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Biological Activities of the Whole Grass Extracts from *Amaranthus viridis* L.

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Amaranthus viridis L. is a traditional medicine in Asia. In this study, the antioxidant activities of petroleum ether, ethyl ether, ethyl acetate, *n*-butanol and water extracts of *A. viridis* L. were evaluated with various antioxidant assays, including DPPH (1,1-diphenyl-2-pricrylhydrazyl) free radical scavenging, xanthine oxidase inhibitory, hydroxyl radical scavenging, lipid peroxidation scavenging and protein damage assay. All the extracts had strong antioxidant activity in the DPPH scavenging, lipid peroxidation scavenging and xanthine oxidase inhibitory. The petroleum ether extract had the highest hydroxyl radical scavenging activity. Its activity was equivalent to quercetin at 0.25 mg/mL. Inhibition effect on α-glucosidase of 0.5 mg/mL extract was also evaluated and the petroleum ether fraction had the highest inhibitory activity. Its activity was slightly lower than that of acarbose at 0.5 mg/mL, indicating that *A. viridis* L. extracts may have antidiabetic activity. Antimicrobial activity (10 mg/mL extract) was also evaluated and the ethyl ether extract had the highest antimicrobial activity. Its activity was slightly lower than that of cefalexin, indicating that *A. viridis* L. extracts may have antimicrobial activity. These results suggest that *A. viridis* L. may have health-enhancing effects.

Key Words: *Amaranthus viridis* L., Antioxidant activity, α-Glucosidase inhibitory activity, Antimicrobial activity.

INTRODUCTION

Amaranthus plants (Amaranthaceae) are spread throughout the world, growing under a wide range of climatic conditions and they are known as grains and leafy edible vegetables[1]. The leaves of amaranth constitute an inexpensive and rich source of protein, vitamin C, carotenoids and dietary fibre[2], minerals like calcium, zinc, iron, phosphorus[3,4] and magnesium[2].

Amaranthus viridis L. (Amaranthaceae) has been used in Chinese traditional system. Traditionally, the plant parts of *A. viridis* L. were used in the treatment of dysentery, enteritis, hemorrhoids and kidney diseases. *A. viridis* L. has been reported for its antiinflammatory, antidiabetic, antihyperlipedemic and antioxidant activities[5,6]. A novel extract including an antifungal and antiproliferative lectin, β-carotene and a ribosome inactivating protein was isolated from *A. viridis* that possess antiviral activity[7-10]. As a vegetable, the young leaves of *A. viridis* are used. However, research on the whole grass was not conclusive. Thus, in this study, we evaluated the antioxidant activities of various extracts using *in vitro* assays, including DPPH (1,1-diphenyl-2-pricrylhydrazyl) free radical scavenging, hydroxyl radical scavenging, lipid peroxidation scavenging, xanthine-xanthine oxidase assays and protein damage assay, as well as α-glucosidase inhibition assays and antimicrobial activity.

EXPERIMENTAL

Preparation of extracts: *A. viridis* L. were collected from Yangzhou and identified by Professor H.Y. Huai (College of Bioscience and Biotechnology, Yangzhou University, China). 250 g dried sample of the powder was subjected to a methanol extraction process for 3 days, produced a crude extract of 16.6 g. The crude extract was extracted sequentially with petroleum ether, ethyl ether, ethyl acetate and *n*-butanol. The final yields of petroleum ether fraction, ethyl ether fraction, ethyl acetate fraction, *n*-butanol fraction and water fraction were 28.9, 3.1, 9.0, 6.0 and 53.6 %, respectively.

DPPH radical scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the extracts were determined by Kilani *et al.*[11] with some modifications. Aliquots (4.0 mL) of varying concentration of the

extract were mixed with freshly prepared DPPH in methanol (final concentration 100 μM) and the absorbance at 514 nm was measured after incubation for 0.5 h in the dark at room temperature. Distilled water was used as control and ascorbic acid as reference compound. Each dilution was assayed in triplicate, the three readings were averaged and the percentage inhibition of activity was calculated as:

$$\left(1-\frac{A_{sample}}{A_{control}}\right)\times 100\%$$

Superoxide anion generation by xanthine-xanthine oxidase assay: Superoxide anion generation by the xanthine-xanthine oxidase system and measured by Choi *et al.*[12] with some modifications. The reaction mixture in a total volume of 5 mL contained 50 mM potassium phosphate buffer (pH 7.8), xanthine (2 mM) and NBT (1.8 mM). Varying concentrations of the extract, in ethanol was added into the mixture. The reaction was initiated by the addition of xanthine oxidase (25 mU/mL) in the same phosphate buffer and the absorbance at 560 nm was measured after incubation for 0.5 h at room temperature. The absorbance (Ab) of formazan chromophore was measured against a blank solution in which xanthine oxidase was replaced by buffer solution.

