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In vitro antioxidant properties of *Rosa roxburghii* aqueous extracts

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Abstract

Rosa roxburghii Tratt, is a wild plant of Southwest China, which has numerous beneficial actions against various diseases. However, whether *R. roxburghii* has antioxidant activity for free radicals-mediated biomolecules damage is still unknown. In the present study, the protective effect of *R.roxburghii* aqueous extracts (RAE) on free radicals induced damage to biological molecules (lipids, DNA and proteins) was investigated. Significant inhibitory effects of RAE on the oxidation of protein and lipid were observed by using Cu^{2+}/H_2O_2- or AAPH-treated bovine serum albumin, BSA and liver homogenate systems. In addition, RAE effectively protected DNA against oxidative damage induced by 1,10-phenanthroline (OP)-Cu^{2+}/ascorbate/H_2O_2. Results showed that RAE possessed strong radical-scavenging and redox abilities as evidenced by several model antioxidant assays including DPPH and ABTS radical scavenging and FRAP (Ferric-reducing antioxidant power). Total polyphenols and flavonoids of one-gram RAE were equivalent to 223.02 ± 3.61 mg of gallic acid and 20.41±1.71 mg of rutin, respectively. The potential bioactivity of *R. roxburghi* extract may associates with its high polyphenols and flavonoids contents.

Keywords: Aqueous extracts, biological molecules, DNA damage, protein oxidation, *Rosa roxburghii*
Abbreviations: AAPH-2,2-Azobis (2-amidinopropane) dihydrochloride; ABTS-2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonicacid); TPTZ-2,4,6-tripyridyl-S-triazine; BHA-butylated hydroxyanisole;BHT-butylated hydroxytoluene; DNPH-2,4-dinitrophenylhydrazine; DPPH-1,1-diphenyl-2-picrylhydrazyl; DTNB-5,5',dithiobis (2-nitrobenzoic acid); FRAP-ferric-reducing antioxidant power; MDA-malondialdehyde; RAE-*R.roxburghii* aqueous extracts; TBARS- thiobarbituric acid reactive substances.

Introduction

Free radicals and oxidative stress are involved in the pathogenic mechanisms of many human diseases including Alzheimer's disease, Parkinson's disease, diabetes mellitus, atherosclerosis, ischemia-reperfusion injury, and also in the aging processes (Pham-Huy et al., 2008). Inducing damage to DNA, proteins, lipids and other biomolecules is proposed as one mechanism of action of free radicals in oxidative stress-related diseases (Cooke et al., 2003; Obulesu et al., 2011). It has been reported that plant-based diet with high intake of antioxidants may lower the risk of oxidative stress-related diseases (Mayne, 2003). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been suspected to cause or prompt negative health effects and their application have been restricted (Branen, 1975). Thus, much attention has been devoted to searching for effective and non-toxic antioxidants from natural products. Natural antioxidants from plant products have been demonstrated to act as free radical scavengers in the prevention of oxidative damage in healthy cells without causing unwanted effects. Therefore, these natural antioxidants are useful in the treatment of many human diseases, including diabetes, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases (Albarracin et al., 2012; Kawaguchi et al., 2011; Singh et al., 2011). Besides the well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices (Kanner et al., 1994; Madsen and Bertelsen, 1995), many other plant species have been investigated in the search for novel antioxidants (Koleva et al., 2002). Many new natural antioxidants have already been isolated from different kinds of medicinal plants, herbs and spices, such as *Curcuma longa*, *Rosmarinus officinalis*, *Salvia officinalis* (Gupta and Sharma, 2006). Some natural antioxidants are already exploited commercially either as antioxidant additives or as nutritional supplements. While more research is needed to explore new natural antioxidants form plants.

