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Free Radical Scavenging Activities of the Extracts from *Evodia rutaecarpa*

WEN QIN[1], BIQUN ZOU[1,2], YE ZHANG[1,*], ZHONGQUAN CHENG[1] and XIANGHUI YI[1,*]

[1]Department of Chemistry, Guilin Normal College, Guangxi 541001, P.R. China
[2]College of Chemistry and Chemical Engineering, Guangxi Normal University, Guangxi 541004, P.R. China

*Corresponding authors: Fax: +86 77 32806321; Tel: + 86 77 32823285; E-mail: zhangye_81@yahoo.com.cn; yixianghui2008@yahoo.com.cn

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Under ultrasound, *Evodia rutaecarpa* herbs were extracted with acetone, ethanol, water and ethyl acetate solvents to offer acetone extract, ethanol extract, water extract and ethyl acetate extract, respectively. Their free radical scavenging activities were evaluated against DPPH, $ABTS^+$, hydroxyl and superoxide anion radicals, respectively. The results showed that all the extracts displayed good radical scavenging activities, while acetone extract even demonstrated better scavenging activity than the commercial antioxidant butylated hydroxytoluene (BHT) in $ABTS^+$ assay, with IC_{50} 4.73 μg/mL and the extract solvents were found to have important effect on their radical scavenging activities.

Key Words: *Evodia rutaecarpa*, Free radical scavenging activity, Ultrasound-assisted extract.

INTRODUCTION

With the development of separation science and technique, isolation of natural radical scavengers or antioxidants from natural plants is becoming more and more popular, since that what may be learned from natural compounds could help to overcome the toxicity problem of synthetic radical scavengers or antioxidants.

Evodia rutaecarpa, a traditional Chinese drug, which is the fruit of *Evodia rutaecarpa (Juss.) Benth* and *Evodia rutaeca (Fuss.) Benth.var. bodinie* (Dode), is mainly used in the treatment of headache, dysentery, abdominal pain, chill limbs, amenorrhage, stomachache, migrains, nausea and hypertension[1]. Modern pharmacological studies have showed that *Evodia rutaecarpa* have sedative, antiseptic, analgesic, antihypersensitive and antianxity activity[2]. Recently, its extracts were found to show good radical scavenging activity against DPPH radical in the initial test and it aroused our interest. Therefore, in this present study, the free radical scavenging activities of the extracts from *Evodia rutaecarpa*, were investigated.

Fresh berrys of *Evodia rutaecarpa* were collected from Guilin city of Guangxi Province (China) in November, 2010. Under ultrasound, the fresh berry (20 g) were extracted with acetone, ethanol, water and ethyl acetate solvents at room temperature for 1.0 h and filtered through Whatman No. 4 filter paper, respectively. Then the four extract solutions were vacuum evaporated at 50 ºC to dryness to offer acetone extract (AE), ethanol extract (EE), water extract (WE) and ethyl acetate extract (AEE) with 15.3, 23.4, 21.7 and 20.5 % yields, respectively. Acetone extract, ethanol extract, water extract and ethyl acetate extract were dissolved in ethanol to the concentrations 0.13, 0.1456, 0.11 and 0.46 mg/mL, respectively and then their UV/VIS spectrums were determined by TU-1901 ultraviolet spectrophotometer. As showed in Fig. 1, it can be found that in the UV/VIS spectra of acetone extract, ethanol extract, water extract and ethyl acetate extract there were about two main peaks, which were ascribed to be evodiamine and rutacarpine, implying that evodiamine and rutacarpine were the main components of acetone extract, ethanol extract, water extract and ethyl acetate extract[3].

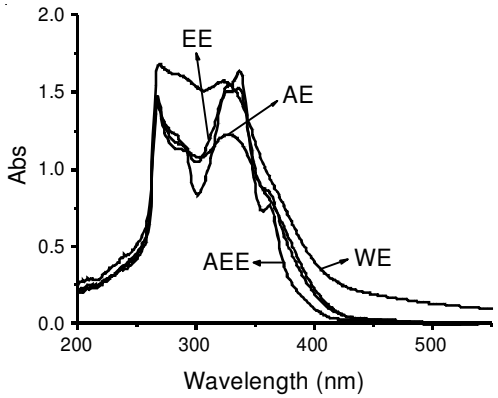

Fig. 1. UV/VIS spectrums of the acetone, ethanol, water and ethyl acetate extracts from *Evodia rutaecarpa*

It is well known that free radicals play an important role as major contributors to aging, as well as degenerative diseases of aging, such as immune system decline, cardiovascular disease, brain dysfunction, cataracts and cancer[4], so free radical scavenging activities are very important in the biological activities. The free radical scavenging activities were determined as showed in Figs. 2-5 according the literatures[5-8]. Significant free radical scavenging activities were evident at all the tested extracts. The values of IC_{50}, the effective concentration at which 50 % of the radicals were scavenged, were calculated to evaluate the radical scavenging activities (Table-1). A lower IC_{50} value indicated greater radical scavenging activity.

