Advance Journal of Food Science and Technology 5(11): 1483-1489, 2013
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: July 17, 2013 Accepted: July 31, 2013 Published: November 05, 2013

Physico Chemical Properties and Antioxidant Activity of Roselle Seed Extracts

Abdoulaye Idrissa Cissouma, Fatoumata Tounkara, Mehdi Nikoo, Na Yang and Xueming Xu
State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R. China

Abstract: The aim of this research was to extract phenolic compounds from defatted Roselle (*Hibiscus sabdariffa* L.) seed and assess their antioxidant potential. Water, ethanol (30%), methanol (30%) and acetone (30%) were used as solvent for extraction. The proximate composition, total phenolic content and extraction yield were analyzed. Antioxidant efficacies of Roselle seed extract were tested by using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), hydroxyl, 2, 2'-azinobis-3-ethylbenzothiaz oline-6-sulfonic acid (ABTS) radicals scavenging capacities and reducing power analysis. Roselle seeds were found to be rich in protein (27.745%), carbohydrates (40.45%) and oil (20.83%). The total phenolic content ranged from 1.66±0.03 to 1.99±0.01 (GAE) mg/g using water and 30% acetone respectively. The highest inhibitory capacity on DPPH and ABTS radicals was observed in 30% acetone extract and was at 3 mg/mL for DPPH and 6 mg/mL for ABTS respectively. Ethanol extract showed the highest hydroxyl radical scavenging ability value of 66.36 at 20 mg/mL, followed by methanol (57.27), acetone (56.36) and water (30). The reducing potential of the different extracts was concentration dependent and increased with increase in concentration. These results indicate that substantial antioxidant activity can be obtained from Roselle seed phenolic compounds extract by using 30% acetone.

Keywords: Antioxidant activity, extraction solvent, proximate composition, roselle seed, total phenolic content

INTRODUCTION

Hibiscus sabdariffa L. or Roselle is one of the most common flower plants grown worldwide. The origin of *H. sabdariffa* L. is not fully known, but it is believed to be native to tropical Africa. Roselle is used to produce jam, jelly and wine. Omobuwajo *et al*. (2000) reported that Roselle seeds are bigger than pearl millet varieties, having average dimension of 5.21 and 2.81 mm. The seeds are somewhat bitter. However, in Africa, they are ground into meal for human food due to their high protein content. They are also roasted to use as a substitute for coffee (Morton, 1987) and contain a substantial amount of oil that resembles that of cotton seed (Mohammed *et al*., 2007).

In ethno-medicine, *H. sabdariffa* L. is used to deal with several health problems, including hypertension, pyrexia, liver disorders and microorganism growth. It is also used as a diuretic, sedative, or digestive (Faraji and Tarkhani, 1999; Chen *et al*., 2003; Akindahunsi and Olaleye, 2003) and is said to have aphrodisiac properties (Duke and DuCellier, 1993). Many medicinal applications of this plant have been developed around the world. Studies by Muhammad and Shakib (1995) have shown that Roselle can prevent cancer, lower blood pressure and improve the digestive system in humans. Its calyx extract has also been used as an effective treatment for patients with kidney stones due to its uricosuric effect (Prasongwatana *et al*., 2008).

Antioxidants are bioactive micro-components present in food, having the ability to retard oxidation. Free radicals have been implicated for aging as well as food and chemical deterioration. Many researchers found that plant polyphenolic compounds scavenge free radicals, thereby protect humans against oxidative damage and diseases (Beecher, 1999; Cicerale *et al*., 2009; Okawa *et al*., 2001). Recently, natural antioxidant α-tocopherol and some synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole and propyl gallate are commonly used to act against free radicals in food and biological systems. However, synthetic antioxidants are under heavy criticism due to their undesirable side effects on human health (Goli *et al*., 2005). In order to minimize or eliminate the use of these artificial antioxidants in food, essential oils or plant extracts have been used as natural antioxidants against the proliferation of microorganisms or for protecting food from oxidation (Bubonja-Sonje *et al*., 2011). For example, Roselle plant protects against Low Density Lipoprotein (LDL)-oxidation and has hypolipidemic effects *in vivo* (Hirunpanich *et al*., 2006). In some instances, it is also used to preserve food. There are many reports published on the efficacy of Roselle

Corresponding Author: Xueming Xu, State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, 214122 Jiangsu, P.R. China, Tel./Fax: +86 51085917100

calyces extract but only limited work has been done on seed extract. The objective of this research was to determine the chemical composition of seed extracts from different solvents. Moreover, in vitro antioxidant assay of seed extract was also performed.

