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Research Article

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Antioxidant activities of extracts from *Cyclocarya paliurus* leaves

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ABSTRACT

Cyclocarya paliurus (Batal.) Iljinsk (C. paliurus) commonly known as 'sweet tea tree' is a medicinal herb, which has been widely used in China as drug formulation in traditional Chinese medicine for the treatment of diabetes mellitus. The aim of this study was to investigate the antioxidant activity of the ethanol extract from leaves of C. paliurus and screen the active fractions separated from the ethanol extract. Five antioxidant methods were established including total reducing power, 2, 2-diphenyl-1- picrylhydrazyl, superoxide anion and hydroxyl radical scavenging activity assay and the inhibitory effect on H_2O_2 induced lipid peroxidation. The results showed that the ethanol extract from leaves of C. paliurus and the four different polar fractions exhibited antioxidant activities at different magnitudes of potency. Among these extracts, the ethyl acetate fraction showed the best antioxidant activity, which was much better than the crude ethanol extract. This research demonstrates that the ethyl acetate fraction from ethanol extract of C. paliurus might be valuable antioxidant natural source and applicable in both medicine and food industry.

Keywords: *Cyclocarya paliurus*, antioxidant, radical scavenging, lipid peroxidation

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INTRODUCTION

Oxidative stress plays a very important role in the process of aging and pathogenesis of numerous diseases like diabetes, cancer, neurodegenerative diseases and respiratory tract disorders and has received extensive concern over the last few decades [1]. Reactive oxygen species (ROS) are among the major sources of primary catalysts initiating oxidative stress injury *in vivo* and *in vitro* [2]. Active oxygen free radicals such as the superoxide anion radical and hydroxyl radical are thought to be linked to the pathogenesis of many diseases. Lipid peroxidation, that involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage. Therefore, antioxidants that prevent damage caused by free radicals are worth of additional investigation [3].

Recently, traditional Chinese herbal medicines show particular superiorities as natural antioxidants with low toxicity. *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*), grown on cloudy and foggy highlands in southern China, is a very important medicinal herb which belongs to the family of Juglandaceae[4]. The leaves of *C. paliurus* taste sweet and are consumed daily as a kind of tea in this region for the treatment of hypertension and diabetes [5]. Extracts from the leaves of *C. paliurus* have been found to possess a variety of bioactivities including antihypertensive activity, hypoglycemic activity, enhancement of mental efficiency and antioxidant activity. Chemical studies have shown that the plant contains protein, polysaccharides, triterpenoids, flavonoids, steroids, saponins and phenolic compounds [6].

In the present study, we investigated the antioxidant potential of the ethanol extract from leaves of *C. paliurus* and its separated products using *in vitro* methods including total reducing power, free radicals clearance and lipid peroxidation inhibition, in the hope of screening active fractions and exploiting natural antioxidants.

EXPERIMENTAL SECTION

Chemicals and Instruments

Male Wistar albino rats weighing between 180-220 g were provided by Weifang Medical Experimental Animal Center and bred in standard animal facility. All experiments on animals were conducted in accordance with and after approval by the Institution Animal Ethics Committees of Weifang Medical University. 2, 2-diphenyl -1-picrylhydrazyl: DPPH (Sigma Co., USA); Superoxide anion, hydroxy free radical and MDA assay kits were all purchased from Jiancheng Bioengineering Institute, Nanjing, China. All other chemicals and reagents used were of analytical grade. UV8000 UV–visible spectrophotometer (Shanghai Precision Instrument Co., Ltd, China.); HC-2518R high-speed refrigerated centrifuge (Anhui Zhongke Zhongjia Scientific Instruments Co., Ltd, China.).

Plant material and preparation of ACP

C. paliurus leaves were collected in Zhangjiajie, Hunan province, China and authenticated by Dr. Chongmei Xu from the Department of Pharmacognosy of Weifang Medical University. The ethanol extract from leaves of *C. paliurus* (ECP) was obtained by ethanol soaking extracting method for three times and seven days each time. The extraction was concentrated and dried under reduced pressure. Then the extracts were dissolved in ultra pure water and extracted by petroleum ether, ethyl acetate and n-butyl alcohol, the remaining was aqueous extract and the four polar extractions were abbreviated as F1, F2, F3 and F4, respectively.

Total reducing power assay

The total reducing powers of the extractions were measured according to the method of Liu [7] with a minor modification. All extracts (2.0 mL) with various concentrations (0-8 mg/mL) were mixed with 0.2 mL of phosphate buffer (0.2 mol/L, pH 6.8) and 2.0 mL of 1% potassium ferricyanide and then the mixture was incubated at 50 ^{o}C for 20 min. After cooling immediately, 2.0 mL of 10% trichloroacetic acid was added and centrifuged (3742 g × 10 min).Five milliliter of the supernatant was mixed with 4.0 mL of distilled water and 1.0 mL of 0.1% ferric chloride. Ten minutes later, the absorbance was measured in triplicate at 700 nm. A higher absorbance indicated a higher reducing power activity.

