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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, in vitro antimicrobial and antioxidant activities of chalcone and flavone derivatives holding allylic substitutions

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Abstract In a wide search program towards new and efficient biological active agents, a series of chalcone and flavone derivatives holding allylic substitutions have been synthesized and tested for their in vitro antibacterial and antifungal activities. The synthesized compounds were tested in vitro against Gram-positive, Gram-negative bacteria, and the yeast *Candidia kefir* in comparison with control drugs (Sulfamethoxazole and Fluconazole). The antioxidant activity of the synthesized allylic chalcones and flavones was also assessed using two methods, including, 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging, and reducing power assays and compared to reference drug Trolox.

Keywords Antimicrobial activity · Antioxidant activity · Chalcones · Flavones · Allylic substitutions

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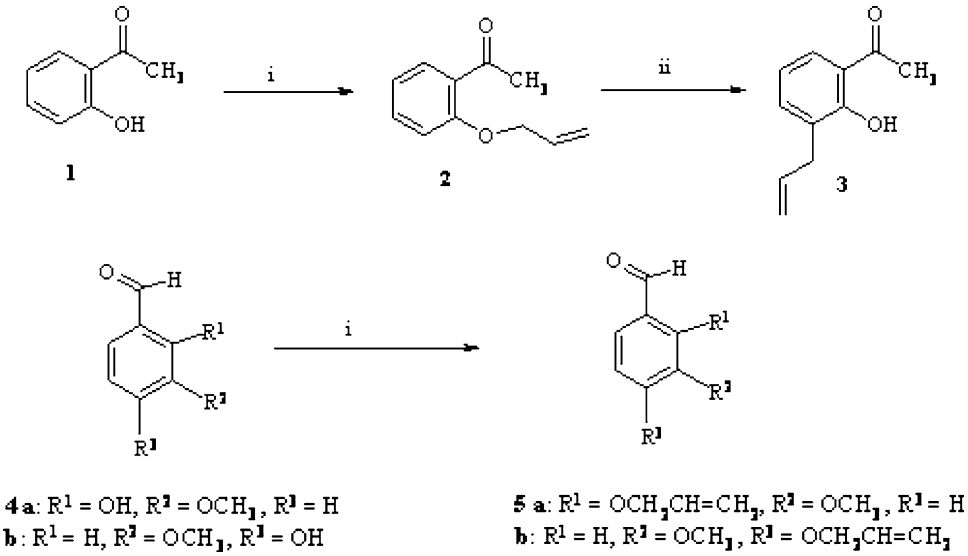
Introduction

Flavonoids comprise a large family of plant-derived polyphenolic compounds that demonstrate diverse biological activities, including the ability to inhibit retroviral transcriptases (Nakane and Ono, 1990), protein-tyrosine kinases (Geahlen *et al.*, 1989), and other enzymes (Hagiwara *et al.*, 1988). These compounds have been found to possess anticancer (Sánchez *et al.*, 2001), chemopreventive (Cassady *et al.*, 1990) and antibacterial (Mughal *et al.*, 2006) activities. In addition, naturally occurring or synthetic flavonoids and natural lignans have been studied (Edwards *et al.*, 1979; Hirano *et al.*, 1989), since they appear to be compounds of low toxicity and some of them apparently have antiproliferative activity against human tumor cells (Hirano *et al.*, 1994). Investigations carried out by Kaul *et al.* (1985) have shown an important structure–activity relationship in this group of natural flavonoids.

Antioxidants are of great interest because of their involvement in important biological and industrial processes. In general, compounds with antioxidant activity have also been found to have anticancer, anti-cardiovascular, anti-inflammation, and many other activities (Cadenas and Packer, 1996). Reactive oxygen species (ROS) and free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging (Chabot *et al.*, 1998). ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radical, are thought to be generated subsequent to the reduction of molecular oxygen in aerobic organisms. Under normal conditions, cells and tissues are protected against ROS by an array of enzyme defense systems, such as superoxide dismutase, catalase, and glutathione peroxidases, in addition to numerous non-enzymatic small molecules distributed widely in the biological system and capable of scavenging free radicals. These molecules

Scheme 1 Reagents and conditions: (i) allyl bromide, K_2CO_3, acetone, (ii) 250°C, N_2



include glutathione, α-tocopherol (vitamin E), vitamin C, β-carotene, and selenium (Jacob and Burri, 1996). In this study, we wish to report chalcones and flavones with allylic substitutions and evaluate their antimicrobial and antioxidants activities (Scheme 1).

