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NOTE

Free Radical Scavenging Activities of Extracts from *Mulberries*

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Under ultrasound, *Mulberries* were extracted with ethanol, acetone and water solvents to offer acetone extract (AE), ethanol extract (EE) and water extract (WE), respectively. Their free radical scavenging activities were evaluated against DPPH, $ABTS^+$ and hydroxyl radicals, respectively. The results showed that all IC_{50} of acetone extract, ethanol extract and water extract were much lower than the standard value 10 mg/mL in these three assays, suggesting all these extracts displayed good radical scavenging activities. In addition, the extract solvents had important effect on their radical scavenging activities.

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

The importance of free radicals and reactive oxygen species (ROS) in the pathogenesis of various chronic diseases, such as carcinogenesis, inflammation, atherogenesis and aging, has attracted considerable attention. Free radical scavengers, as well as antioxidants are currently forged as the drug candidates to counter these diseases. Isolation of natural radical scavengers or antioxidants from natural plants has become an important method, since that what may be learned from natural compounds could help to overcome the toxicity problem of synthetic radical scavengers or antioxidants. Previous study has also demonstrated that isolation of good natural radical scavengers or antioxidants from natural plants is feasible[1,2].

Mulberries, the fruit of *Morus alba L.*, which is a traditional Chinese drug, is mainly used in the treatment of swimmy tinnitus, physically and mentally fatigued, early graying hair, thirst, diabetes with heat, diarrhea with blood deficient *etc.*[3]. Modern pharmacological studies have showed that *Mulberries* have reducing blood lipid, antimutagenic and antiviral activity[4]. However, there is no available information relating to the ultrasonic extraction, as well as the antioxidant and radical-scavenging activities of this species extracted by ultrasonic. The aim of the present study was to evaluate the antioxidant and radical-scavenging activities of ethanol extract, acetone extract and water extract of *Mulberries* that extracted by ultrasonic. The extracts were investigated by several methods establishing *in vitro*, such as 1,1-diphenyl-2-picryhydrazyl (DPPH) radical-scavenging assay, 2,2'-azino*bis*-(3-ethylbenzthiazoline-6-sulfonate) cation ($ABTS^+$) radical-scavenging assay and hydroxyl radical-scavenging assay.

Mulberries were collected from Guilin city of Guangxi Province (China) in May, 2010. Under ultrasonic, the *Mulberries* berry (20 g) were extracted with ethanol, acetone and water solvents at room temperature for 2.0 h and filtered through Whatman No. 4 filter paper, respectively. Then the three extract solutions were vacuum evaporated at 50 ºC to dryness to offer ethanol extract, acetone extract and water extract with 2.8, 2.1 and 1.9 % yields, respectively.

In vitro antioxidant activities were measured against DPPH[5], $ABTS^{+6}$ and hydroxyl radicals[7], respectively, according to the literatures[5-8] with a little modification. The values of IC_{50}, the effective concentration at which 50 % of the radicals were scavenged, were calculated to evaluate the antioxidant activities. A lower IC_{50} value indicated greater antioxidant activity. IC_{50} values of lower than 10 mg/mL usually implied effective activities in antioxidant properties[5]. The tested results were shown in Figs. 1-3 and Table-1.

DPPH radical scavenging activity evaluation, which could offer rapid techniques for screening the radical scavenging activity of the antioxidants or radical scavenger, is a classical assay in radical scavenging activity studies. As showed in Fig. 1 and Table-1, IC_{50} of ethanol extract (EE), acetone extract (AE) and water extract (WE) were found to be 0.17, 0.18 and 0.19 mg/mL, respectively. Evidently, they were further lower than the standard value 10 mg/mL[5], indicating that all the extracts exhibited good potent inhibition of DPPH radical. The order

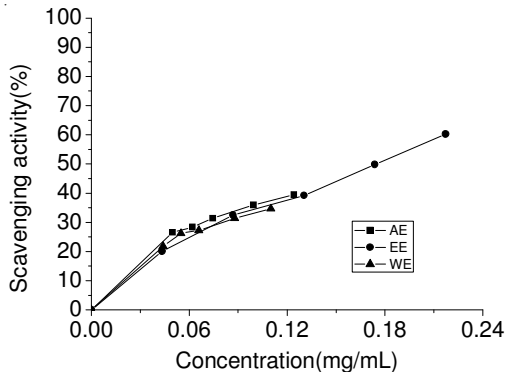

Fig. 1. DPPH radical-scavenging activities of the extracts of *Mulberries*. Values are means ± SD of three determinations

