African Journal of Pharmacy and Pharmacology Vol. 6(45), pp. 3118-3123, 8 December, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.408
ISSN 1996-0816 © 2012 Academic Journals

Full Length Research Paper

Antioxidant and *a*-glucosidase inhibitory activity of red raspberry (Harrywaters) fruits *in vitro*

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Accepted 28 September, 2012

[2,2-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid] Diammonium salt (ABTS) radical scavenging and ferric reducing antioxidant power (FRAP) assay were used to analyze the antioxidant activity of red raspberry (Harrywaters, HW) fruits with 2,6-Di-tert-butyl-4-methylphrnol (BHT) as positive controls and α-glucosidase inhibitory activity of HW extracts with acarbose as positive control *in vitro*. HW fruits had free radical scavenging activity and iron-reduction activity. ABTS radical scavenging activity of petroleum ether extract (HWPE), ethyl acetate extract (HWEA) and *n*-butanol extract (HWBU) with IC_{50} values of 54.09, 57.88 and 47.74 μg/mL, respectively, were weaker than that of BHT (IC_{50} = 7.72 μg/mL). HWPE exhibited higher ferric reducing antioxidant power with 367.57 ± 2.77 μmol/g as Trolox equivalent than that of HWEA and HWBU, but weaker than that of BHT with1581.68 ± 97.41 μmol/g as Trolox equivalent. HWEA (IC_{50} = 67.7 μg/mL) had the best α-glycosidase inhibitory activity, followed by HWPE (IC_{50} = 163.1 μg/mL) and HWBU (IC_{50} = 292.2 μg/mL). Their α-glycosidase inhibitory activity were far higher than that of acarbose (IC_{50} = 1103.01 μg/mL) as positive control. Results indicated that HW had a certain extent antioxidant activity and good α-glycosidase inhibitory activity *in vitro*, and active compounds should be isolated and identified in the next work.

Keywords: Red raspberry (Harrywaters), antioxidant activity, α-glucosidase inhibitory activity.

INTRODUCTION

Raspberry (*Rubus* spp), belonging to the Rosaceae family, is a high nutritional and medicinal value fruit (Wang, 2001). Its fruits, seeds, roots and leaves can be used as medicine, which have the effects of heat-clearing and detoxicating, expelling wind and dampness, benefiting kidney and fixing sperm (Patel et al., 2004).

Phytochemical research showed that the main chemical compositions had phenols including anthocyanidin, flavones and ellagic acid (Versari et al., 1997), ursolic acid analogues (Bowen-Forbes et al., 2009), ketone (Gallois, 1982) and volatility compounds (Wang, 2003). Pharmacological investigations showed that raspberry had a variety of biological effects, such as anti-inflammatory, anti-bacterial (Ryan et al., 2001), anti-cancer (Ravai, 1996; Zhang et al., 2007), hypoglycemic (Wang et al., 2003), antiobesity (Morimoto et al., 2005), antiproliferation (Juranic et al., 2005) and antioxidation (Viljanenk et al., 2004; Gülçin et al., 2011).

The antioxidant activity of red raspberry fruits concerning inhibiting lipid oxidation, scavenging hydroxyl and DPPH radical have been reported, for example, extracts of red raspberry can inhibit lipid oxidation *in vitro* (Viljanenk et al., 2004). Anthocyanins from red raspberry fruit have good effects of scavenging hydroxyl radical and superoxide anion radical, and also have some inhibiting lipid peroxides effect in lard (Sun et al., 2009). Ellagic acid in the red raspberry has strong reducing power, it can effectively inhibit the oxidation of lard and scavenge hydroxyl radicals and DPPH radical (Li et al., 2010). To the best of our knowledge, scavenging ABTS radical and ferric reducing antioxidant activity and α-glucosidase inhibitory activity of red raspberry (Harrywaters) have not been reported *in vitro*, and therefore, our results can be

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evaluated for the first time about the activity of red raspberry (Harrywaters) *in vitro*.

MATERIALS AND METHODS

Plant

Harrywaters fruits (HW) were collected in Fengqiu County, Henan Province, China, in August 2011 and identified by Associate Professor Changqin Li. Voucher specimen was deposited in the Institute of Chinese Materia Medica, Henan University.

