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Original article

Isoeugenol-based novel potent antioxidants: Synthesis and reactivity

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ABSTRACT

We are attempting to develop the novel phenolic synthetic antioxidants aimed at retarding the effects of free radicals and oxidants. The phenolic compounds (**7**–**12**) were synthesized by Friedel-Crafts alkylation of isoeugenol (**1**) and phenol derivatives (**2**–**6**) and their structures were determined by spectroscopic methods. All the synthesized phenolic compounds (**7**–**12**) except **12** are new. Antioxidant and radical scavenging activities of synthesized compounds (**7**–**12**) were determined by using various in vitro assays such as 1,1-diphenyl-2-picrylhydrazyl free radicals (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals ($ABTS^{+}$), and superoxide anion radicals (O_2^{-}) scavenging, ferric reducing antioxidant power (FRAP) and total antioxidant activity by ferric thiocyanate. The antioxidant activities of compounds were compared with standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trolox as positive controls. The results showed that the synthesized compounds, especially **10** and **11**, had better properties than standard antioxidants (BHT, BHA and trolox).

1. Introduction

Antioxidants are chemical compounds that can quench reactive radical intermediates formed during the oxidative reactions. The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines [1–3]. These antioxidants act usually as both through chain transfer and chain termination reagent [1]. The first step of the reactive radicals termination by this type of antioxidants is hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate [1,2]. Small amounts of antioxidants are added into most synthetic polymers to prevent or retard oxidation and to increase the service lifetimes of the products [1,2,4,5]. Free radicals and active oxygen species have been related with cardiovascular and inflammatory diseases, and even with a role in cancer and aging [6,7]. Efforts to counteract the damage caused by these species are gaining acceptance as a basis for novel therapeutic approaches and the field of preventive medicine is experiencing an upsurge of interest in medically useful antioxidants [8,9].

Phenolic derivatives are one of the groups of antioxidants [10] that have been studied by many research groups. A great number of examples have been described in the literature, such as caffeic acid and its analogs, which are known to have antiviral, anti-inflammatory and antiatherosclerotic properties [11], resveratrol with known anticancer and heart protecting effects [12] and olive oil phenols, particularly hydroxytyrosol, which inhibits human low-density lipoprotein (LDL) oxidation (a critical step in atherosclerosis) [13] inhibits platelet aggregation [14] and exhibits anti-inflammatory [15] and anticancer properties [16].

Phenols have been utilized extensively for food preservation. Synthetic phenolic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) or butylated hydroxyquinone (TBHQ) possess good antioxidant capacity [17]. The main structural feature responsible for the antioxidative and free radical scavenging activity of phenolic derivatives is the phenolic hydroxyl group. Phenols are able to donate the hydrogen atom of the phenolic OH to the free radicals, thus stopping the propagation chain during the oxidation process [18]. The presence of a second hydroxyl group at the *ortho*-position, to give a catechol ring, also lowers the O–H bond-dissociation enthalpy and increases the rate of H-atom transfer to peroxyl radicals [19,20]. A third hydroxyl group in the phenolic ring increases the antioxidant capacity further [21]. Another structural feature that may increase the antioxidant capacity is the primary hydroxyl group on the alkyl chain of antioxidants such as tyrosol and hydroxytyrosol. In fact, hydroxytyrosol is a better antioxidant when added to olive oil than caffeic acid and homoprotocatechuic acid [22,21] all of them containing the same *ortho*-diphenolic structure. In addition, the alkyl chain connecting the phenolic ring and the carboxylic or alcohol group in phenolic

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derivatives may stabilize the radical formed during oxidation, and its contribution remains uncertain [23–25]. Actually, some phenolic antioxidants contain an alkenyl chain for this connection, such as in caffeic acid or sinapic acid, and this functionality may also be important to stabilize the radical formed [24,9].

Isoeugenol [2-methoxy-4-(1-propenyl)phenol, **1**] is a natural compound and is used in perfumes, soaps, detergents, air fresheners and as a flavoring agent in cosmetics and food products. Also, isoeugenol has a propenyl moiety and the beneficial properties such as antioxidant and anti-inflammatory [26–29].

The aim of this study is to synthesized steric hindered novel phenolic compounds (**7**–**12**) using the isoeugenol as alkylating agent and investigated their antioxidant capacity and radical scavenging effects. Determination of antioxidant and radical scavenging activities were evaluated by different in vitro antioxidant tests such as DPPH, $ABTS^+$, O_2^- scavenging activities, reducing power activity (FRAP), and total antioxidant activity by ferric thiocyanate methods. The antioxidant and radical scavenging activities of the compounds (**7**–**12**) were compared with the BHA, BHT and trolox for positive controls.

2. Chemistry

The synthesis of the tested compounds (**7**–**12**) was carried out according to the slightly modified Friedel-Crafts alkylation method [30]. The reaction of phenol derivatives (**2**–**6**) (phenol, ethoxypyrocatechol, 2-*iso*-propylphenol, pyrocatechol and resorcinol) and isoeugenol (**1**) in ratio of 2:1 in the presence of aluminum isopropoxide ($Al(O\text{-}i\text{-}Pr)_3$) as catalyst gave products (**7**–**12**) (Scheme 1). All synthesized compounds except compound **12** [31] are novel.

