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Isolation and Structural Characterization of Curcuminoids with Spectral and Chromatographic Techniques

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Abstract

Curcuminoids are the active ingredients of *Curcuma longa L.* and are one of the most researched subjects owing to their biological activities. This study focuses on the structural analysis of curcuminoids isolated from turmeric roots using NMR spectroscopy. Turmeric rhizomes were extracted with methanol and hexane. Curcuminoids were isolated using column chromatography, and preparative HPLC-UV. The structures of the isolated compounds were characterized using FT-IR, UV-Vis, and GC-MS as well as NMR. Spectral and physicochemical data showed that isolated curcuminoids (ar-turmeron, curcumin, demethoxycurcumin, and bisdemethoxycurcumin) were obtained entirely from the turmeric rhizomes. When both isolation methods are compared, it was concluded that the prep-HPLC method is efficient and practical, while column chromatography is cheap and easy. In both methods, efficient and pure curcuminoids could be easily obtained by using the solvent mixtures specified in this study.

Keywords: Turmeric, Curcuminoid, Isolation, NMR, Chromatography

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Introduction

Traditionally used medicinal plants contain plenty of bioactive molecules having medicinal and pharmacological properties. Turmeric (*Curcuma longa L.*) is a rhizomatous species distributed widely in tropical and subtropical regions of the world, including Southeast Asia and India [1].

Since ancient times, dried rhizomes have been commonly used as spices, food preservatives, and coloring materials. It has also been reported that curcumin rhizomes have antioxidant, antimicrobial, anticarcinogenic, thrombo-suppressive, cardiovascular, anticoagulant, hypoglycemic, antiarthritic and antidiabetic properties. They promote menstruation and relieve pain [2-4].

Curcuma species contain active ingredients such as curcuminoids, sesquiterpenes, alkaloids, and monoterpenes. Curcuminoids, which constitute approximately 3-5% of turmeric, are considered one of these active ingredients [5]. Curcuminoids are curcumin's (CUR) derivatives such as demethoxy-curcumin (DMC), α-turmerone, and cyclocurcumin. Each of these active ingredients has various pharmacological properties, both separately and in combination. CUR is the most active ingredient, and its pharmacological effectiveness (lowers blood cholesterol, suppresses type II diabetes, rheumatoid arthritis, and neurological diseases) has been

proven in many studies [6-11]. It is at least ten times more active than vitamin E [12].

In addition to CUR, other derivatives have critical properties. For example, bisdemethoxycurcumin (BDMC) and DMC effectively protected PC-12 and HUVEC cell lines [13]. DMC has almost as active an antioxidant effect as CUR [14], and it is more stable and active than CUR under physiological conditions (pH >7.30) [15]. It also shows anticancer activity against various types of cancers [16-18]. Moreover, DMC is more effective against breast cancer than other curcuminoids [19].

BDMC is more stable than DMC and CUR under physiological conditions [20]. It has been reported that BDMC can alleviate renal fibrosis [21], effective in different types of cancers [22-24].

It has been shown that ar-turmerone stimulates peripheral blood mononuclear cell proliferation and cytokine production [25] and is effective in various cancer types [26-28]. In addition, it has been reported to be effective against psoriasis and neurodegenerative diseases [29, 30].

Because of their various pharmacological effects, curcuminoids have considerable potential in many different industries, including food, cosmetics, and pharmaceuticals. Therefore, it is crucial to obtain and evaluate curcuminoids separately. There are studies in which preparative HPLC [31, 32], flash column chromatography [33, 34], preparative thin layer chromatography (TLC) [35, 36], preparative counter-current chromatography [37] and column chromatography [31, 32] were applied separately to *Curcuma longa* species [38, 39]. However, to our knowledge, no study has compared these two methods using the same method. Therefore, this study aimed to develop a suitable method for the separation, purification, and characterization of curcuminoids that are abundant in turmeric. Curcuminoids were successfully separated using prep-HPLC-UV and column chromatography (Fig. 1). Structural characterization of the isolated curcuminoids was performed using ^{1}H and ^{13}C nuclear magnetic resonance spectrometry (NMR), mass spectrometry (MS), ultraviolet-visible spectrometry (UV-Vis), and infrared spectrometry (FT-IR).

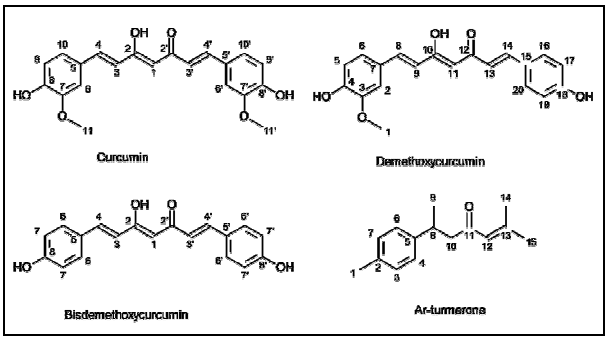

Figure 1. **Isolated curcuminoids from turmeric (*Curcuma Longa L.*)**

Material and Methods

Instrumentations, Chemicals and Reagents

A Thermo Dionex Ultimate 3000 preparative HPLC-UV and an Agilent PLRP-S C18 (150 x 4.6 mm, 5 µm) reverse phase column were used for instrumental isolation of the curcuminoids. Glass column and Merck 70-230 mesh ASTM silica gel 60 were used to separate the curcuminoids with the column chromatography. Merck F254 silica-imprinted aluminum plates were used to isolate curcuminoids using thin-layer chromatography (TLC). Bruker DPX–400 High-Performance Digital FT-NMR Spectrometer, Perkin Elmer Spectrum One FT-IR Spectrometer and Thermo Scientific TSQ Quantum Access Max Mass Spectrometer (ESI-MS) were used for qualitative analysis. NMR-grade and HPLC-grade solvents, that is, methanol, hexane,

chloroform, acetic acid, and acetonitrile DMSO-D6, were purchased from Sigma Aldrich and were obtained from Sigma-Aldrich and Merck Corporation. Analytical standard CUR (purity 95%) was purchased from Alfa Aesar.