Rosa roxburghii Tratt, belonging to *Rosa* genus of Rosaceae family, is a wild plant of the rose family originates from Southwest China. *R. roxburghii* contains notable amounts of ascorbic acid, polyphenols, vitamin E, trace elements, superoxide dismutase (SOD) and so on (An et al., 2005). *R. roxburghii* has been shown to improve the antioxidant status and immune function in humans, resulting in beneficial effects on diseases such as atherosclerosis, cancer, diabetes (Hu et al., 1994; Ma et al., 1997). While the health promoting effects of *R. roxburghii* are well documented (van der Westhuizen et al.,2008; Zhang et al.,2001), there is no published data about the effects of *R.roxburghii* extract on oxidative stress-mediated damage of biological molecules. In addition, although *R.roxburghii* is composed of several vital components such as polysaccharide, vitamin C and vitamin E (Ma et al., 1997), its free radical scavenging activities were

not researched in detail. Thus, the aim of this study was to research the free radical-scavenging activity of RAE and effect of RAE on oxidative damage of biological molecules (lipids, DNA and proteins). In addition, the total quantity of phenols and flavonoids were determined. The possible protective mechanism of RAE was also discussed.

Results

Effects of RAE on protein oxidation induced by Cu^{2+}/H_2O_2

As a biomarker of oxidative protein damage in aging and in various diseases, carbonyl content was measured. The results in Fig 1a showed that, the carbonyl content significantly increased from 2.18 ± 0.14 to 4.17 ± 0.27 nmol mg^{-1} when liver homogenate was incubated with 25 mM H_2O_2 and 0.1 mM $CuSO_4$; however, the carbonyl content in RAE pretreated-groups significantly decreased compared to the Cu^{2+}/H_2O_2-treated group. The susceptibility of thiols to oxidation can lead to the formation of disulfides and higher oxidation products, often with loss of biological activity. In this study, the addition of 25 mM H_2O_2 and 0.1 mM Cu^{2+} to liver homogenate resulted in a significant decrease in thiol content, from 4.85 ± 0.08 to 1.38 ± 0.11 nmol thiol mg^{-1} protein. With preincubation of RAE at 37 °C, the decrease of thiol contents was significantly inhibited in a dose-dependent way (Fig 1b).

Effects of RAE on lipids peroxidation induced by Cu^{2+}/H_2O_2

In this study, the effect of RAE on liver lipid peroxidation, as estimated by measurement of TBARS and conjugated dienes, has been studied. Fig 1c showed the level of TBARS increased significantly in the Cu^{2+}/H_2O_2 treated group compared with the control group. The formation of TBARS was inhibited by the pre-treatment of RAE. RAE reduced TBARS by 26.80% and 41.28%, at 0.5 mg mL^{-1} and 1.0 mg mL^{-1}, respectively. The absorbance ratio of A_{233}/A_{215}, was defined as the "oxidation index" and used as a relative measurement of double bond conjugation (Kantar et al., 1992). As shown in Fig 1d, after treatment with Cu^{2+}/H_2O_2, the "oxidation index" in liver homogenate was increased to 0.339 ± 0.018, compared with 0.223 ± 0.049 in the control group. RAE lowered the formation of conjugated dienes (CD) in a dose-dependent way and 1 mg mL^{-1} of RAE decreased the formation of CD by 34.74% (Fig 1d).

Effects of RAE on DNA damage induced by OP-Cu^{2+} /ascorbate/H_2O_2

In the presence of Cu^{2+} and ascorbic acid, 1,10-Phenanthroline (OP) is able to induce the degradation of DNA by oxidative mechanism, and carbonyl species produced from DNA degradation can be identified by red color of the thiobarbituric acid (TBA) complex (Yang et al., 2000; Zhao et al., 2008). The absorbance at 532 nm was significantly increased in the OP-Cu^{2+}/ascorbate/H_2O_2 treated group with comparison to the control group, and the absorbance value at 532 nm was decreased for the samples with the pre-treatment of RAE (Fig 2a). The results demonstrated RAE decreased formation of TBARS in DNA samples induced by OP-Cu^{2+}/ascorbate/H_2O_2. The percentages of inhibition were 57.29 % and 66.91%, at 10 mg mL^{-1} and 15 mg mL^{-1}, respectively (Fig 2b). Our data indicated that RAE inhibited OP-Cu^{2+}/ascorbate/H_2O_2-induced DNA damage; however, the protective mechanism needs further investigation.

