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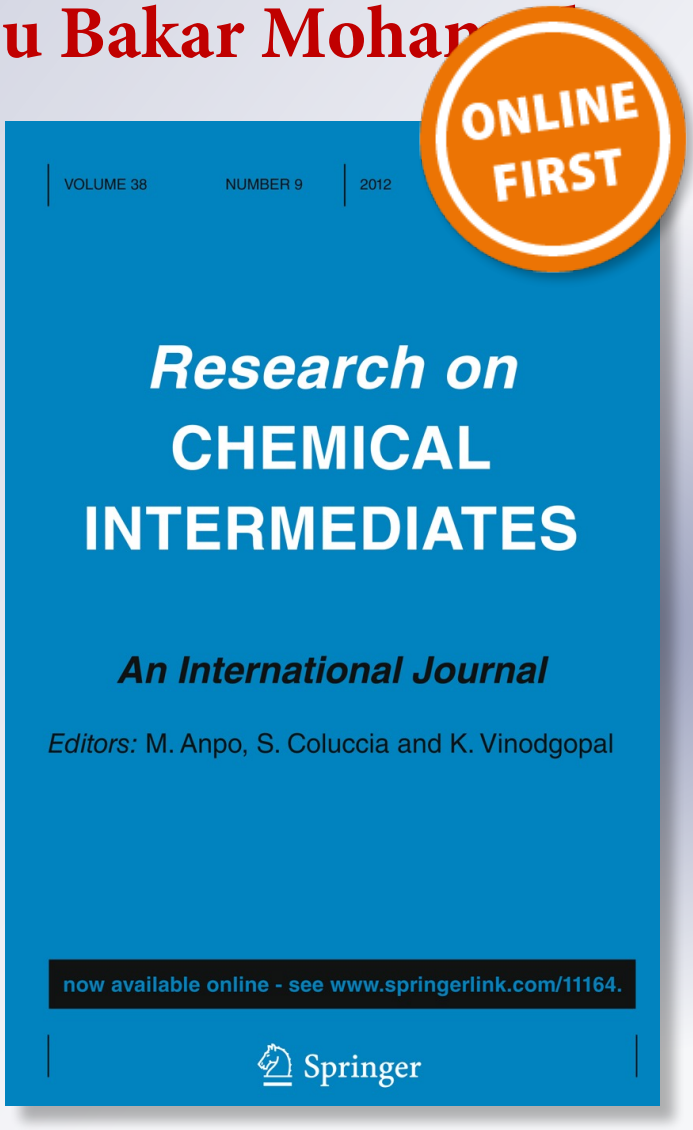

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Curcuminoids as antioxidants and theoretical study of stability of curcumin isomers in gaseous state

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Abstract The antioxidant activity of three extracted curcuminods, namely, curcumin [(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5dione] (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), were studied with the DPPH, hydrogen peroxide and nitric oxide radical methods, and compared with the known antioxidant ascorbic acid. Structures for the extracted curcuminods are proposed on the basis of spectroscopic evidence. Curcumin molecule stability was studied in the gaseous state, and it has two isomers [the diketone form, curcumin(I), and the enol form, curcumin(II)] through the theoretical study by relying on the results of density functions theory (DFT). The results of each of the total energy and the high occupied molecular orbital (HOMO) to curcumin(I) are more stable than to curcumin(II). The increase of the amount of total energy is −0.01301141 a.u., or equivalent, −8,164.783 cal mol^{-1}. The HOMO level is −0.35865 eV, also the thermodynamic values (the change in entropy ΔS and the change in enthalpy ΔH) of the isomerization conversion form curcumin(II) to curcumin(I) spontaneous and endothermic reaction, of ΔS and ΔH are −3.136 cal mol^{-1} K^{-1} and −0.673 kcal mol^{-1}, respectively. The results showed that the wavelength for greatest absorption (λ max) of the enol form curcumin(II) is longer than curcumin(I). This is due to the formation of a new double bond which leads to the association distribution of the electronic density along the molecule in the enol form.