Plant Material

Turmeric (*Curcuma longa L.*) rhizomes used in this study were purchased from herbalists. After proper cleaning, it was dried in the open air out of the sun. The samples were brought to a constant weight and ground in an electric grinder until they turned into powder. It was stored such that it did not absorb moisture until the extraction process.

Extraction Procedure of Curcuminoids from Curcuma Longa L.

Powdered turmeric (25 g) was extracted in 50 mL methanol under reflux for 24 h. The extracts were filtered through a filter paper and stored in a refrigerator at 4°C. Methanol (50 mL) was then added to the residue and extracted again. This process was repeated three times. The extracts obtained at the end of the 1st, 2nd, and 3rd days were combined. It was evaporated at 40 °C in a rotary evaporator until approximately 30 mL of extract residue remained. The extracted turmeric contains oleoresins complexed with curcuminoids. Oleoresins are soluble in hexane but curcuminoids are insoluble in hexane [38]. Hence, liquid-liquid extraction was performed by adding 30 mL of hexane to the residue to separate oleoresins from curcuminoids. The methanol phase was dried using a rotary evaporator to obtain a crude extract.

Identification of Curcuminoids by TLC

The crude extract and standard CUR solutions (5%), prepared by dissolving in methanol, were loaded onto TLC plates. Chloroform: methanol (12:0.5) was used as the mobile phase. Retention factor (Rf) values were calculated for each curcuminoid. The mobile phase mixture determined by TLC was used to isolate the curcuminoids by column chromatography.

Isolation of curcuminoids by column chromatography

The glass column was packed with 50 g of silica gel in a mobile phase to isolate the curcuminoids. Crude curcuminoid extract (1 g) was loaded onto the column, and curcuminoids were separated by column chromatography using a mobile phase (chloroform: methanol, 12:0.5). TLC was applied to eluted fractions and standard curcumin. In the TLC chromatogram, as shown in Fig. 2, "S" denotes standard CUR and when compared to it, fraction C2 contains CUR, and fraction C4 contains BDMC. Other fractions contain DMC and BDMC. For this reason, column chromatography was repeated to obtain DMC with the same solvent mixture and fraction C3 containing DMC. A summary of the fractionation of the curcuminoids is shown in Fig. 3. Fractions (C1, C2, C3, and C4) were dried in a vacuum oven until they reached a constant weight to ensure complete drying. It was stored in a dark and dry place, and its structure was determined using ^{1}H-NMR and ^{13}C-NMR spectrometry.

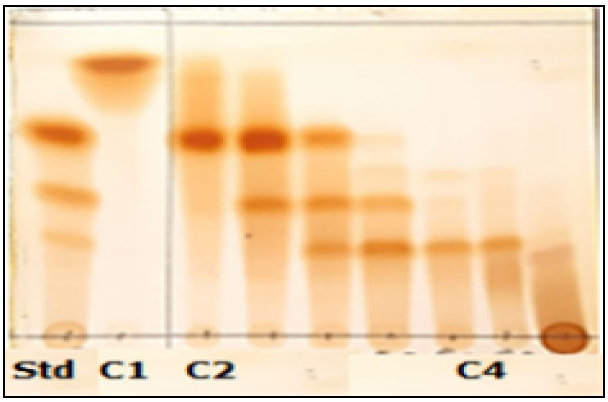

Figure 2. **TLC chromatograms of the fractions obtained by column chromatography (mobile phase: chloroform: methanol, 12:0.5)**

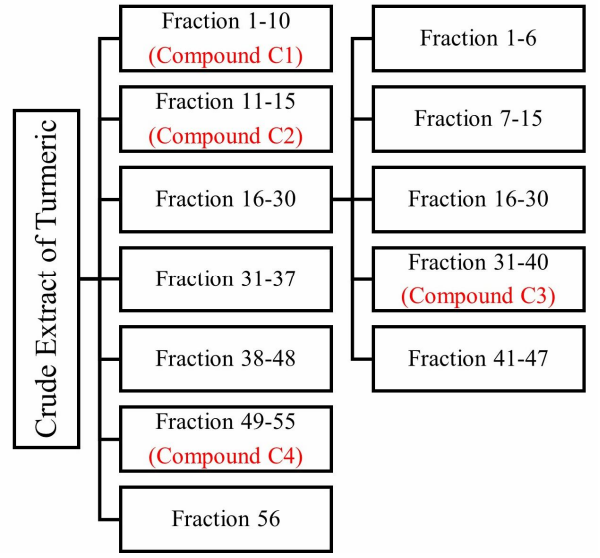

Figure 3. **Fractions obtained from the turmeric (*Curcuma longa L.*) plant by column chromatography: compounds C1, C2, C3, and C4.**

Isolation of Curcuminoids by Preparative HPLC-UV

At this stage, the isolation process was carried out using preparative HPLC-UV. The mobile phase and device conditions are listed in Tables 1 and 2, respectively. Better separation of curcuminoids was observed at 280 nm, and therefore, it was decided to work with this wavelength. Fractions showing the three largest peaks, estimated to be CUR, DMC, and BDMC, were coded as P1, P2, and P3, respectively. The collected fractions were dried using a rotary evaporator and stored away from moisture until structural analysis. Structural characterization and qualitative analysis were performed using NMR, UV, MS, and IR spectroscopies.

Table 1. Mobile phase conditions for curcuminoids by preparative HPLC-UV.

Time (min)	%70 Acetonitrile (%A)	%2 Acetic Acid (%B)
0-3	50	50
3-10	60	40
10-20	70	30
20-30	100	0
30-31	50	50
31-41	50	50

Table 2. HPLC-UV conditions for curcuminoid isolation.

