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FULL PAPER

Experimental and theoretical investigations of the antioxidant activity of 2,2′-methylenebis(4,6-dialkylphenol) compounds

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The antioxidant activity of two primary antioxidants, 2,2′-methylenebis(4-methyl-6-*tert*-butylphenol) (MMBPH2) and 2,2′-methylenebis(4,6-di-*tert*-butylphenol) (MDBPH2), has been studied using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The synthesized compounds have been successfully characterized systematically using elemental analyses, infrared, ^{1}H NMR and ^{13}C NMR spectra and GC–MS. Importantly, it has been found that the compound MMBPH2 in particular is more active in DPPH radical scavenging. In addition, density functional theory calculations (B3LYP) have been used to predict the antioxidant activity and predict structural geometries of the compounds in the gas phase.

KEYWORDS

antioxidants, dimethoxymethane, diphenylpicrylhydrazyl, DFT calculations

1 | INTRODUCTION

Phenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity.[1] Herbs are used in many domains, including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances and cosmetics.[2] Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic and antimutagenic effects and anticarcinogenic potential.[3]

Free radicals are molecules, ions or atoms with unpaired electrons in their outermost shell of electrons.[4,5] These species, which are constantly formed in the human body, can become toxic when generated in excess or in the presence of a deficiency in naturally occurring antioxidant defenses. High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells and tissues.[6–8]

In previous studies, reactive oxygen species, such as superoxide radicals, hydroxyl radicals and peroxyl radicals, have been reported as natural byproducts of the normal metabolism of oxygen in living organisms with important roles in signaling.[9,10] In addition, phenols with antioxidant activity could scavenge reactive chemical species as well as minimize oxidative damage resulting from excessive light exposure.[11] Various methods are currently used to assess the antioxidant activity of phenolic compounds. $ABTS^{+\bullet}$ (2,2'azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) or $DPPH^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) radical scavenging methods are common spectrophotometric procedures for determining the antioxidant activities of components.[12]

Therefore, in the study reported here, the antioxidant activity of 2,2′-methylenebis(4-methyl-6-*tert*-butylphenol) (MMBPH2) and 2,2′-methylenebis(4,6-di-*tert*-butylphenol) (MDBPH2) was investigated using the DPPH method and using the Gaussian 03 program with density functional theory (DFT/B3LYP) using 6-31G(d) basis set for geometry optimization. MMBPH2 and $MDBPH_2$ are effective sterically hindered phenolic primary antioxidants, which are widely used to protect rubbers, oils, fats, adhesives and waxes from ageing.

2 | MATERIALS AND METHODS

2.1 | Materials

2-*tert*-Butyl-4-methylphenol, 2,4-di-*tert*-butylphenol, dimethoxymethane, *n*-heptane, sulfuric acid 98% and ethanol were obtained from Merck. Paraformaldehyde was obtained from Panreac. DPPH was obtained from Sigma Aldrich.

Melting points were determined with an Electrothermal 9200. Elemental analyses (C, H and N) were carried out with a Heraeus instrument (Vario EL). Purity determination was carried out using HPLC (Shimadzu HPLC-SPD-M20 A) with a Chromolith-Performance HPLC column RP-8e (100 × 4.6 mm). The flow rate was 1 ml min^{-1}. The chromatograms were recorded at 280 nm (UV–visible). MS was carried out with a Shimadzu GC–MS mass spectrometer. The flow rate was 1 ml /min^{-1}. The gradient profile was: 90 ° C (3 min); 280 °C (20 min). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Bruker Advance spectrometer. Infrared (IR) spectra were obtained with a FTIR Unicam, Galaxy Series 5000 type spectrometer using KBr discs.

2.2 | Preparation of MMBPH2 and MDBPH2

2.2.1 | Dimethoxymethane method

Amounts of 0.025 mmol of sulfuric acid, 0.4 mmol of dimethoxymethane and 0.5 mmol of 2-*tert*-butyl-4-methylphenol (or 2,4-di-*tert*-butylphenol) were added to a reactor with a thermometer, a condenser and a stirrer. The contents were stirred for 2 h at 60–70 °C. Then the stirrer was removed and the reaction mixture was left in an ice chest or refrigerator overnight. Then petroleum ether was added and stirred until the complete dissolution of the reaction mixture and then the sulfuric acid was separated. The petroleum ether and unreacted dimethoxymethane were distilled off from the reaction mixture. The residue was purified with 280 ml of an ethanol–water mixture (3:1 v/v) at 75 °C, with stirring for 1 h. After that the resulting mixture was cooled to 20 °C, filtered, washed with 160 ml of ethanol–water mixture and filtered. The residue was dried for 3 h at 85 °C.

