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Study on the Relationship between the Structure and Antioxidant Activities of Chalcones*1

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ABSTRACT

The purpose of this study is to examine the relationship between antioxidant activities and chemical structures of various chalcones. Twenty-two chalcones were assessed for their radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Of 22 compounds tested, the most active on DPPH radical was 2′,4-dihydroxy-3,3′5-trimethoxy-5′-propylchacone (4) (72.6% at 100 ppm). It was followed by 3′,4′-dihydroxy-3,4,5-trimethoxy-6′-methylchalcone (6), 2′,4,4′-trihydroxy-3-methoxy-5-propenylchalcone (7) and 2′,4,4′-trihydroxy-3,5-dimethoxychalcone (13). Based on the results, we concluded that the scavenging activity is controlled by the number and the position of the substitution in the compound.

Keywords : *chalcone, antioxidant activity, structure-activity relationships*

1. INTRODUCTION

Chalcones are a group of compounds widely present in higher plants (Star and Mabry, 1971). They are phenolic substances which were consist of two phenyl rings in trans configuration, separated by three carbons, of which two are connected by double bond and the third is a carbonyl group. They belong to the largest class of plant secondary metabolites and it is believed that they provide to counteract reactive oxygen species (ROS) in order to prevent molecular damage (Yayli *et al.*, 2005). Chalcones have been reported to be anti-inflammatory, analgestic and antipyretic (Satyanarayana and Rao, 1993 : Khatib *et al.*, 2005). They are also known to possess antioxidant activity at various extents.

Antioxidants are used widely in food, drug and cosmetic field. These antioxidants help to maintain the quality of many food products by preventing oxidation and can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Javanmardi *et al.*, 2003). Oxidation can be defined as the transfer of electrons from one atom to another and its occur-

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rence in living organisms is known to cause damage to DNA, protein and lipids (Maxwell S. J., 1995). This damage appears to be one of the major ageing factors in living organisms.

The ability of flavonoids to act as antioxidants and their structure-antioxidant activity relationships have been extensively established (Rice-Evans *et al.*, 1996; Modak *et al.*, 2005; Park *et al.*, 2004). However, the antioxidant activity of chalcones and relationships between the activity and chemical structure are not well known (Haraguchi *et al.*, 1998). Therefore, in this study, we measured antioxidant activities of chalcones by their radical scavenging activity using the DPPH assay and focused on the relationship between antioxidant activity of chalcones and their chemical structures.

2. MATERIALS and METHODS

2.1. Materials

Twenty-two chalcones were tested for their antioxidant activities (Fig. 1). These compounds were isolated from natural resources and identified by spectroscopic methods including EI-MS (JEOL JMS-6000W), ^{1}H-, ^{13}C- and 2D-NMR (Varian UI 500) analysis.

2.2. Antioxidant Activity Test

The antioxidant activity was measured by the DPPH method according to the procedure of Cotelle *et al.* (2002). Ethyl alcohol soluble fraction (0.5 ㎖) of samples at various concentrations (50, 100 and 500 ppm) were added to a solution of DPPH in EtOH (100 ㎛, 3 ㎖) and the reaction mixture were shaken vigorously. After incubating the mixtures for 10 min at room temperature, the remaining amounts of DPPH were determined by colorimetry (852A Diode Array Spectrophotometer, Hewlett Packard Co.) at 517 nm. The mixture of 0.5 ㎖ EtOH with a solution of 3 ㎖ DPPH was used as control.

3. RESULTS and DISCUSSION

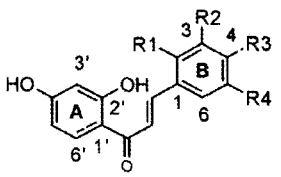
3.1. The Structures of Chalcones

The structures of the compounds used in this study are presented in Fig. 1. These chalcones are substituted at various positions with $-CH_3$, $-OCH_3$, -OH, $-OCH_2CH_3$ and $-OC_6H_5$.