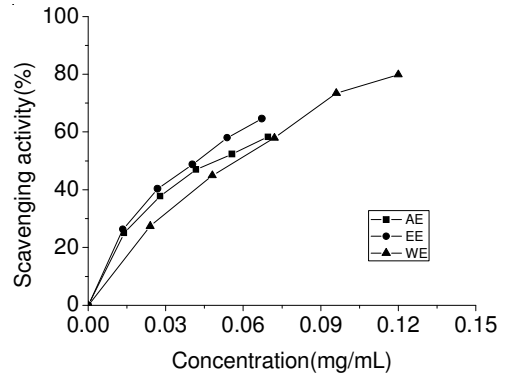

Fig. 2. $ABTS^+$ radical-scavenging activities of the extracts of *Mulberries*. Values are means ± SD of three determinations

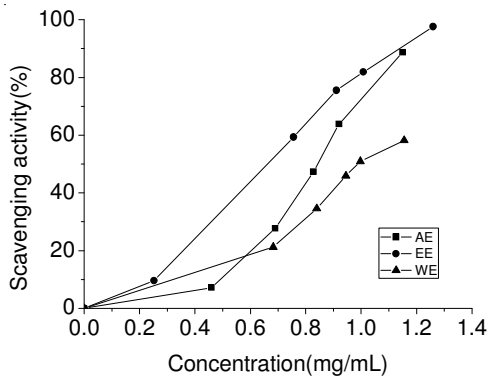

Fig. 3. Hydroxyl radical-scavenging activities of the extracts of *Mulberries*. Values are means ± SD of three determinations

TABLE-1
IC_{50} OF THE EXTRACTS FROM MULBERRIES (mg/mL)

	Acetone extract	Ethanol extract	Water extract
$DPPH^{\bullet}$	0.18	0.17	0.19
$ABTS^{+\bullet}$	0.052	0.044	0.060
$OH^{\bullet}$	0.83	0.67	1.02

of scavenging activity of tested was: ethanol extract > acetone extract > water extract. The above observation suggested that the extract solvents have minimal influence on DPPH radical scavenging activity.

$ABTS^+$ radical evaluation assay is another excellent tool for determining the antioxidant and radical scavenging activity[6]. IC_{50} of acetone extract, ethanol extract and water extract on $ABTS^+$ radical were found to be 0.052, 0.044 and 0.060 mg/mL (Fig. 2 and Table-1) and much lower than 10 mg/mL[5], demonstrating their good potent inhibi-tion of $ABTS^+$ radical. Clearly, the order of scavenging activity of tested was: ethanol extract > acetone extract > water extract. Based on the above observation, it could be concluded that the extract solvents had more important influence on the $ABTS^+$ radical scavenging activity than on $DPPH^{\bullet}$.

The radical scavenging activities were also tested in the present study using hydroxyl radicals generated by Fenton reagent[8]. As shown in Fig. 3 and Table-1, all the extracts exhibited good activity in an amount dependent manner and their IC_{50} values were much lower than 10 mg/mL[5], indicating their good radical scavenging activities on hydroxyl radicals. The highest scavenging activity was found to be ethanol extract and its IC_{50} was determined to be 0.67 mg/mL, while that of acetone extract and water extract were found to be 0.83 and 1.02 mg/mL, respectively. Obviously, scavenging activities of the three extracts decreased in the order of ethanol extract, acetone extract and water extract. The results indicated the extract solvents had important effect on hydroxyl radical scavenging activities.

Conclusion

The above studies showed that the extract solvents had important effect on their radical scavenging activities. In all the three assays, ethanol extract (EE) showed the best scavenging activity, while acetone extract (AE) displayed moderate and water extract (WE) demonstrated the lowest, respectively. On the basis of the above studies, it could be summarized that it is feasible to isolate natural radical scavengers or antioxidants from *Mulberries*.

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