3. Results and discussion

3.1. Structural characterization of compounds 7–12

Structural confirmation of synthesized compounds was done using NMR, GC/MS, IR and UV–VIS spectra. In ^1H NMR spectra of the synthesized phenol derivatives (**7**–**12**), the dibenzylic proton resonates as a triplet between 3.46 and 4.22 ppm with a coupling constant of $J = 7.6$–8.8 Hz. The $-CH_2-$ protons give an AB system as a multiplet at ca. 2.00 ppm, and the methyl protons resonates as a triplet at ca. 1.00 ppm with a coupling constant of $J = 7.6$–7.2 Hz. All spectral findings indicated the proposed structures. Similarly, IR spectral data of the compounds **7**–**12** confirmed the presence of phenol –OH (3469-3413 and 2962–2958 cm^{-1}). Finally, the structures of the synthesized phenolic compounds (**7**–**12**) were supported by their EI mass spectra.

3.2. Antioxidant activity evaluation

The antioxidant activities of the synthesized phenolic compounds (**7**–**12**) were evaluated by several in vitro methods in order to compare the results and to establish some structure–antioxidant activity relationships for each method. The evaluation study was carried out at various concentrations and in comparison with the standard antioxidants.

3.2.1. DPPH radical scavenging activity

The DPPH radical scavenging activity assay is a simple method for measuring the antioxidant ability to trap free radicals. The scavenging effects of compounds **7**–**12** and isoeugenol are shown in Table 1. Three controls, BHT, BHA and trolox, are also included. Compounds **10** and **11**, containing a catechol moiety, showed the highest activity (IC_{50}=5.80 μM). Compounds **7**–**9** and **12** showed higher activity than BHA (IC_{50}=9.55 μM), BHT (IC_{50}=11.50 μM) and isoeugenol (IC_{50}=40.67 μM), but lower activity than trolox (IC_{50}=5.58 μM).

3.2.2. $ABTS^+$ radical cation scavenging activity

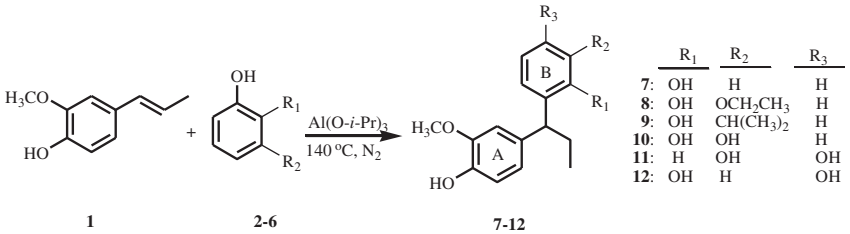
The $ABTS^+$ assay is a widely used method for measuring the antioxidant ability to trap free radicals [32]. The $ABTS^+$ radical cation scavenging capacity of the synthesized compounds **7**–**12** is shown in Table 1. The IC_{50} values of compounds **7**–**12** were in the range 7.00–10.07 μM. All compounds showed higher activity than BHT (IC_{50} = 10.23 μM) and BHA (IC_{50} = 10.94 μM), and almost the same activity of isoeugenol (IC_{50} = 8.84 μM) and trolox (IC_{50} = 8.00 μM), with the exception of compound **12** (IC_{50} = 10.07 μM). Compounds **8** and **9** (IC_{50} = 7.19 and 7.00 μM, respectively) showed the highest activity.

3.2.3. Superoxide radical anion (O_2^-) scavenging activity

Superoxide anion radical is normally initially formed, and its effects can be magnified because it produces other kinds of free radicals and oxidizing agents [33]. The superoxide anion radical (O_2^E) scavenging activities of synthesized compounds **7**–**12** are shown in Table 1. All compounds, with the exception of **12** (IC_{50} = 15.29 μM), exhibited higher activity than isoeugenol, BHA, BHT and trolox. Compounds **10** and **11** showed the highest activity (IC_{50} = 6.17 and 6.61 μM, respectively).

3.2.4. Ferric reducing antioxidant power (FRAP)

Ferric reducing power was determined using the iron(III) to iron(II) reduction assay. FRAP values (absorbances at $\lambda = 700$ nm) of compounds **7**–**12**, isoeugenol and standard antioxidants are shown in Table 2. At low concentration (10 μg/mL), compounds **10** and **11**, containing an *ortho*-dihydroxy function, showed the best reducing power (absorbance value = 0.49 and 0.43, respectively), while the reducing power of the other synthesized compounds, isoeugenol, trolox, BHA and BHT were similar, absorbance values ranging from 0.21 to 0.32. The reducing power of all compounds and standards showed an increase by rising concentrations. At high concentration (40 μg/mL), the reducing power of compounds **10** (0.73) and **11** (0.64), trolox (0.72) and BHT (0.60), were comparable, while BHA showed the highest value (0.94). FRAP values of compounds **7**–**9** and **12** were in the range 0.24–0.47.



