Free Radical Scavenging Activities of Extracts from Pomelo

YONGZHI LIAO¹, WEN QIN², PENGWEI LI², YE ZHANG^{2*} and XIANGHUI YI^{2*}

¹Guangxi Academy of Fishery Sciences, Nanning 530021, P.R. China

²Department of Chemistry, Guilin Normal College, Guangxi 541001, P.R. China

*Corresponding authors: Fax: +86 77 32806321; Tel: +86 77 32823285; E-mail: zhangye81@126.com; yixianghui2008@163.com

Received: 8 February 2014;

Accepted: 5 May 2014;

Published online: 30 September 2014;

AJC-16158

Under ultrasound, pomelo peel (PP) and pomelo flesh (PF) were extracted with acetone, ethanol, water and ethyl acetate solvents at 70 °C to offer acetone extract (PAE), ethanol extract (PEE), water extract (PWE), ethyl acetate extract (PAEE, acetone extract (FAE), ethanol extract (FEE), water extract (FWE) and ethyl acetate extract (FAEE), respectively. Their free radical scavenging activities were evaluated and compared with the common antioxidant butylated hydroxytoluene, employing DPPH[•] assay, ABTS^{•+} assay, O₂^{•-} assay and OH[•] assay. The results showed that all the extracts displayed good radical scavenging activities, with IC₅₀ less than standard value 10 mg/mL. It is important to point out that all these eight extracts demonstrated better scavenging activity than the commercial antioxidant BHT in OH[•] assay. The extract solvents were found to have important effect on their radical scavenging activities.

Keywords: Pomelo, Free radical scavenging activity, Ultrasound-assisted extract.

INTRODUCTION

Recently, isolation of good natural radical scavengers/antioxidants from edible fruits have greatly attracted bioorganic chemists' interest and become a hot research topic in bioorganic chemistry, due to the non-toxicity of fruits. In fact, screening for natural radical scavengers/antioxidants from edible fruits has become one of the important and efficient methods.

Pomelo (*Citrus grandis*), the rutaceae citrus fruit, is rich in important vitamin C, flavonoids, coumarins and limonoids and has been mainly used in the prevention of and treatment of hypertension, atherosclerosis, heart cerebrovascular disease, diabetes, etc.¹⁻⁴. It is well known that vitamin C, flavonoids and coumarins should lead to good antioxidant activity^{5,6}, so it is to expect that pomelo extracts may exhibit good antioxidant activity. However, to our best of knowledge, there is no available information relating to the antioxidant and radical scavenging activities of pomelo extracted by ultrasonic. So the aim of the present study was to assay the *in vitro* antioxidant and radical-scavenging activities of ethanol extract, acetone extract, water extract and ethyl acetate extract of pomelo that extracted by ultrasonic, employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, 2,2'-azinobis-(3-ethylbenz-thiazoline-6-sulfonate) cation (ABTS^{•+}) radical-scavenging assay, superoxide radical-scavenging assay and hydroxyl radical-scavenging assay.

EXPERIMENTAL

Pomelos were purchased in Guilin city of Guangxi Province in September, 2012 and they were separated as pomelo peel (PP) and pomelo flesh (PF). Under ultrasound, PP (20 g) were extracted with acetone, ethanol, water and ethyl acetate solvents at 70 °C for 1 h and filtered through Whatman No. 4 filter paper, respectively. Then the four extract solutions were vacuum evaporated at 50 °C to dryness to offer four extracts: acetone extract (PAE), ethanol extract (PEE), water extract (PWE) and ethyl acetate extract (PAEE) with 7.5, 8.9, 12.6 and 5.5 % yields, respectively. Pomelo flesh was then treated with the same procedure to offer acetone extract (FAE), ethanol extract (FEE), water extract (FWE) and ethyl acetate extract (FAEE) with 9.3, 13.4, 11.5 and 7.9 % yields, respectively.