İnjection Volume	170 µL
Column temperature	25 ºC
Flow rate	1 mL/min
Wavelength	280 nm

Results and Discussions

Structural Characterization of Curcuminoids Compounds with TLC

It was determined that the most suitable solvent mixture to isolate CUR and curcuminoids by TLC was methanol: chloroform (0.5:12). The TLC chromatogram obtained with this solvent mixture is shown in (Fig. 2). The calculated Rf values are 0.96, 0.89, 0.78, 0.56. In the studies in the literature, similar chromatograms were obtained in TLC chromatograms applied to raw turmeric extract [39, 40]. Based on the literature, the 2nd organic compound is CUR, the 3rd organic compound is DMC, and the 4th organic compound is BDMC. When we compare the prep-HPLC results, we can conclude that the three major components of turmeric are visible in the chromatogram. In addition, it was determined that four different organic compounds, one of which is CUR, can be obtained using this mobile phase solution (chloroform: methanol) in column chromatography. Thus, we aimed to obtain at least four organic components using this mixture for column chromatography.

Structural Characterization of Compound C1 (ar-turmerone) by NMR

Compound C1 was isolated as a light-yellow oil. The TLC chromatogram of the standard substance did not show a spot similar to that of the A3 fraction. There were 5 -C- or -CH_2- groups and 8 -CH_3 and -CH- groups in the ^{13}C-APT NMR spectrum. Carbonyl carbons absorb in the range of δ 160-250. Therefore, the negative peak at δ 199.52 belongs to the quaternary carbon of the C=O

group. Alkene and aromatic carbons absorb in the range of δ 110-145. The peaks concentrated in this range indicated the presence of aromatic rings and double bonds in the structure. Three of the carbons on the negative side should be quaternary or methylene, and the three on the positive side should be methine. Alkanes absorb in the range δ 0-70. Therefore, the peak at δ 52.20 is the methylene peak and there must be one methylene group in the structure (Table 3). It shows a $-CH_3-CH_2-CH_2-$ group in the multiplet six-peak structure found at δ 3.19 in the ^{1}H NMR spectrum of A3 compound (Fig. 4). Methyl, methylene, and methine protons absorb in the range of δ 0.0-0.2. From this point of view, we can say that the protons that peak in the area higher than δ 2.5 are alkyl groups. The doublet peak at δ 1.15 indicates that an alkyl group is attached to the -C-H group. In addition, the singlet peaks at δ 1.84, 2.02 and 2.26 indicate that each alkyl group is attached to a quaternary (-C-) carbon. The range of δ 6.0-9.0 is the aromatic proton region. The quaternary peak (δ 7.11) in this region indicates the presence of an aromatic ring in the structure. Doublet peaks at δ 2.67 and δ 2.71 suggest that there is geminal proton coupling in the structure due to non-magnetic equivalent protons. Owing to adjacent π bonds, non-magnetic equivalent doublet peaks cleave again, creating peak abundance. In other words, the $-CH_2-$ protons produce both vicinal and geminal coupling and form the double peak of a double peak. When all of these findings were evaluated, compound C1 was determined to be ar-turmerone.

Table 3. **^{13}C-APT NMR spectral data of isolated curcuminoids by column chromatography and prep-HPLC-UV.**

^{13}C	Column Chromatography				Prep-HPLC		
	C1	*C2*	*C3*	*C4*	*P3*	*P2*	*P1*
1	22.59	101.33	56.12	101.42	100.82	55.95	101.12
2	135.26	183.69	123.71	183.69	183.10	123.52	183.09
3	129.29	123.63	148.45	121.27	123.58	148.23	121.23
4	127.07	141.19	149.81	140.84	141.23	149.06	140.84
5	143.93	126.78	116.12	126.30	126.97	115.96	126.37
6	127.07	111.73	111.64	130.80	111.18	111.21	130.82
7	129.29	148.44	126.78	116.38	148.24	126.95	116.34
8	35.15	149.81	141.19	160.27	149.04	141.18	159.77
9	27.55	116.14	121.48	-	115.97	121.42	-
10	52.20	121.53	183.76	-	121.46	183.18	-
11	199.52	56.13	101.42	-	55.97	101.02	-
12	124.43	-	183.62	-	-	182.98	-
13	154.46	-	121.28	-	-	121.18	-
14	21.04	-	140.85	-	-	140.90	-
15	20.68	-	126.27	-	-	126.41	-
16	-	-	130.83	-	-	130.80	-
17	-	-	116.37	-	-	116.32	-
18	-	-	160.29	-	-	159.55	-