Effects of RAE on Cu^{2+}/H_2O_2 and AAPH-induced protein oxidation and carbonylation

Oxidative damage to proteins was indicated by increased carbonyl formation and the occurrence of protein fragmentation and polymerization. The fragmentation of BSA was observed during exposure to Cu^{2+}/H_2O_2 or AAPH (upper panels in Fig 3a & b). RAE exhibited strong inhibitory effect against BSA fragmentation in a dose-dependent way in both systems. Protein carbonyl content of BSA was measured by western blot analysis to examine the oxidative stress status. As shown in Fig 3a (bottom panel) and Fig 3b (bottom panel), RAE showed a concentration-dependent inhibitory effect on Cu^{2+}/H_2O_2 or AAPH induced BSA carbonylation.

In vitro antioxidant activity of RAE

Three *in vitro* model assays including DPPH, ABTS and FRAP assays were used to evaluate the antioxidant activity of RAE. As shown in Fig 4, RAE scavenged DPPH and ABTS radicals in a concentration-response manner. RAE showed scavenging activities on DPPH radical (IC_{50} 74.93 μg mL^{-1}) and ABTS radical (IC_{50} 153.83 μg mL^{-1}), while the IC_{50} values of trolox were 2.46 μg mL^{-1} and 17.11 μg mL^{-1}, respectively. The TEAC values of RAE in scavenging DPPH and ABTS radical were 31.588 mg g^{-1} and 109.53 mg g^{-1}, respectively. FRAP assay is a commonly used method for assessing total antioxidant status and can be applied to both aqueous and alcohol extracts of plants. The antioxidant capacity of RAE obtained from the FRAP assay was in good accordance with $FeSO_4$ (R^2=0.996) and the antioxidant capacities of RAE were 1.36±0.07 mmol Fe^{2+} g^{-1} RAE.

Total polyphenol and flavonoid contents

Total polyphenolic and flavonoid content were determined spectrophotometrically. Total amounts of polyphenols and flavonoids in RAE were 223.02 ± 3.61 mg gallic acid equivalents (RAE) per gram and 20.41±1.71 mg of rutin equivalent per gram respectively.

Discussion

Biomolecules, including lipids, proteins and DNA, are biotargets for ROS-mediated oxidative injury. Oxidative damage to these molecules involves in various and numerous pathological processes, including inflammation, atherosclerosis, cancer and neurodegenerative diseases (Cooke et al., 2003; Halliwell, 2007; Obulesu et al., 2011). Therefore, it is essential to identify and characterize compounds that have protective activity against oxidative damage of biomolecules. *R.roxburghii*, a rare fruit crop originated in China, has recently been labeled as one promising new fruit crop in China (Wen and Deng, 2005). In this study, the effect of *R.roxburghii* aqueous extracts on liver injury, lipid peroxidation, protein oxidation and DNA damage induced by free radicals was investigated. Lipid peroxidation plays a major role in oxidative stress-related diseases (Guéraud et al., 2010; Negre-Salvayre et al., 2010). It has been reported that *R.roxburghii* Tratt juice prevented the oxidative modification of low density lipoprotein by copper ions in terms of TBARS content and the formation of conjugated dienes (Zhang et al., 2001). In this study, oxidative damage to liver was induced by Cu^{2+}/H_2O_2. In Cu^{2+}/H_2O_2 reaction system, hydroxyl radical can be formed