MMBPH2: yield 70%; m.p. 127–130 °C; purity ≥99% (HPLC). MDBPH2: yield 84%; m.p. 147–150 °C; purity ≥99% (HPLC).

2.2.2 | Paraformaldehyde method

Amounts of 0.006 mmol of sulfuric acid, 200 ml of *n*-heptane, 0.5 mmol of 2-*tert*-butyl-4-methylphenol (or 2,4-di-*tert*-butylphenol) and 0.275 mmol of paraformaldehyde were added to a reactor with a thermometer, a condenser and a stirrer. The contents of the reactor were stirred for 2 h at 85–93 °C. Then the obtained reaction mixture was cooled to 20 °C and the sulfuric acid separated. The *n*-heptane was distilled off from the reaction mixture. The residue was purified with an ethanol–water mixture (3:1 v/v) in the same way as described for the dimethoxymethane method.[11]

MMBPH2: yield 69.7%; m.p. 127–129 °C; purity ≥99% (HPLC). MDBPH2: yield 85%; m.p. 149–150 °C; purity ≥99% (HPLC).

2.3 | Characterization

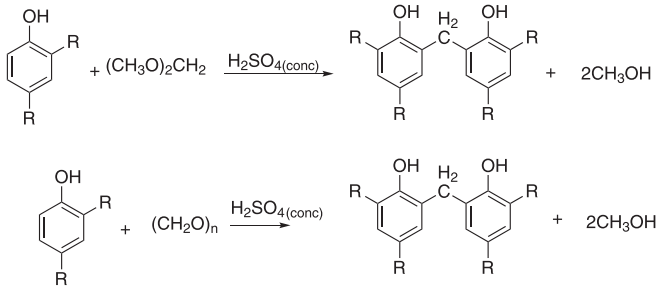
MMBPH2. Anal. Found (calcd) for $C_{23}H_{32}O_2$ (%): C, 80.10 (81.13); H, 8.42 (9.47). IR (cm^{-1}): 1232.9 (C—O), 1477.6 (C═C), 3121.1 (C—H), 3393 (—OH). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.44 (s, 18H), 2.3 (s, 6H), 3.93 (s, 2H), 5.86 (s, 2H, OH-Ar), 7 (s, 4H, Arom). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 150.07–126.4 (Ar-*C*), 34.36 (Ar-*C*H_2-Ar), 32.14 (Ar-*C*H_3), 30.01 (*C*$(CH_3)_3$), 20.91 (*C*H_3). GC–MS: 177 (100%), 57 (32.1%), 105 (14.3%), 77 (7%), 106 (4%).

MDBPH2. Anal. Found (calcd) for $C_{29}H_{44}O_2$ (%): C, 81.1 (82.02); H, 9.43 (10.44). IR (cm^{-1}): 1199.6 (C—O), 1478.2 (C═C), 3121.1 (C—H), 3531.5 (—OH). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.32 (s, 18H), 1.45 (s, 18H), 3.97 (s, 2H), 5.92 (s, 2H, OH-Ar), 7.16 (d, J = 5.3 Hz, 2H (near to CH_2), Ar), 7.24 (d, J = 5.3 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz, δ, ppm): 149.9–122.57 (Ar-*C*), 34.65 (Ar-*C*H_2-Ar), 32.54 (*C*$(CH_3)_3$), 31.61 (3*C*H_3 (near to OH)), 30.07 (3*C*H_3). GC–MS: 177 (100%), 57 (100%), 219 (57.14%), 105 (7.14%).

3 | RESULTS AND DISCUSSION

MMBPH2 and MDBPH2 were prepared by two referential methods: the first one uses dimethoxymethane as condensing agent for 2,4-dialkylphenols[10] and the second one uses paraformaldehyde.[11] The reaction for both methods was carried out in the presence of sulfuric acid (Scheme 1).