Keywords Antioxidant · Curcumin · DPPH · HOMO · LUMO

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Introduction

Plants produce many antioxidants to control the oxidative stress caused by sunbeams and oxygen, and they can represent a source of new compounds with antioxidant activity [1]. Curcumin and its derivatives have been shown to have a strong anti-inflammatory activity in carragenin- and caoline-induced edema and formaline-induced arthritis with a corticosteroid-like and a strong antioxidant activity [2]. Curcumin and its derivatives have a protective effect on liver against CCl_4 and D-galactosamine [3]. It has been observed that curcumin decreases high cholesterol levels, like statine. Curcumin and its derivatives have shown antitumor activity in in vitro tests [4]. Turmeric antioxidant protein (TAP) has been isolated from aqueous extract of turmeric. TAP prevents Ca–ATPase from inactivation in the presence of promoters of lipid peroxidation (LPO) as well as the depletion of thiol (SH) content during peroxidation. The antioxidant activity is probably mediated through the protection of the SH group of the enzyme. Similar results have been obtained through reagents which reduce thiol groups [5]. Curcumin (C), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) protect normal human keratinocytes from xanthine hypoxanthine oxidase injury [6]. Curcumin can exist in several tautomeric forms, including a 1,3-diketo form and two equivalent enol forms. The enol form is more energetically stable in the solid phase and in solution [7]. This compound can be used for boron quantification in the curcumin method. The aromatic ring systems, which are polyphenols, are connected by two α,β-unsaturated carbonyl groups. The diketones form stable enols or are easily deprotonated and form enolates, while the α,β-unsaturated carbonyl is a good Michael acceptor and undergoes nucleophilic addition [8].

Results and discussion

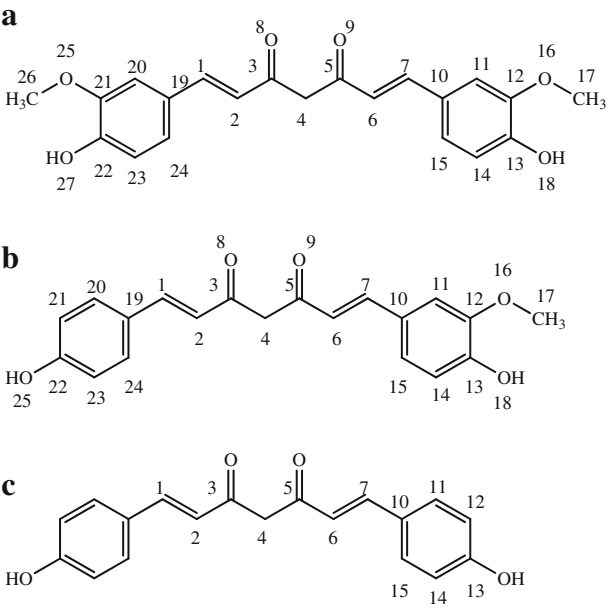
Chemistry

Curcumin, DMC, and BDMC were synthesized and characterized by spectroscopic methods UV–Vis, FT-IR, and NMR (Scheme 1).

Computational studies

DFT

Curcumin molecule has two isomers: the diketone form isomer, curcumin(I), and the enol form isomer, curcumin(II), as shown in Fig. 1. The stability of these forms from the accounts of quantum using the density functions theory (DFT) are shown in Table 1, the curcumin(I) form being more stable than the curcumin(II) form, which is evidenced by the value of the total energy calculated for them. The energy of the curcumin(I) form is more than the curcumin(II) form at about −0.01301141 a.u. or −8,164.783 cal mol^{-1}, and the isomerization conversions between the two forms were clarified in reactions (1 and 2) as shown in Fig. 1. Thermodynamic



Scheme 1 Structure of curcuminoides; **a** curcumin (C), **b** desmethoxycurcumin (DMC), **c** bisdesmethoxycurcumin (BDMC)

results showed that the reaction (1) is spontaneous, because of the a positive value of the entropy change ($\Delta S = 3.136$ cal mol^{-1} K^{-1}) so that the curcumin(I) form is more stable than the others, and the positive value of enthalpy change ($\Delta H = 0.673$ kcal mol^{-1}) clarifies that the reaction (1) is endothermic, while reaction (2) is not spontaneous and exothermic, with values of ΔS and ΔH of -3.136 cal mol^{-1} K^{-1} and -0.673 kcal mol^{-1}, respectively.

As it turns out, the values of the high occupied molecular orbital (HOMO) for the two forms show that the curcumin(I) form is more stable than the curcumin(II) form by -0.35865 eV. In solid state physics, a band gap, also called an energy gap, is an energy range in a solid where no electron states can exist; the gap energy generally refers to the energy difference (in electron volts) between the low unoccupied molecular orbital (LUMO) and the HOMO in insulators and semiconductors. This is equivalent to the energy required to free an outer shell electron from its orbit about the nucleus to become a mobile charge carrier, able to move freely within the solid material. In this research, all molecules were studied in gas phase isolates where the energy gap is an approximation. Energy gap values of the two forms, curcumin(I) and curcumin(II), are 3.75271 and 3.36005 eV, respectively, and this indicates that the thin molecules in the gas phase are insulators for the electrical conductivity. Figure 2 shows the HOMO electronic distribution for the two forms. Other accounts of wavelength for greatest absorption (λ_{max}) of the two forms, curcumin(I) and curcumin(II), are 330.38 and 369.00 nm, respectively, with the curcumin(II) form having a wavelength longer than the curcumin(I) form due to the