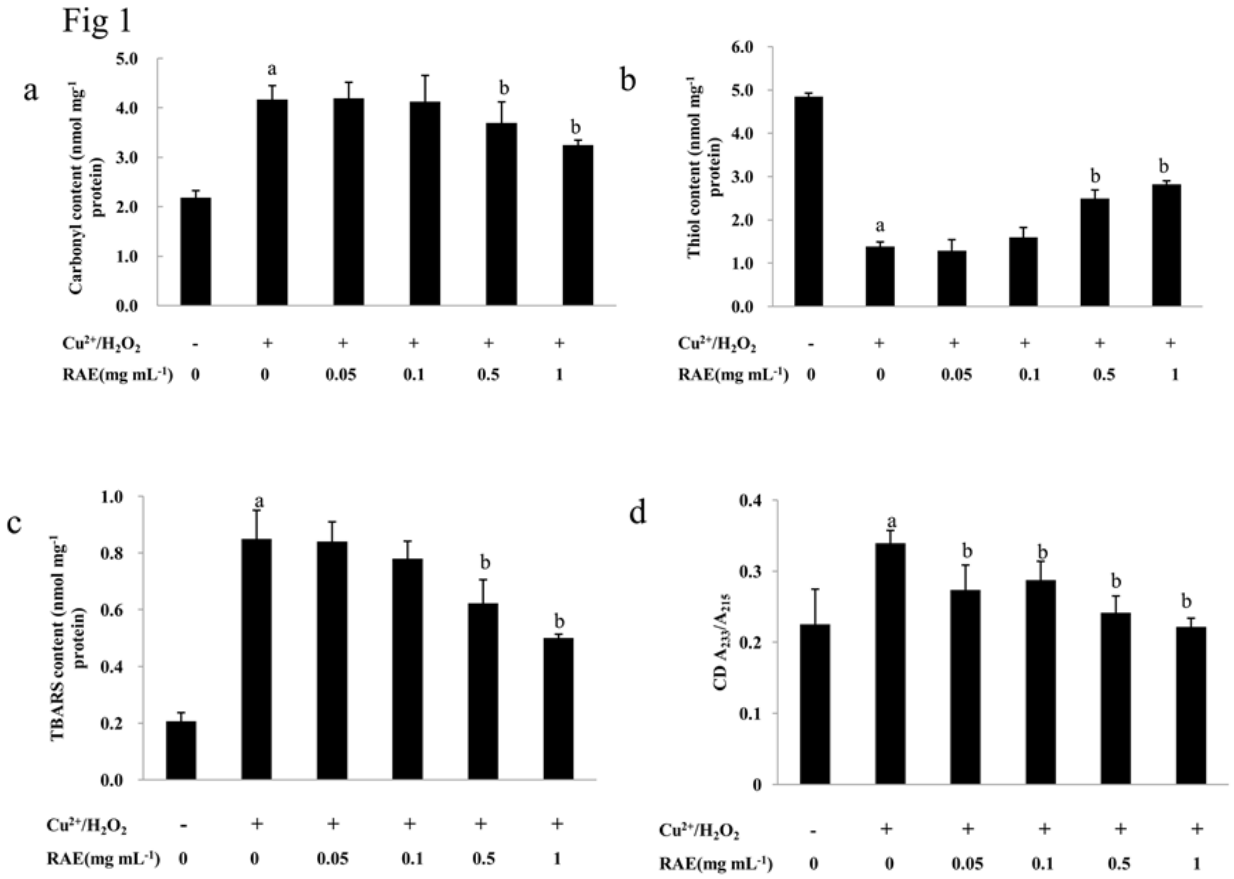

Fig1. Effect of the RAE on liver oxidation injury by Cu^{2+}/H_2O_2. Liver homogenate was preincubated with or without RAE at 37 °C for 30 min, and then 0.1 mM Cu^{2+} and 25 mM H_2O_2 (final concentration) were added, the mixture was further incubated at 37 °C for 30 min. Carbonyl content (a), thiol content (b), TBARS (c) and conjugated dienes (d) were measured as described in Materials and Methods. Each value presents the mean ± SD (n=3). $^{a}p < 0.05$ versus normal control group; $^{b}p < 0.05$ versus Cu^{2+}/H_2O_2 -treated group.