MATERIALS AND METHODS

Materials: Roselle (*H. sabdariffa* L.) seeds were obtained from Koutiala, southern region of Republic of Mali and the seeds were transported to Wuxi, China through DHL®. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide 30%, 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Folin-Ciocalteu's reagent, gallic acid were obtained from Sangon Biotech (Shanghai) Co., Ltd. and Shanghai Sunny Biotech CO., Ltd., China. All other chemicals and standards used were of analytical grade quality.

Preparation of defatted roselle seed flour: Roselle seeds were cleaned by removing dust, stones and plant debris. The seeds were milled using a laboratory scale hammer mill and the resulting powder was sieved through a 60 mesh screen until fine powder was obtained. Thereafter, the powder was defatted with n-hexane, following a small-scale hexane extraction method described by Tzeng *et al*. (1990). The oil-free flour was desolventized and stored in a desiccator at room temperature for subsequent use.

Proximate analysis: Proximate analysis is an important index for identification and classification of the nutritional value of a food material. The seeds are regarded as a by-product of Roselle leaves processing and used in West Africa as raw material for making Bikalga known as datou in Mali, a kind of fermented condiment.

The proximate analysis of Roselle seed was determined according to AOAC (2000). The moisture content was determined by drying in an oven at 105°C until a constant weight was obtained. Ash was determined by weighing the incinerated residue obtained at 550°C for 8-12 h. Total nitrogen and the protein content were determined based on the Kjeldahl method using the conversion factor of 6.25. The total lipid in samples was determined by Soxhlet method. The total available carbohydrate was determined by difference, subtracting the sum of percentage of moisture, crude fat, crude protein and ash contents from 100%. Triplicate samples were analyzed for each sample.

Minerals composition: Samples were digested in 100 mL micro-Kjeldahl flask with $HNO_3/HClO_4$ until the solution became colorless. The samples were cooled and diluted to volume in a 25 mL volumetric flask with 0.1 M HCl. Sodium, potassium, calcium, magnesium, iron, zinc, manganese and copper were measured by atomic absorption spectrophotometry, (Garcia *et al*., 1972) using a Varian spectra atomic absorption spectrophotometer (Varian SpectrAA220, Varian, Palo Alto, CA).

Fatty acid analysis: Fatty acids content in Roselle seeds were determined according to the method of Ceirwyn (1995). Fat was extracted with methyl ether and prepared directly by treatment with sodium methoxide. Gas Chromatography/Mass Spectra (GC/MS) system was used to identify and quantify the fatty acids. A FINNIGAN TRACE MS gas chromatograph/mass spectra equipped with a 30 m×0.25 mm Ov-1701 column was used. Column flow rate was 0.8 mL/min with helium as the carrier gas, split was 64 mL/min and the source temperature was 270°C. The fatty acid methyl esters were identified by comparison with the retention times of NU CHECK Inc. standards (Elysian, 1L) and quantified by internal normalization.

Polyphenol extraction: Polyphenolic extract yields of plant matter are substantially influenced by the nature of the extracting solvent owing to the presence of different bioactive compounds of different chemical properties and polarities that may or may not be soluble in a particular solvent. Thus differences in polarity coupled with safety reasons influenced the choice of the four solvents used for the study.

The extraction was carried out in a 250 mL beaker by the following method: (to 20 mL of different solvents: de-ionized water, 30% acetone, 30% ethanol and 30% methanol (v/v) was added 1 g of defatted Roselle seed flour). Laboratory scale magnetic stirrer was used to gently stir. The phenolic extracts were separated from the solid mass by filtration using Watman's No. 1 filter paper. Each filtrate obtained was collected into a graduated cylinder and the volume adjusted to 20 mL with the same extracting solvent in order to estimate the total polyphenol content and assay for antioxidant activity.

