumin content, the synthesized samples were dissolved in a solvent. Then the concentration was determined by UV-Vis spectroscopy to find the best sample with the highest curcumin content. As seen in Figure 9 and Table 1, the best sample containing the highest curcumin level was synthesized at 70 °C.

Curcumin with the highest yield was synthesized using the electrochemical method proposed in this study. The main advantages of this method are high speed, repeatability, and the lower number of synthesis steps. The purity of the

synthesized curcumin was significantly improved by the proposed synthesis method, and curcumin was synthesized selectively.

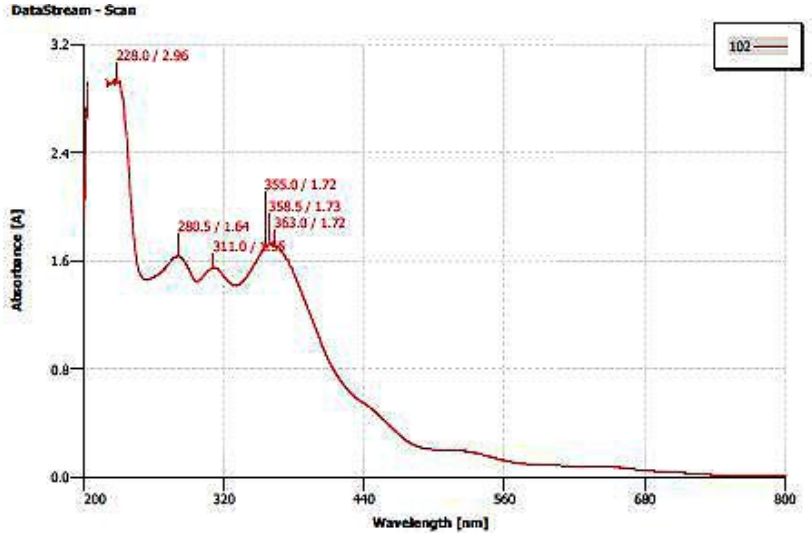

Figure 9: The UV-Vis spectrum for the best-synthesized curcumin

The necessary preparations performed before the synthesis of curcumin were (1) preparation of tri-butyl borate, (2) provision of the best synthesis conditions for the electrochemical technique, and (3) optimization of the synthesis time. Curcumin was synthesized at different temperatures, and the synthesis conditions were optimized. Decantation and ultra-sonication were used for the extraction of ethyl acetate. The solvent volume and type, and the synthesis time and temperature were optimized. The highest curcumin yield with the highest impurity was obtained at an optimal temperature of 70 °C. The synthesized curcumin was washed with ethanol. The major advantages of the magnetic stirrer used in the synthesis of curcumin are less mixing time, less solvent consumption, and the maximum curcumin yield. The synthesized curcumin was evaporated at the optimal temperature, dried in an oven, and then powdered. The peaks obtained from the HPLC exhibited the high purity of the synthesized curcumin like the standard curcumin. The UV-Vis spectrum of the sample was recorded at a wavelength of 200-800 nm, and the obtained peaks confirmed the high purity of the synthesized curcumin which was close to that of the standard curcumin. The FTIR spectrum of the powder dispersed in KBr showed the presence of identical functional groups in the synthesized and standard curcumin. No additional interfering compound was found in the synthetic curcumin relative to the standard curcumin. These results were close to those for the standard curcumin.

Conclusion

In this research study, the curcumin was synthesized using a novel electrochemical method at various temperatures. The product was extracted by ethanol through decantation and rotary evaporation. This simple but time-consuming process eventually resulted in a high-purity product. Curcumin with the highest purity was obtained at 70 °C for 18 h. The proposed synthesis method improved the yield while reducing the costs and development time. The reaction is carried out at relatively milder conditions than earlier reported methods. Innovation in the method of preparation of curcumin at low temperatures has not been mentioned in an article.

Acknowledgment

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Conflict of Interest

We have no conflicts of interest to disclose.

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