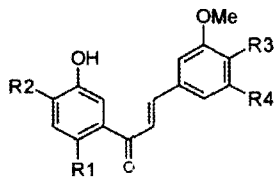
3.2. Antioxidant Activity of Chalcones

The antioxidant activities of twenty-two chalcones were evaluated according to the free radical scavenging activity assay. Free radical scavenging activity of chalcones was evaluated by the colorimetrical decrease in the absorbance of DPPH due to the chemical trapping of unfaired electron. The results are shown in Fig. 2. The scavenging activities of chalcones increased with the concentrations from 50 ppm to 500 ppm. Among the compounds, 2′,4-dihydroxy-3, 3′,5-trimethoxy-5′-propylchacone (4) showed highest scavenging activity (72.6% at 100 ppm) on DPPH radical. Other compounds such as 3′,4′-dihydroxy-3,4,5-trimethoxy-6′-methylchalcone (6), 2′,4,4′-trihydroxy-3-methoxy-5-propenylchalcone (7) and 2′,4,4′-trihydroxy-3,5-dimethoxychalcone (13) have also high antioxidant activity (51.6%, 50.9% and 50.6%, respectively, at 100 ppm). These four compounds which were above 50% of antioxidant activities at 100 ppm have three substitutions in common on C-3, C-4 and C-5 with -OH, -OMe or $-CHCHCH_3$.

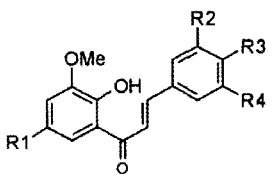
According to Guo *et al.* (1999), it was reported that an *ortho*-trihydroxyl group in the B ring of (-) epigallocatechin was more efficient radical scavenger than an *ortho*-dihydroxyl group in the B ring of (-) gallocatechin. Nanjo *et al.*,



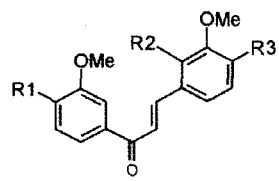
1 : R1 = H, R2 = OMe, R3 = OCH2CH3, R4 = H
2 : R1 = H, R2 = OMe, R3 = OCHCH3CH3, R4= H
3 : R1 = H, R2 = OMe, R3 = OCHCH3CH3, R4 = CHCHCH3
7: R1 = H, R2 = OMe, R3 = OH, R4 = CHCHCH3
11: R1 = H, R2 = H, R3 = OH, R4 = H
12: R1 = H, R2 = OMe, R3 = OH, R4 = H
13: R1 = H, R2 = OMe, R3 = OH, R4 = OMe
14: R1 = OH, R2 = H, R3 = H, R4 = H
15: R1 = OH, R2 = OMe, R3 = H, R4 = H



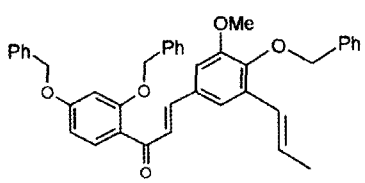
5 : R1 = CH3, R2 = OMe, R3 = OH, R4 = CHCHCH3
6 : R1 = CH3, R2 = OH, R3 = OMe, R4= OMe
19: R1 = H, R2 = OMe, R3 = OH, R4 = H



4 : R1 = CH2CH2CH3, R2 = OMe, R3 = OH, R4 = OMe
10: R1 = CH2CHCH2, R2 = OMe, R3 = OMe, R4= H
21: R1 = CH2CHCH2, R2 = OMe, R3 = OH, R4 = H
22: R1 = H, R2 = OMe, R3 = OH, R4 = H



17 : R1 = OH, R2 = OH, R3 = H
18 : R1 = OH, R2 = H, R3 = OH
20 : R1 = OMe, R2 = H, R3 = OH



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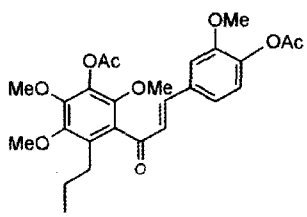

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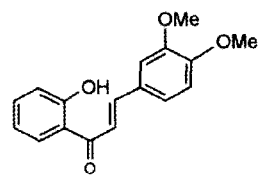

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Fig. 1. Chemical structures of chalcones used in this experiment.

(1996) also suggested that the presence of *ortho*-trihydroxyl group in the B ring contributes to the strong radical scavenging activity of the compounds.

3.3. Structure Relationships of Antioxidant Activity

In this study, we attempt to investigate whether the substitution of various groups can change and improve the antioxidant activities of the chalcones.