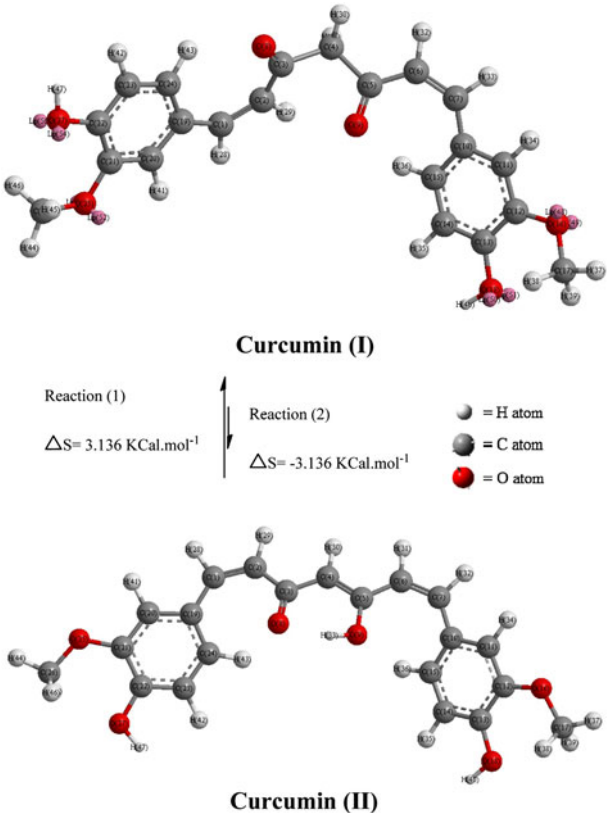


Fig. 1 The optimized structure for curcumin isomers (isomer diketone, curcumin(I), and isomer enol, curcumin(II), and values of ΔS and ΔH

Table 1 Some physical values are calculated of curcumin isomers

Subject	Curcumin(I)	Curcumin(II)
Total energy (a.u.)	−1,263.19644594	−1,263.18343453
Entropy (S) (cal mol^{-1} K^{-1})	187.960	184.824
Enthalpy (H) (kcal mol^{-1})	250.033	249.360
E_{HOMO} (eV)	−5.89861	−5.53996
E_{LUMO} (eV)	−2.14589	−2.17990
Gap energy ($E_{LUMO} - E_{HOMO}$) (eV)	3.75271	3.36005
λ_{Max} ($E_{LUMO} - E_{HOMO}$) (nm)	330.38	369.00
Heat capacity (C_v) (Cal.mol^{-1} K^{-1})	97.265	97.987
Point group	C1	C1
Dipole moment (Debye)	4.5752	4.7636