Chemicals

2,6-Di-tert-butyl-4-methyiphenol (BHT) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) from Acros organics, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) from Fluka, α-Glucosidase, 4-Nitrophenyl-α-D-glucopyranoside (PNPG) and acarbose were purchased from the United States Sigma company, Dimethyi sulfoxde (DMSO) from Tianjin Rich yu fine chemical co., LTD.

Preparation of extracts

The fresh Harrywaters fruits (2.75 kg) were extracted three times with methanol at room temperature, every time for 3 days, after evaporation of solvent *in vacuo* to get the total extract. The total extract was suspended in water and extracted with petroleum ether, EtOAC (ethyl acetate) and *n*-BuOH (*n*-butanol), respectively. The solution was concentrated under reduce pressure to yield petroleumether extract 109.2 mg (HWPE), EtOAC extract 8.2 g (HWEA) and *n*-Butanol extract 100.2 g (HWBU).

Antioxidant activity using ABTS assay

ABTS assay was carried out as described by Wei et al., (2012), Kang and Wang (2010) and Gülçin (2011). Extracts were made into 2.0 mg/mL as preliminary screening concentration with methanol, and diluted into in turn 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/mL. 0.15 mL sample were mixed with 2.85 mL ABTS radical working liquid. After 10 min response period at room temperature in dark, the absorbance of the resulting solution and blank (with same chemicals, except for the sample) were recorded against BHT as positive control. Absorbance of ABTS was measured at 734 nm using UV-2000 type Ultraviolet-visible Spectrophotometer. ABTS radical scavenging rate was calculated in the following way:

ABTS radical scavenging rate (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Where A_{blank} is the absorbance of ABTS itself and A_{sample} is the absorbance of the samples on ABTS.

According to the above scavenging rates, IC_{50} value was calculated by origin 6.0 software, which represented the concentration of the extracts (tested samples) that caused 50% reduction of ABTS radical.

Ferric reducing ability of plasma (FRAP) reducing activity assay

FRAP assay for extracts was determined according to the method of Kang et al. (2011) and Gülçin et al. (2010). Extracts were made into a series of concentrations using methanol: 2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL. 0.2 mL samples were mixed with 3.8 mL TPTZ working liquid freshly preparated. After 30 min response period at 37°C, the absorbance of the resulting solution were recorded against BHT as the positive control. Absorbance was measured at 734 nm using UV-2000 type Ultraviolet-visible Spectrophotometer. At the same time with trolox as reference, results were expressed in μmol Trolox equivalents (TE)/g sample.

α-Glucosidase inhibition assay

Making standard curve

According to the reaction system, 1000 μmol/L PNP was prepared in phosphoric acid buffer with pH value of 6.8, and then made into seven different concentrations: 400, 300, 200, 150, 100, 50, 25 and 5.0 μmol/L. Seven different concentrations were taken in 160 μL, respectively, mixed with 80 μL of 0.2 mol/L Na_2CO_3 solution. Resulting solution absorbance was measured at 405 nm using Multiskan MK3 type microplate reader from United States Thermo Electron company. In the end, a group set of three parallels took the mean values of absorbances, absorbance values as the vertical axis and PNP concentrations as abscissa were used to make the standard curve.

Determination of α-Glucosidase activity

According to the reaction system, 112 μL of potassium phosphate buffer (pH 6.8), 20 μL of 0.2 U/mL α-glucosidase and 8 μL of DMSO were mixed. After 15 min response period at 37°C in LCH-150 Biochemical Incubator from Shanghai-Heng Technology Co., Ltd. 20 μL of 2.5 mmol/L PNPG prepared in the same buffer was added to the above reaction system, mixed and continued to react for 15 min at 37°C. Finally, after the 80 μL of 0.2 mol/L Na_2CO_3 solution was added, absorbance values were measured at 405 nm. The definition of enzyme activity unit was determined under the conditions of 37°C and pH value of 6.8, as hydrolysis of the substrate to produce 1 μmol of nitrophenol per minute that required the amount of enzyme.