To examine the influence of methoxyl group on antioxidant activities of chalcones, the activities of compound 11, 12. 13, 14, and 15 were assessed. As shown in Fig. 3, the order of the radical scavenging activity against DPPH radicals is compound 13 > compound 12 > compound

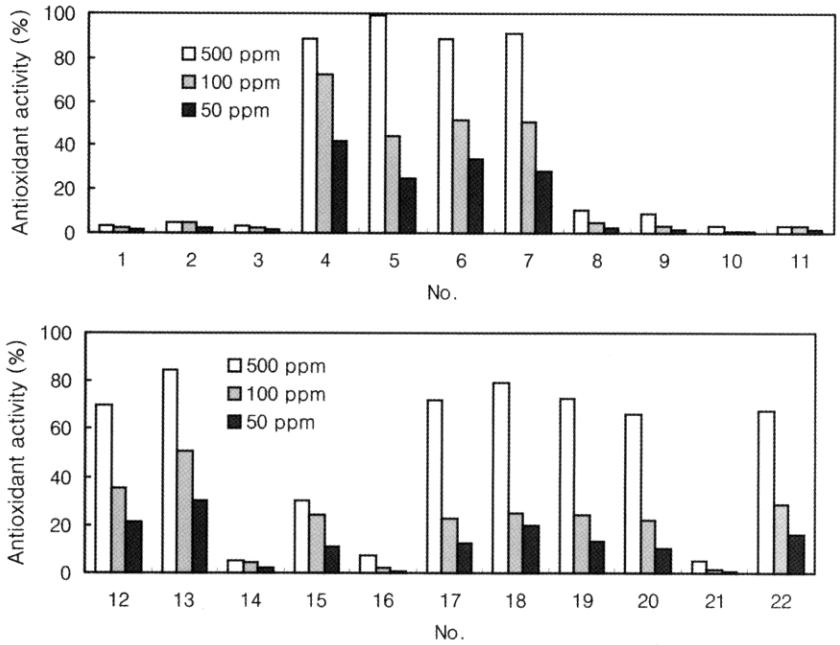


Fig. 2. The antioxidant activity of chalcones on DPPH radical.

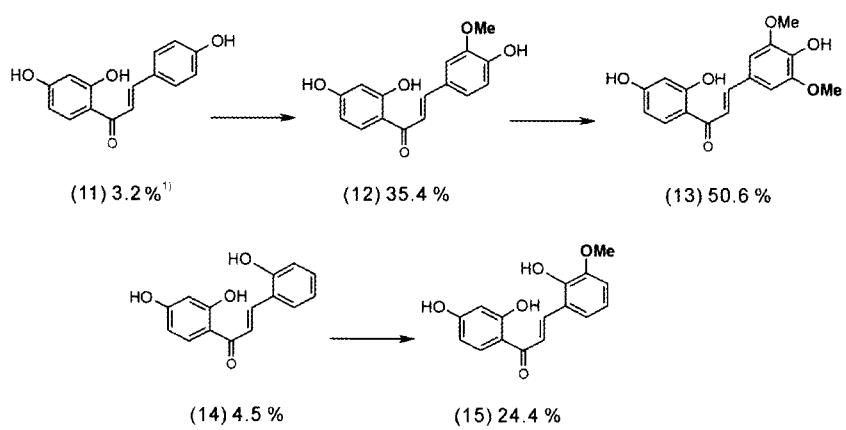
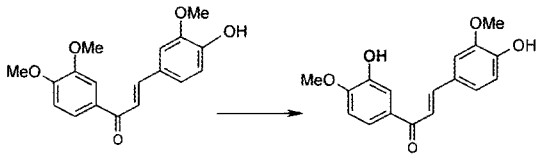


Fig. 3. The influence of the methoxylation in the B ring on the antioxidant activity of the chalcones. [1] The antioxidant activity was measured by the DPPH method at 100 ppm.

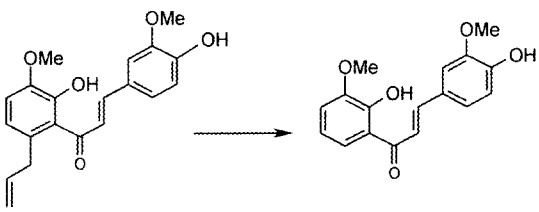
15 > compound 14 > compound 11, under the same experimental condition. From the results, we can assumed that additional insertions of the methoxyl groups at the 3 and 5 position in the B ring contribute to their scavenging activities. Compound 13, which has two methoxyl groups on C-3 and 5 showed the highest radical scavenging activity (50.6% at 100 ppm) whereas compound 11 (no methoxyl group) exhibited the lowest scavenging activity value (3.2% at 100 ppm) on DPPH.