Results and discussion

Antimicrobial activity

Microbiological results showed that the synthesized chalcones and flavones holding allylic substitutions possessed a broad spectrum of antibacterial activity against Gram-positive and Gram-negative microorganisms using conventional agar-dilution method (Baron and Finegold, 1990). The MICs (minimum inhibitory concentration) values were determined by comparison to sulfamethoxazole as reference drug and are presented in Table 1. All the chalcones and flavones indicated poor antibacterial activities having MIC values higher than 512 μg/ml, and showing less potency than the control drugs (Sulfamethoxazole). Moreover, the synthesized compounds also possessed antifungal activity against the yeast *C. kefir* showing MIC values > 512 μg/ml compared to fluconazole (Table 1). However, it is seems that allylic chalcones are more potent than allylic flavones. Perhaps the reason is that allylic flavones have low solubility than the allylic chalcones. This shows the necessity of hydrophilic substitutions for having better results.

Antioxidant activity

The antioxidant activity was assessed using two methods, including, 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging (Blois, 1958), and reducing power (Oyaizu, 1986) assays according to the methods described in the literature (Table 2). The results of antioxidant activity in both methods are approximately similar to each other. All the synthesized compounds exhibited good antioxidant properties. They were less potent than Trolox as the reference. It should be noted that when allylic chalcones are cyclized to allylic flavones, the antioxidant activity is decreased. This is due to the existence of the free hydroxyl group in the chalcone (Table 2). The compounds **6a**, **6b**, **7a**, and **7b** have good antioxidative activity with a major activity for **6b** (IC_{50} 85 μM). Overall, the result of DPPH assay was relatively consistent with that of reducing power assay. The potencies for the antioxidative activity of the test compounds compared to the reference drugs are in the following order: Trolox > **6b** > **6a** > **7b** > **7a**.

Conclusions

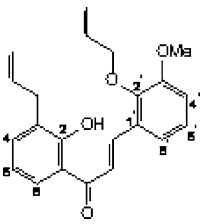
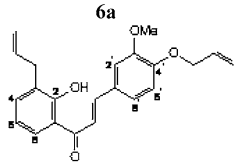
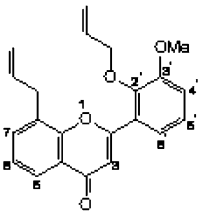
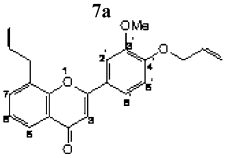
In summary, we have reported biological evaluation of chalcones and flavones with allylic substitutions, which represent antioxidant and antimicrobial activities. All the synthesized chalcones and flavones showed poor antimicrobial activity. A moderate to good antioxidant activity was observed by reducing power assay and DPPH radical scavenging method.

Experimental

Typical procedure for synthesis of allylic chalcone (**6a**)

To a solution of 3-allyl-2-hydroxyacetophenone (**3**) (10 mmol) and 2-allyloxy-3-methoxy benzaldehyde (**5a**) (10 mmol) in

Table 1 The MICs (in μg/mL) values of allylic chalcones and flavones against bacteria and fungus

Compound	*E. coli*	*S. aureus*	*S. epidermidis*	*Candidia kefir*	Yield (%)[a]
 **6a**	512	>1000	>1000	512	74
 **6b**	512	>1000	>1000	512	72
 **7a**	>512	>1000	>1000	512	18
 **7b**	>512	>1000	>1000	>512	17
Fluconazole	–	–	–	4	–
Sulfamethoxazole	4	4	4	–	–

[a] Yield of isolated product after purification

EtOH (20 ml) was added a 60% of KOH (10 ml) solution dropwise at 0°C. The reaction mixture was stirred at 0°C for 1 day. Cold water was added and the reaction mixture was neutralized with cold acetic acid. The yellow precipitate was collected, washed with water and recrystallized from EtOH to yield *β*-(2′-allyloxy-3′-methoxyphenyl)-1-(3-allyl-2-hydroxyphenyl) propenone (**6a**) as orange needles; mp = 86–89°C (yield 74%) (Scheme 2).

