

ANTIOXIDANT PHENOLIC COMPOUNDS FROM CHINESE WHITE OLIVE (*CANARIUM ALBUM L.*)

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Abstract

To find antioxidant compounds from Chinese white olive (CWO) based on bioassay-guided method, the ethanol extract and corresponding 4 fractions were evaluated for their antioxidant activities by DPPH and ABTS assay. The results revealed that the AcOEt fraction had the most potent antioxidant activity. Further, six compounds isolated from the AcOEt fraction, and their structure were identified as (-)-epicatechin gallate (i), ellagic acid (ii), gallic acid (iii), (-)-epigallocatechin (iv), (-)-epigallocatechin gallate (v) and kaempferol (VI) by MS and NMR spectrum. Compounds I, IV and V were isolated and identified from CWO for the first time. All the compounds (I-VI) exhibited potent antioxidant activity. Among them, compound III showed best antioxidant activity in both DPPH and ABTS⁺ assay. The phenolic compounds from CWO with potent antioxidant activities were responsible for the antioxidant activity of CWO extract.

Introduction

Chinese white olive (CWO, *Canarium album L.*), usually called Ganlan or Qingguo in China, is a well-known Chinese fruit tree belonging to the Burseraceae. It is indigenous to the southeast area of China, and then has been introduced to other Asian tropical and semi-tropical regions (Xiang *et al.* 2014). The fresh fruit of CWO is light colored and edible, with low oil and rich nutrition. Most fruits are generally processed in food industry to beverage, candy and confections. The dried fruit is also used as traditional medicine for treatment of faulcitis, stomatitis, hepatitis, and toxicosis in China (Xiang and Wu 2017). According to modern pharmacological investigations, CWO fruits possess some pharmacological properties such as antibacterial (Xiang *et al.* 2013), anti-alcoholic and hepatoprotective (Zhu *et al.* 2010), anti-HIV activities (Duan *et al.* 2013) and antiglycation (Kuo *et al.* 2015). However, the fundamental research of CWO is rather poor. Particularly, there was no report on discovery of active compounds based on activity tracking from CWO.

Oxidative modification of DNA, proteins, lipid and small cellular molecules by reactive oxygen species (ROS) increases the risk of a wide range of common degenerative diseases such as osteoporosis, cancer and cardiovascular diseases. Antioxidants are of great importance in terms of reducing ROS so as to lower the incidence rate of degenerative diseases. Increasing dietary intake of natural antioxidants, such as polyphenolic compounds and antioxidant vitamins, has been shown to have an inverse association with the risks of cardiovascular diseases, inflammatory conditions, neurodegenerative diseases, diabetes mellitus and cancer (Xiang and Wu 2017). Phytochemical investigations revealed that phenolic compounds have been regarded as representative components of the plant. However, there was no report on discovery of antioxidant

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phenolic compounds based on activity tracking. Therefore, the aim of this study was to find antioxidant phenolic compounds based on bioassay-guided method from CWO using DPPH[·] and ABTS⁺ assay and separation by chromatography.

Materials and Methods

Ethanol, PE (60 - 90°C), AcOEt, n-butanol, methanol, chloroform (CHCl₃) (all analytical grade) were purchased from Chongqing East Chemical Industry Co., Ltd. (Chongqing, China). Sephadex LH-20 was the product of GE Healthcare Bio-Sciences AB (Uppsala, Sweden). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (AB TS) were purchased from Sigma Chemical Co. (Missouri, USA). Silica gel (200 - 300 mesh) for column chromatography and silica gel GF₂₅₄ for TLC were obtained from Qingdao Marine Chemical Company (Qingdao, China). The plant materials were collected in September, 2016 from Jiangjin, Chongqing municipality and identified as the dried fruits of *Canarium album* (Lour.) Raeusch by Professor Ren S.G., College of Bioengineering, Chongqing University, China.