RESULTS AND DISCUSSION

According the literatures⁷⁻¹⁰, their free radical scavenging activities were determined as showed in Figs. 1 to 4. Significant free radical scavenging activities were clear at all the tested extracts. The values of IC₅₀, the effective concentration at which 50 % of the radicals were scavenged, were calculated to evaluate the radical scavenging activities (Table-1). A lower IC₅₀ value implied greater radical scavenging activity and IC₅₀ values of less than 10 mg/mL usually indicated effective activities in antioxidant properties¹¹.

It can be seen in Fig. 1 that PAE, PEE, PWE, PAEE, FAE, FEE, FWE and FAEE, showed evident radical scavenging activities in DPPH[•] assay. As showed in Table-1, IC₅₀ values of PAE, PEE, PWE, PAEE, FAE, FEE, FWE and FAEE were found to be 1473.4, 2148.5, 576.9, 606.7, 638.9, 580.3 and 590.9 µg/mL, respectively. Since that their IC₅₀ values were further lower than the standard value 10 mg/mL¹¹, it could concluded that all these above extracts exhibited potent inhibition of DPPH radical. The order of scavenging activity of tested in this assay was: PWE > FWE > FAE > PAEE > FEE > FAEE > PAE > PEE. In addition, extract solvents were found to have important effects on the DPPH radical scavenging activities. The order for pomelo peel was: water > ethyl acetate > acetone > ethanol, while that for pomelo flesh was: water > ethyl acetate > ethanol > acetone.

Fig. 1. DPPH radical-scavenging activities of the extracts from pomelo

Fig. 2 showed that PAE, PEE, PWE, PAEE, FAE, FEE, FWE and FAEE exhibited clear radical scavenging activities in ABTS^{•+} assay. Table-1 displayed that IC₅₀ values of PAE, PEE, PWE, PAEE, FAE, FEE, FWE and FAEE were found to be 213.4, 252.6, 136.1, 125.8, 198.4, 210.8, 161.3 and 129.1 µg/mL, respectively. Obviously, their IC₅₀ values were lower than the standard value 10 mg/mL¹¹, it was thus to conclude that these eight extracts showed good inhibition of ABTS^{•+} radical. The order of scavenging activity of these extracts in this assay was listed as follow: PAEE > FAEE > PWE > FWE > FAE > FEE > PAE > PEE. In addition, extract solvents also showed important influence on the ABTS^{•+} radical scavenging activities. The order for pomelo peel was: ethyl acetate > water > acetone > ethanol, while that for pomelo flesh was: ethyl acetate > water > acetone > ethanol.

As showed in Fig. 3, PAE, PEE, PWE, PAEE, FAE, FEE, FWE and FAEE exhibited marked radical scavenging activities

Fig. 2. ABTS^{•+} radical-scavenging activities of the extracts from pomelo

Fig. 3. Superoxide anion radical-scavenging activities of the extracts from pomelo

in superoxide anion assay. Table-1 showed that IC₅₀ values of PAE, PEE, PWE, PAEE, FAE, FEE, FWE and FAEE were 1346.7, 1486.4, 2570.9, 1866.6, 1584.5, 1304, 2930.6 and 2151.1 µg/mL, respectively. It was worth to point out that all the values were less than the standard value 10 mg/mL¹¹, showing their good inhibition of superoxide anion radical. The order in this assay was listed as follow: FEE > PAE > PEE > FAE > PAEE > FAEE > PWE > FWE. Based on the above observation, extract solvents were found to display important effect on the superoxide anion radical scavenging activities. The order for pomelo peel was: acetone > ethanol > ethyl acetate > water, while that for pomelo flesh was: ethanol > acetone > ethyl acetate > water.