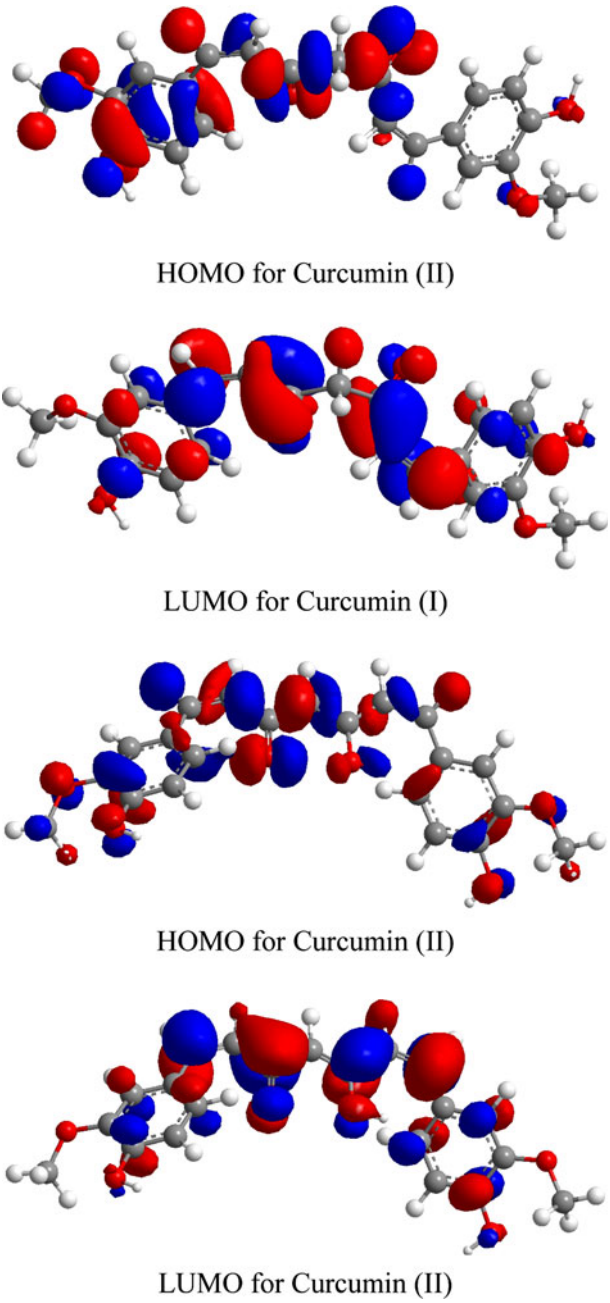


Fig. 2 The distribution of electron density of high occupied molecular orbital (HOMO) for curcumin isomers

increasing number of double bonds. The two forms have no symmetry at all; the symmetry element is the identity, E. Such a molecule belongs to the C1 point group.

Atomic charges

Atomic charges for curcumin(I) The theoretical studies for curcumin(I) (Fig. 1) revealed that the atomic charges have been affected by the presence of the ring substituent. The calculated atomic charges for curcumin(I) are indicated in Table 2. It can be seen that the highest atomic charge is at O(9) −0.601253, and the next charge value is at O(8) −0.59497. These results clearly indicated that these two atoms are the most reactive sites toward the reactions and bonding with the metals. The calculated bond and twist angles and 3D geometrical structure indicated that this molecule is not planar and the C(1)–C(2) stereochemistry is (*Z*) and C(6)-C(7) stereochemistry is also (*Z*).

Atomic charges for curcumin(II) The calculated atomic charges for curcumin(II) (Fig. 1) are shown in Table 3. The results showed that the highest atomic charge is at O(6) −0.272) and the next charge value is at O(19) −0.265. These results showed clearly that these two atoms are the most reactive sites toward the reactions and bonding with the metals. The determined bond angles, twist angles and 3D geometrical structure indicate that this molecule is not planar and the C(1)–C(2) stereochemistry is (*Z*), the C(4)–C(5) stereochemistry is (*Z*), and the C(6)–C(7) stereochemistry is also (*Z*).

Pharmacology

The role of antioxidants is to remove free radicals. One important mechanism through which this is achieved is by donating hydrogen to free radicals in its reduction to non-reactive species. Addition of hydrogen would remove the odd

Table 2 Atomic charges for curcumin(I)

Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C(1)	0.103589	C(13)	0.237841	O(25)	−0.215415	H(37)	0.0283376
C(2)	−0.150755	C(14)	−0.101175	C(26)	0.0721537	H(38)	0.0253561
C(3)	0.418882	C(15)	−0.0409182	O(27)	−0.225689	H(39)	0.0236807
C(4)	−0.144631	O(16)	−0.220386	H(28)	0.0203535	H(40)	0.20511
C(5)	0.436704	C(17)	0.0731618	H(29)	0.0278899	H(41)	0.0178036
C(6)	−0.148781	O(18)	−0.226868	H(30)	0.0606096	H(42)	0.0236737
C(7)	0.107737	C(19)	−0.000788361	H(31)	0.0602958	H(43)	0.0239483
O(8)	−0.59497	C(20)	−0.0709134	H(32)	0.0294167	H(44)	0.028283
O(9)	−0.601253	C(21)	0.176714	H(33)	0.0203645	H(45)	0.024393
C(10)	−0.000176837	C(22)	0.239736	H(34)	0.0181256	H(46)	0.0246909
C(11)	−0.0723124	C(23)	−0.10102	H(35)	0.0237434	H(47)	0.205166
C(12)	0.177291	C(24)	−0.0429334	H(36)	0.023933		

Table 3 Atomic charges for curcumin(II)

Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C(1)	0.0598649	C(13)	0.227781	O(25)	−0.216891	H(37)	0.0282634
C(2)	−0.125409	C(14)	−0.104949	C(26)	0.0724203	H(38)	0.0248218
C(3)	0.336475	C(15)	−0.0403251	O(27)	−0.23533	H(39)	0.0241927
C(4)	−0.231694	O(16)	−0.216174	H(28)	0.0208374	H(40)	0.205359
C(5)	0.33606	C(17)	0.0724717	H(29)	0.0239221	H(41)	0.0181434
C(6)	−0.1085	O(18)	−0.23331	H(30)	0.023495	H(42)	0.023806
C(7)	0.0604165	C(19)	0.000919371	H(31)	0.028195	H(43)	0.0231597
O(8)	−0.72118	C(20)	−0.0812611	H(32)	0.0221486	H(44)	0.0283796
O(9)	−0.211244	C(21)	0.174435	H(33)	0.205129	H(45)	0.0241509
C(10)	0.00527056	C(22)	0.226517	H(34)	0.0183779	H(46)	0.0248569
C(11)	−0.0849284	C(23)	−0.104476	H(35)	0.0242502	H(47)	0.205034
C(12)	0.175069	C(24)	−0.0485212	H(36)	0.0199702		

electron feature which is responsible for radical reactivity. Free radicals have been a subject of significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many workers. It has been proven that free radicals play an important role in the pathogenesis of certain diseases and aging. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [9, 10]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, and therefore attention has been paid to naturally occurring antioxidants. Compounds C, DMC, and BDMC were screened for in vitro antioxidant activity using (1,1-diphenyl-2-picrilhydrazyl) DPPH, nitric oxide, and hydrogen peroxide. They show good antioxidant activity against all methods (Figs. 3, 4, 5). The hydrogen-donating activity, measured using DPPH radicals as hydrogen acceptor, showed that significant association could be found between the concentration of the novel molecule and the percentage of inhibition. Through the DPPH test, compounds C, DMC, and BDMC were shown to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The method was based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction [11]. The nitric oxide assay has been widely used to evaluate the effectiveness of the free radical scavenging on various antioxidant substances. Nitric oxide generated as a result of decomposition of sodium nitroprusside in aqueous medium interacts with oxygen at physiological pH to produce nitrite ions. The nitrite ions were subjected to diazotization followed by azo coupling reaction to yield an azo dye measured by an absorption band at 540 nm. The scavenging ability of the synthesized compounds C, DMC, and BDMC was compared with ascorbic acid as a standard. A nitric oxides radical inhibition study showed that the synthesized compounds were a potent scavenger of nitric oxide. The compounds C, DMC, and BDMC inhibited nitrite formation by competing with oxygen to react directly with nitric oxide and also to

inhibit its synthesis. Scavengers of nitric oxide competed with oxygen, leading to the reduced production of nitric oxide [12, 13]. Antioxidant mechanisms of curcumin have been examined in this study. There are two postulated mechanisms for the reaction of compounds C, DMC, and BDMC as antioxidants as shown in Schemes 2 and 3. The first mechanism depends on the α-hydrogen atom in keto-form, (curcumin(I), where this atom was under the influence of two effects, namely resonance and inductive. The resonance effect of α-hydrogen makes the release of hydrogen as a free radical easy, while the inductive effect on carbonyl oxygen pushes the electrons toward a carbon-free radical, resulting in the molecule becoming stable. The second mechanism depends on the proton of hydroxyl group in the enol form, curcumin(II).

Experimental

The chemicals used were supplied by Sigma-Aldrich. The FT-IR spectra in the range (4,000–400) cm^{-1} were recorded as a KBr disc on a FTIR 8300 Shimadzu Spectrophotometer. The ^{1}H-NMR spectra were obtained on a Bruker -DPX 300 MHz spectrometer with TMS as internal standard. Elemental microanalysis was carried out using a model 5500-Carlo Erba C.H.N elemental analyzer instrument. A Gallenkamp M.F.B.600.010 F melting point apparatus was used to measure the melting points of all the prepared compounds.

Synthesis of curcumioides

Curcumin (C), DMC, and BDMC, were synthesized according to Ref. [14], and the structures of the compounds were confirmed with elemental analyses and spectral analyses (IR, UV–Vis, ^{1}H-NMR, and ^{13}C-NMR).