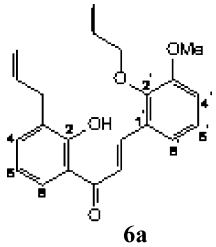
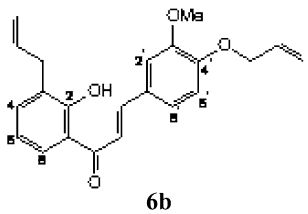
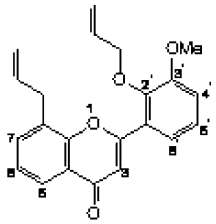
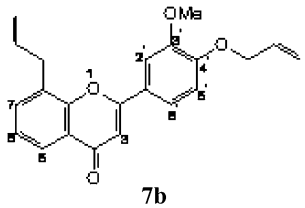
Typical procedure for synthesis of allylic flavone (**7a**)

To a solution of *β*-(2′-allyloxy-3′-methoxyphenyl)-1-(3-allyl-2-hydroxyphenyl) propenone (**6a**) (1 mmol) in DMSO (5 ml) was added I_2 (254 mg, 1 mmol), and the reaction mixture was heated in an oil bath at 130°C for 30 min. After cooling, the reaction mixture was diluted with water and the iodine was removed by washing with a saturated solution of sodium thiosulfate. The product 2-(2′-allyloxy-3′-methoxyphenyl)-8-allyl-4-chromone (**7a**) was then extracted with ethyl acetate and purified by column chromatography (hexane/ethyl acetate, 9:1).

2-Allyloxy-3-methoxy benzaldehyde (**5a**): $R_f = 1.2$ (*n*-Hexane–Ethyl acetate 80: 20); IR (KBr) *v* 3087 (stretch CH sp^2 aromatic), 3041 (stretch CH, sp^2 allylic), 2986 and 2926 (stretch CH_3, sp^3), 2863 and 2770 (stretch CHO), 1710 (stretch C=O), 1648 (stretch C=C allylic), 1602 and 1493 (stretch C=C aromatic), 1240 and 1022 (stretch C–O–Ar), 1000 and 937 (bends C=C allylic OOP), 758 and 653 (1, 2, 3 position).

4-Allyloxy-3-methoxy benzaldehyde (**5b**): $R_f = 1.7$ (*n*-Hexane–Ethyl acetate 80:20); IR (KBr) *v* 3361 (C=O, overtone), 3088 (stretch CH, sp^2 aromatic), 3003 (stretch CH, sp^2 allylic), 2940 (stretch CH_3, sp^3), 2835 and 2729 (stretch CHO), 1683 (stretch C=O), 1642 (stretch C=C, allylic), 1588 and 1507 (stretch C=C, aromatic), 1268 and 1041 (stretch C–O–Ar), 997 and 934 (bends = C–H propenyl OOP), 870 and 810 (1, 3, 4 position).

Table 2 Antioxidant activities of allylic chalcones and flavones in comparison to Trolox

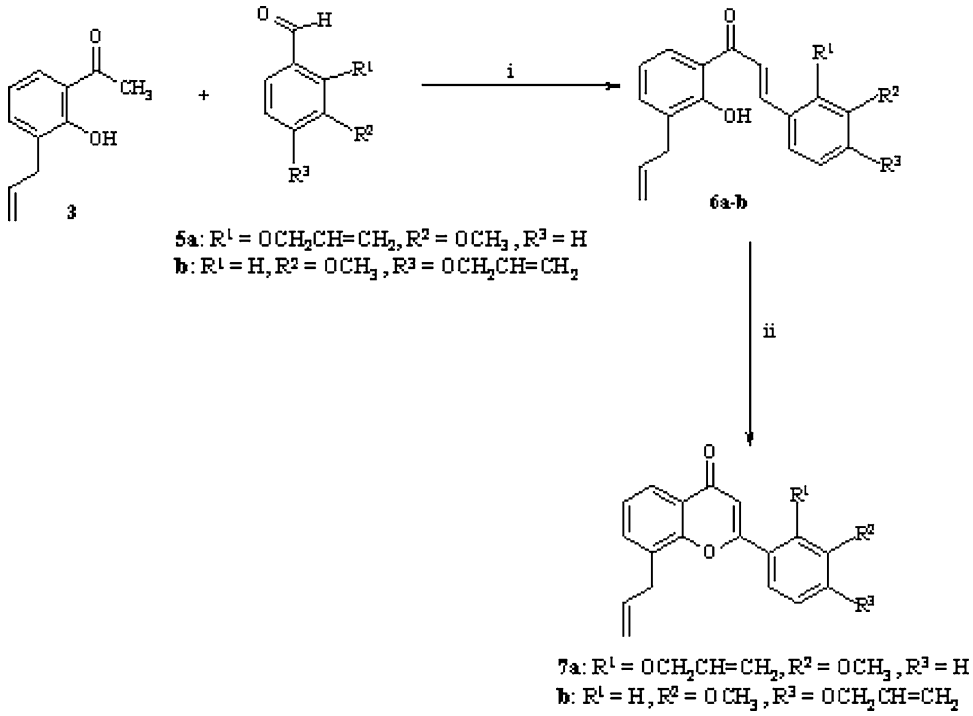
Entry	Compound	DPPH radical scavenging activity (IC_{50}, μM)	Reducing power (Fe^{2+} μM)
1	 **6a**	163	1.87
2	 **6b**	85	2.96
3	 **7a**	320	1.11
4	 **7b**	270	0.57
5	Trolox	36	41.93