Total Polyphenolic compound content determination (TP): The amount of total polyphenol content in the Roselle seed extracts was calorimetrically estimated using the Folin-Ciocalteu reagent method according to Sultana *et al*. (2009), with slight modification. Briefly, 0.04 mL of each crude extract was mixed with 0.2 mL Folin- Ciocalteu reagent in a 10 mL test tube. The mixture was allowed to stand for 5 min before addition of 0.5 mL sodium carbonate (20%). The volume was adjusted to 5 mL with distilled water and vortexed for 10 sec. The mixture was incubated at 45°C for 30 min and cooled to room temperature before reading the absorbance at 750 nm using a UV spectrophotometer (Model: UV-1800, China-Shanghai

Mapada Instruments Co., Ltd.). The amount of TP contents was expressed as Gallic Acid Equivalent (GAE) mg/g Roselle seed on dry weight basis. All samples were analyzed three times and the results were averaged.

Scavenging effect on hydroxyl (HO•) radical: The hydroxyl radical scavenging effects of Roselle seed extract was assayed using the method of Halliwell *et al*. (1987) with slight modification. The reagents were added to a test tube in the following order: 0.4 mL KH_2PO_4-KOH buffer (pH 7.4), 0.1 mL sample solution with concentrations (10, 15, 20 mg/mL) and 0.1 mL of 1 mM EDTA, 10 mM H_2O_2, 60 mM 2-deoxy-Dribose, 2 mM ascorbic acid and 1 mM $FeCl_3$ (0.1 mL distilled water was used as control instead of $FeCl_3$). The reaction solution was incubated at 37°C for 1 h. The reaction was stopped by addition of 1 mL of 20% TCA. The color was developed by addition of 1 mL of 1% TBA into the reaction tubes, which were placed in boiling water for 15 min. The tubes were cooled to room temperature and then the absorbance was read at 532 nm. For each concentration, samples were prepared in triplicate and the antioxidant activity of each was measured in duplicate. The scavenging effects were calculated by the following equation:

$$\text{Radical scavenging activity (\%)} = (1 - Ac/As) \times 100$$

where,
As : The absorbance of the samples at 560 nm
Ac : The absorbance of the control at 560 nm

Reducing power: The reducing power of Roselle seed powder extract was measured according to the method of Wu *et al*. (2003) with some modifications. Briefly, freeze dried samples at concentrations of 0.5, 1 and 1.5 mg/mL were dissolved in a 0.2 M phosphate buffer (pH 6.6). An aliquot (2.5 mL) of sample solution was then added to 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min, followed by addition of 2.5 mL of 10% (w/v) Trichloroacetic Acid (TCA). After incubation 2.5 mL of distilled water and 2.5 mL of 0.1% (w/v) $FeCl_3$ were added to the mixture. The absorbance of the solution was then recorded immediately at 700 nm. The blank of each sample was prepared by adding distilled water instead of $FeCl_3$. Net increase in absorbance of the reaction mixture indicates increased reducing power.

DPPH radicals scavenging activity assay: The scavenging effect of Roselle seed powder extract on DPPH free radical was measured according to the method of Shahidi *et al*. (2006) with little modification.

Freeze-dried samples were dissolved in 0.1 mM DPPH, initially dissolved in 95% ethanol at serial concentrations of (1, 2 and 3 mg/mL). An aliquot (0.1 mL) of each sample solution was added to 1.9 mL of ethanolic DPPH solution (50 µM) and the mixture was shaken and left for 30 min at room temperature. The absorbance of resulting solution was read at 517 nm. A lower absorbance represents a higher DPPH scavenging activity. The scavenging effect was expressed as shown in the following equation:

$$\text{DPPH scavenging activity (\%)} = 100\% \times [Abs_{control} - (Abs_{sample} - Abs_{blank}) / Abs_{control}]$$

where, Abs_{sample} is the absorbance of the different Roselle seed extracts with DPPH, $Abs_{control}$ is the absorbance of DPPH without any Roselle seed extracts, Abs_{blank} is absorbance of Roselle seed extracts without DPPH. The EC_{50} value (the median effective concentration that causes a decrease in the initial radical concentration by 50%) is a parameter widely used to measure the antiradical efficiency. The lower the EC_{50} value, the higher the free radical-scavenging ability (Zhang *et al*., 2006).