DPPH radical scavenging activity

The radical scavenging effects on DPPH radical of samples were estimated according to the method of Fu [8] with a minor modification. Briefly, DPPH was dissolved and diluted with absolute alcohol to be a concentration of 0.2 mmol/L. The extracts were dissolved with 95% alcohol at different concentrations. During the reaction, the same volume of DPPH solution was added, and the solution was placed away from light for 15 min. The absorbance was detected immediately at 515 nm wavelength. The scavenging rate was calculated as follows:

DPPH radical scavenging rate (%) = $[(A_{control} - A_{sample})/A_{control}]\times 100\%$, where the control solution contained DPPH solution and 95% alcohol. All tests were performed in triplicate and the mean of absorbance was used in the equation above.

Superoxide anion and hydroxyl radical scavenging activity

The anti-superoxide anion and hydroxyl radical assay kits were used to determine the extracts' capabilities of scavenging superoxide anion and hydroxyl radicals.

The scavenging effects were calculated by the following equation:

Scavenging effect (%) = $(1- A_{sample}/ A_{control}) \times 100\%$.

Capability of inhibiting lipid peroxidation

The inhibition of lipid peroxidation was assayed according to the method of Zhang [9] with a minor modification. Fresh liver was separated from healthy Wistar rats and 10% homogenate was made in ice bath. Different concentrations of the extracts were added into 1.5 ml of the homogenate, then 0.1 ml of H_2O_2 was added and the mixture was incubated at 37 °C for 30 min. MDA contents were assessed by measuring the absorbance value at wavelength of 532 nm.

Statistical analysis

The experimental results were subjected to variance analysis using SPSS 16.0 and expressed as mean ± SD.

RESULTS AND DISCUSSION

Total reducing power assay

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. As shown in Fig.1, the reducing power of the samples increased with increasing concentrations. Moreover, the fraction F2 exhibited the highest reducing power, followed by F3, F1 and F4 and the reducing power of ECP was closed to F3. The results indicated that the crude extract and the four polar fractions all possess an antioxidant activity and could provide a natural material for separating and purifying natural antioxidant components efficiently.

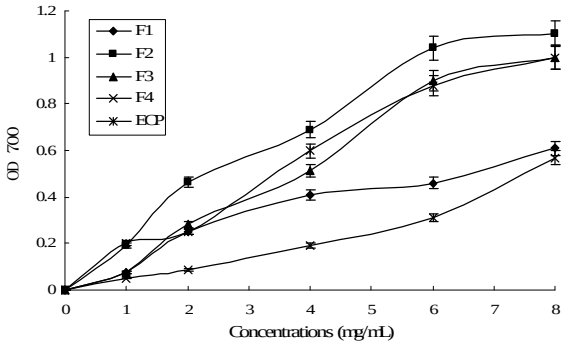

Fig. 1 Antioxidant activity of extracts from *C. paliurus* leaves

DPPH radical scavenging activity

The DPPH radical has been widely used to estimate free radical scavenging activities of antioxidants [10], such as plant extracts and natural and synthetic compounds. The DPPH radical-scavenging abilities of the extracts from *C. paliurus* leaves were examined. As shown in Fig. 2 all extracts exhibited a dose-dependent DPPH radical scavenging activities at the concentrations raging from 0 to 1 mg/mL and the inhibition is ordered as F2 > F3 > ECP > F1 > F4.

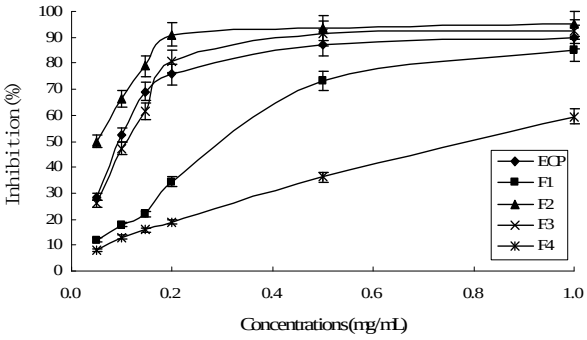

Fig. 2 Inhibitory effect on DPPH radical of extracts from *C. paliurus* leaves

Superoxide anion and hydroxyl radical scavenging activity

Superoxide anion radical was easily transformed from molecular oxygen and cause oxidative stress damage in cellular tissue [11]. Scavenging activity of superoxide anion radicals is widely used in antioxidant study. The hydroxyl radical, more likely to be produced in vivo, is considered to be the most reactive and poisonous free radical. This radical can easily cross cell membranes and cause damage to DNA and protein in living organisms [12]. In this research the anti-superoxide anion and hydroxyl radical assay kits were used with the superiority of simple and easy. The IC_{50} values of the extracts on superoxide anion and hydroxyl radicals were detected, and the results were listed in Table 1. Table 1 indicated that the extracts could well scavenge $O_2^{-}\cdot$ and HO·, and the inhibition was ordered as F2 > F3 ≥ ECP > F1 > F4. Hence, the extracts were advantageous in scavenging hydroxyl radicals