The ethanol extract from fruits of CWO was obtained by heating at 70°C for 8 hrs in a water bath (H-6, Ronghua, Jiangsu, China) using 80% ethanol as solvent after soaking 15 days. After removal of solvent under vacuum with rotary evaporator (RE-5205, Yarong, Shanghai, China), most of the residue was dispersed by water and extracted with PE (4 × 2 L, each 24 hrs), AcOEt (4 × 2 L, each 24 hrs) and n-butanol (4 × 2 L, each 24 hrs) successively to obtain 4 fractions, namely PE, AcOEt, n-butanol and water fraction. The DPPH and ABTS⁺ assay was performed according to literature (Xiang *et al.* 2017) and Vitamin C (Vc) as positive control group.

The EtOAc fraction was dissolved in EtOAc and extracted with 5% sodium bicarbonate aqueous solution, 5% sodium carbonate aqueous solution and 2% sodium hydroxide aqueous solution successively to give four subfractions B₁ - B₄. Subfraction B₁ was purified over silica gel with CHCl₃-MeOH to obtain four mixtures M₁ - M₄. Mixture M₁ was purified over silica gel (Qingdao Marine, Shandong, China) with PE- acetone repeatedly to afford compounds I and II. Mixture M₃ was purified over silica gel with CHCl₃ - MeOH repeatedly to afford compounds III and IV. Subfraction B₂ was purified over silica gel with CHCl₃ - MeOH to obtain three mixtures M₅ - M₇. Mixture M₆ was purified over silica gel with CHCl₃ - MeOH and Sephadex LH-20 (GE, Uppsala, Sweden) repeatedly to afford compound V. Mixture M₇ was purified over silica gel with CHCl₃-MeOH and sephadex LH-20 repeatedly to afford compound VI. The NMR data were recorded on Bruker spectrometers (AV-500, Germany) using DMSO-d₆ as solvent and TMS as internal reference. ESI-MS and HR-ESI-MS were performed with Mat-212 and Micromass Auto Spec Q-TOF spectrometers (Micromass, U.K.), respectively.

Results and Discussion

The antioxidant activity of the extracts of CWO was reported by Ho and Luo (2015). However, there was no report on discovery of antioxidant fraction and compounds based on bioassay-guided method from CWO. There are so many methods to evaluate the potential antioxidant activity due to different mechanisms. DPPH[·] and ABTS⁺ assays are both especially effective way to test the antioxidant activity. In the DPPH[·] assay, there existed a good linear correlation between antioxidant activities and concentrations of ethanol extract and four fractions of CWO in certain range of concentrations, while, they arose a high concentrations, the antioxidant activities tended to vary gently (Fig. 1). The SC₅₀ value as follow: 0.038 mg/ml for Vc used as positive control and 4.429 mg/ml for ethanol extract. It is obvious that the ethanol extract of CWO expressed good DPPH radical scavenging activity. Among the four fractions, the AcOEt exhibited the best DPPH scavenging activity with the minimal SC₅₀ value (1.654 mg/ml),

meanwhile, the water fraction exhibited the worst activity with the maximal SC_{50} value (8.826 mg/ml). The PE fraction owned some radical scavenging activity in DPPH[·] system with the good SC_{50} value (4.484 mg/ml), though it is weaker than n-butanol fraction with the good SC_{50} value (3.908 mg/ml).

In order to evaluate antioxidant activity more credibly, ABTS⁺ system for determination of total antioxidant capacity was adopted. In the ABTS⁺ assay, there existed a good linear correlation between antioxidant activities and concentrations of ethanol extract and four fractions in certain range of concentrations. Based on the ABTS⁺ scavenging activities in Fig. 2. It is obvious that the ethanol extract of CWO expressed good ABTS⁺ radical scavenging activity, though it is weaker than Vc. Among the four fractions, the AcOEt exhibited the best ABTS⁺ scavenging activity with the minimal SC_{50} value (0.477 mg/ml), meanwhile, the water fraction exhibited the worst activity.