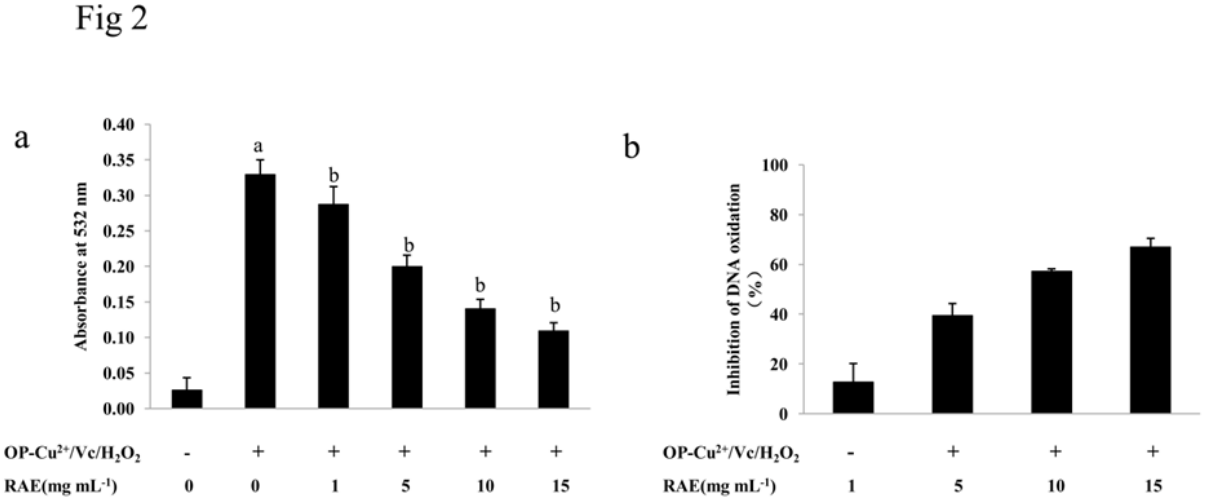

Fig 2. Effects of RAE on DNA damage induced by OP-Cu^{2+}/ascorbate/H_2O_2. (a) the absorbance at 532 nm. (b) the inhibition ratio was calculated. Each value represents the mean ± SD (n=3). $^{a}p < 0.05$ versus normal control group; $^{b}p < 0.05$ versus OP-Cu^{2+}/ascorbate/H_2O_2 -treated group.

from H_2O_2 through the reduction of Cu(II) to Cu(I) by H_2O_2 and the Fenton-type reaction between Cu(I) and H_2O_2. Inhibitive effect of RAE on lipid peroxidation was assessed by the amount of TBARS and conjugated dienes produced. Similar with previous report (Zhang et al., 2001), RAE inhibited the formation of TBARS and conjugated dienes in the Cu^{2+}/H_2O_2 treated-liver homogenates. Besides, RAE inhibited the formation of protein carbonyls and decrease of thiol group in the liver homogenates, which were induced by hydroxyl radical. According to the FRAP assay results, RAE may inhibit the formation of hydroxyl radical generated by Fenton-type reaction between Cu(I) and H_2O_2. Therefore, our results indicate that RAE might have a protective effect on ROS-mediated protein and lipids damage. DNA is another important biological molecule for ROS-mediated oxidative damage (Cooke et al., 2003). ROS can cause extensive base modification and single strand breakage in both mitochondrial and genomic DNA (Zhao et al., 2005). Some antioxidants have potential to protect DNA against oxidative damage (Dorman and Hiltunen, 2011; Zhao et al., 2005). van der Westhuizen et al evaluated the genoprotective effect *R. roxburghii* fruit extract using the comet assay (van der Westhuizen et al., 2008). In the present study, the effect of RAE on OP-Cu^{2+}/ascorbate/H_2O_2-induced DNA damage was also examined. The suggested mechanism of this reaction was as following: $Cu^{I}(OP)_2$ reduces O_2 and is regenerated by ascorbate. The formed superoxide can react with hydrogen ion to form H_2O_2. H_2O_2 reacts with Cu(I) to produce a copper 'oxo' complex, which induces DNA damage. During the DNA damage, more than 20 carbonyl species are formed eventually, which can be estimated quantitatively by measurement of TBARS (Zhao et al., 2005). Similar with the previous reports (van der Westhuizen et al., 2008), our data indicate that RAE inhibits OP-Cu^{2+}/ascorbate/H_2O_2-induced DNA damage, which is similar with the previous reports (van der Westhuizen et al., 2008); however, the protective mechanism needs further investigation. Protein carbonyl derivatives, which are used as the most general indicator and the most common marker of protein oxidation, are generated by direct oxidation of amino acid residues, particularly proline, arginine, lysine and threonine, or by secondary reaction with the primary oxidation products of sugars and lipids (Dalle-Donne et al., 2006; Ogino and Wang, 2007). Carbonyl modification of proteins may lead to the structural alternation and functional inactivation of many enzyme proteins. AAPH, a water-soluble azo compound, decomposes at physiological temperature producing alkyl peroxyl radicals (Liégeois et al., 2000). Hydroxyl radical generated in Cu^{2+}/H_2O_2 system and peroxyl radical generated from AAPH were used to induce BSA oxidation in the present study. Exposure of BSA to Cu^{2+}/H_2O_2 or AAPH results in accumulation of protein carbonyls. RAE potential inhibits the protein carbonyls in a dose-dependent manner in both inducing systems, indicating RAE has great inhibitory effect on different free radicals induced protein carbonyls accumulation. The inhibitory effect of RAE might operate by scavenging the hydroxyl radical generated in Cu^{2+}/H_2O_2 system and peroxyl radical generated from AAPH. DPPH, ABTS and FRAP assays have been commonly used to estimate the total antioxidant capacities of various biological specimens for their good reproducibility and easy quality control (Lee et al., 2006; Thaipong et al., 2006). Therefore, the *in vitro* antioxidant properties of *R.roxburghii* aqueous extracts were investigated by the DPPH, ABTS and FRAP assays. Our results show that the RAE exhibits strong scavenging effects on DPPH and ABTS radicals. The antioxidant mechanism of DPPH radical scavenging is based on the acceptance of proton