^{13}C-APT NMR, 101 MHz, DMSO-d_6, δ in ppm

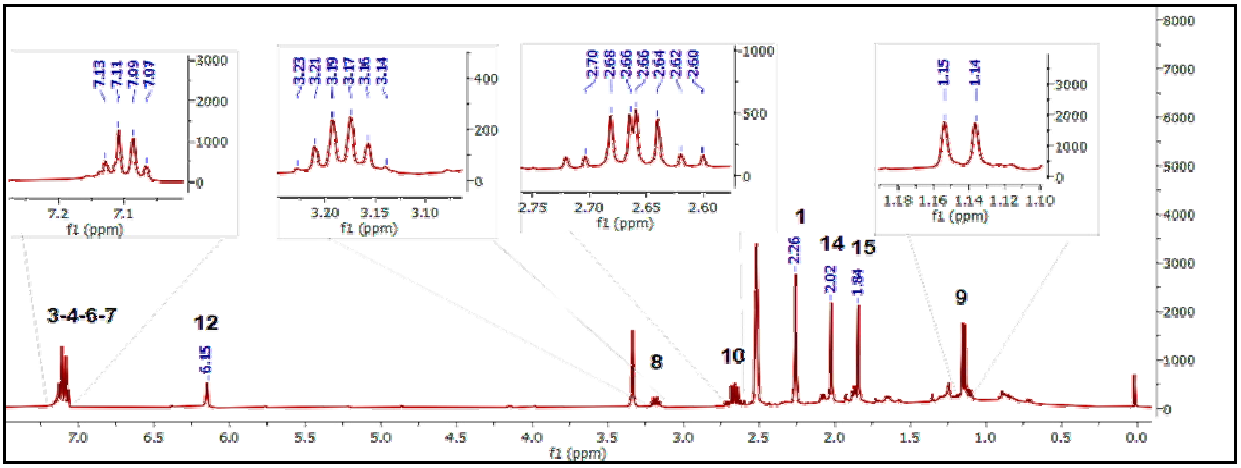

Figure 4. **^{1}H NMR spectrum of ar-turmerone (C1), DMSO-d_6, δ in ppm.**

Structural Characterization of Compound C2 and P3 (Curcumin) by NMR

The spectra of compound C2 isolated by column chromatography and compound P3 isolated by prep-HPLC were identical. There are methylene and quaternary carbons in the positive area, and methine and methyl carbons in the negative area. There were four quaternary carbon peaks in the positive area, which should be in the structure of CUR. The -CH carbon peaks at δ 101.33 - δ 100.82 in the spectra indicate that the structure is in the enol form. The peaks located at δ 55.97 - δ 56.13 in the high area belong to the electron-donating methoxy (-OCH_3) group carbons. Since the carbons attached to the electronegative atom give a peak in the low area, the peaks in the lowest area in the spectra, δ 183.69 − δ 183.10, are C=O carbons (Table 3). Eight different proton peaks were observed in the ^{1}H NMR spectra of B3 and C compounds, and the spectra were the same (Fig. 5).

The largest and sharpest singlet peak at δ 3.37- δ 3.8 in the high area, represents hydrogens in the –OCH_3 group, which has two in CUR. Considering the peak integrations, it can be said that this peak is a 6H peak; therefore, the molecule contains two -OCH_3 groups. Because the electron density decreases around the H atom (such as -OH, -NH, and-SH) attached to an electronegative atom, with the inductive effect, the chemical shift of such protons is in the low area. Therefore, the small singlet peak at δ 9.7-δ 9.66 indicates the Ar-OH proton. Aromatic protons peak in the range of δ 6.0-9.0. As the electron density around the proton increases, the shielding increases. Consequently, proton peaks appear in the high field. The order of electron-donating power of Class 1 substituents is OH >OCH_3> CH. Hence, the protons at δ 6.83, close to the -OH group, gave a double peak in the high field region, and the protons at δ 7.34, close to the -OCH_3 group, gave a single peak in the low field region. These findings confirm that the construct is CUR. The largest and sharpest singlet peak at δ 3.37- δ 3.8 in the high area, represents hydrogens in the –OCH_3 group, which has two in CUR. Considering the peak integrations, it can be said that this peak is a 6 H; therefore, the molecule contains two -OCH_3 groups. Because the electron density decreases around the H atom (such as -OH, -NH, and -SH) attached to an electronegative atom, with the inductive effect, the chemical shift of such protons is in the low area.

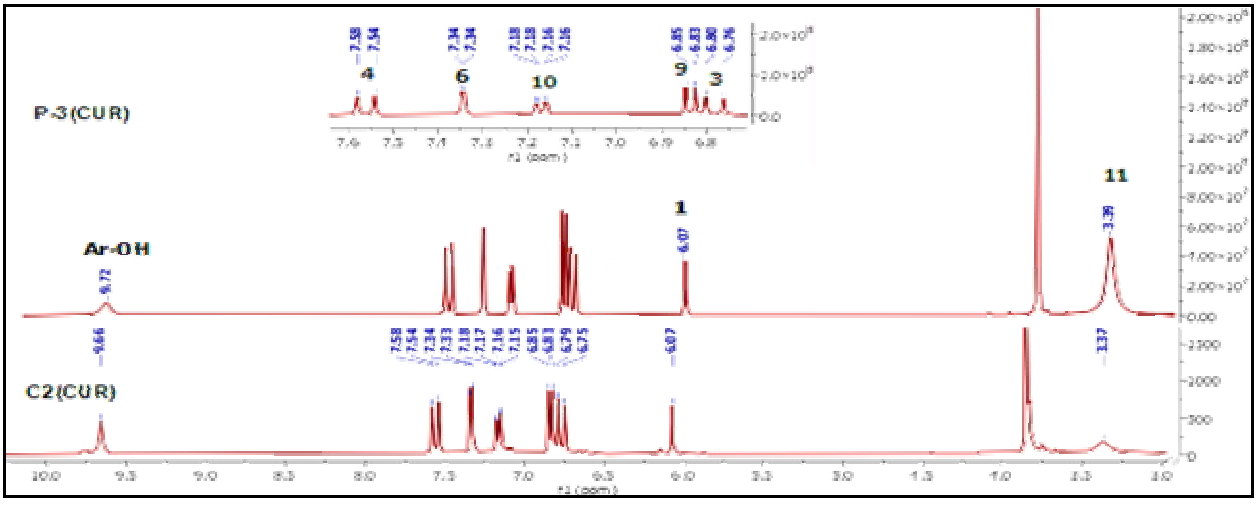

Figure 5. **^{1}H NMR Spectrum of curcumin (C2 and P3), DMSO-d_6, δ in ppm. Top spectrum obtained by prep-HPLC, bottom spectrum obtained by column chromatography.**

Therefore, the small singlet at δ 9.7-δ 9.66 indicates the Ar-OH proton. Aromatic protons peak in the range of δ 6.0-9.0. As the electron density increases around the proton, the shielding increases. Consequently, proton peaks appear in the high field. The order of electron-donating power of class 1 substituents is OH > OCH_3> CH. For this reason, the protons at δ 6.83, close to the -OH group, gave a double peak in the high field, and the protons at δ 7.34, close to the -OCH_3 group, gave a single peak in the low field. All these findings confirm that the construct is curcumin.

Structural Characterization of Compound C3 and P2 (Demethoxycurcumin) by NMR

The ^{13}C-APT NMR spectra of C3 and P2 were the same. The presence of seven quaternary or methylene carbon peaks in the positive area of the spectra confirmed that the fraction was DMC. The peaks around δ 183 seen in the lowest area in the spectra belong to C=O quaternary carbons showing enol-keto tautomerism. DMC contains an-OCH_3 group on the benzene ring. OCH_3 is an electron-donating group that provides electrons to the benzene ring on which it is located. For this reason, the benzene ring with -OCH_3 on it and the -C-OH carbons attached to the benzene ring do not appear in different areas. The peak at δ 160.29–δ 159.55 belongs to the C-OH carbon at the non-methoxy-bonded end of DMC, and the peak at δ 149.81–δ 148.23 belongs to the C-OH carbon at the methoxy-bonded end. The peak at δ 56.12-δ 55.95 in the highest area of the spectrum belongs to the methoxy group carbons. The peaks around δ 140 in the lowest area in the negative region indicate alkenyl groups (Table 3).