The radical scavenging effects were also evaluated in the present study using hydroxyl radicals generated by Fenton

TABLE-1
IC₅₀ OF THE EXTRACTS FROM POMELO

Entry	PAE	PEE	PWE	PAEE	FAE	FEE	FWE	FAEE	BHT
DPPH (µg/mL)	1473.4	2418.5	567.9	606.7	660.3	638.9	580.3	590.9	14.5
ABTS (µg/mL)	213.4	252.61	136.1	125.8	198.4	210.8	161.3	129.1	8.1
O ₂ ⁻ (µg/mL)	1346.7	1486.4	2570.9	1866.6	1584.5	1304.0	2930.6	2151.17	55.9
OH (mg/mL)	0.387	0.887	0.305	0.069	0.805	0.863	0.705	0.811	5437.7

reagent¹². As shown in Fig. 4, all the extracts displayed good activity in an amount dependent manner. It can be seen from Table-1, PAEE showed the best hydroxyl radical scavenging activity in this assay with IC_{50} of 0.069 mg/mL, while IC_{50} values of PAE, PEE, PWE, FAE, FEE, FWE and FAEE were found to be 0.387, 0.887, 0.305, 0.805, 0.863, 0.705 and 0.811 mg/mL, respectively. It was important to point out that all these seven extracts showed better hydroxyl radical scavenging activity than the common antioxidant butylated hydroxytoluene (BHT). Scavenging activities of these eight extracts decreased in the order of PAEE, PWE, PAE, FWE, FAE, FAEE, FEE and PEE. The results indicated that extract solvents had the important effect on hydroxyl radical scavenging activities and ethyl acetate should be the best extract solvent in this assay.

On the basis of the above observation, it could be summarized that it is feasible to isolate natural radical scavengers or antioxidants from pomelo and this study may provide important guiding opinions for the comprehensive utilization pomelo. In addition, the extract solvents had important effect on their radical scavenging activities.

ACKNOWLEDGEMENTS

This study was supported by the Fund of Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization (No. FPRU2011-6; FPRU2013-6), the Guilin Scientific Research and Technological Development Project (20120108-6; 20110106-2), the Guangxi Natural Science Foundation (No. 2014GXNSFBA118050) and Guangxi Department of Education Research Project (No. 2013YB283; 2013YB284).

Fig. 4. Hydroxyl radical-scavenging activities of the extracts from pomelo

REFERENCES

1. N. Guthrie, K. Morley, S. Hasegawa, G.D. Manners and T. Vandenberg, in eds.: M.A. Berhow, S. Hasegawa and G.D. Manners, *Inhibition of Human Breast Cancer Cells by Citrus Limonoids*, American Chemical Society, Washington, D.C., Vol. 758, pp. 164-174 (2000).
2. W.E. Bronner and G.R. Beecher, *J. Chromatogr. A*, **705**, 247 (1995).
3. L.C. Sadler, J. Davis and D. Dezman, *J. Food Sci.*, **55**, 1460 (1990).
4. S. Nagy, *J. Agric. Food Chem.*, **28**, 8 (1980).
5. O. Benavente-García, J. Castillo, F.R. Marin, A. Ortuño and J.A. Del Río, *J. Agric. Food Chem.*, **45**, 4505 (1997).
6. E. Middleton and C. Kandaswami, *Food Technol.*, **48**, 115 (1994).
7. Y. Zhang, B.Q. Zou, K. Wang, Y.M. Pan, H. Liang, X.H. Yi and H.S. Wang, *Med. Chem. Res.*, **21**, 1341 (2012).
8. B. Terry, J.B. Terry and A. Wolk, *J. Intern. Med.*, **250**, 280 (2001).
9. J. Sun, Y.F. Chu, X. Wu and R.H. Liu, *J. Agric. Food Chem.*, **50**, 7449 (2002).
10. A. Baldi, M.K. Pandit and P. Ranka, *Int. J. Pharm. Biol. Arch.*, **3**, 157 (2012).
11. Y.L. Lee, M.T. Yen and J.L. Mau, *Food Chem.*, **104**, 1 (2007).
12. Y.M. Pan, X.P. Zhang, H.S. Wang, Y. Liang, J.C. Zhu, H.Y. Li, Z. Zhang and Q.M. Wu, *Food Chem.*, **105**, 1518 (2007).