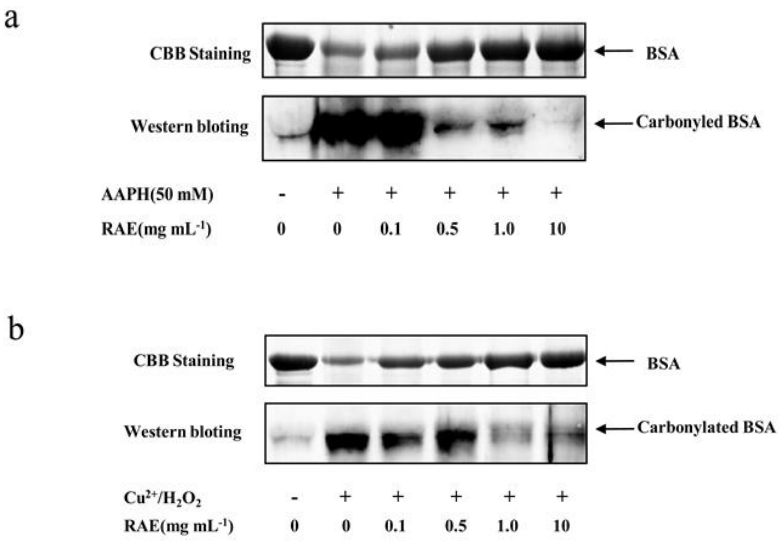

Fig 3. Effects of RAE on fragmentation and carbonylation of BSA induced by ROS. BSA samples were induced AAPH (a) or Cu^{2+}/H_2O_2 (b) with or without pretreatment of RAE as described in Materials and Methods. Fragmentation and carbonylation of BSA were detected using CBB staining and western blot with rabbit anti-DNP polyclonal antibody. The arrows in (a) and (b) shown the positions of native or oxidated BSA (upper panel) and carbonylated BSA (bottom panel).

by the DPPH radical. Therefore, the result of DPPH assay is indicative of the hydrogen donating ability of RAE. The basis of ABTS assay is to monitor the decay of the radical-cation $ABTS^{\cdot+}$ produced by the oxidation of ABTS caused by the addition of antioxidants. The result indicates that RAE has a strong scavenging power for the ABTS radical and may be explored as a potential antioxidant. The antioxidant power of RAE was also measured by the FRAP method, which is a simple and effective method for measuring the ability of antioxidants in plant samples to act as reducing agents (Moon and Shibamoto, 2009). However, the FRAP value of RAE is lower than many fruits (Fu et al., 2011). Recent studies have demonstrated that polyphenols and flavonoids found in the fruits and vegetables may act as antioxidants, and the antioxidant capacity of which were supposed to have a positive linear correlation with the contents of phenolic or flavonoid (Hazra et al., 2010; Kedage, et al., 2007). In this work, total amounts of polyphenols and flavonoids in RAE were measured. The total phenolic contents were high in RAE (223.02 ± 3.61 mg of GAE g^{-1} of RAE). The total phenolic contents in *R. roxburghii* fruit extract was measured at 64.9 g of GAE L^{-1} (van der Westhuizen et al., 2008). The preparation processes of these two samples are different, so it is difficult to compare these data. Many polyphenol compounds have been found in *R.roxburghii*, including quercetin, gallic acid and catechin. These polyphenols and flavonoids compounds may contribute to the antioxidant action of *R. roxburghii*. It should be pointed out that polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones.