When the ^{1}H NMR spectra (Fig. 6) of the C3 and P2 compounds were examined, the equivalent sharp singlet peaks around δ 10 showed protons attached to the –OH groups in the aromatic ring. The two peaks indicate two different -OH groups. In DMC, a methoxy group is attached to one of its aromatic rings. Because the methoxy group is electron-donating, the -o and -p protons are shielded and resonate at a lower frequency than they should.

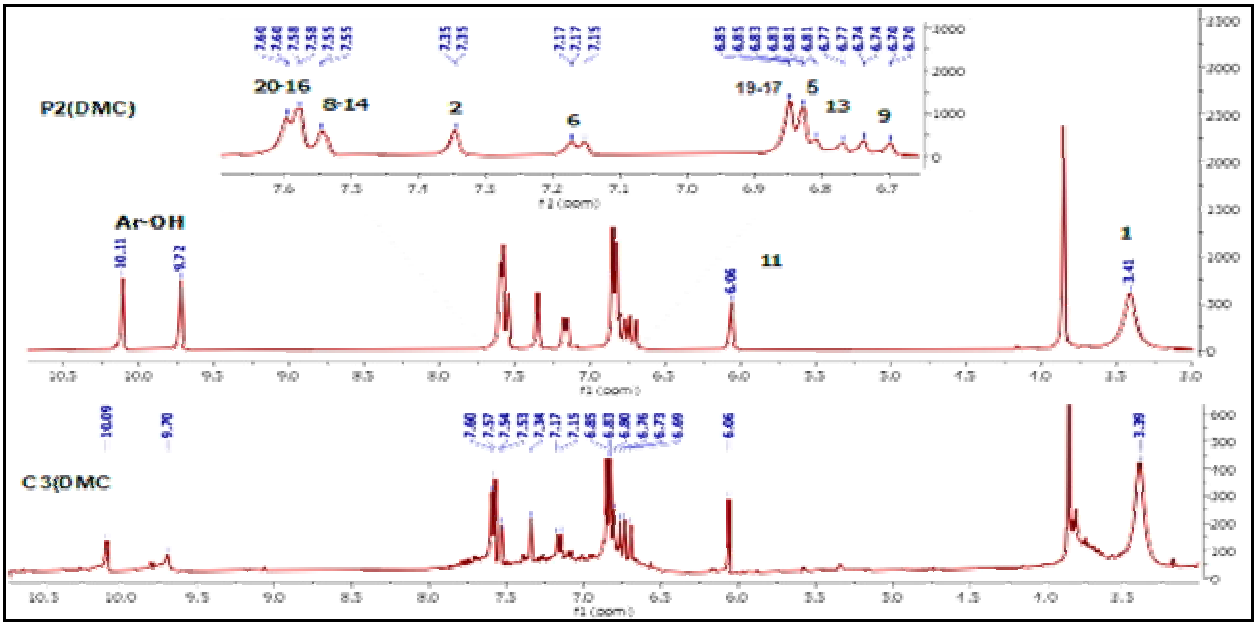

Figure 6. **^{1}H NMR Spectrum of demethoxycurcumin (C3 and P2), DMSO-d_6, δ in ppm. Top spectrum obtained by prep-HPLC, bottom spectrum obtained by column chromatography.**

Thus, the -OH protons in the methoxy-bonded aromatic ring appeared in a larger area than those in the unbonded ring. The electron-donating methoxy group is attached to the ring, shifting the–OH proton to a larger area than the other proton. The singlet peak around δ 3 corresponds to the $–OCH_3$ proton group. The singlet peak at δ 6.06 is the protons entering the enol-keto equilibrium. Based on this information, it was confirmed that the molecule was demethoxycurcumin.

Structural Characterization of Compound C4 and P1 (Bisdemethoxycurcumin) by NMR

Similar to CUR, BDMC is a symmetric molecule. There are six quaternary carbons in its structure and three peaks in the positive area in the ^{13}C-APT NMR spectrum of C4 and P1 compounds belonging to the quaternary carbons. Carbons bonded to electronegative atoms peak at a low field. Therefore, the peak at approximately δ 183 corresponds to C=O carbons. The peak around δ 160 corresponds to the C-OH carbons attached to the aromatic rings. The peak at δ 101.42 in the negative direction belongs to the α carbon between the two carbonyl atoms. The negative direction indicates that the carbon is in the -CH- structure, and the molecule is in the enol form. The methoxy carbon peak, which was observed around δ 55 in the CUR and DMC spectra, was not observed in this spectrum. This indicated that there was no methoxy group in the structure and no methoxy group in the BDMC molecule (Table 3).

In the spectra of C4 and P1 compounds (Fig. 7), the singlet peak structure observed around δ 6 belongs to the $-CH_2$ protons participating in enol-keto tautomerism. The -OH protons appear in the lowest area owing to electronegativity and hydrogen bonding. Therefore, the singlet peak observed at approximately δ 10 corresponds to -OH protons. Broadening of this peak was observed in the chromatogram of the C4 compound. This is because the protons attached to the -OH group are shared with other molecules owing to the formation of intramolecular and intermolecular H-bonds.