The scavenging activity of the compound 15



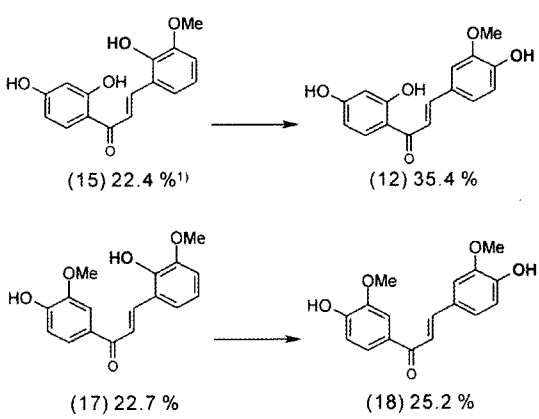
(20) 21.9 %[1)] (19) 24.5 %

Fig. 4. The influence of the presence of hydroxyl group and methoxyl group on the antioxidant activity of the chalcones. [1)] The antioxidant activity was measured by the DPPH method at 100 ppm.



(21) 1.2 %[1)] (22) 28.5 %

Fig. 5. The influence of the presence of allyl groups on the antioxidant activity of the chalcones. [1)] The antioxidant activity was measured by the DPPH method at 100 ppm.



(15) 22.4 %[1)] (12) 35.4 %

(17) 22.7 % (18) 25.2 %

Fig. 6. The influence of the position of hydroxyl groups on the antioxidant activity of the chalcones. [1)] The antioxidant activity was measured by the DPPH method at 100 ppm.

was higher than that of the compound 14, suggesting that the presence of a methoxyl group at the 3 position played an important role in scavenging free radicals (Fig. 3). Additional methoxyl groups 3 and 5 position of ring B increase the antioxidant activities of chalcones.

Pietta (2000) reported that the radical scavenging activity of flavonoids depends on the structure and the substituents of ring B. The major factors for radical-scavenging capability were found to be the presence of a catechol group in the ring B, which has better electron-donating properties. Yu *et al.* (2005) also suggested that flavonoids which have only one hydroxyl group in ring B diminish the antioxidant activity.

From the results of Fig. 4, we can assumed that the presence of a hydroxyl group on ring A of chalcone increases the antioxidant activity. Comparison of compound 20 (21.9%) with compound 19 (24.5%) shows that methoxylation of the 3′-hydroxyl group in the ring A has a strongly suppressive influence on the antioxidant activity. In the case of isoflavones, similar results were observed. According to Pietta (2000), the antioxidant activity of genistein (4′,5,7-trihydroxy-isoflavone) is higher than that of biochanin A (5,7-dihydroxy-4′-methoxy isoflavone), which has methoxyl group on C-4′. From the results, it is likely that the methoxylation diminishes the antioxidant activity.

Although the presence of hydroxyl group on ring A was found to increase the antioxidant activity, the presence of allyl group on ring A decreases the antioxidant activity (Fig. 5).

The antioxidant activities of chalcones were also affected by the position of hydroxyl group on ring B. Compound 12 and 18 which have the hydroxyl group on C-5 have higher antioxidant activity than compound 15 and 17 which have the hydroxyl group on C-3 (Fig. 6). As the steric hindrance of compound 12 and compound 18 is smaller than that of compound 15 and compound 17, the stability of compound 12 and compound 18 is better than that of

compound 15 and compound 17, respectively. Therefore, the rates of the reactions of compound 12 and compound 18 with free radicals are faster than those of the reactions of their corresponding compounds with free radicals. Because of the above reasons, the compound 12 and compound 18 are more effective scavengers in comparison with compound 15 and compound 17, respectively. Guo *et al.* (1999) reported that the differences between sterical structures played an important role in the ability to scavenge large free radicals, such as the free radicals generated from DPPH radical and compared the scavenging abilities of EGCG with GCG, suggesting that the differences between the sterical structures of the catechins and their epimers play a important role.

Besides these results about antioxidant activities of chalcones and their relationships between activity and structures, the bioavailability and the antioxidant activity of chalcones in vivo still need further study.

4. CONCLUSIONS

In order to find out the structure-relationship of antioxidant activity of chalcones, 22 chalcones were used for DPPH free radical scavenging effect test. Based on the results, we conclude that the number and the position of hydroxyl and methoxyl groups are the important determinants of antioxidant activity. The addition of the methoxyl groups to the 3 and 5 position in the B ring is likely to influence their scavenging activities and thus to enhance their antioxidant activity. The antioxidant activities of chalcones were also affected by the position of hydroxyl group on ring B. The differences between sterical structures played an important role in their ability to scavenge large free radicals.

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