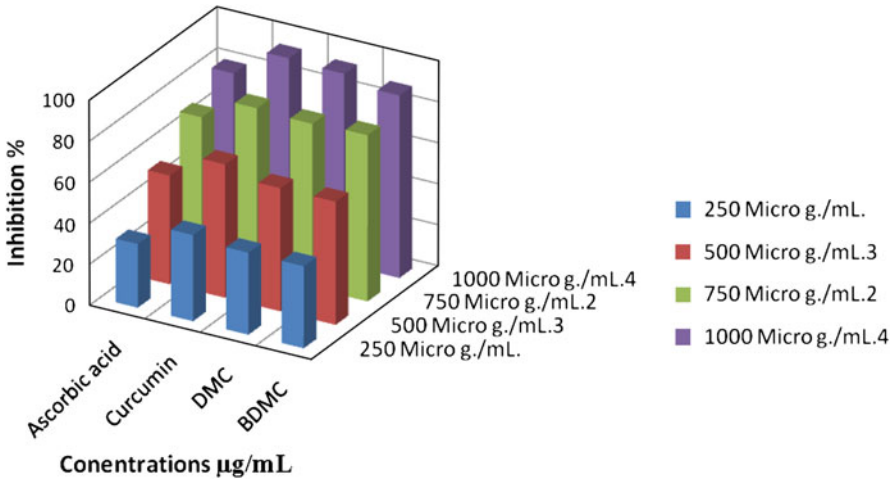


Fig. 3 Effect of curcuminoids C, DMC, and BDMC toward 1,1-diphenyl-2-picrilhydrazyl (DPPH)

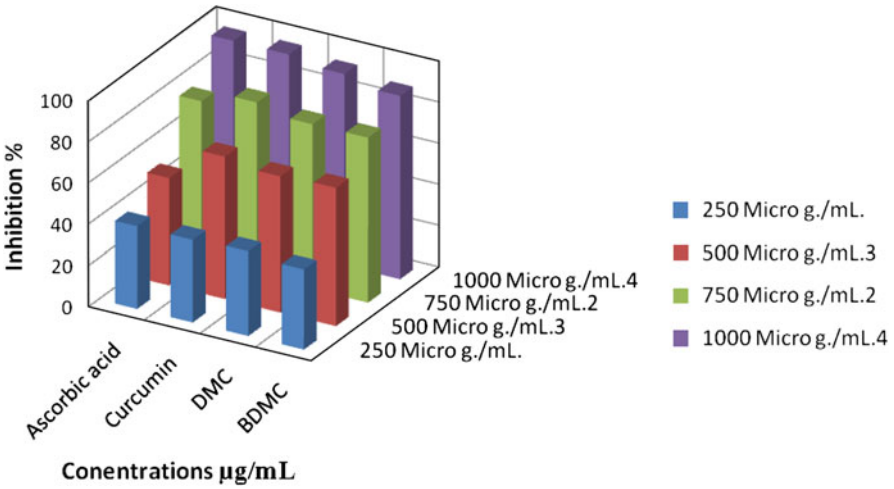


Fig. 4 Effect of curcuminoids C, DMC, and BDMC toward nitric oxide

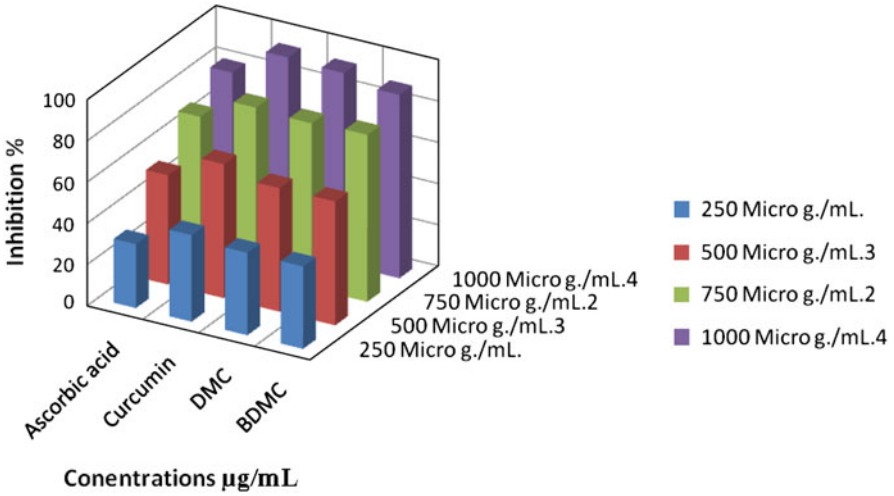
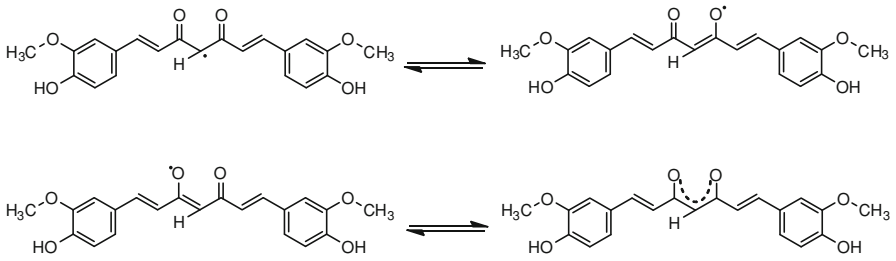