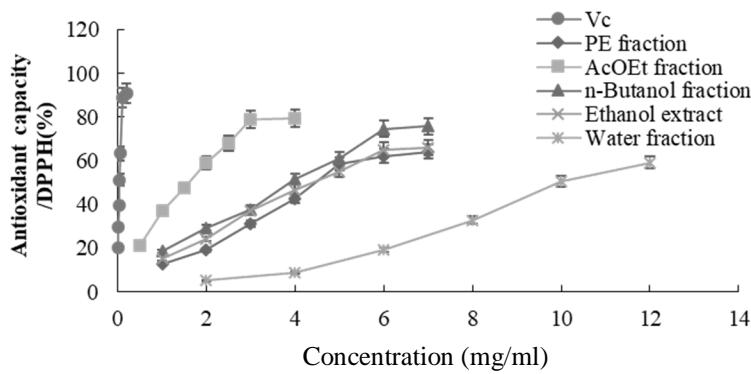


Fig. 1. DPPH[·] scavenging activities of ethanol extract and 4 fractions of CWO.

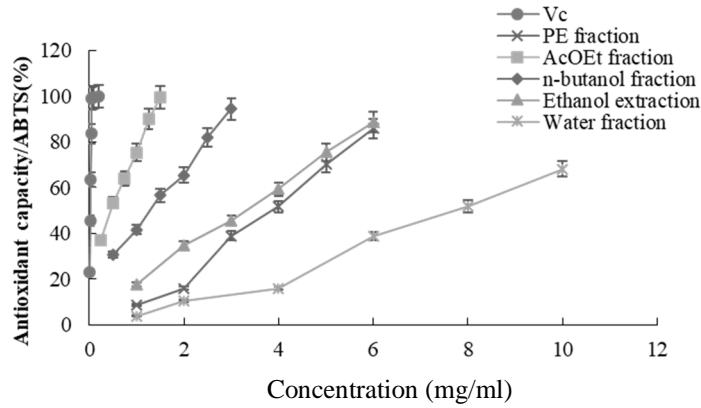


Fig. 2. ABTS⁺ scavenging activities of ethanol extract and 4 fractions of CWO.

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of the many human diseases, including cellular damage, cancer, aging and atherosclerosis (Zheng *et al.* 2013). Although oxidation could be prevented in foods by the addition of synthetic antioxidants such as BHT, BHA and TBHQ, but more attention has recently been paid to natural antioxidants because of potential toxicities and carcinogenic effects of these synthetic antioxidants (Wang 2014). The antioxidant activity of CWO was reported, but most of them focused on the activity owning main compositions, such as tannins, total polyphenols (Zhang

et al. 2008), total polysaccharide (Zhu *et al.* 2013), and so on. In fact, the tannins, total polyphenols and total polysaccharide obtained from CWO were impure and even low in content. Therefore, antioxidant activity may also come from other components. It is very important to separate the extract systematically for seeking the antioxidant compound. Among the four fractions, the AcOEt fraction exhibited the best antioxidant activity with the minimal SC₅₀ value, so it is the most likely to find antioxidant compound from the fraction.

Compound **I**, a white powder with good solubility in ethanol and ethyl acetate, FeCl₃ reaction was positive. It exhibited a quasi-molecular ion peak at *m/z* 443 [M+H]⁺ by ESI-MS and the molecular formula C₂₂H₁₈O₁₀ (Fig. 3) was determined by HRESI-MS (*m/z* 443.0833 [M+H]⁺). All the NMR spectral data were consistent with Fu *et al.* (2012), and compound **I** was identified as (-)-epicatechin gallate and first found from CWO.

Compound **II**, a light-yellow powder with good solubility in ethanol and ethyl acetate, FeCl₃ reaction was positive. It exhibited a quasi-molecular ion peak at *m/z* 301 [M-H]⁻ by ESI-MS and the molecular formula C₁₄H₆O₈ was determined by HRESI-MS (*m/z* 300.9985 [M-H]⁻). All the NMR spectral data were consistent with those reported by Shi *et al.* (2006), and compound **II** was identified as ellagic acid.