Hydroxyl radical scavenging activity: The effect of extracts on hydroxyl radicals was determined by using the deoxyribose method[13].

Linoleic acid peroxidation assay: The effect of extracts on linoleic acid peroxidation was assayed by using the Choi *et al.*[12] method.

Protection from protein damage: The effect of different fractions on protein oxidation was carried out according to the method of Hu *et al.*[14].

Rat intestinal α-glucosidase inhibitory activity: α-Glucosidase inhibitory activity was assayed by Kim *et al.*[15].

Agar diffusion assay: The agar diffusion assay was done as described by Ye and Ng[16]. Inhibitory activity was scored visually as follows: –, no inhibition; +, very weak inhibition; ++, low inhibition with a little clear zone near the rim of the colony; +++ strong inhibition, with large clear zone near the rim of the colony and ++++, very strong inhibition, with no growth near the rim of the colony.

Statistical analyses: All tests were carried out independently in triplicate (n = 3). Data are expressed as the mean ± standard derivation (SD). The results were processed using Excel 2003 (Microsoft, Redmond, WA, USA)

RESULTS AND DISCUSSION

Amaranthus plants have been reported as one of many vegetables which are rich in antioxidant components. Carotenoids, ascorbic acid, flavonoids and phenolic acids might be some of the components able to contributing to their antioxidant activity[17].

Xanthine-xanthine oxidase assay: Xanthine oxidase catalyses the oxidation of hypoxanthine or xanthine to uric acid, during the oxidation an equivalent rate of superoxide radical is produced[18]. A xanthine oxidase inhibitor without any additional superoxide scavenging activity will produce the reduction in the rate of NBT. The superoxide radical scavenging activities of different extracts are shown in Table-1. The order of superoxide radical scavenging activity was: ethyl acetate > ethyl ether > *n*-butanol > water > petroleum ether. As shown in Table-1, the maximum inhibition of NBT reduction with ethyl acetate fraction (60 %) and minimum inhibition of NBT reduction with the petroleum ether fraction (42 %) was reached at 250 μg/mL.

TABLE-1
INHIBITORY ACTIVITY OF XANTHINE OXIDATION AND LINOLEIC ACID PEROXIDATION AND HYDROXYL RADICAL AND SCAVENGING CAPACITY OF DPPH BY PLANT FRACTIONS*

Fractions	DPPH	Xanthine oxidation	Hydroxyl radical	Linoleic acid peroxidation
Petroleum ether	70.3	42.0	84.8	29.3
Ethyl ether	80.4	56.0	64.0	38.4
Ethyl acetate	78.9	60.0	56.0	33.3
n-Butanol	50.7	48.4	53.0	33.9
Water	54.3	48.1	36.0	26.6
Quercetin	100.0	77.0	85.0	63.7

*Per cent of inhibition and scavenging at 250 μg/mL as a mean of triplicate experiments.

Hydroxyl radical scavenging activity: Hydroxyl radical is recognized as a DNA-damaging agent with physiological significance. DNA is a sensitive biotarget for free radical-mediated oxidative damage[19]. Oxidative DNA lesions, which accumulate with age, are important contributors to the aging process. As shown in Table-1, the fractions from *A. viridis* L. exhibited inhibition of hydroxyl radicals. The petroleum ether and ethyl ether fractions of *A. viridis* L. showed considerable hydroxyl radical scavenging activity. At 0.25 mg/mL, the petroleum ether and ethyl ether fractions quenched 85 and 64 % of the hydroxyl radicals in the reaction mixture, respectively, which was comparable to quercetin which quenched 85 % at 0.25 mg/mL.