	R_1	R_2	R_3
7:	OH	H	H
8:	OH	OCH_2CH_3	H
9:	OH	$CH(CH_3)_2$	H
10:	OH	OH	H
11:	H	OH	OH
12:	OH	H	OH

2: R_1= OH, R_2 = H; **3**: R_1 = OCH_2CH_3, R_2 =H; **4**:R_1 =$CH(CH_3)_2$, R_2 = H; **5**: R_1 =OH, R_2 =OH; **6**: R_1 =H, R_2 OH

Scheme 1. General procedure for preparation of test compounds (**7**–**12**).

Table 1
Concentration required for 50% scavenging (IC_{50}) of DPPH, $ABTS^+$, O_2^E radical scavenging activities of synthesized compounds (**7**–**12**), isoeugenol and the standard antioxidants: BHA, BHT, and trolox. and; Inhibition of Lipid Peroxidation (%) of synthesized compounds (**7**–**12**), isoeugenol and the standard antioxidants.

Test compounds	Scavenging activity (IC_{50}, μM)			% Inhibition lipid peroxidation					
	DPPH	$ABTS^+$	O_2^-	6	12	18	24	30	36
				Incubation (h)					
7	7.83	8.53	10.50	32 ± 3.9	75 ± 3.1	90 ± 1.7	94 ± 0.6	97 ± 0.1	96 ± 0.4
8	6.19	7.19	13.71	37 ± 3.7	77 ± 4.7	89 ± 1.7	96 ± 0.8	97 ± 0.2	96 ± 0.6
9	6.30	7.00	10.10	38 ± 3.8	78 ± 1.9	88 ± 1.9	95 ± 0.9	97 ± 0.1	96 ± 0.8
10	5.80	8.43	6.17	34 ± 6.7	74 ± 2.4	90 ± 2.7	94 ± 0.8	97 ± 0.1	96 ± 0.2
11	5.80	8.28	6.61	37 ± 1.4	78 ± 7.6	90 ± 5.1	96 ± 1.4	98 ± 0.6	97 ± 0.5
12	6.71	10.07	15.29	36 ± 4.6	75 ± 4.7	85 ± 3.2	94 ± 0.8	97 ± 0.4	94 ± 0.3
Isoeugenol	40.67	8.84	42.26	28 ± 4.5	42 ± 3.2	90 ± 1.4	96 ± 2.1	91 ± 3.2	82 ± 0.7
Trolox	5.84	8.00	61.32	35 ± 3.5	70 ± 5.1	87 ± 6.8	89 ± 2.1	92 ± 0.3	86 ± 1.0
BHA	9.55	10.94	15.22	44 ± 2.7	78 ± 4.7	91 ± 2.3	96 ± 0.9	98 ± 0.2	97 ± 0.5
BHT	11.50	10.23	28.45	47 ± 3.0	81 ± 4.3	92 ± 1.4	97 ± 0.9	98 ± 0.1	98 ± 0.2

3.2.5. Determination of inhibition of lipid peroxidation

Lipid peroxidation contains a series of free radical-mediated chain reaction processes and is also associated with several types of biological damage. The role of free radicals and ROS is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging and atherosclerosis [34]. The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation which is the primary product of lipid peroxidation. Total antioxidant activity of the synthesized compounds (**7**–**12**), isoeugenol, BHA, BHT and trolox was determined by the ferric thiocyanate method in the linoleic acid system.

Almost all compounds showed high antioxidant activity in this assay. The solutions of all compounds (**7**–**12**), isoeugenol and standards (BHT, BHA, Trolox) were prepared within the same concentrations (100 μg/mL) in this test. Test results are given in Table 1. All the synthesized compounds showed the ability to inhibit lipid peroxidation; at the end of incubation period (36 h) in linoleic acid emulsion system, the inhibition values were in the range 94–96%. The activities of these compounds were greater than that of isoeugenol (82 ± 0.7) and trolox (86 ± 0.4%) and similar to BHA (97 ± 0.5%) and BHT (98 ± 0.2%) at the same concentrations.

Isoeugenol, a methoxyphenol with a short hydrocarbon chain, is found in some vegetables such as monkey orange [35], it also acts as an antioxidant [36,37] and is used as a fragrant food additive. In the recent studies, the antioxidant properties of isoeugenol were analyzed on the basis of the protective effect on metal-mediated lipid peroxidation. Antioxidant activity of isoeugenol has been explained by its methoxyphenolic structure [38]. In the present study, all synthesized compounds have 2-metoxy-4-propylphenol moiety (isoeugenol unit). All antioxidant assays showed that all compounds **7**–**12** have almost a parallel and/or higher activity than standard antioxidants. Our results are consistent with previous studies [36,37]. It is concluded that our synthesized compounds had high antioxidant capacity due to these compounds which have isoeugenol unit.

Moreover, the parent methoxyphenol is intramolecularly hydrogen bonded [39]. It is estimated that this hydrogen bond stabilizes to the parent compound by 4 kcal mol^{-1}, which opposes the electronic effect of the methoxy group [40].

The synthesized compounds (**7**–**12**) in this work just become only distinct by substitute phenol ring (B ring), so the differences of their antioxidant activities had been discussed correlating with structure of this phenol ring.