As evident from Table 1, the compound MMBPH2 (Fig. 1) is an effective antioxidant comparing with gallic acid at 1 mmol l^{-1} concentration, and it is more effective by 35% than MDBPH2 (Fig. 1), even when MDBPH2 is used at a concentration of more than 20 mmol l^{-1}. From the theoretical study which used density functional theory (DFT/B3LYP) it is found that the two compounds are stable and have asymmetric stereochemical structures. The *tert*-butyl groups at *para* position in MDBPH2 lead to stereochemical hindrance preventing the compound from making an intramolecular hydrogen bond which means this compound has less ability



DPPH scavenging activity (%)=[(A_B-A_A)]/AB.100

SCHEME 1 Synthesis of MMBPH2 and MDBPH2.

TABLE 1 Results of DPPH tests

	MDBPH2			MMBPH2			Gallic acid
Concentration (mmol l^{-1})	28.3	23.6	21.2	1.47	1.18	1.12	1
DPPH (%)	±57.9 0.5	±54.5 0.25	50.7 ± 0.5	82.9 ± 0.45	76.6 ± 0.61	75.76 ± 0.38	74.89

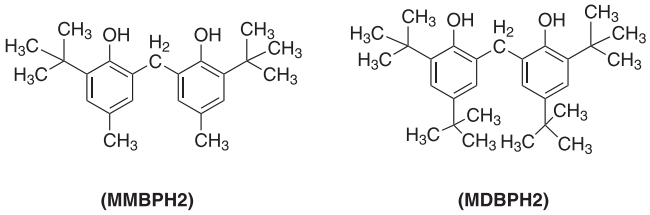


FIGURE 1 Chemical structures of MMBPH2 and MDBPH2

to donate a hydrogen radical to free radicals. For this reason, MMBPH2 is more effective than MDBPH2 as an antioxidant.

3.1 | Study of Antioxidant Activity using DPPH Method

Gallic acid was used as phenolic reference to study the antioxidant activity of the two compounds MMBPH2 and MDBPH2.

A series of standards of gallic acid were prepared (Table 2). Five normal solutions with different concentrations (0.2, 0.4, 0.6, 0.8, 1 mmol l^{-1}) (Fig. 2) were prepared from a 10 mmol l^{-1} solution of gallic acid, using ethanol as diluent. An amount of 6 ml of 45 μg ml^{-1} DPPH solution was added to 100 μl of each of the normal solutions of gallic acid. The mixed solution was incubated at room temperature for 30 min in the dark, and then the absorbance of the reaction mixture was read at 517 nm and the remaining DPPH was calculated. The free radical scavenging activity is expressed as follows:

$$\text{DPPH scavenging activity } (\%) = \frac{A_B - A_A}{A_B} \times 100$$

where A_B is the absorbance of the blank (EtOH) and A_A is the absorbance of the sample. All experiments were performed in triplicate.

Three solutions with different concentrations of the two compounds were prepared using ethanol as diluent. An amount of 6 ml of 45 μg ml^{-1} DPPH solution was added to 100 μl of each of the three solutions of the two compounds. The mixed solution was incubated at room temperature for 30 min in the dark, and then the absorbance of the reaction mixture was measured at 517 nm. Table 1 shows the results of the DPPH test, and the difference in the antioxidant activity between the two compounds.

TABLE 2 Series of standards of gallic acid

Absorbance	1.374	1.142	0.927	0.707	0.523	0.345
Concentration (mmol l^{-1})	0	0.2	0.4	0.6	0.8	1