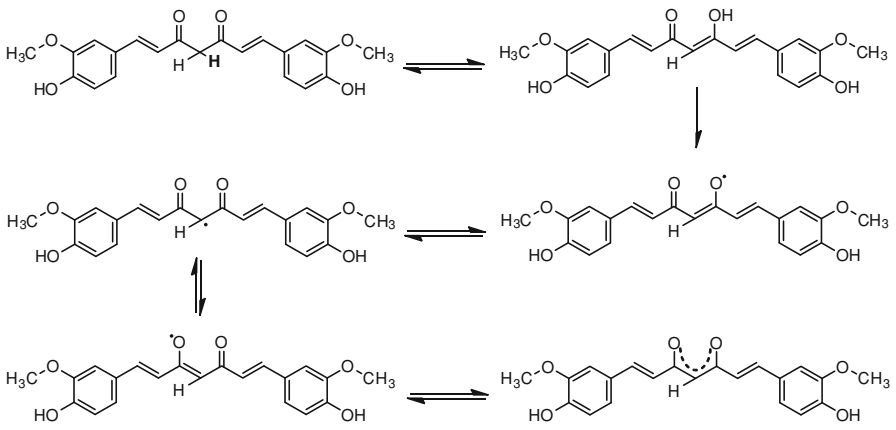
Fig. 5 Effect of curcuminoids C, DMC, and BDMC toward hydrogen peroxide

Curcumin

Thin layer chromatography (TLC) solvent systems were chloroform:benzene:methanol (80:15:5) and the R_f value was 0.69. Yield 65 %; M.P. 185 °C. UV–Vis in methanol, λ_{max}: 267, 415, and 431 nm. FT-IR spectrum, ν, cm^{-1}: 3,250–3,400 (O–H), 1,623.4 (C=O), 1,587.3 (C=C), 1,460.0 (C=C aromatic), 716.2, 755.0 and 821.7 (C=C–H aromatic). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 3.72 (3H, s, CH_3); 5.79 (2H, d, C–H); 6.72 (1H, d, $J = 10.1$ C–H alkene); 7.01 (1H, d, $J = 7.3$ C–H alkene); 8.32 (1H, d, OH); 7.31 (2H, d, C–H aromatic); 7.46 (2H, d,



Scheme 2 Postulated mechanism for using of curcumin(I) as antioxidant



Scheme 3 Postulated mechanism for using of curcumin(II) as antioxidant

C–H aromatic); 7.62 (1H, dd, $J = 1.8, 11.5$, C–H aromatic). **^{13}C** NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 44.6 (C17); 99.6 (C4); 102.1 (C11); 105.3 (C12); 120.0 (C13); 122.8 (C14); 124.2 (C15); 139.6 (C10); 145.9 (C7); 177 (C6); 190.8 (C5). Elemental analysis. Found, %: C 67.10; H 4.88; $C_{21}H_{20}O_6$. Calculated, %: C 68.47; H 5.47.

Desmethoxycurcumin

Thin layer chromatography solvent systems were chloroform:benzene:methanol (80:15:5) and the R_f value was 0.5. Yield 45 %; M.P. 172 °C. UV–Vis in methanol, λ_{max}: 422 nm. FT-IR Spectrum, v, cm^{-1}: 3,210 (O–H), 1,640.1 (C=O), 1,621.2 (C=C), 1,543.7 (C=C aromatic). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (J, Hz): 3.33 (3H, s, CH_3); 5.60 (1H, s, C–H); 5.93 (2H, d, C–H alkene); 6.75 (2H, d, C–H alkene); 6.98 (1H, d, OH); 7.23 (1H, t, C–H aromatic); 7.51 (2H, d, C–H aromatic); 7.59 (1H, d, C–H aromatic). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 53.1 (C17); 100.7 (C4); 102.1 (C11); 102.5 (C2-); 102.9 (C21); 131.2 (C12); 126.4 (C13); 122.2 (C14); 122.6 (C15); 126.9 (C22); 122.9 (C23); 123.1 (C24); 141.7 (C19); 141.3 (C10); 141.9

(C1); 143.3 (C7); 145.5 (C2); 146.2 (C6); 189.2 (C3); 189.5 (C5). Elemental analysis. Found, %: C 69.82; H 4.96; $C_{20}H_{18}O_5$. Calculated, %: C 70.99; H 5.36.