The main structural feature responsible for the antioxidative and free radical scavenging activity in the case of phenolic derivatives is the phenolic hydroxyl group. Phenols are able to donate the hydrogen atom of the phenolic OH to the free radicals, thus they stop the propagation chain during the oxidation process. This effect is modulated by the ring substituents, so that electron-withdrawing groups increase the bond-dissociation enthalpy, due to the stabilization of the phenol by a polar structure that leaves a positive charge on the OH group. Consequently, electron-donating groups (alkyl, methoxy or ethoxy) produce a reduction of the bond-dissociation enthalpy due to the stabilization of the phenoxyl radical by mesomeric structures bearing a positive charge on the substituent. This is the case in the presence of a second hydroxyl group at the *ortho*-position, yielding a catechol ring that also lowers the OH bond-dissociation enthalpy and increases the rate of H-atom transfer to peroxyl radicals [19]. In fact, early comparisons between tyrosol and hydrotyrosol [41] showed a much better radical scavenging capacity for hydrotyrosol than for tyrosol. Also, Son and Lewis [42] compared a radical scavenging efficiency of caffeic and ferulic acid. They observed that inhibition of DPPH radicals was almost double for caffeic acid containing a di-*ortho* phenolic motif than for ferulic acid where a methoxy group replaces the OH group at position *ortho*. Again, this tendency is confirmed in a food matrix, where the Rancimat test shows that caffeic acid protects an oil matrix much better then ferulic acid, yielding a three times longer induction time [43].

Similar results were obtained in our group during comparing the radical scavenging and antioxidant activities of phenolic compounds (**7**–**12**) that contain either a phenolic motif (**7**), or a ethoxypyrocatechol ring (**8**), or a 2-isopropyl phenolic motif (**9**), or a di-*ortho* phenolic motif (catechol) (**10** and **11**), or a di-*meta*

Table 2
Total ferric reducing power (FRAP) of different concentrations (10–40 μg/mL) of synthesized compounds (**7**–**12**), isoeugenol and the standard antioxidants. Values are expressed as mean ± standard deviation of three replicate assays.

Concentration (μg/mL)	Fe^{3+}-Fe^{2+} reducing ability (Absorbance 700 nm) of test compounds, isoeugenol and standards									
	7	**8**	**9**	**10**	**11**	**12**	Isoeugenol	Trolox	BHA	BHT
10	0.26	0.21	0.22	0.49	0.43	0.27	0.32	0.23	0.30	0.27
20	0.27	0.22	0.20	0.54	0.55	0.32	0.37	0.38	0.54	0.39
30	0.30	0.25	0.20	0.58	0.58	0.36	0.41	0.56	0.73	0.50
40	0.34	0.28	0.24	0.73	0.64	0.47	0.43	0.72	0.94	0.60

phenolic motif (resorcinol) (**12**). When the antioxidant activity was tested in reducing capacity, we observed a much higher activity for the phenolic compounds containing the di-*ortho* phenolic motif than either of the two other groups. In the case of inhibition of lipid peroxidation, assay for **7**, **8**, **10** and **11** was a quite similar data, in contrast to the radical scavenging efficiency observed.

The most significant finding is that the remarkable differences in the DPPH radical scavenging, superoxide anion radical scavenging activity and reducing power are exhibited depending on the substitution patterns of two hydroxyl groups on B ring. The *ortho*-(i.e. 2′, 3′, compound **10**) and *ortho*-(i.e. 3′, 4′, compound **11**) substitution patterns show much stronger activity than *meta*-(i.e. 2′, 4′, compound **12**) substitution pattern and the others. These dramatic differences could be interpreted in terms of the capability of the hydroxyl groups of phenols to react with radicals and the subsequent formation of the quinone structures (**15** and **19**) (Scheme 2). When the molecules react with the radicals, they are readily converted into the phenoxy radicals due to the high reactivity of phenolic hydroxyl groups of the compounds [44].

Since the *ortho*-dihydroxylated (i.e. catechol structure, compounds **10** and **11**) benzene ring system are generally known to be very efficient systems to delocalize electrons, but not for *meta*-dihydroxylated system (i.e. resorcinol structure, compound **12**), the phenoxy radicals occurring at the *ortho*-(i.e. catechol structure) benzene ring subsequent quinine structures (Scheme 2) than *meta*-system. The central role of the catechol structure in the enhancement of antioxidant activity has been reported for other classes of polyphenolic antioxidants [45].

Compound **9** (α-isopropyl phenol) showed the highest ABTS radical cation scavenging activity. The introduction of bulky substituents (e.g., *tert*-butyl) that optimally shield the hydrogen atom of the hydroxy group enhances the stability of the phenoxyl radicals formed and, correspondingly, their inhibiting activity [46]. Previous studies were exclusively carried out in an attempt to compare the antioxidant activities of propofol and its structurally related compounds, including constitutional isomers and phenyl analogs. Tsuchiya et al. [47] observed that inhibition of DPPH radicals and superoxide anion were in order propofol (2,6-diisopropylphenol) > 2-isopropyl-phenol > 4-isopropylphenol, respectively. In comparison between propofol isomers, it is apparent that an additional 6-isopropyl group contributes to increasing the inhibitory effect greatly more than 4-isopropyl groups [47].