DPPH radical scavenging activity: It was reported that DPPH is the method of choice for evaluating the free radical scavenging activity of natural compounds[20]. As shown in Table-1, the maximum inhibition of DPPH radicals by ethyl ether fraction (80.4 %) and minimum inhibition of DPPH radicals by the *n*-butanol fraction (50.7 %) was reached at 250 μg/mL. These results were consistent with the previous observation that *Amaranthus* varieties[21] contained radical scavenging agents that could directly react with and quench stable DPPH radicals. The ability of an *A. paniculatus* extract to act as a free radical scavenger or hydrogen donor was reported[17]. Moreover, the antioxidative properties of the ethyl acetate extract of *A. lividis* on scavenging DPPH were found to be superior to those of *A. cruentus*[21].

The DPPH scavenging activities of the extracts, expressed as an SC_{50} value, ranged from 70 to 144 μg/mL. The ethyl acetate extract exhibited the strongest antioxidant activity (SC_{50} value of 70 μg/mL), followed by the ethyl ether extract (SC_{50} value of 74 μg/mL) and the *n*-butanol extract (SC_{50} value of 144 μg/mL) which showed the weakest activity. The *A. lividis* extracts showed stronger DPPH radical scavenging activities compared to the DPPH radical scavenging activity of the methanolic extract of *A. retroflexus* (IC_{50} value of 92.7 μg/mL)[22].

Linoleic acid peroxidation inhibitory activity: ROS produced within the cell might be harmful, for example attacking polyunsaturated fatty acids and damaging the membrane lipid bilayer[23]. Linoleic acid is a component of the cellular membrane structure[24]; it was chosen to test the capacity of the extracts to inhibit lipoperoxidation because it is an essential fatty acid afforded by food. Table-1 shows that the extracts of *A. viridis* L. exhibited inhibition of linoleic acid peroxidation. The inhibition of linoleic acid peroxidation was assayed by the TBARS test. All the extracts, demonstrated linoleic acid peroxidation inhibitory activity and among those, ethyl ether extract was the most efficient, while water fraction was the least efficient (Table-1). It is noteworthy that the ethyl ether extract produced the best inhibitory effect of linoleic acid peroxidation among *A. viridis* L. extracts, which was also very active to scavenge DPPH free radicals and to inhibit xanthine-xanthine oxidase reaction.

Protection against protein damage: The oxidative protein damage induced by free radicals has been shown to play a significant role in several pathological events and in aging[25]. Hydroxyl radical is recognized as a protein-damaging agent with physiological significance. The protein damage was induced by the Fe^{3+}/H_2O_2/ascorbic acid system. The ethyl ether and ethyl acetate extracts restored the BSA band intensity, ethyl acetate extract showed slightly greater protein protection than that of ethyl ether extract (Fig. 1).

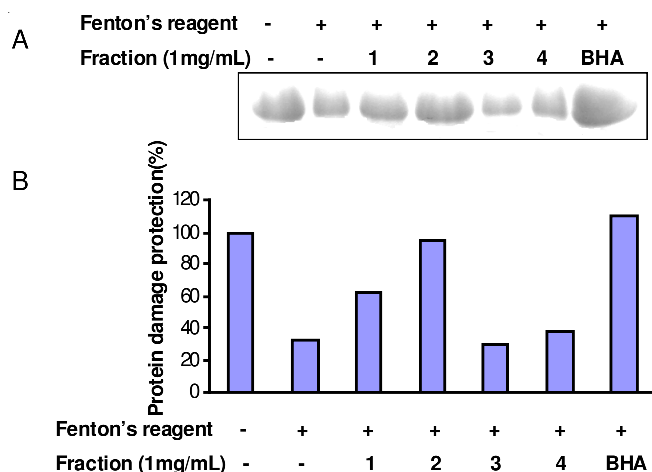

Fig. 1. (A) SDS-PAGE profile of the BSA protein treated with Fe^{3+}/H_2O_2/ ascorbic acid system in the presence of the fractions of *A. viridis* L. (1 mg/mL). (B) Histogram showing the protective effect densitometric measurements. Lanes 1-4, protein incubated with Fenton's reagent in the presence of ethyl ether extract, ethyl acetate extract, petroleum ether extract and *n*-butanol extract