Test method

The extracts of HW were dissolved in DMSO, and stored at 4°C in refrigerator. The α-glucosidase inhibitory activity of the extracts was assessed according to the 96 microplate screening method reported by Kang et al. (2011) and Kang and Wang (2010). Absorbance (A) was detected at 405 nm. Enzymatic inhibition data were expressed as IC_{50} values (concentration of inhibitor required for 50% inhibition against *α*-glucosidase). The inhibitory rates (%) were calculated according to the formula: $[1 - (OD_{test} - OD_{blank}) / (\text{control } OD_{test} - \text{control } OD_{blank})] \times 100\%$. All reactions were carried out with three replications. Acarbose was used as positive control.

RESULTS

Antioxidant activity

Antioxidant activity of extracts from Harrywaters fruits and positive control that determined by ABTS and FRAP assays were showed in Table 1 and Figure1. In ABTS assays, in the same screening concentration at 100 μg/mL, the scavenging rates of HWPE, HWEA and

Table 1. Antioxidant activity of the different extracts of Harrywaters fruits.

Extracts	ABTS assay		FRAP assay
	Scavenging rate (%)	IC_{50} (μg/mL)	Trolox equivalent (μmol/g)
HWPE	78.62±0.071	54.09	367.57±2.77
HWEA	69.07±0.36	57.88	258.9±11.69
HWBU	84.1±0.85	47.74	177.23±4.20
BHT		7.72	1581.68±97.41

BHT is positive control.

Table 2. The α-glucosidase inhibitory activity of extracts.

Extracts	Preliminary screening		IC_{50} (μg/mL)
	Concentrations (μg/mL)	Inhibition rates (%)	
HWPE	1500	98.64±0.66	163.1
HWEA	1500	99.42±0.31	67.7
HWBU	1500	99.18±1.0	292.2
Acarbose	1500	57.26±0.39	1103.01

Acarbose is positive control.

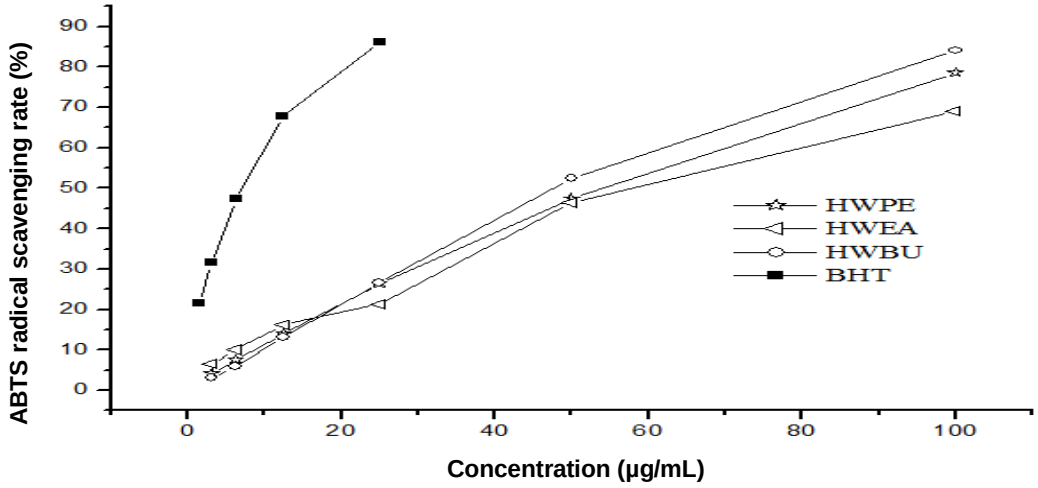

Figure 1. Effect of concentrations of extracts on ABTS free radical.

HWBU were 78.62 ± 0.071, 69.07 ± 0.36 and 84.1 ± 0.85%, which were greater than 50%, the antioxidant activity of three extracts (IC_{50} = 54.09, 57.88 and 47.74 μg/mL, respectively) were weaker than that of BHT (IC_{50} = 7.72 μg/mL).

In Figure 1, the ABTS radical scavenging rates of three extracts were lower than that of BHT. In the experiment concentration range, ABTS radical scavenging rates of three extracts had positive dose-effect relationship with concentrations, that is, with the increasing of the extracts concentrations, the ABTS free radical scavenging rates increased. In Table 1, results indicated that Ferric reducing activity of HWPE (Trolox Equivalent = 367.57 ± 2.77 μmol/g) was the strongest in the three extracts, but was still lower than that of BHT (Trolox Equivalent = 1581.68 ± 97.41 μmol/g).