ABTS radicals scavenging activity assay: ABTS radical scavenging activity was determined as described by Phanturat *et al*. (2010). ABTS free radical ($ABTS^{•+}$) was generated by reacting 7.4 mM ABTS and 2.6 mM potassium persulphate at a ratio of 1:1 (v/v). The mixture was stored in the dark for 12 h at room temperature. Prior to assay, $ABTS^{•+}$ solution was diluted with methanol to obtain an absorbance of 1.1 (±0.02) at 734 nm. To initiate the reaction, 150 µL of freeze dried sample at different concentrations (2, 4 and 6 mg/mL, respectively) was mixed with 2850 µL of $ABTS^{•+}$ solution. The absorbance was then read at 734 nm after 2 h of incubation in dark, at room temperature. The blank was prepared in the same manner, except that distilled water was used instead of the sample. The percentage reduction of $ABTS^{•+}$ to ABTS was calculated according to the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Statistical analysis: All analyses were performed in triplicate. Data were analyzed using Statistical Package for Social Science (SPSS) version 17.0 software package. Results were analyzed statistically using a 2 sample test, assuming equal variances and one-way Analysis of Variance (ANOVA).The difference was considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Proximate chemical composition: The proximate analyses of dried whole Roselle seed extracts are presented in Table 1. From the results, solid content was the most important factor. The carbohydrate, fat, protein, ash and moisture content were 40.45, 20.83, 27.32, 4.67 and 6.79%, respectively.

Table 1: Proximate composition of roselle seeds powders

Parameter (%)	Mean±S.D.
Protein	27.32±0.38
Moisture	6.79±0.14
Fat	20.83±0.55
Ash	4.67±0.21
Carbohydrate	40.45±0.60

Mean values in rows are significantly different ($p<0.05$); S.D.: Standard deviation

Table 2: Minerals composition of roselle seeds powders (µg/g)

Parameter (µg/g)	Mean±S.D.
Zinc	120.74±0.21
Potassium	19799.33±0.58
Iron	80.17±0.49
Magnesium	4113.11±0.76
Sodium	434.32±2.83
Calcium	2155.08±0.55

Mean values in rows are significantly different ($p<0.05$); S.D.: Standard deviation

Table 3: Fatty acids analysis of roselle seeds oils (contents are area %)

Fatty acid (%)	Roselle seed oil
Saturated	
Myristic acid	0.2411
Palmitic acid	18.7637
Arachidic acid	0.5910
Stearic acid	4.8624
Total	24.4500
Unsaturated	
Palmitoleic acid	0.3233
Oleic acid	33.0752
Linoleic acid	35.1653
α-linolenic acid	1.4539
Total	70

Minerals: The mineral contents of Roselle seed are given in Table 2.

The main macro-minerals in Roselle seed were potassium (K) (19799 µ/g), magnesium Mg (4113 µ/g), calcium Ca (2155 µ/g), sodium Na (434 µ/g), zinc (Zn) (121 µ/g) and iron (Fe) (80 µ/g)). These nutrients are essential in preventing deficiency diseases. For instance, magnesium, is required for the activation of more than 300 enzymes in the body, use of some vitamins and minerals, normal function and structure of the arteries, heart, kidney and bone (Sleelig, 1980) and for the neuromuscular system (Durlach, 1988).

Fatty acid analysis: Fatty acid composition of Roselle seed oil is depicted in Table 3. The seeds contained a considerable amount of fatty acids. The total content constituted of 70% unsaturated and 24.45% saturated fatty acids. Oleic and linoleic acids had the highest values of 33.07 and 35.16%, respectively. Among the unsaturated fatty acids (Table 3), palmitoleic acid content was the lowest (0.32%), whereas palmitic acid (18.76%) was the highest saturated fatty acids (Table 3). The fatty acid composition shown the high content in unsaturated fatty acid, especially linoleic acid (35.16%), thus, is indicating the nutritional benefits of Roselle seeds. Linoleic acid has beneficial effects on blood lipids, lowering blood pressure and serum cholesterol (Savage, 2001; Enujiugha and Akanbi, 2008). The test showed no significant difference ($p<0.05$) among the sample in terms of fatty acid content and composition.

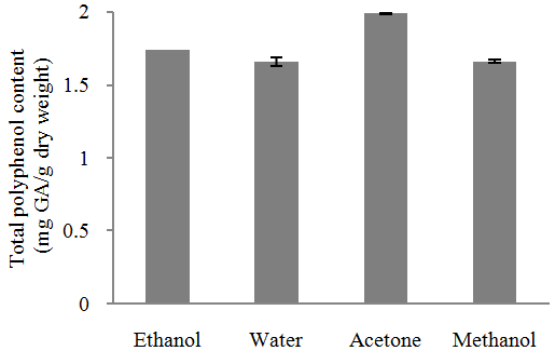

Fig. 1: Total phenolic contents (milligrams of GAE per gram of Roselle seed powder) of different solvent extracts from Roselle seed powder
Vertical bars represent the standard deviation for each data point; Mean values are significantly different ($p<0.05$)

Total phenolic content: Results obtained in this study reveal that the level of total phenolic compounds in Roselle extracts is considerable (Fig. 1).