2-Allyloxy acetophenone (**2**): $R_f = 0.7$ (*n*-Hexane–Ethyl acetate 95:5); IR (KBr) *v* 3333 (C=O, overtone), 3080 (stretch CH, sp^2 aromatic), 3000 (stretch CH, sp^2 allylic), 2982 (stretch CH_3, sp^3), 1672 (stretch CHO), 1595 and 1482 (stretch C=C, aromatic), 1237 and 1011 (stretch C–O–Ar), 993 and 912 (bends = CH oop), 757 (1, 2 position).

3-Allyl-2-hydroxy acetophenone (**3**): $R_f = 1.3$ (*n*- Hexane-Ethyl acetate 85:15); IR (KBr) *v* 3368 (OH), 3070 (stretch CH, sp^2 aromatic), 3017 (stretch CH, sp^2 allylic), 2975 (stretch CH_3), 1683 (stretch C=O), 1640 and 1440 (stretch C=C, aromatic), 1247 and 1010 (stretch C–O–Ar), 979 and 916 (bends =CH OOP), 754 and 660 (1, 2, 3 position).

β-(2′-Allyloxy-3′-methoxyphenyl)-1-(3-allyl-2-hydroxy phenyl) propenone (**6a**): $R_f = 0.5$ (*n*-Hexane–Ethyl acetate 90:10); mp = 86–89°C; IR (KBr) *v* 3080 (stretch CH, sp^2 aromatic), 3010 (stretch CH, sp^2 allylic), 2975 (stretch CH_3 sp^3), 1640 (stretch C=O), 1571 and 1480 (stretch C=C, aromatic), 1274 and 1103 (stretch C–O–Ar); ^{1}H-NMR (DMSO-d_6): δ 3.364-3.386 (d, 2H, –CH_2–), 3.837 (s, 3H, OCH_3), 4.528–4.547 (d, 2H, –OCH_2–), 5.029–5.406 (m, 4H cis, =CH_2), 5.344–5.406 (dd, 2H trans, =CH_2) 5.912–6.024 (m, 2H, –CH=), 6.925–6.976 (t, 1H, C_5 H), 7.155–7.176 (d, 1H, C_4 H), 7.166–7.192 (d, 1H, $C_{4'}$H), 7.430–7.455 (d, 1H, $C_{6'}$H),7.658–7.689 (t, 1H, $C_{5'}$H), 8.015–8.067 (d, $J = 15.6$, H_α), 8.136–8.188 (d, $J = 15.6$, H_β), 8.172–8.199 (d, 1H, C_6H), 13.3 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ 33.388

Scheme 2 Reagents and conditions: (i) KOH, (ii) I_2/DMSO



(–CH_2–), 56.350 (–OCH_3), 74.322 (–OCH_2–), 115.89 (=CH_2), 116.496 (=CH_2), 118.443 ($C_{4'}$), 119.064 ($C_{6'}$), 119.799 (–C_5), 120.054 ($C_{5'}$), 122.245 ($C_{1'}$), 124.775 (C_α), 128.678 (C_1), 128.960 (C_3), 129.442 (C_6), 134.448 (C_4), 136.658 (–CH=), 137.048 (–CH=), 140.024 (C_β), 147.584 ($C_{2'}$), 153.231 ($C_{3'}$), 161.229 (C_2), 194.667 (C=O).