4. Conclusions

In conclusion, a series of novel phenolic compounds (**7**–**12**) has been synthesized by the reaction of phenol derivatives (**2**–**6**) (phenol, ethoxypyrocatechol, 2-*iso*-propylphenol and pyrocatechol) with isoeugenol (**1**). The synthesized compounds (**7**–**12**) showed significant ferric ion reducing power, lipid peroxidation inhibition and radical scavenging activity on DPPH, $ABTS^{+}$, and O_2^{-} radicals. Also antioxidant activity of all compounds showed an increase by rising concentrations.

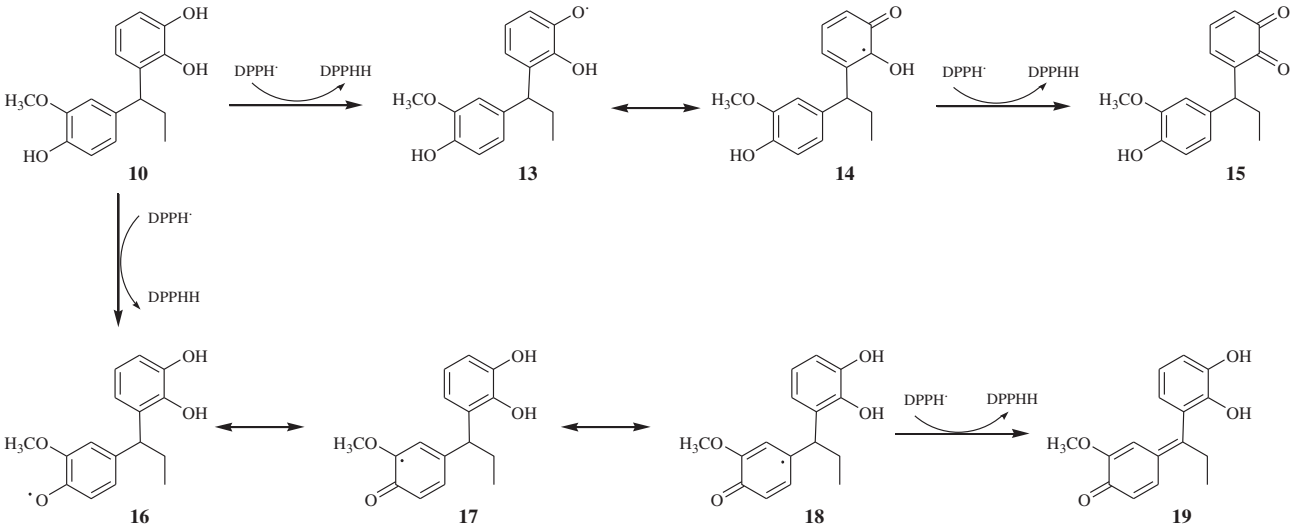
5. Experimental

All the reagents were used as purchased from commercial suppliers without further purification. Melting points were measured on Electrothermal 9100 apparatus and a re uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Brucker Advance III instrument (400 MHz). As internal standards served TMS (δ 0.00) for ^1H NMR and $CDCl_3$ (δ 77.0) for ^{13}C NMR spectroscopy *J* values are given in Hz. The multiplicities of the signals in the ^1H NMR spectra are abbreviated by s (singlet), d (doublet), t (triplet), q (quartet), h (heptuplet), m (multiplet), br (broad) and combinations thereof. Elemental analyses were obtained from a LECO CHNS 932 Elemental Analyzer. GC–MS analyses were obtained from a Perkin Elmer Clarus 500 GC–MS. The isolation of the products was performed by column chromatography using silica gel Merck 60 (230–400 mesh, 0.04–0.063 mm). Unless otherwise noted, all reactions were carried out under a nitrogen atmosphere with commercial reagents and solvents.

5.1. Chemistry

5.1.1. General procedure for the synthesis of compounds 7–12

A two-necked flask equipped with a thermometer and N_2 gas inlet was charged with phenols (2 equiv.) and heated up to 140 °C and added Al(O-*i*-Pr)$_3$ (20% mol). After catalyst and phenol were completely dissolved, the solution was treated with isoeugenol (1 equiv.). The reaction was carried out through keeping the



Scheme 2. Proposed rationale for strong activity of *ortho*-dihydroxylated compound.

temperature at 140 °C for 3 h. When the reaction was complete, the mixture was cooled, diluted with ethyl acetate, and treated with HCl solution (5%) to compose the catalyst. Then, the mixture was washed with saturated $NaHCO_3$ solution and water until rinsing was neutral. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated, and the crude products were purified by column chromatography (on a silica gel column) using *n*-hexane/EtOAc (9:1) as eluent. The products were crystallized from *n*-hexane/EtOAc (9:1).