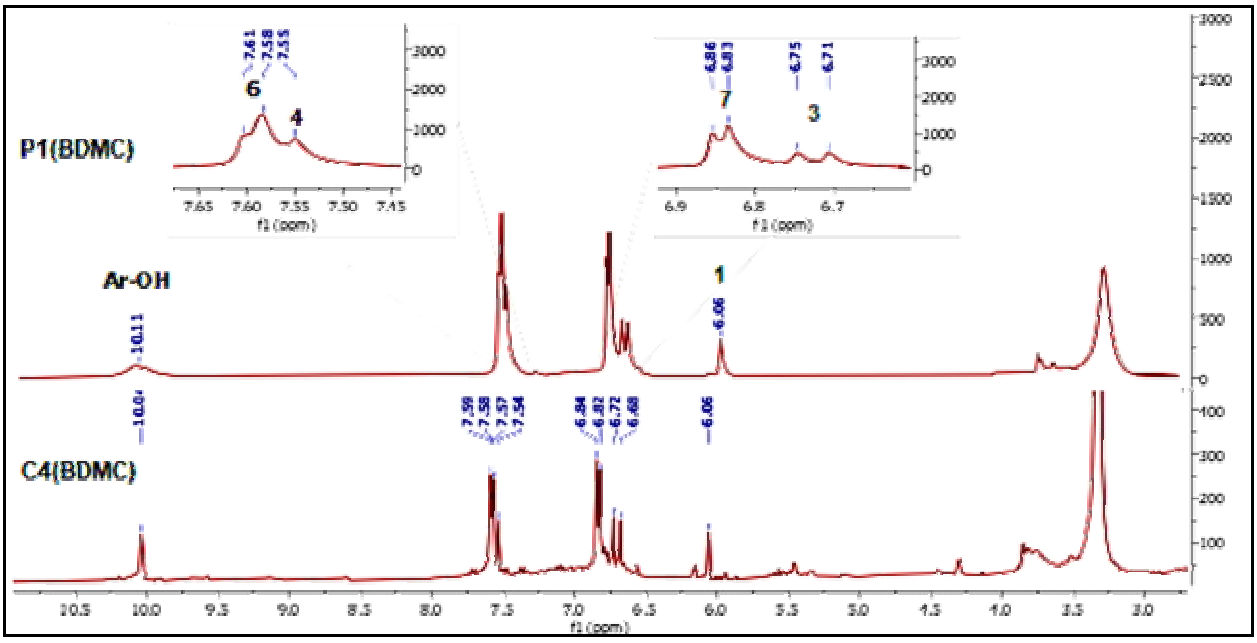

Figure 7. **^{1}H NMR Spectrum of bisdemethoxycurcumin (C4 and P1), DMSO-d_6, δ in ppm. Top spectrum obtained by prep-HPLC, bottom spectrum obtained by column chromatography.**

The -OH group provides electrons to the ring through a mesomeric effect. The electron density is concentrated at the -o and -p positions. Thus, the -o protons in the structure are shielded more than the -m protons and give a peak in a higher area. Therefore, the doublet peaks around δ 6 correspond to the-o carbons. The methoxy peak, around δ 3-3.5 in the ^{1}H NMR spectra of CUR and DMC, was not observed in the spectrum of the C4 and P1 compounds, confirming that the structure is BDMC. Based on these findings, we can conclude that C4 and P1 are bisdemethoxycurcumin.

Qualitative Analysis of Curcuminoids

The maximum absorption wavelengths at which the isolated curcuminoids were determined using prep-HPLC-UV. CUR, DMC and BDMC solid crude extracts were evaluated by analyzing the FT-IR spectra. Molecular weights were determined by evaluating the ESI-MS spectra. The physical properties of the isolated curcuminoids, Rf, Rt, m/z, λmax, IR absorption bands, and NMR spectral data results are summarized below.

Ar-Turmerone (C1): Viscous light-yellow oil ($CHCl_3$). Rf 0.96 (TLC). ^{1}H NMR (400 MHz, DMSO) δ 7.10 (q, J = 8.1 Hz, 1H), 6.17 – 6.12 (m, 0H), 3.23 (s, 0H), 3.18 (q, J = 7.1 Hz, 0H), 2.73 – 2.59 (m, 1H), 2.26 (s, 1H), 2.02 (d, J = 0.9 Hz, 1H), 1.84 (d, J = 1.0 Hz, 1H), 1.15 (d, J = 6.9 Hz, 1H). The ^{1}H and ^{13}C-NMR spectral data (DMSO), are shown in Table 3 and Fig. 4.

Curcumin (C2 and P3): Orange powder (MeOH), Rf 0,89 (TLC), Rt 18.9 (prep-HPLC). ESIMS: m/z 367.00 $[M-H]^{+}$ (calculated for $C_{21}H_{20}O_6$, 368,38). UV (MeOH) λmax 426.2 nm. IR absorption bands (cm^{-1}): 3407 (O-H, str); 2822 (Ar-OCH_3); 1626 (α-β unsaturated C=O); 1581-1521 (aromatic ring C=C). ^{1}H NMR (C2) (400 MHz, DMSO) δ 9.66 (s, 0H), 7.58 (s, 0H), 7.54 (s, 0H), 7.34 (d, J = 2.0 Hz, 0H), 7.18 (d, J = 2.0 Hz, 0H), 7.15 (d, J = 2.0 Hz, 0H), 6.85 (s, 0H), 6.83 (s, 0H), 6.79 (s, 0H), 6.75 (s, 0H), 6.07 (s, 0H), 3.35 (s, 1H). ^{1}H NMR (P3) (400 MHz, DMSO) δ 9.72 (s, 1H), 7.56 (d, J = 15.8 Hz, 1H), 7.34 (d, J = 2.0 Hz, 1H), 7.17 (dd, J = 8.2, 2.1 Hz, 1H), 6.84 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 15.8 Hz, 1H), 6.07 (s, 1H), 3.39 (s, 6H). The ^{1}H and ^{13}C NMR spectral data (DMSO) are shown in Table 3 and Fig. 5.