β-(4′-Allyloxy-3′-methoxyphenyl)-1-(3-allyl-2-hydroxyphenyl) propenone (**6b**): $R_f = 0.55$ (*n*-Hexane–Ethyl acetate 85:15); mp = 88–85°C; IR (KBr) *v* 3083 (stretch CH, sp^2 aromatic), 3011 (stretch CH, sp^2 allylic), 2923 (stretch CH_3, sp^3), 1638 (stretch C=O), 1596 and 1509 (stretch C=C, aromatic), 1258 and 1063 (stretch C–O–Ar); ^{1}H-NMR (DMSO-d_6): δ 3.351–3.385 (d, 2H, –CH_2), 3.870 (s, 3H, CH_3), 4.608–4.635 (d, 2H, OCH_2), 5.016–5.112 (m, 2H cis, =CH_2), 5.233–5.444 (m, 2H trans, =CH_2), 5.909–6.113 (m, 2H, =CH–), 6.921–6.958 (d, 1H, C_4H), 6.998–7.008 (d, 1H, $C_{5'}$H), 7.050 (d, 1H, $C_{6'}$H), 7.381–7.450 (t, 1H, C_5H), 7.586–7.595 (d, 1H, $C_{2'}$H), 7.793–7.870 (d, $J = 22.98$ Hz, H_α), 7.931–8.007 (d, $J = 23.01$ Hz, H_β), 8.225–8.272 (dd, 1H, C_6H), 13.454 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ 33.385 (–CH_2–), 56.333 (–OCH_3–), 69.341 (–OCH_2–), 111.812 (–CH_2=), 113.414 (–CH_2=), 116.482 ($C_{2'}$), 118.363 ($C_{5'}$), 118.834 ($C_{6'}$), 118.993 ($C_{1'}$), 119.751 (C_5), 125.066 (C_α), 127.896 (C_1), 128.866 (C_3), 129.389 (C_6), 133.835 (C_4), 136.665 (CH=), 136.849 (CH=), 146.403 (C_β), 149.744 ($C_{4'}$), 151.072 ($C_{3'}$), 161.201 (C_2), 194.559 (C=O).

2-(2′-Allyloxy-3′-methoxyphenyl)-8-allyl-4-chromone (**7a**): $R_f = 0.5$ (*n*-Hexane–Ethyl acetate 70:30); mp = 103–105°C; IR (KBr) *v* 3083 (stretch CH, sp^2 aromatic), 2986 (stretch CH, sp^2 allylic), 2923 (stretch CH_3), 1645 (stretch C=O), 1603 and 1516 (stretch C=C, aromatic), 1276 and 1143 (stretch C–O–Ar); ^{1}H-NMR (DMSO-d_6): δ 3.658–3.680 (d, 2H, –CH_2–), 3.875 (s, 1H, –OCH_3), 4.503–4.522 (d, 2H, –OCH_2–), 5.054–5.282 (m, 4H, =CH_2), 5.851–5.962 (m, 2H, –CH=), 6.853 (s, 1H, C_3H), 7.243–7.309 (t and d, 2H, C_6H and $C_{6'}$H), 7.384–7.457 (dd and dd, 2H, $C_{4'}$H, $C_{5'}$H), 7.642–7.666 (dd, 1H, C_7H), 7.913–7.945 (dd, 1H, C_5H); ^{13}C-NMR (DMSO-d_6): δ 33.699 (CH_2), 56.538 (OCH_3), 73.959 (OCH_2), 112.05 (CH_2=), 116.277 (CH_2=), 117.178 ($C_{4'}$), 118.169 ($C_{6'}$), 120.836 ($C_{5'}$), 123.433 (C_6), 123.706 ($C_{1'}$), 125.087 (C_5), 125.573 (C_3), 126.655 (C_{10}), 130.044 (C_8), 134.255 (CH=), 134.655 (CH=), 136.178 (C_7), 146.356 ($C_{2'}$), 153.508 ($C_{3'}$), 154.395 (C_2), 161.275 (C_9), 177.605 (C_4).