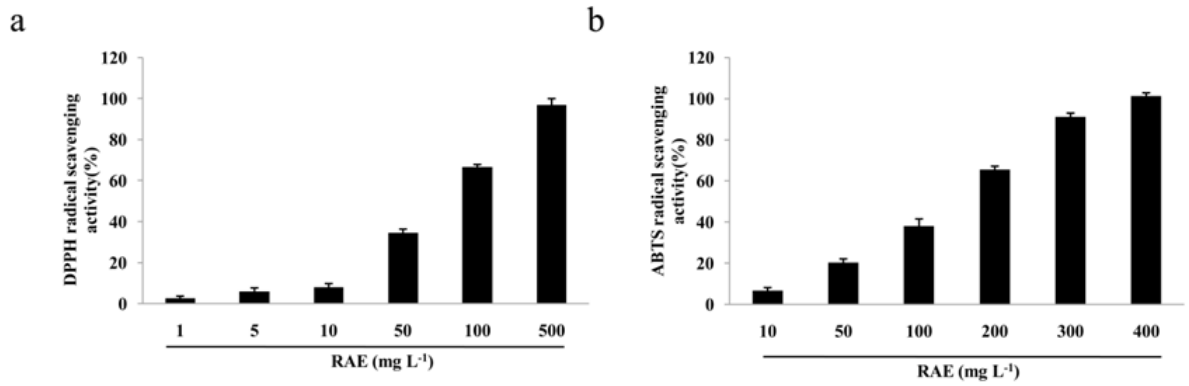

Fig 4. Scavenging effects of the RAE on DPPH radical (a) and ABTS radical (b). The details are described in Materials and methods. Results are mean ± SD (n=3).

Materials and methods

Plant materials

The *R.roxburghii* fruits were purchased from Kaifeng Jinweikang Biotechnology Co., Ltd, Kaifeng, China.

Animals

Adult healthy Wistar rats, weighted between 200 and 300 g, were supplied by the Experimental Animal Center of Xi'an Jiao tong University (Xi'an, China). This experiment followed the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health, and was approved by Northwest A&F University Animal Care and Use Committee.

Preparation of R. roxburghii aqueous extracts

The air-dried *R. roxburghii* fruits were smashed into powder, which was macerated in hot water (45 ^{o}C) for 2 h. Then the samples were extracted with 10 volumes of distilled water at 100 ^{o}C for 2 h. After vacuum filtration, the filtrate was collected and the residue was re-extracted under the same conditions twice again. The combined filtrates were concentrated using a rotary evaporator (Buchi R-200, Flawil, Switzerland) at 45 ^{o}C, and the resultant extract was freeze-dried in a lyophilizer (Ilshin Freezer, FD5508, Yang Ju Si, Korea). The lyophilized powder was stored d in dark at 4 ^{o}C before being used for the bioactivity tests (Hsu et al., 2006).

Homogenate preparation and oxidation by Cu^{2+}/H_2O_2

Six rats were killed by neck fracture. The livers rapidly excised and chilled on crushed ice and thereafter these were rinsed in ice-cold saline [0.9% (w/v) NaCl]. The whole rat liver was homogenised with 0.1M phosphate buffer saline at pH 7.4 containing protease inhibitor (Zhao et al., 2006). After centrifugation at 10000 rpm for 15 min at 4 ^{o}C, the obtained supernatant liver homogenates were pre-incubated with or without RAE at 37 ^{o}C for 30 min, and then 25 mM H_2O_2 and 0.1 mM $CuSO_4$ (final concentration) were added, the mixture was further incubated in 37 ^{o}C water bath for 30 min to induce peroxidation.

Detection of protein carbonyl contents and thiol contents

The oxidative damage to protein in liver homogenate was measured by the qualification of carbonyl groups based on the reaction with DNPH as described previously by Levine *et al* (Levine et al., 1990). Results were expressed as nmol carbonyl mg^{-1} protein. The concentration of thiol groups in homogenate was quantitated using DTNB (Ellman 1959; Väänänen et al., 2005) and results were expressed as nmol thiol mg^{-1} protein. The protein concentration in liver homogenate was measured using the BCA Protein Assay kit (Pierce, Rockford, IL, USA).