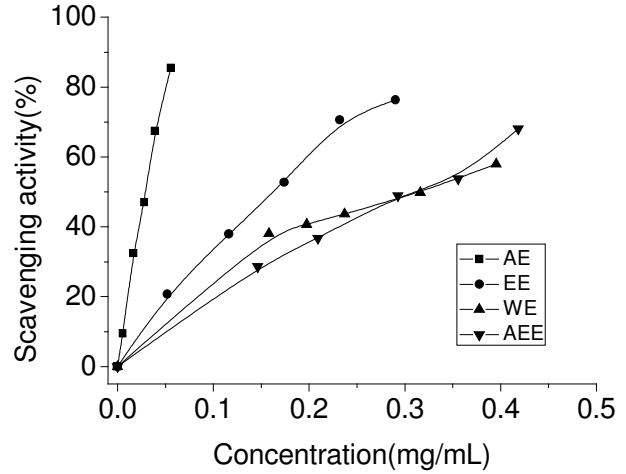

Fig. 2. DPPH radical-scavenging activities of the extracts of *Evodia rutaecarpa*. Values are means ± SD of three determinations

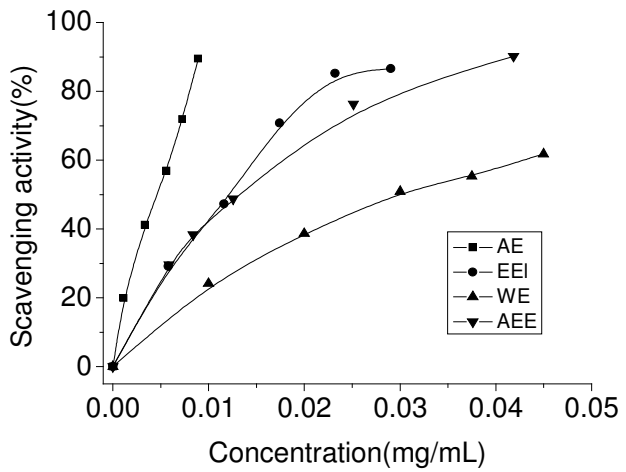

Fig. 3. ABTS radical-scavenging activities of the extracts of *Evodia rutaecarpa*. Values are means ± SD of three determinations

DPPH radical scavenging activity evaluation, which is classical assays in radical scavenging activity studies, usually offer rapid techniques for screening the radical scavenging activity of the extracts. As showed in Table-1, the IC_{50} of all the extracts were further lower than the standard value[5] 10 mg/mL[5], indicated that all the extracts exhibited potent inhibition of DPPH radical. The order of scavenging activity of tested was: acetone extract > ethanol extract > water extract > ethyl acetate extract. On the basis of this observation, it can be concluded that DPPH radical scavenging activity was greatly affected by the extract solvents.

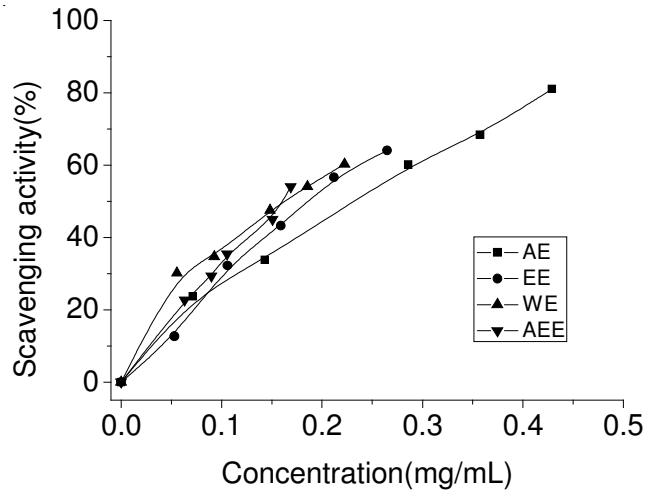

Fig. 4. Superoxide anion radical-scavenging activities of the extracts of *Evodia rutaecarpa*. Values are means ± SD of three determinations