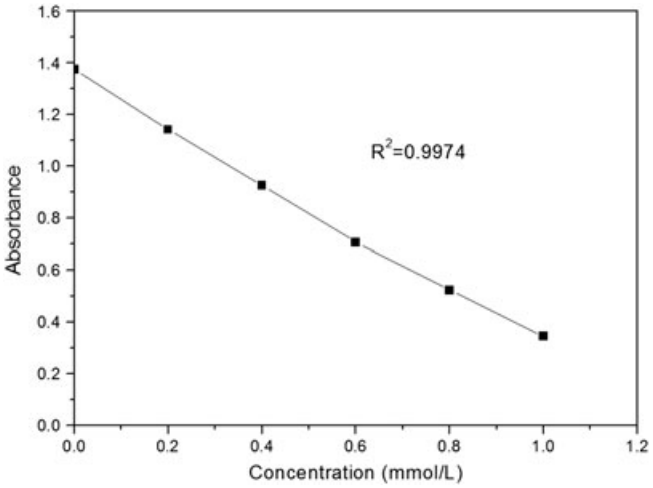


FIGURE 2 Series of standards of gallic acid

3.2 | Theoretical Study

The theoretical treatment of MMBPH2 and MDBPH2 included in this work was performed using the DFT/B3LYP approach implemented in the Gaussian 03 series of programs.[13,14] It aimed to find a scientific explanation for the results of the DPPH test. Standard pseudopotentials developed in Toulouse were used to describe the atomic cores.

3.2.1 | Optimized geometries

The geometries of MMBPH2 and MDBPH2 were optimized using analytic gradients. These results (Figs 3 and 4; Table 3) show that the two compounds have C1 symmetry. Hydroxyl group O(1)—H(1) (which is connected to carbon atom C(4)) and hydroxyl group O(2)—H(2) (which is connected to

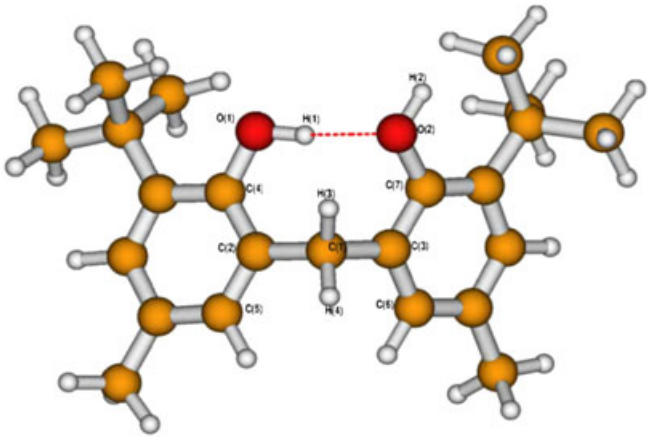


FIGURE 3 DFT/B3LYP optimized geometry of MMBPH2

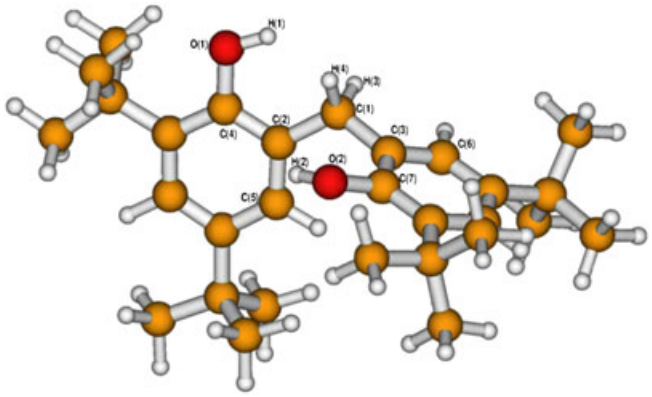


FIGURE 4 DFT/B3LYP optimized geometry of MDBPH2

TABLE 3 Selected geometrical parameters (bond lengths, Å; angles, °) for MMBPH2 and MDBPH2 optimized at the DFT/B3LYP level (see Figs 3 and 4 for labeling of the atoms)

MMBPH2 C_1	MDBPH2 C_1	Geometrical parameters
1.106	1.101	C(1)–H(3)
1.110	1.101	C(1)–H(4)
1.535	1.531	C(1)–C(2)
1.521	1.531	C(1)–C(3)
1.380	1.373	C(4)–O(1)
1.379	1.393	C(7)–O(2)
0.971	0.981	O(1)–H(1)
0.976	0.970	O(2)–H(2)
4.188	1.800	O(2)–H(1)
120.1	121.3	C(2)–C(4)–O(1)
118.6	113.7	C(3)–C(7)–O(2)
109.3	112.3	C(4)–O(1)–H(1)
108.8	110.3	C(7)–O(2)–H(2)
119.6	121.9	C(1)–C(2)–C(4)
119.6	121.1	C(1)–C(3)–C(7)
102.4	169.3	O(1)–H(1)–O(2)
23.8	38.2	O(1)–H(1)–O(2)–H(2)
–98.2	22.4	O(1)–C(4)–O(2)–C(7)