Bisdesmethoxycurcumin

Thin layer chromatography solvent systems were chloroform: benzene:methanol (80:15:5) and the R_f value was 0.42. Yield 30 %; M.P. 230 °C. UV–Vis in methanol, λ_{max}: 411 nm. FT-IR spectrum, ν, cm^{-1}: 3,175 (O–H), 1,640.7 (C=O), 1,620.7 (C=C), 1,454.2 (C=C aromatic). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 6.02 (2H, d, C–H); 6.65 (1H, d, C–H alkene); 6.68 (1H, d, C–H alkene); 7.70 (1H, d, OH); 7.22 (2H, d, C–H aromatic); 7.28 (2H, d, C–H aromatic); 7.61 (2H, dd, $J = 1.8$, 11.5, C–H aromatic). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 80.3 (C4); 129.3 (C10); 124.3 (C11); 124.6 (C12); 125.1 (C14); 125.5 (C15); 167.4 (C13); 148.7 (C7); 165.3 (C6); 189.2 (C5). Elemental analysis. Found, %: C 74.71; H 4.94; $C_{19}H_{16}O_4$. Calculated, %: C 74.01; H 5.23.

The calculation method

Gaussian 03, Revision C.01 [15] was used for the calculation of ground-state geometry optimized to a local minimum without any symmetry restrictions using basis set 3–21G [16, 17]. The Becke three-parameter hybrid (B3) [18, 19] exchange functional in combination with the Lee–Yang–Parr (LYP) [20] correction functional (B3LYP) was used for all geometry optimizations, thermodynamic functions at conditions (temperature = 298.150 K, and pressure = 1.0 Atm), HOMO and LUMO distribution, and some physical properties for all molecules.

Pharmacology

(2,2-diphenyl-1-picrylhydrazyl) (DPPH) radical scavenging activity The DPPH radical scavenging activities of the test samples compounds C, DMC, and BDMC were evaluated according to Soares et al. [21]. Initially, 0.1 mL of the samples at concentrations of 250, 500, 750, and 1,000 μg mL^{-1} was mixed with 1 mL of 0.2 mM DPPH that was dissolved in methanol. The reaction mixture was incubated in the dark for 20 min at 28 °C. The control contained all reagents without the sample while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using the UV–Vis spectrophotometer. The DPPH radical scavenging activity of ascorbic acid was also assayed for comparison. The percentage of DPPH radical scavenger was calculated using Eq. (1).

$$\text{Scanvenging effect}\,(\%) = \frac{A_0 - A_1}{A_0} \times 100 \qquad (1)$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards.

Nitric oxide scavenging activity Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide spontaneously; it interacts with oxygen to produce nitrite ions, which can be estimated by the use of the GriessIllosvoy reaction [22]. In the present investigation, the GriessIllosvoy reagent was modified using naphthylethylenediaminedihydrochloride (0.1 % w/v) instead of 1-naphthylamine (5 %). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL), and compounds C, DMC, and BDMC (250, 500, 750, and 1,000 μg mL^{-1}) or standard solution (0.5 mL) was incubated at 25 °C for 150 min. After the incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33 % in 20 % glacial acetic acid) and allowed to stand for 5 min to complete diazotization. Then, 1 mL of naphthylethylenediaminedihydrochloride (1 %) was added, mixed, and allowed to stand for 30 min. A pink-colored chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank. Ascorbic acid was used as standard. Nitric oxide percentage scavenging activity was then calculated using Eq. (1).

Hydrogen peroxide scavenging activity A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (250, 500, 750, and 1,000 μg mL^{-1}) of synthesized compounds (or ascorbic acid) were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide [23, 24]. Hydrogen peroxide percentage scavenging activity was then calculated using Eq. (1).

Conclusion

In this study, curcuminoids, namely, curcumin (C), DMC, and BDMC have been successively synthesized and characterized by using various spectroscopic methods and elemental analysis techniques. Two postulated mechanisms have been proposed for the action of the compounds as antioxidants. The antioxidant activity of the compounds was initially tested and showed that the compounds have improved properties compared to ascorbic acid. We have carried out quantum chemistry calculation using the DFT method to study the stability of curcumin isomers in the gaseous state, The results showed that the isomer diketone, curcumin(I), is more stable than the isomer enol, curcumin(II), as was proven by calculations (total energy, energy of HOMO, the distribution of electron density, change in entropy and the change in enthalpy), so that the DFT can be relied on in distinguishing between stabilizing isomers.

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