Demethoxycurcumin (C3 and P2): Orange crystal (MeOH), Rf 0.78 (TLC), Rt 18.3 (prep-HPLC). ESIMS: m/z 336.69 $[M-H]^{+}$ (calculated for $C_{20}H_{18}O_5$, 338.35). UV (MeOH) λ_{max} 419.9 nm. IR absorption bands (cm^{-1}): 3328 (O-H, str); 2925 (Ar-OCH_3); 1622 (α-β unsaturated C=O); 1569 (aromatic ring C=C); 1427 (-CH_2-C=O); 1260 (C-O-C); 1231 (C-O); 960 (CH=CH-C=O); 825 (1,4-disubstituted). ^{1}H NMR (C3) (400 MHz, DMSO) δ 10.09 (s, 1H), 9.70 (s, 0H), 7.59 (d, J = 8.9 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.34 (d, J = 2.0 Hz, 1H), 7.16 (d, J = 8.4 Hz, 0H), 6.86 – 6.82 (m, 1H), 6.80 (s, 0H), 6.76 (s, 0H), 6.73 (s, 0H), 6.69 (s, 0H), 6.06 (s, 1H), 3.39 (s, 12H). ^{1}H NMR (P2) (400 MHz, DMSO) δ 10.11 (s, 1H), 9.72 (s, 1H), 7.59 (d, J = 8.8 Hz, 1H), 7.55 (s, 0H), 7.34 (d, J = 1.9 Hz, 0H), 7.16 (d, J = 8.2 Hz, 0H), 6.84 (d, J = 8.7 Hz, 1H), 6.79 (d, J = 15.8 Hz, 0H), 6.71 (d, J = 15.9 Hz, 0H), 6.06 (s, 0H), 3.41 (s, 3H). The ^{1}H and ^{13}C NMR spectral data (DMSO) are shown in (Table 3) and (Fig. 6), respectively. Bisdemethoxycurcumin (C4 and P1): Orange crystal (MeOH), Rf 0.56 (TLC), Rt 17.4 (prep-HPLC). ESIMS: m/z 306.73 $[M-H]^{+}$ (calculated for $C_{19}H_{16}O_4$, 308.33). UV (MeOH) λmax 473.8 nm. IR absorption bands (cm^{-1}): 3183 (O-H, str); 1620 (α-β unsaturated C=O); 1509 (aromatic ring C=C); 1600 (alkenyl C=C); 1427 (-CH_2-C=O); 1231 (C-O); 960 (CH=CH-C=O); 828 (p-disubstituted). ^{1}H NMR (C4) (400 MHz, DMSO) δ 10.04 (s, 1H), 7.61 – 7.56 (m, 3H),

7.54 (s, 0H), 6.83 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 15.9 Hz, 1H), 6.06 (s, 1H). ^{1}H NMR (P1) (400 MHz, DMSO) δ 10.10 (s, 0H), 7.59 (d, J = 8.7 Hz, 1H), 7.55 (s, 0H), 6.83 (t, J = 10.6 Hz, 1H), 6.73 (d, J = 15.9 Hz, 0H), 6.06 (s, 0H). The ^{1}H and ^{13}C NMR spectral data (DMSO) are shown in Table 3 and Fig. 7, respectively.

Conclusion

In this study, we successfully separated four curcuminoids from C. longa rhizomes using two known methods. We performed a structural analysis of these four compounds using NMR, BDMC, DMC, and CUR. They are structurally similar, and their different structural properties were elucidated by MS, UV-Vis, and FT-IR spectroscopy. The solvent mixtures used for column chromatography were curcuminoid-specific. However, the device conditions and gradient elution system used for prep-HPLC-UV could be applied in similar studies. Prep-HPLC is time-saving, efficient, and practical, but column chromatography is inexpensive and environmentally friendly. With the application of both the methods and solvent mixtures, curcuminoids of high quantity and purity can be obtained.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. J. Luo, M. Li and G. Yang, *Mitochondrial DNA Part B,* 4:2 (2019) 3044. doi:10.1080/23802359.2019.1666665
2. L. Labban, *Int. J. Pharm. Biomed. Sci*., 5:1 (2014) 17. https://scholar.google.com/scholar?hl=tr&as_sdt=0%2C5&q=labban+&btnG=
3. Y. Zhang, R. Chen and W. Xu, *Int. J. Clin. Exp. Med.,* 14:1 (2021) 164. https://www.webofscience.com/wos/woscc/full-record/WOS:000702307900017
4. Z. Yu, Y. Yan and Y. Lou, *Trop. J. Pharm. Res.,* 20:2 (2021) 269. doi:10.4314/tjpr.v20i2.7
5. P. Basnet and N. Skalko-Basnet, *Molecules,* 16 (2011) 4567. doi:10.3390/molecules16064567
6. K. K. Soudamini, M. C. Unnikrishnan, K. B. Soni and R. Kuttan, *Indian J. Physiol. Pharmacol.,* 36 (1992) 239. https://ijpp.com/IJPP%20archives/1992_36_4/1992_36_4_toc.php
7. B. P. Suresh and K. Srinivasan, *Mol. Cell Biochem.,* 181 (1998) 87. doi:10.1023/A:1006821828706
8. S. D. Deodhar, R. Sethi and R. C. Srimal, *Indian J. Med. Res.,* 71 (1980) 632. https://research.ebsco.com/c/mwdjhe/results?q=AN%2091259731
9. C. Natarajan and J. J. Bright, *J. Immunol.,* 168:12 (2002) 6506. doi:10.4049/jimmunol.168.12.6506
10. M. Garcia-Alloza, L. A. Borrelli, A. Rozkalne, B. T. Hyman and B. J. Bacskai, *J. Neurochem.,* 102 (2007) 1095. doi:10.1111/j.1471-4159.2007.04613.x
11. B. Jagatha, R.B. Mythri, S. Vali and M.M. Bharath, *Free Radic. Biol. Med.,* 44 (2008) 907. doi:10.1016/j.freeradbiomed.2007.11.011