carbon atom C(7)) are asymmetric, which is because compound MMBPH2 has an intramolecular hydrogen bond between H(1) and O(2), with a length of 1.800 Å, whereas compound MDBPH2 has *tert*-butyl groups at the *para* position in each of the two aromatic rings. The *tert*-butyl groups cause stereochemical hindrance and force the two parts of MDBPH2 (the two aromatic rings) to turn around the methylene group (CH_2) just to retain the hybrid sp^3 orbital for carbon atom C(1) which makes the distance between H(1) and O(2) (Fig. 4) to be 4.188 Å (instead of the 1.800 Å in MMBPH2).[15] The optimized geometrical parameters are summarized in Table 3. According to the previous study we can state the following reasons as to why MMBPH2 is more effective than MDBPH2 as an antioxidant:

1. Stereochemical structure. Figure 4 shows the bulky stereochemical structure of the *tert*-butyl groups at *para* position to the hydroxyl groups in MDBPH2 decreases the ability of the hydroxyl groups to donate a hydrogen radical to the free radicals in oxidation reactions and decreases the ability of these free radicals to reach the hydrogen radical of the hydroxyl group; this situation does not occur for MMBPH2.[15]
2. Intramolecular hydrogen bond.[14] The intramolecular hydrogen bond in MMBPH2 (Fig. 3) stabilizes the phenoxy radical of the compound. This will make the hydroxyl groups in this compound have more of an ability to donate a hydrogen radical to free radicals than the hydroxyl groups in MDBPH2 which does not have an intramolecular hydrogen bond, it just having an intermolecular hydrogen bond (Fig. 5).
3. Inductive effect of the alkyl groups.[14] The positive inductive effect of the *tert*-butyl groups (+I) is stronger than the (+I) of the methyl groups. This will make the hydroxyl groups in MMBPH2 have more of an ability to donate a hydrogen radical to free radicals than the

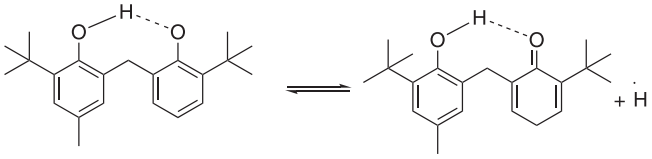


FIGURE 5 Intramolecular hydrogen bond in MMBPH2

TABLE 4 Calculated harmonic vibrational frequencies and IR intensities (Km mol^{-1}) at the DFT/B3LYP level for MMBPH2 and MDBPH2

MDBPH2			MMBPH2			
Experimental (IR)	B3LYP		Experimental (IR)	B3LYP		
Vibration (cm^{-1})	Vibration (cm^{-1})	Intensity (Km mol^{-1})	Vibration (cm^{-1})	Intensity (Km mol^{-1})	Vibration (cm^{-1})	
3393	38013078	93325	3531.5	297920	38123593	—OH
2953	310030873087	5073166	2955.6	575878	300730183083	—CH aliphatic
—	30202951	187176	—	751223	308830883044	—CH aliphatic (methylene group)
—	1642163616251622	3021	—	2119	1650164616311626	Benzene
1232.9	1256	111	1199.6	58	1273	—C=O
—	3161318032143245	14483	—	17151711	3143315431673194	H—benzene

hydroxyl groups in MDBPH2 which has *tert*-butyl groups in *para* and *ortho* positions.

3.2.2 | Vibrational frequency calculations

The harmonic vibrational frequencies of the different stationary points of the potential energy surface have been calculated at the same level of theory in order to identify the local minima.

The vibration frequency of the ─OH bond in MDBPH2 has two theoretical values (3812 and 3593 cm^{-1}). The theoretical value which has the high intensity is in agreement with the experimental value obtained from IR analysis: 3531.5 cm^{-1} (Table 4).[15,16]

4 | CONCLUSIONS

We synthesized MMBPH2 and MDBPH2. In this study, the two compounds were studied experimentally and theoretically. Also, it has been found that MMBPH2 is more effective than MDBPH2 as an antioxidant because of the *tert*-butyl groups at *para* position in MDBPH2 which lead to stereochemical hindrance.

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