5.1.2. 4-(1-(2-Hydroxyphenyl)propyl)-2-methoxyphenol (**7**)

Colorless crystals, Yield (25%), M.p. 99–101 °C ^{1}H NMR (400 MHz, $CDCl_3$, ppm): $\delta = 7.28$ (d, $J = 8.0$ Hz, 1H), 7.12 (dt, $J = 7.5$, 1.6 Hz, 1H), 6.95 (t, $J = 7.5$ Hz, 1H), 6.87 (d, $J = 8.0$ Hz, 1H), 6.80 (dd, $J = 8.0$, 1.6 Hz, 1H), 6.76 (bd, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 1.6$ Hz, 1H), 5.56 (s, 1H, −OH), 4.85 (s, 1H, −OH), 4.00 (t, $J = 7.6$ Hz, 1H), 3.82 (s, 3H, $-OCH_3$), 2.06 (m, 2H), 0.94 (t, $J = 7.6$ Hz, 3H, $-CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): $\delta =$ 153.6, 146.7, 144.1, 135.9, 131.2, 127.8, 127.3, 120.8, 120.5, 116.1, 114.3, 110.6, 55.8, 46.1, 27.7, 12.6. IR (KCl, cm^{-1}): 3469, 3378, 3012, 2973, 2954, 2929, 2869, 1515, 1502, 1457, 1378, 1276, 1255, 1226, 1149, 1130, 1037, 796, 757, 638, 553. Anal. Calcd for $C_{16}H_{18}O_3$: C, 74.39; H, 7.02. Found: C, 73.97; H, 6.92. GC/MS (*m/z*): 258 (M^+, 20%), 229 (100%, $-CH_2CH_3$), 197 (15%, $-OCH_3$), 77 (10%, C_6H_5). UV–Vis: λ (log ε) 239 (1.79), 242 (1.70), 278 (2.17).

5.1.3. 4-(1-(2-Hydroxy-3-ethoxyphenyl)propyl)-2-methoxyphenol (**8**)

Colorless crystals, Yield 40%, M.p. 162–164 °C ^{1}H NMR (400 MHz, d_6-DMSO, ppm): $\delta = 8.66$ (s, 1H, −OH), 8.16 (s, 1H, −OH), 6.82 (s, 1H), 6.77 (dd, $J = 7.2$, 1.6 Hz, 1H), 6.73–6.67 (m, 4H), 4.15 (t, $J = 8.0$ Hz, 1H), 4.02–3.94 (m, 2H, -OCH_2CH_3), 3.71 (s, 3H, $-OCH_3$), 1.97–1.90 (m, 2H), 1.32 (t, $J = 7.2$ Hz, 3H, $-OCH_2CH_3$), 0.82 (t, $J = 7.2$ Hz, 3H, $-CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO, ppm): $\delta =$ 147.6, 146.7, 144.9, 144.2, 136.5, 132.5, 120.4, 119.6, 119.1, 115.5, 112.7, 110.5, 64.4, 56.1, 44.6, 27.9, 15.1, 13.3. IR (KCl, cm^{-1}): 3432, 2962, 2931, 2867, 1610, 1511, 1469, 1251, 1203, 1126, 1052, 806, 754, 559. Anal. Calcd for ($C_{18}H_{22}O_4$): C, 71.50; H, 7.33. Found: C, 70.98; H, 7.27. GC/MS (*m/z*): 302 (M^+, 20%), 273 (100%, $-CH_2CH_3$), 243 (5%, $-CH_2CH_3$), 213 (10%, $-OCH_3$), 77 (5%, C_6H_5). UV–Vis: λ (log ε) 240 (1.32), 281 (1.31).

5.1.4. 4-(1-(2-Hydroxy-3-isopropylphenyl)propyl)-2-methoxyphenol (**9**)

Colorless crystals, Yield (64%), M.p. 75–77 °C ^{1}H NMR (400 MHz, d_6-DMSO, ppm): $\delta =$ 8.65 (s, 1H, −OH), 8.05 (s, 1H, −OH), 6.99 (d, $J = 7.6$ Hz, 1H), 6.95 (d, $J = 7.2$ Hz, 1H), 6.84 (s, 1H), 6.76 (t, $J = 7.6$ Hz, 1H), 6.67 (s, 2H), 4.22 (t, $J = 7.6$ Hz, 1H), 3.71 (s, 3H, $-OCH_3$), 3.34–3.25 (h, $J = 6.8$ Hz, 1H, $-CH(CH_3)_2$), 1.97–1.85 (m, 2H), 1.13 (t, $J = 7.2$ Hz, 6H), 0.83 (t, $J = 6.8$ Hz, 3H, $-CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO, ppm): $\delta =$ 151.4, 147.6, 144.9, 136.4, 135.6, 133.3, 124.9, 123.4, 120.5, 120.2, 115.6, 112.9, 56.1, 44.4, 28.5, 26.5, 23.6, 23.3, 13.2. IR (KCl, cm^{-1}): 3390, 3255, 2964, 2929, 2867, 2836, 1598, 1523, 1459, 1446, 1274, 1257, 1207, 1184, 1128, 1035, 782, 746, 565. Anal. Calcd for ($C_{19}H_{24}O_3$): C, 75.92; H, 8.05. Found: C, 75.62; H, 7.75. GC/MS (*m/z*): 300 (M^+, % 20), 271 (100%, $-CH_2CH_3$), 239 (5%, $-OCH_3$), 197 (10%, $-CH(CH_3)_2$), 77 (5%, C_6H_5). UV–Vis: λ (log ε) 239 (2.08), 242 (1.76), 277 (2.07).