Antimicrobial activity of *A. viridis* L. fractions: As shown in Fig. 2, the *A. viridis* L. extracts exhibited the antimicrobial activity towards the different microbials (*Escherichia coli, Staphyloccocus aureus* and *Salmonella sp.*). The activity of the *A. viridis* L. fractions was evaluated by agar diffusion assay against several pathogenic bacteria (Table-2). Among the 3 species of microbial tested, ethyl ether extract showed the strong inhibitory activity and ethyl acetate extract showed the low inhibitory or weak inhibitory activity. A 30-residue antimicrobial peptide Ar-AMP was isolated from seeds of *A. retroflexus* that inhibited the growth of *Helinthosporum sativum, Fusarium culmorium, Botrytis cinerea* and *Alternaria consortiale*[26]. In this study, the ethyl ether extract of *A. viridis* L. showed marked antimicrobial activity.

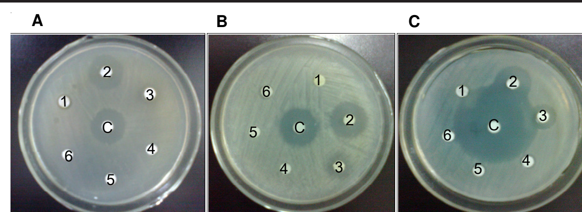

Fig. 2. Inhibitory activity of plant extractions toward *Escherichia coli* (A), *Staphyloccocus aureus* (B) and *Salmonella sp.* (C). (1) Petroleum ether extract; (2) Ethyl ether extract; (3) Ethyl acetate extract; (4) *n*-Butanol extract; (5) Water extract; (6) Methanol; (C) Cefalexin (concentration 10 mg/mL)

TABLE-2
INHIBITORY SPECTRUM OF FRACTION OF AMARANTH, AS DETERMINED BY AGAR DIFFUSION ASSAY

Fungi	Inhibitory activity					
Escherichia coli	–	+++	+	–	–	+++
Staphyloccocus aureus	–	+++	+	–	–	+++
Salmonella sp.	–	+++	++	–	–	++++++

α-Glucosidase inhibition: Inhibitors of intestinal α-glucosidase are used in the treatment of type 2 diabetes[27]. *A. viridis* L. has nutritional and functional properties that probably need a more in depth investigation. To determine if *A. viridis* L. possess α-glucosidase inhibitory activity, we studied the effect of *A. viridis* L. extracts in α-glucosidase inhibition assays. All the extracts, except the *n*-butanol and water extracts, demonstrated α-glucosidase inhibitory activity (Table-3) which increased steadily with increasing sample concentration. In particular, the petroleum ether extract at a concentration of 500 μg/mL inhibited α-glucosidase activity by 65 %. This result was slightly lower than that of acarbose (α-glucosidase inhibitor), at 100 μg/mL. Most of the extracts of *A. viridis* L. had α-glucosidase inhibitory activity, indicating that they contain effective α-glucosidase inhibitors that may have potential antidiabetic activity.

TABLE-3
EFFECT OF DIFFERENT EXTRACTS OF *Amaranthus viridis* L. ON RAT INTESTINAL α-GLUCOSIDASE INHIBITION ASSAY*

Extracts	Concentration (μg/mL)	Inhibition (%)
Petroleum ether	250	40.0 ± 1.9
	500	65.0 ± 2.2
	1000	92.5 ± 2.3
Ethyl ether	250	22.5 ± 1.9
	500	35.0 ± 3.2
	1000	55.0 ± 1.2
Ethyl acetate	250	20.0 ± 2.2
	500	30.0 ± 1.8
	1000	55.0 ± 3.2
n-Butanol	250	10.0 ± 1.1
	500	13.7 ± 2.1
	1000	26.2 ± 4.3
Water	250	5.7 ± 0.3
	500	12.8 ± 1.9
	1000	25.0 ± 2.7
Acarbose	500	99.15 ± 1.1

*Acarbose at the concentration of 500 μg/mL was used as control. Values represent the mean ± SD (n = 3).

Conclusion

The extracts of *A. viridis* L. showed different biological activities. The extracts had different levels of antioxidant activity in different assays (including superoxide anion scavenging, hydroxyl radical scavenging, linoleic acid peroxidation scavenging, DPPH free radical scavenging and protein damage protection assay). The ethyl ether and ethyl acetate extracts exhibited higher anti-α-glucosidase activity and antimicrobial activity. These results indicate that *A. viridis* L. could be considered as a functional food ingredient and pharmaceutical.

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