Total phenolic content of different Roselle extract varied significantly ($p<0.05$) ranging from 1.99±0.01 to 1.66±0.03 mg GAE/g for 30% acetone and water extract respectively. The extract prepared using 30% acetone showed the highest phenolic content, while the lowest was observed in the aqueous extract with 1.99±0.01 and 1.66±0.03 mg GAE/g, respectively with a significant difference ($p<0.05$) when compared with other tested solvent extracts (Fig. 1). This study showed that Roselle seed extracts obtained using different solvents, had different total phenolic contents. The differences observed in yields could be related to the polarity of particular solvent used in the extraction. Acetone (30%) yielded the highest contents and water the lowest. From these results, the yields of phenolics followed the following order: 30% acetone>30% ethanol >30% methanol >water. The results are in agreement with the findings of John *et al*. (2012) for cocoa shell polyphenols. This indicates that phenolic compounds of Roselle seed are better extracted with acetone than all the other solvents tested.

Hydroxyl radical scavenging activity: Hydroxyl radical is an extremely reactive oxygen species, capable of modifying almost every molecule in the living cells. In addition, hydroxyl radicals are capable the quick initiation of lipid peroxidation process by subtracting hydrogen atoms from unsaturated fatty acids (Kappus *et al*., 1991; Aruoma, 1998).The ability of Roselle seed extracts to scavenge hydroxyl radicals was evaluated and results are shown in Fig. 2. All extracts were capable of scavenging hydroxyl radicals. At a

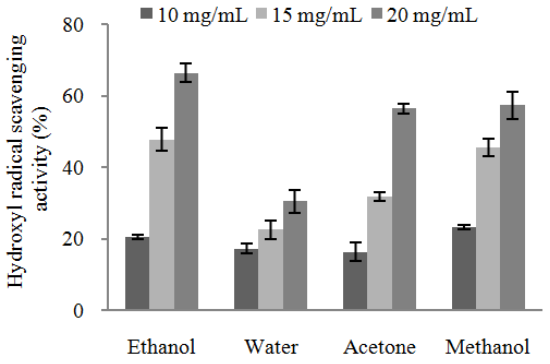

Fig. 2: Hydroxyl radical scavenging activities (percent) of different solvent extracts from roselle seed powder
Vertical bars represent the standard deviation for each data point each value is expressed as mean±S.D. of three determinations

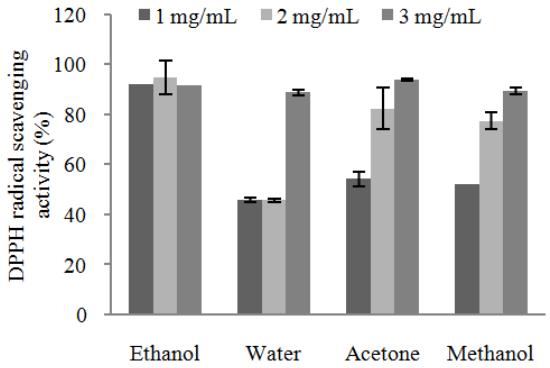

Fig. 3: DPPH radical scavenging activities (percent) of different solvent extracts from Roselle seed powder
Vertical bars represent the standard deviation for each data point; Each value is expressed as mean±S.D. of three determinations

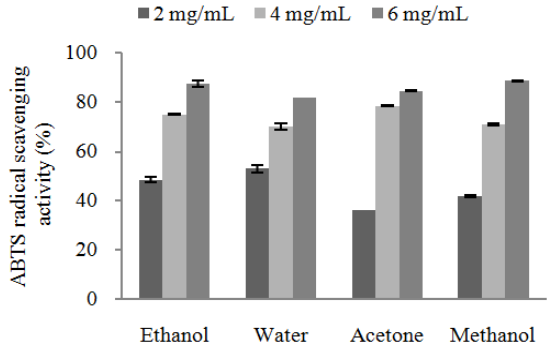

Fig. 4: ABTS radical scavenging activities of different solvent extracts from roselle seed powder
Vertical bars represent the standard deviation for each data point; Each value is expressed as mean±S.D. of three determinations