α-Glucosidase inhibition activity

In Figure 2, α-glucosidase inhibition rates of three extracts of HW were far greater than that of acarbose as positive control with the same concentration at 1500 μg/mL in the preliminary screening, and the inhibition rates of three extracts were close to each other. Table 2 showed that HWEA (IC_{50} = 67.7 μg/mL) had the best α-

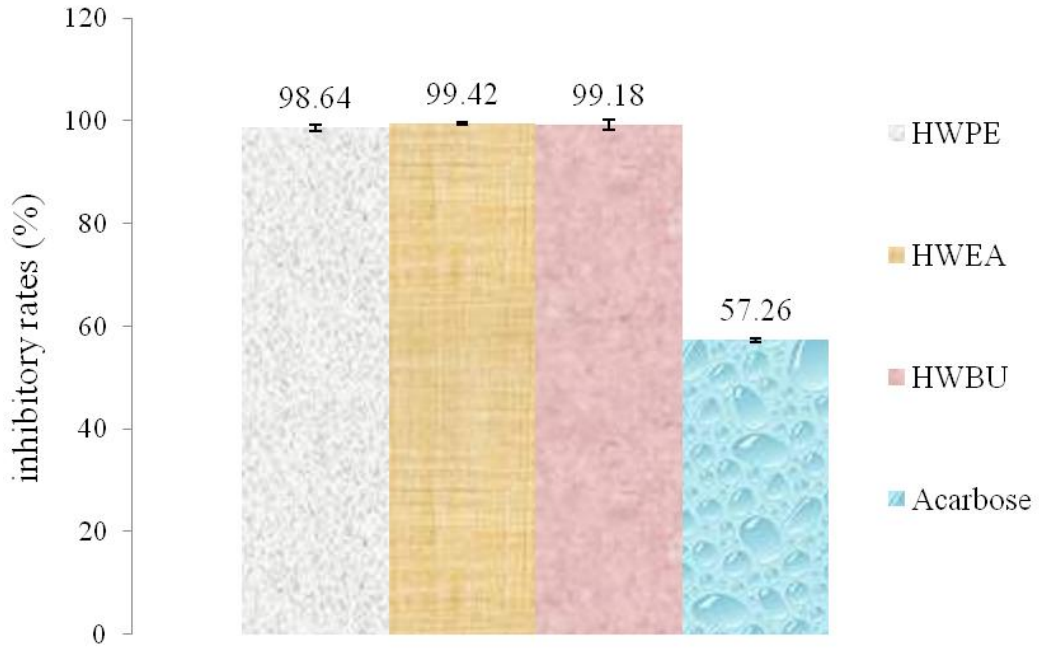

Figure 2. The comparison of α-glucosidase inhibitory activity of extracts

glycosidase inhibitory activity, followed by HWPE (IC_{50} = 163.1 μg/mL) and HWBU (IC_{50} = 292.2 μg/mL). Their inhibitory activity were far higher than that of acarbose (IC_{50} = 1103.01 μg/mL). Figure 3 showed that when the concentration was lower than 0.1875 mg/mL, α-glucosidase inhibition activity of HWPE and HWE had dose-dependent manner. With the concentration increasing, the inhibition rate was also increasing. Thereafter, with the concentration increasing, the inhibition rate increased slowly, which were close to saturation. In any concentration, the inhibition rate of HWEA was larger than that of HEPE and HWBU.

DISCUSSION

Active oxygen and in particular, free radicals, are considered to induce oxidative damage in biomolecules and to play an important role in aging, cardiovascular diseases, cancer, and inflammatory diseases. In addition, they are also well known to be major causers of material degradation and food deterioration (Kang et al., 2011; Tohma and Gülçin, 2010). Consequently, antioxidants are now known to be prospective protective or therapeutic agents.

Anthocyanins, flavonoids and tannins are the main components of the phenolic compounds in red raspberry. Anthocyanins have strong antioxidant capacity and the inhibition of lipid peroxidation (Jeong et al., 2010; Gülçin et al., 2005). Flavonoids and tannins have the effect of antioxidant and scavenging oxygen free radical (Wang et al., 2011), but for the red raspberry (Harrywaters) produced in Fengqiu, Henan, whether contains phenolic compounds, needs to be studied further.