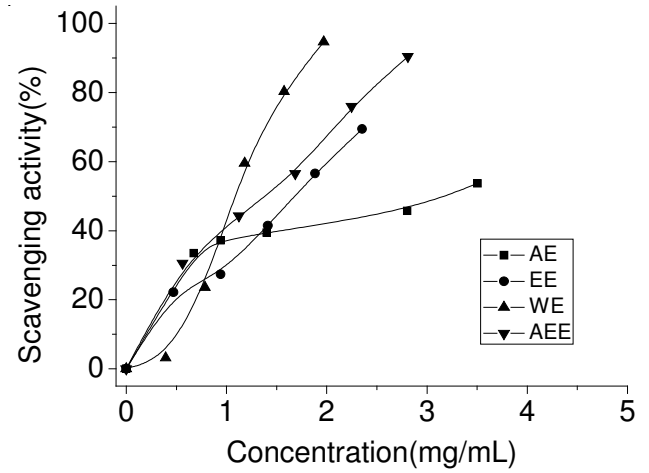

Fig. 5. Hydroxyl radical-scavenging activities of the extracts of *Evodia rutaecarpa*. Values are means ± SD of three determinations

TABLE-1
IC_{50} OF THE EXTRACTS FROM *Evodia rutaecarpa*

	AE	EE	WE	AEE	BHT
$DPPH^{\bullet}$ (μg/mL)	30.36	170.16	299.96	306.00	14.50
$ABTS^{+\bullet}$ (μg/mL)	4.73	13.49	32.48	17.41	8.150
$O_2^{-\bullet}$ (μg/mL)	245.11	193.38	166.12	158.12	ND
$OH^{\bullet}$ (mg/mL)	2.86	1.67	1.10	1.42	ND

*Not done.

$ABTS^{+}$ radical cation decolourization assay is an excellent tool for determining the antioxidant and radical activity of hydrogen-donating antioxidants and of chain breaking antioxidants[6]. The IC_{50} of acetone extract, ethanol extract, water extract and ethyl acetate extract on ABTS radical were comparable with that of commercial antioxidant butylated hydroxytoluene. It can be seen that from Table-1 all the extracts displayed good potent inhibition of $ABTS^{+}$ radical and IC_{50} of acetone extract (4.73 μg/mL) was much smaller than that of butylated hydroxytoluene (8.15 μg/mL), demonstrating that scavenging activities of acetone extract was stronger than that of butylated hydroxytoluene. Obviously, the order of scavenging activity of tested was: acetone extract > butylated hydroxytoluene > ethanol extract > ethyl acetate extract > water extract. The extract solvents also had important effect on the

$ABTS^+$ radical scavenging activity. Based on the above observation, the extracts acetone extract, ethanol extract, water extract and ethyl acetate extract should be attributed to hydrogen-donating antioxidants or chain breaking antioxidants.

Superoxide anion radical is an initial radical and plays an important role in the formation of other reactive oxygen-species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems[7]. Significant superoxide anion radical scavenging activities were evident at all the tested concentrations of the extracts. The IC_{50} (Table-1), were found to be 245.11, 193.38, 166.12 and 158.12 μg/mL for acetone extract, ethanol extract, water extract, ethyl acetate extract, respectively. Evidently, the order of the superoxide anion radical-scavenging activity was ethyl acetate extract > water extract > ethanol extract > acetone extract. The results suggested that the extracts display good scavenging effect on superoxide anion radical generation that could help prevent or ameliorate oxidative damage and the extract solvent effect on superoxide anion radical scavenging activity was less than that in $DPPH^\bullet$ and $ABTS^{+\bullet}$ assays.

The radical scavenging effects were also examined in the present study using hydroxyl radicals generated by Fenton reagent[8]. As shown in Table-1, all the extracts exhibited moderate activity in an amount dependent manner. The highest scavenging activity was found to be water extract and its IC_{50} was determined to be 1.10 mg/mL, while that of acetone extract, ethanol extract and ethyl acetate extract were 2.86, 1.67 and 1.42 mg/mL, respectively. Scavenging activities of the four extracts decreased in the order of water extract, ethyl acetate extract, ethanol extract and acetone extract. The results indicated that water extract possessed higher scavenging activities than other extracts and the extract solvents had the minimal effect on hydroxyl radical scavenging activities.

On the basis of the above observation, it could be concluded that the key components of the extracts from *Evodia rutaecarpa* were evodiamine and rutacarpine, which may contributed to their good radical scavenging activities, while the extract solvents had important effect on their radical scavenging activities.

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