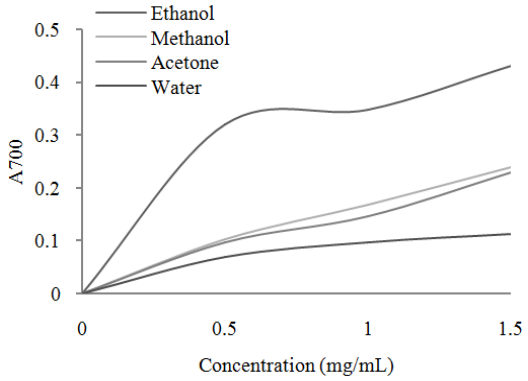

Fig. 5: Reducing power (absorbance at 700 nm) of different solvent extracts from roselle seed powder

concentration of 20 mg/mL, the 30% ethanol exhibited the strongest hydroxyl scavenging activity, followed by 30% methanol, 30% acetone and water extract. The hydroxyl radical scavenging activity was increased significantly ($p<0.05$) between 30% ethanol and 30% methanol extracts prepared from defatted Roselle seed powder. Roselle seed extracts obtained from ethanol, methanol, acetone and water extracts exhibited 66.36, 57.27, 56.36 and 30% hydroxyl radical scavenging activities respectively. These results show that Roselle seed extracts obtained from different solvents may exhibit an effective hydroxyl radical scavenging activity. Ethanol extract showed the higher OH scavenging activity (Fig. 2), but its ability to scavenge DPPH (Fig. 3) and ABTS (Fig. 4) was weaker than of the other solvent extracts. It could be that the phenolic compounds in different extracts had different mechanism against diverse free radicals scavenging activity. These results also demonstrated that 30% ethanol might be a more suitable solvent for extracting antioxidants from defatted Roselle seed powder resulting in higher hydroxyl scavenging activity, but weaker DPPH and ABTS scavenging abilities.

Reducing power: In this analysis, the presence of antioxidants caused the reduction of the Fe_3^+/ferricyanide complex to the ferrous form and the yellow color of the test solution changed to various shades of green and blue depending on the reducing power of each compound. Figure 5 shows the reducing power of different solvent extracts at different concentrations (0.5, 1, 1.5 mg/mL) from the defatted Roselle seed flour. Different solvent extracts showed significant ($p<0.05$) differences in their reducing power, indicating that Roselle seed reducing power can also be influenced by choice of extraction solvent. The reducing power of different solvent extracts increased with increase in amount of sample concentration (0.5, 1, 1.5 mg/mL). All the concentrations showed high activity. The reducing power of the different extracts was in the following order: 30% acetone >30% ethanol >30% methanol and water. This data indicates that Roselle seed extracts are capable of donating electrons, which can react with free radicals to convert them to stable products and strongly inhibit radical chain reaction.

Table 4: IC_{50} value (mg/mL) of roselle seeds extracts

	IC_{50} (mg/mL)	
Sample	ABTS	DPPH
Ethanol	2.17±.03[b]	0.84±0.15[b]
Water	2.49±0.19[a]	1.24±0.02[a]
Acetone	0.76±0.08[d]	0.65±0.03[b]
Methanol	1.68±0.01[c]	0.69±0.04[b]

IC50 value, the effective concentration at which the antioxidant activity was 50% and it was obtained by extrapolation from linear regression analysis; Data is expressed as mean±standard deviation of three determinations; Mean with different superscript letters (a, b, c, d) within the column indicate significant difference ($p<0.05$)

DPPH radical scavenging activity: DPPH free radical test is based on the exchange of a proton between the antioxidant and the stable DPPH free radical and shows absorption at 517 nm. In principle, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine, which can be observed by a rapid color change from purple to yellow and can be monitored spectrophotometrically (Matthäus, 2002). The results for 30% acetone, 30% methanol, 30% ethanol and aqueous extracts are shown in Fig. 3. The scavenging ability of all the crude extracts on DPPH radicals exhibited concentration dependence. Their scavenging capacity increased with increase in concentration. Thirty percent acetone extract exhibited the highest DPPH scavenging activity, followed by 30% methanol, 30% ethanol and water. The values obtained were 93.83, 92, 89 and 88%, respectively at a concentration of 3 mg/mL. In addition, the acetone extract had the highest scavenging activity at all concentrations. Statistical analysis revealed that the difference between the 30% acetone extract and the other samples was significant ($p<0.05$). Similar results were also found with respect to IC_{50} values as indicated in Table 4. Acetone had the least value (0.65 mg/mL), followed by methanol (0.69 mg/mL), ethanol (0.84 mg/mL) and water (1.24 mg/mL). A lower IC_{50} value means better efficiency of antioxidant activity of the sample. From these results acetone extract was the most potent solvent.