Compound **III**, a white powder with good solubility in ethanol and ethyl acetate, FeCl₃ reaction was positive. It exhibited a quasi-molecular ion peak at *m/z* 169 [M-H]⁻ by ESI-MS and the molecular formula C₇H₆O₅ was determined by HRESI-MS (*m/z* 169.0145 [M-H]⁻). All the NMR spectral data were consistent with those reported by Shi *et al.* (2006), and compound **III** was identified as gallic acid.

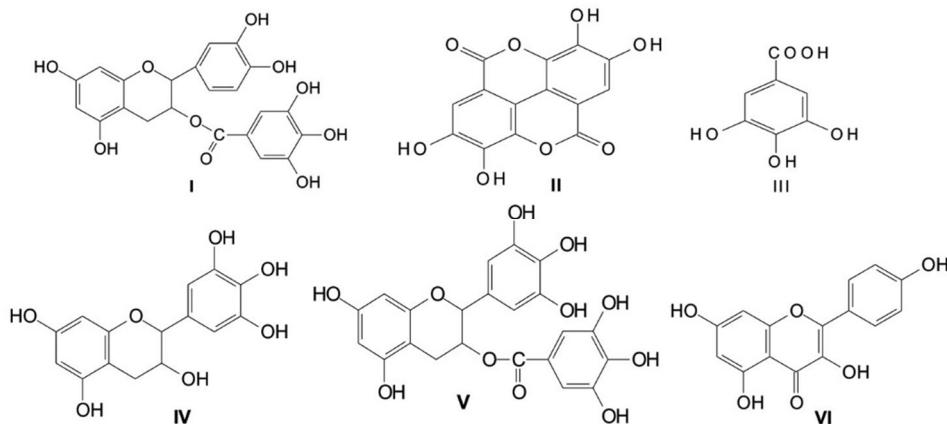


Fig. 3. Chemical compositions from antioxidant fraction of CWO.

Compound **IV**, a white powder with good solubility in ethanol and water, FeCl₃ reaction was positive. It exhibited a quasi-molecular ion peak at *m/z* 307 [M+H]⁺ by ESI-MS and the molecular formula C₁₅H₁₄O₇ was determined by HRESI-MS (*m/z* 307.0717 [M+H]⁺). All the NMR spectral data were consistent with those reported by Jia *et al.* (1998) and compound **IV** was identified as (-)-epigallocatechin and first found from CWO.

Compound **V**, a white powder, FeCl₃ reaction was positive. It exhibited a quasi-molecular ion peak at *m/z* 459 [M+H]⁺ by ESI-MS and the molecular formula C₂₂H₁₈O₁₁ was determined by HRESI-MS (*m/z* 459.3373 [M+H]⁺). All the NMR spectral data were consistent with Zhao *et al.* (2012), and compound **V** was identified as (-)-epigallocatechin gallate and first found from CWO.

Compound VI, a yellow powder, the color of FeCl_3 reaction was yellow green. It exhibited a quasi-molecular ion peak at m/z 287 [$\text{M}+\text{H}^+$] by ESI-MS and the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_6$ was determined by HRESI- MS (m/z 287.0468 [$\text{M}+\text{H}^+$]). All the NMR spectral data were consistent with those reported by Hu *et al.* (2003), and compound VI was identified as kaempferol.

In the DPPH[·] assay, there existed a good linear correlation between antioxidant activities and concentrations of all the compounds (I-VI) from antioxidant fraction of CWO in certain range of concentrations, while they arose a high concentration, the antioxidant activities tended to vary gently. The SC_{50} value as follow: 0.038 mg/ml for Vc used as positive control, 0.194 mg/ml for compound I, 0.055 mg/ml for compound II, 0.026 mg/ml for compound III, 0.102 mg/ml for compound IV, 0.060 mg/ml for compound V and 0.206 mg/ml for compound VI. Based on the DPPH scavenging activities in Fig. 4. It is obvious that all the compounds (I-VI) from antioxidant fraction of CWO expressed potent DPPH[·] radical scavenging activity, especially the compound III is better than Vc used as positive control widely. Furthermore, there are two compounds (II and V) exhibiting similar DPPH[·] scavenging activity with Vc. Even, the SC_{50} value of compound VI with the worst activity is only 0.206 mg/ml. So, all the compounds (I-VI) showed good radical scavenging activity in DPPH[·] system.