Measurement of thiobarbituric acid reactive substances and conjugated dienes

Cu^{2+}/H_2O_2-mediated lipid peroxidation in liver homogenates was monitored by measuring thiobarbituric acid reactive substances (TBARS) and conjugated diene formation. TBARS were determined using the method described (Ohkawa et al., 1979) and the amount of TBARS was expressed as nmol MDA mg^{-1} protein. Peroxidation-generated conjugated dienes were measured by the increase in the A_{233}/A_{215} absorbance ratio (Anitha Nandhini et al., 2002).

DNA damage induced by OP-Cu^{2+} /ascorbate/H_2O_2

The effect of RAE on OP-Cu^{2+}/ascorbate/H_2O_2-induced DNA damage was performed in 2 mL Tris-HCl buffer (pH7.0) containing 0.30 mg calf thymus DNA (ctDNA), 0.1 mM $CuSO_4$, 0.2 mM 1,10-phenanthroline and 1.75 mM ascorbic acid with or without different concentration of RAE. After 1.5 h incubation in a shaking water bath at 37 ^{o}C, 2 mL of TBA (1%, W/V) and 2 mL of TCA (28%, W/V) were added into the reaction mixture and heated at 100 ^{o}C for 10 min. The mixture was cooled and the absorbance was measured at 532 nm (Handayani and Arty, 2008). The mixture without RAE was used as blank. TBARS content was used to evaluate the protective of RAE on DNA damage and the inhibition ratio (%) was also calculated according to the formula:

$$\text{Inhibition } (\%)=(A_0- A_1)/A_0\times100$$

Where A_0 was the absorbance of the control group (blank,

without RAE) and $A_1$was the absorbance in the presence of RAE.

Protein oxidation and carbonylation induced Cu^{2+}/H_2O_2 or AAPH

BSA (0.5 mg mL^{-1}) was incubated with Cu^{2+} (0.1 mM) and H_2O_2 (2.5 mM) in the presence or absence of RAE. Reactions were performed in opened tubes placed in a water bath at 37 ^oC for 30 min with frequent shaking. In the AAPH reaction system, BSA (0.5 mg/ml) was incubated with AAPH (50 mM) in the presence or absence of RAE. Reactions were performed in closed tubes placed in a shaking water bath at 37 ^oC for 4 h (Mayo et al., 2003). After 10% SDS-PAGE electrophoresis, the protein was stained with 0.1% CBB R-250. Carbonylation of BSA was studied by Western blot using anti-DNP antibody (Robinson et al., 1999). Rabbit anti-DNP polyclonal antibody (D9656) and goat anti-rabbit IgG-HRP (sc-2004) were purchased from Sigma (St. Louis, MO, USA) and Santa Cruz Biotechnology, Inc. (CA, USA), respectively.

DPPH, ABTS and FRAP assays of RAE

In vitro antioxidant activity of RAE were investigated using DPPH, ABTS and FRAP assays according to the methods described by previous studies (Loizzo et al., 2009; Yang et al., 2009). Trolox was used as a positive control in above assays. FRAP assay was assessed using a a previously reported method (Benzie and Strain, 1996) and the FRAP values were expressed in mmol Fe(II) per gram of sample (mmol g^{-1}).

Determination of total polyphenol and flavonoid content

The total phenolics content (TPC) and total flavonoid contents in RAE were determined according to the Folin-Ciocalteau method (Chun et al., 2003) and aluminum chloride colorimetricassay (Lin et al., 2007). Gallic acid and Rutin equivalents were used for these parameters.

Statistical analysis

All data are expressed as means ± standard deviation (SD) of triplicate experiments. Student's t-test for independent samples was used to test for differences at a significance level of $p < 0.05$. All statistical analyses were performed with SPSS 16.0 statistical package (SPSS Inc., Chicago, USA).

Conclusion

In summary, the present study indicates that RAE can scavenge radicals of biological interest and prevent damage to DNA, lipids, and proteins from oxidative stress. Due to the abundant resources and easy availability, *R.roxburghii* should be regarded as a source of natural antioxidant due to the strong antioxidant activity of their aqueous extracts.

Acknowledgments

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