2-(4′-Allyloxy-3′-methoxyphenyl)-8-allyl-4-chromone (**7b**): $R_f = 0.53$ (*n*-Hexane–Ethyl acetate (80: 20); mp = 108–109°C; IR (KBr) *v* 3083 (stretch CH, sp^2 aromatic), 3007 (stretch CH, sp^2 allylic), 2923 (stretch CH_3), 1648 (stretch C=O), 1595 and 1493 (stretch C=C, aromatic), 1272 and 1098(stretch C–O–Ar); ^{1}H-NMR (DMSO-d_6): δ 3.314 (s, 3H, H_2O as impurity), 3.752 (s, 2H, –CH_2), 3.873–3.885(d, 2H, –OCH_3), 4.651(s, 1H, CH_2) 5.085-5.133 (t, 2H cis, =CH_2), 5.262–5.443 (dd, 2H trans, =CH_2), 6.066–6.124 (m, 2H, –CH=), 7.020–7.039 (d, 1H, C_3H), 7.115–7.163 (dd, 1H, $C_{6'}$H), 7.392–7.435 (dd, 1H, $C_{5'}$H), 7.564-7.582 (d, 1H, $C_{2'}$H), 7.630–7.650 (dd, 2H, $C_{7,6'}$H), 7.886–7.921 (d, 1H, $C_{5'}$H); ^{13}C-NMR (DMSO-d_6): δ 33.941 (–CH_2), 56.241 (–OCH_3), 69.370 (–OCH_2–) 106.084 (=CH_2), 110.009

(=CH_2), 113.650 ($C_{2'}$), 116.966 ($C_{5'}$), 118.443 ($C_{6'}$), 120.094 (C_6), 123.477 (C_5), 123.853 (C_{10}), 124.185 (C_8), 125.476 (C_3), 129.864 ($C_{1'}$), 133.770 (=CH–), 134.669 (=CH$_{'}$), 136.329 (C_7), 149.671 ($C_{4'}$), 151.189 ($C_{3'}$), 154.022 (C_2), 162.730 (C_9), 177.630 (C_4).

Microbial strains

The synthesized compounds were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli* and the yeast *Candidia kefir* which were provided by Department of Microbiology (Faculty of Medicine, Tabriz University Medicinal Sciences, Tabriz, Iran).

Antimicrobial assay

Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland Standard by preparing bacterial suspension of 3–5 well-isolated colonies of the same morphological type selected from an agar plate culture. The cultures were further diluted 1000-fold to get an inoculum size of 1.5×10^5 CFU/ml. The test compounds (50 mg) were dissolved in DMSO (0.5 ml) and the solution was diluted with water (4.5 ml) to get a stock solution of 10,000 mg/ml of each compound. Further progressive double dilution with Muller–Hinton broth was performed to obtain the required concentrations of 1000, 512, 256, 128, 64, 32, 16, 8, 4, 2 μg/ml. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. Our results indicated that all the test compounds tested in this study demonstrated lower activity against both Gram-positive and Gram-negative microorganisms than the reference drug. Standard antibiotic (sulfamethoxazole) was also diluted in the same manner. Each microwell in a series of 12 microwells was inoculated with 75 microliter of the serial dilutions, and then 75 microliter of the bacterial suspension was added. After overnight incubation in 37°C, growth was surveyed.

DPPH radical-scavenging activity assay

The compounds were dissolved in appropriate solvent mixed with 1 ml of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in ethanol, and final volume was adjusted to 2 ml. Mixtures were variously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using a UV–VIS spectrophotometer. 1 ml of 0.2 mM DPPH diluted in 1 ml of ethanol was used as control. Neutralization of DPPH radical was calculated using the equation: $S\ (\%) = 100\ (A_o - A_s)/A_o$, where A_o is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the test sample. Results were compared to activity of *L*-ascorbic acid and BHA. The IC_{50} values represented the concentration of the test compounds that caused 50% inhibition are shown in Table 2.

Reducing power assay

0.13 ml of samples in different concentrations (25, 50, 100, 250, 500, 1000 μg/ml) in phosphate buffer (0.2 mM, pH 6.6) were mixed with 0.125 ml of potassium ferric cyanide (1% w/v) and incubated at 50°C for 20 min. Afterwards, 0.125 mL of trichloroacetic acid (TCA) (10% w/v) was added to the mixture to terminate the reaction. Then the solution was mixed with 1.5 ml ferric chloride (1% w/v) and the absorbance was measured at 700 nm. The capability of antioxidant activity was calculated using the following equation: Inhibition concentration $(\%) = 1 - A_{sample}/A_{control}$. IC_{50} is inhibition concentration defined as the concentration inhibitory 50% radical generation or scavenging 50% radical generated. The IC_{50} values of the compounds are presented in Table 2.

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