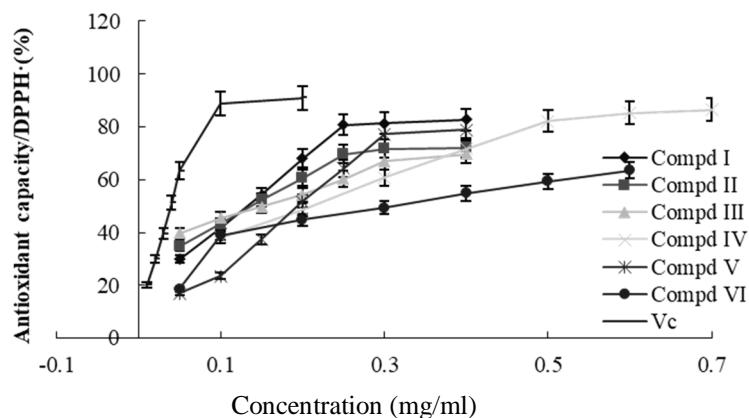


Fig. 4. DPPH scavenging activities of compounds I-VI from antioxidant fraction of CWO.

In order to evaluate antioxidant activity of all the compounds (I-VI) more credibly, ABTS⁺ system for determination of antioxidant capacity also has been adopted. In the ABTS⁺ assay, there existed a good linear correlation between antioxidant activities and concentrations of all the compounds (I-VI) in certain range of concentrations similarly. Based on the ABTS⁺ scavenging activities in Fig. 5. It is obvious that all the compounds (I-VI) from antioxidant fraction expressed potent ABTS⁺ radical scavenging activity with the very good SC_{50} value (0.023 - 0.040 mg/ml), which is in accordance with the previous DPPH assay, similarly the ABTS⁺ scavenging activity of compound III is better than that of Vc according to the SC_{50} value. The former is 0.023 mg/ml, and the latter is 0.028 mg/ml.

All the compounds (I-VI) exhibited very good antioxidant activity in the present study, especially the antioxidant activity of compound III is better than that of Vc used as positive control widely. The results were consistent with that phenolic compounds including flavones are well-known antioxidants (Peng *et al.* 2018). Phenolic compounds are secondary metabolites widely found in fruits, mostly represented by flavonoids and phenolic acids. Studies have shown

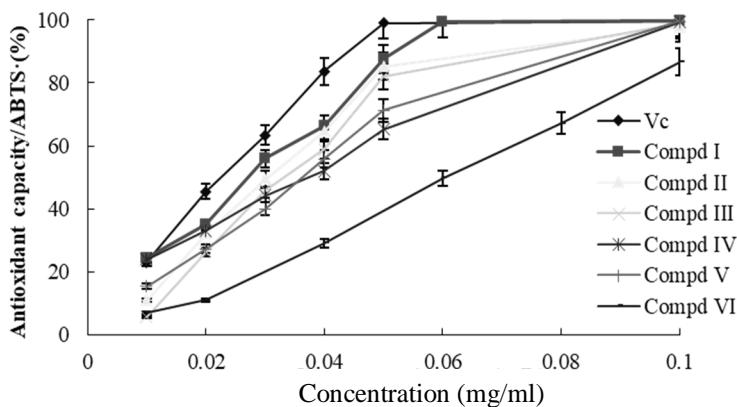


Fig. 5. ABTS⁺ scavenging activities of compounds I-VI from antioxidant fraction of CWO.

the importance of the regular consumption of fruits, especially for preventing diseases associated with oxidative stress. From the present result it may concluded that all the compounds (I-VI), especially compound III obtained from the CWO fruit could be used for good natural antioxidants. Phenolic compounds from CWO with potent antioxidant activities were responsible for the antioxidant activity of CWO extract.

Acknowledgments

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