5.1.5. 3-(1-(4-Hydroxy-3-methoxyphenyl)propyl)benzen-1,2-diol (**10**)

Colorless crystals, Yield (35%), M.p. 103–105 °C ^{1}H NMR (d_6-DMSO, ppm): 9.21 (s, 1H, −OH); 8.63 (s, 1H, −OH); 8.06 (s, 1H, −OH); 6.79 (s, 1H); 6.64–6.52 (m, 5H); 4.10 (t, $J = 7.6$ Hz, 1H); 3.70 (s, 3H, $-OCH_3$); 1.97–1.86 (m, 2H); 0.80 (t, $J = 7.2$ Hz, 3H, $-CH_3$); ^{13}C NMR (d_6-DMSO, ppm): δ 147.6, 145.2, 144.8, 143.2, 136.6, 132.8, 120.42, 119.0, 118.2, 115.5, 113.0, 112.8, 56.1, 44.6, 28.0, 13.3. IR (KCl, cm^{-1}): 3473, 3403, 3369, 3243, 2967, 2956, 2929, 2869, 1600, 1521, 1477, 1278, 1122, 1027, 966, 800, 761, 632. Anal. Calcd for ($C_{16}H_{18}O_4$): C, 70.02; H, 6.61. Found: C, 69.78; H, 6.44. GC/MS (*m/z*): 274 (M^+, 30%), 245 (100%, $-CH_2CH_3$), 213 (13%, $-OCH_3$), 77 (10%, C_6H_5). UV–Vis: λ (log ε) 239 (1.24), 280 (1.19).

5.1.6. 4-(1-(4-Hydroxy-3-methoxyphenyl)propyl)benzen-1,2-diol (**11**)

Colorless crystals, Yield (55%), M.p. 127–129 °C ^{1}H NMR (400 MHz, d_6-DMSO, ppm): $\delta = 8.66$ (s, 2H, −OH), 8.59 (s, 1H, −OH), 6.73 (s, 1H), 6.64 (d, $J = 8.0$ Hz, 1H, A part of AB system), 6.60–6.58 (m, 3H, ArH), 6.49 (dd, $J = 8.0$, 1.6 Hz, 1H, B part of AB system), 3.70 (s, 3H, $-OCH_3$), 3.46 (t, $J = 7.6$ Hz, 1H), 1.89–1.82 (m, 2H), 0.77 (t, $J = 7.2$ Hz, 3H, $-CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO, ppm): $\delta =$ 147.7, 145.3, 144.9, 143.6, 137.2, 137.1, 119.9, 118.5, 115.7, 115.7, 115.4, 112.4, 56.1, 51.8, 28.8, 13.2. IR (KCl, cm^{-1}): 3440, 3361, 3253, 2958, 2931, 2873, 1602, 1513, 1427, 1353, 1268, 1027, 794, 779, 557. Anal. Calcd for ($C_{16}H_{18}O_4$): C, 70.02; H, 6.61. Found: C, 70.25; H, 6.55. GC/MS (*m/z*): 274 (M^+, 20%), 245 (100%, $-CH_2CH_3$), 213 (5%, $-OCH_3$), 77 (5%, C_6H_5). UV–Vis: λ (log ε) 239 (1.24), 280 (1.19).

5.1.7. 4-(1-(4-Hydroxy-3-methoxyphenyl)propyl)benzene-1,3-diol (**12**)

Colorless crystals, Yield (65%), M.p. 88–90 °C ^{1}H NMR (400 MHz, d_6-DMSO, ppm): $\delta = 9.02$ (s, 1H, −OH), 8.93 (s, 1H, −OH), 8.60 (s, 1H, −OH), 6.87 (dd, $J = 8.4$, 2.8 Hz, 1H), 6.74 (s, 1H), 6.63–6.57 (m, 2H), 6.22 (s, 1H), 6.13 (d, $J = 8.4$ Hz, 1H), 3.93 (t, $J = 8.0$ Hz, 1H), 3.69 (s, 3H, $-OCH_3$), 1.89–1.81 (m, 2H), 0.77 (t, $J = 5.6$ Hz, 3H, $-CH_3$). ^{13}C NMR (100 MHz, d_6-DMSO, ppm): $\delta =$ 156.3, 155.7, 147.5, 144.7, 137.2, 128.1, 122.8, 120.3, 115.5, 112.7, 106.5, 102.8, 56.0, 44.0, 28.2, 13.3. IR (KCl, cm^{-1}): 3444, 3396, 3288, 2964, 2954, 2931, 2871, 1743, 1617, 1604, 1513, 1446, 1432, 1375, 1251, 1199, 1128, 1031, 971, 842, 813, 640, 557. Anal. Calcd for ($C_{16}H_{18}O_4$): C, 70.02; H, 6.61. Found: C, 69.80; H, 6.65. GC/MS (*m/z*): 274 (M^+, 30%), 245 (100%, $-CH_2CH_3$), 213 (15%, $-OCH_3$), 77 (10%, C_6H_5). UV–Vis: λ (log ε) 239 (1.83), 240 (1.85), 243 (1.82), 282 (2.11).