Table 1 IC_{50} of extracts from *C. paliurus* leaves on O_2^{-} and HO· ($\bar{x} \pm s$, n = 5)

	F1	F2	F3	F4	CE
O_2^{-}	11.66 ± 0.75	6.14 ± 0.01	7.24 ± 0.42	14.00 ± 0.98	8.01± 0.75
HO·	0.22 ± 0.02	0.09 ±0.01	0.11 ±0.01	0.33 ±0.02	0.11 ±0.01

Capability of inhibiting lipid peroxidation

Lipid peroxidation is an oxidative change of polyunsaturated fatty acids in the cell membranes that generates numbers of degradation products. malondialdehyde (MDA), one of the products of lipid peroxidation, has been widely studied as an indicator of lipid peroxidation and oxidatives tress[13]. As shown in Fig. 3, excellent inhibition was found for the fraction F2 which was much better than the crude extract. The inhibition was ordered as F2 > CE > F3 > F1 > F4.

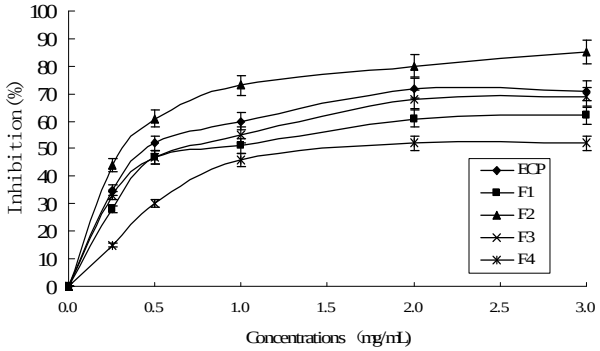

Fig. 3 Inhibitory effect on lipid peroxidation of extracts from *C. paliurus* leave

CONCLUSION

In summary, it was demonstrated that *C. paliurus* leave possesses effective antioxidant activity, which included total reducing power, free radicals clearance and lipid peroxidation inhibition, especially the ethyl acetate fraction. This part might possibly be a valuable antioxidant natural source and applicable in both medicine and food industry.

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REFERENCES

[1] R Upadhyay; JK Chaurasia; KN Tiwari; K Singh. *Appl. Biochem. Biotechnol*. **2013**, DOI 10.1007/s12010-013-0487-5.
[2] YE Jeon; YS Lee; SS Lim; SJ Kim; SH Jung; YS Bae; JS Yi; IJ Kang. *J. Korean Soc. Appl. Biol. Chem*., **2009**, 52 (5), 472-479.
[3] HL Yang; SC Chen; KY Lin; MT Wang; YC Chen; HC Huang; HJ Cho; L Wang; KJ.S Kumar; YC Hseu. *J. Ethnopharmacol.*, **2011**, doi: 10.1016/j.jep.2011.06.017
[4] HK Urihara; SA Sami; HS Hibata; H Fukami; TT Anaka. *Biol. Pharm. Bull.*, **2003**, 26 (3) 383-385.
[5] S Li; J Li ; XL Guan; J Li; SP Deng; LQ Li; MT Tang; JG Huang; ZZ Chen; RY Yang. *Fitoterapia*, **2011**, 82, 1081-1085.
[6] JH Xie; X Liu; MY Shen; SP Nie; HZ; Chang Li; DM Gong; MY Xie. *Food Chemistry*, **2013**, 136, 1453-1460.
[7] J Liu, C.-g. Meng, Y.-h. Yan, Y.-n. Shan, J. Kan, C.-h. Jin, *Int. J. Biol. Macromol.*, **2015**, http://dx.doi.org/10.1016/j.ijbiomac.2015.11.027.
[8] R Fu; YT Zhang; YR Guo; QL Huang; T Peng; Y Xu; L Tang; F Chen. *J. Ethnopharmacol*, **2013**, 147, 517-524.
[9] WJ Zhang; HX Chen; ZS Wang; GS Lan; LK Zhang. *J. Food Sci. Technol.*, **2013**, 50(6),1122– 1129.
[10] WX Zhang; D Song; D Xu; TT Wang; L Chen; JY Duan. *Carbohydr. Polym.*, **2015**, 133, 154–162.
[11] ZJ Wang; JH Xie; MY Shen; W Tang; H Wang; SP Nie; MY Xie. *Carbohydr. Polym.*, **2016**, 136, 988-994.
[12] DM Liu; JW Sheng; ZJ Li; HM Qi; YL Sun; Y Duan; WF Zhang. *Int. J. Biol. Macromol.*, **2013**, 56, 1-5.
[13] R Yazdanparast; A Ardestani. *J. Med. Food.*, **2007**,10(4), 667-674.