ABTS and FRAP methods are based on the spectrophotometric determination to determine antioxidant activity of extracts which are widely used for determination of total antioxidant activity *in vitro* from different angles (Gülçin, 2012). The nature of ABTS method does not directly reflect the activity of the tested substances, it is only used to characterize the ability of reaction of the test samples and oxidation of ABTS, rather than blocking the oxidation process; actually, the actual determination of the FRAP method is the ability of ferric restoring divalent iron (Liu et al., 2009). Therefore, the combination of two methods can more fully reflect the antioxidant activity. Because of the different reaction mechanisms and reaction conditions, the antioxidant result of two methods is different. It can be seen that the antioxidant activity of plants is related to plant sources, the type and polarity of extraction solvent, and also related to the measurement system (Kang et al., 2003).

α-Glucosidase inhibitors can be competitive to inhibite α-glucosidase activity in the small intestine, and can slow down or inhibit the absorption of glucose in the intestine. Thus, it can effectively reduce the peak of post-prandial glycemia, adjust blood sugar levels, reduce the stimulation of high glucose on the pancreas, improve insulin sensitivity, protect the pancreas function and effectively prevent and improve the occurrence and development of diabetic complications (Zhang and Lu, 2004). In addition, α-glucosidase inhibitor can inhibit the

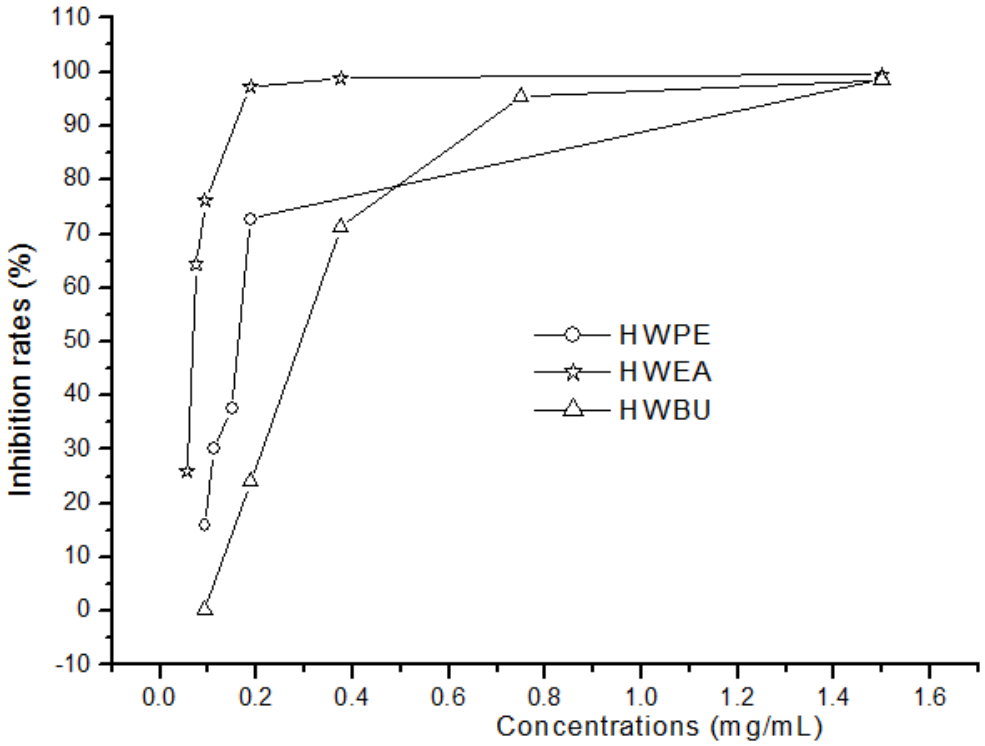

Figure 3. The concentrations of extracts effects on inhibitory activity of α-glucosidase

process of protein glycosylation and lipid glycosylation, it also has anti-cancer, anti-viral and immune stimulating activity (Xu et al., 2005). From the above results, α-glucosidase inhibitory activity of the different extracts from HW has some differences. Different solvents that are used to extract plant have some effects on its activity, three extracts of HW have good α-glucosidase inhibitory activity, and active compounds should be carried out in the future.

ACKNOWLEDGEMENTS

This work was supported by the key project of Science and Technology Department, Henan, China (102102310019) and the Key Project of Science and Technology in Zhengzhou City (20120684).

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