12. S. M. Khopde, K. I. Priyadarsini, P. Venkatesan and M. N. Rao, *Biophys. Chem.,* 80:2 (1999) 85. doi:10.1016/S0301-4622(99)00070-8
13. D. S. H. L. Kim, S. Y. Park and J. Y. Kim, *Neurosci. Lett.,* 303 (2001) 57. doi:10.1016/S0304-3940(01)01677-9
14. G. K. Jayaprakasha, L. J. Rao and K. K. Sakariah, *Food Chem.,* 98:4 (2006) 720. doi:10.1016/j.foodchem.2005.06.037
15. M. Hatamipour, M. Ramezani, S. A. S. Tabassi, T. P. Johnston and A. Sahebkar, *J. Cell Physiol.,* 234:11 (2019) 19320. doi:10.1002/jcp.28626
16. C. Y. Lin, C. C. Hung, C. N. W. Charles, H. Y. Lin, S. H. Huang and M. J. Sheu, *Phytomed.,* 53 (2019) 28. doi:10.1016/j.phymed.2018.08.005
17. F. S. Chueh, J. C. Lien, Y. C. Chou, W. W. Huang, Y. P. Huang, Y. J. Huang, Y. J. Kuo, W. N. Huang, S. Y. Sheng, H. Y. Tung, H. Y. Chen and S. F. Peng, *In Vivo,* 34:5 (2020) 2469. doi:10.21873/invivo.12062
18. G. J. Lee, H. Lim, J. Y. Seo, K. R. Kang, D. K. Kim, J. S. You, J. S. Oh, Y. S. Seo and J. S. Kim, *Transl. Cancer Res.,* 11:5 (2022) 1064. doi:10.21037/tcr-21-2410.
19. A. Simon, D. P. Allais, J. L. Duroux, J. P. Basly, S. Durand-Fontanier and C. Delage, *Cancer Lett.,* 129:1 (1998) 111. doi:10.1016/S0304-3835(98)00092-5
20. V. Basile, E. Ferrari, S. Lazzari, S. Belluti, F. Pignedoli and C. Imbriano, *Biochem. Pharmacol.,* 78:10 (2009) 1305. doi:10.1016/j.bcp.2009.06.105
21. F. Q. Jin, Y. Jin, J. X. Du, L.Y. Jiang, Y. Zhang, Z. Y. Zhao, B. Yang, P. H. Luo and Q. J. He, *Eur. J. Pharmacol.*, 847 (2019) 26. doi:10.1016/j.ejphar.2019.01.012
22. S. Yodkeeree, W. Chaiwangyen, S. Garbisa and P. Limtrakul, *The J. Nutr. Biochem.,* 20:2 (2009) 87. doi:10.1016/j.jnutbio.2007.12.003
23. H. F. Pei, Y. Yang, L. Cui, J. Yang and X.C. Li, *Sci. Rep.,* 6 (2016) 28773. doi:10.1038/srep28773
24. M. Boonrao, S. Yodkeeree, C. Ampasavate, S. Anuchapreeda and P. Limtrakul, *Arch. Pharm. Res.,* 33:7 (2010) 989. doi:10.1007/s12272-010-0703-6
25. G. G. L. Yue, B. C. L. Chan, P. M. Hon, M. Y. H. Lee and K. P. Fung, *Food Chem. Toxicol.,* 48:8-9 (2010) 2011. doi:10.1016/j.fct.2010.04.039
26. S. B. Cheng, L. C. Wu, Y. C. Hsieh, C. H. Wu, Y. J. Chan L. H. Chang, C. M. J. Chang, S. L. Hsu, C. L. Teng and C. C. Wu, *J. Agric. Food Chem.,* 60 (2012) 9620. doi:10.1021/jf301882b
27. S. Y. Park, Y. H. Kim, Y. Kim and S. J. Lee, *J. Cell. Biochem.,* 1130:12 (2012) 3653. doi:10.1002/jcb.24238.
28. M. J. Ji, J. Choi, J. Lee and Y. Lee, *Int. J. Mol. Med.,* 14:2 (2004) 253. doi:10.3892/ijmm.14.2.253
29. Y. L. Li, Z. Y. Du, P. H. Li, L. Yan, W. Zhou, Y. D. Tang, G. R. Liu, Y. X. Fang, K. Zhang and C. Z. Dong, *Int. Immunopharmacol.,* 64 (2018) 319. doi:10.1016/j.intimp.2018.09.015.
30. S. Y. Park, M. L. Jin, Y. H. Kim, Y. H. Kim and S. J. Lee, *Int. Immunopharmacol.,* 14:1 (2012) 13. doi:10.1016/j.intimp.2012.06.003.
31. S. Mollayi, S. Tamhidi, H. Hashempour and A. Ghassempour, *Acta Chromatographica,* 27 (2015) 387. doi:10.1556/AChrom.27.2015.2.13
32. R. C. Lantz, G. J. Chen, A. M. Solyom, S. D. Jolad and B. N. Timmermann, *Phytomedicine,* 12:6-7 (2005) 445. doi:10.1016/j.phymed.2003.12.011
33. N. Ahmad, R. Ahmad, A. A. Naqvi, M. A. Alam, M. Ashafaq, Z. Iqbal and F. J. Ahmad, *J. Liq. Chromatogr. Relat.*

Technol., 40:3 (2017) 133. doi:10.1080/10826076.2017.1293549
34. G. K. Jayaprakasha, G. A. N. Gowda, S. Marquez and B. S. Patil, *J. Chromatogr. B,* 937 (2013) 25. doi:10.1016/j.jchromb.2013.08.011
35. D. K. Yadav, K. Sharma, A. Dutta, A. Kundu and A. Awasthi, *J. AOAC Int.,* 10:3 (2017) 586. doi:10.5740/jaoacint.17-0057
36. O. D. Dhingra, G. N. Jham, R. C. Barcelos, F. A. Mendonça and I. Ghiviriga, *J. Essent. Oil Res.,* 19:4 (2007) 387. doi:10.1080/10412905.2007.9699312
37. Y. Zhou, C. Wang, R. Wang, L. Lin and Z. Yin, *Sep. Sci. Technol.,* 52:3 (2017) 497. doi:10.1080/01496395.2016.1251461
38. V. Gokhul, S. Yuvapriya, M. Chandramohan and P. Muthukumaran, *Int. J. Pharm. Res. Allied Sci.,* 4:2 (2015) 79. https://ijpras.com/issue/2015-volume-4-issue-2
39. N. Maithilikarpagaselvi, M. G. Sridhar and R. Sripradha, *J. Young Pharm.,* 12:2 (2020) 113. doi:10.5530/jyp.2020.12.23