5.2. Antioxidant activity

5.2.1. DPPH radical scavenging activity

The free radical scavenging activities of phenolic compounds were measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) using the method of Blois [48]. Briefly, 0.1 mM solution of DPPH in ethanol was prepared and 1 mL of this solution was added to 3 mL of ethanolic phenolic compounds at different concentrations (10–40 μg/mL). The mixture was shaken vigorously and allowed to be kept stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The radical scavenging activities of the samples were expressed in terms of IC_{50} (concentration required for a 50% decrease in absorbance of DPPH radical). A lower IC_{50} (μM) value indicates greater antioxidant activity.

5.2.2. $ABTS^+$ radical scavenging activity

Radical scavenging activity was evaluated according to the protocol of Nenadis, Wang, Tsimidou & Zhang [49] which was appropriately adjusted. To quench the $ABTS^+$, a blue-green chromophore was used with a characteristic absorption at 734 nm; an antioxidant is added to a pre-formed ABTS radical solution, and after a fixed time period, the remaining $ABTS^+$ is quantified spectrophotometrically at 734 nm. The $ABTS^+$ was produced by reacting ABTS (2 mM) in water with potassium persulfate (2.45 mM, $K_2S_2O_8$), stored in the dark at room temperature for 12 h. Then, to $ABTS^+$ solution (0.5 mL) was added solution of the synthesized compounds (**7**–**12**) in ethanol at various concentrations (1.5 mL, 10–30 μg/mL). After the solution was mixed for 30 min, the absorbance was

recorded, and the percentage of the radical scavenging was calculated for each. For preparation of the calibration curve, various concentrations of $ABTS^+$ (0.033–0.33 mM) were used. The $ABTS^+$ concentration (mM) was calculated from the following calibration curve (r^2: 0.9899). The $ABTS^+$ radical scavenging activities of the samples were expressed in terms of IC_{50} (μM).

5.2.3. Superoxide anion radical scavenging activity

The method of Zhishen [50] was adopted as follows. All solutions (riboflavin (3×10^{-5} M), methionine (1×10^{-2} M) and nitroblue tetrazolium (NBT) (1×10^{-4} M)) were prepared in a 0.05 M phosphate buffer (pH 7.8). The total reaction mixture (3 mL) was illuminated at 25 °C for 40 min. The photochemically-reduced riboflavins generated O_2^E, which reduced NBT to form blue formazan. The absorbance of the unilluminated reaction mixture as control was measured at 560 nm, as control. Then, compounds (10–40 μg/mL) were added to the reaction mixture. The O_2^E radical scavenging activities of the samples were expressed in terms of IC_{50} (μM).

5.2.4. Ferric-reducing/antioxidant power assay

Ferric ion (Fe^{3+}) reducing power was evaluated according to the protocol of Oyaizu [51] with adjustments. 1 mL of diluted phenolic compounds was mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and $K_3Fe(CN)_6$ (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 10,000g for 10 min. The upper layer of the solution (2.5 mL) was mixed with deionized water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. BHT, BHA and trolox were used as positive controls. A higher absorbance value indicates greater reducing power ability.

5.2.5. Determination of inhibition of lipid peroxidation

The method of Mitsuda [52] was adopted as follows. The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. A 0.5 mL ethanol solution of 100 μL of synthesized compounds was mixed with a linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0) and phosphate buffer (2.5 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C in the dark to accelerate the peroxidation process. During incubation, analysis was performed at every 6 h. Aliquots of 100 μL were taken at different intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (4.7 mL, 75%), an ammonium thiocyanate sample solution (100 μL, 30%) and ferrous chloride (100 μL, 0.02 M in 3.5% HCl). After 3 min, the absorbance at 500 nm was read at 500 nm. BHT, BHA and trolox were used as reference compounds. A control was performed with linoleic acid without the test compounds. All data reported were the average of triplicate analyses. Percentage inhibition of lipid peroxide generation was calculated using the following formula: % Inhibition $= [(A_c - A_s)/A_c] \times 100$ where A_s is the absorbance value of the tested sample and A_c is the absorbance value of the control sample.

5.3. Statistics

IC_{50} values from the in vitro data were calculated by regression analysis. Each experiment was repeated three times. The data were expressed as means ± standard error (SE) and analyzed by SPSS (version 13.0). One-way analysis of variance (ANOVA) and Scheffe multiple comparisons were carried out to test for any significant differences between the means. Differences among the means at the 5% confidence level were considered significant. Correlation coefficients (r) to determine the relationship between variables were calculated using the Bivariate correlation statistical function.

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Abbreviations

°*C:* centigrade
min: minute
h: hour
mL: milli Liter
μM: micro molar
mg/mL: milli gram per milli Liter
g/mL: gram per milli Liter
%: percentage
IC_{50}*:* 50 percent inhibition concentration
nm: nano meter
mM: milli molar
ABTS: 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
DPPH: 1,1-diphenyl-2-picrylhydrazyl
LDL: low-density lipoprotein
FRAP: total ferric reducing power
BHA: butylated hydroxyanisole
BHT: butylated hydroxyl toluene
TBHQ: tertiary butylated hydroxytoluene
ROS: reactive oxygen species
RSA: radical scavenging activity
LPI: lipid peroxidation inhibition
SD: standard deviation
<: less than