ABTS radical scavenging activity: ABTS scavenging activities of Roselle seed extracts are presented in Fig. 4. The results indicated that Roselle seed extracted by four different solvent have ABTS radical scavenging activity at the concentrations of 2-6 mg/mL. There was a significant ($p<0.05$) in the concentration of ABTS due to the scavenging capacity of each sample: acetone, methanol, ethanol and water extract. The scavenging effect on ABTS radical was in the following order: 30% acetone >30% methanol >30% ethanol >water and the values were 97.01, 91.16, 90.43 and 85.06%, respectively at a concentration of 6 mg/mL. Similar to DPPH results, the highest and the lowest ABTS scavenging activity was in the acetone and water extracts. Similar results were also found with respect to IC_{50} values (Table 4). Acetone had the least value (0.76 mg/mL), followed by methanol (1.68 mg/mL), ethanol (2.17 mg/mL) and water (2.49 mg/mL). It may be concluded that the acetone extract might be the appropriate solvent for extracting antioxidants with higher ABTS scavenging activity.

CONCLUSION

The results of this study show that Roselle seed is of high nutritional value and therefore can be a cheap potential source of many nutrients, especially protein and polyphenols. It was also revealed that 30% acetone effectively extracts polyphenols better than the other three solvents. Further investigations on the relationship between phenolic content and antioxidant activity of Roselle seed extract is needed, with particular emphasis on the identification of the bioactive compounds responsible for the biological activity.

ACKNOWLEDGMENT

This study was financially supported by National "Twelfth Five-Year" Plan for Science and Technology Support (2012BAD37B06) and National "Twelfth Five-Year" Plan for Science and Technology Support (2012BAD37B07). Authors are grateful to Alima Traore and Idrissa K Cissouma for their helpful advice.

REFERENCES

Akindahunsi, A.A. and M.T. Olaleye, 2003. Toxicological investigation of aqueous-methanolic extract of the calyces of *Hibiscus sabdariffa* L. J. Ethnopharmacol., 89: 161-164.

AOAC, 2000. Official Method of Analysis of AOAC. 17th Edn., Association of Official Analytical Communities, Arlington, VA, U.S.A.

Aruoma, O.I., 1998. Free radicals, oxidative stress and antioxidants in human health and disease. J. Am. Oil Chem. Soc., 75: 199-212.

Beecher, G.R., 1999. Phytonutrients' role in metabolism: Effects on resistance to degenerative processes. Nutr. Rev., 57: S3-S6.

Bubonja-Sonje, M., J. Giacometti and M. Abram, 2011. Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. Food Chem., 127: 1821-1827.

Ceirwyn, J.S., 1995. Analytical Chemistry of Foods. Blackie Academic and Professional, London, pp: 71-135.

Chen, C.C., J.D. Hsu, S.F. Wang, H.C. Chrang, M.Y. Yang, E.S. Kao, Y.O. Ho and C.J. Wang, 2003. *Hibscus sabdariffa* extrac inhibit the development of atherosclerosis in cholesterol-fed rabbits. J. Agric. Food Chem., 18: 5472-5477.

Cicerale, S., X.A. Conlan, S.A. Jinclair and R.S. Keast, 2009. Chemistry and health of olive oil phenolics. Crit. Rev. Food Sci., 49: 218-236.

Duke, J.A. and J.L. DuCellier, 1993. CRC Handbook of Alternative Cash Crops. 1st Edn., CRC Press Publisher, Boca Raton, Florida.

Durlach, J., 1988. Magnesium in Clinical Practice. Libbey Eurotext, London, Paris, pp: 360.

Enujiugha, V.N. and C.T. Akanbi 2008. Quality evaluation of canned fermented oil bean seed slices during ambient storage. Afr. J. Food Sci., 2: 54-59.

Faraji, M. and A. Tarkhani, 1999. The effect of sour tea (*H. sabdariffa*) on essential hypertension. J. Ethnopharmacol., 65: 231-236.

Garcia, W.J., C.W. Blessin and G.E. Inglett, 1972. Mineral constitutents in corn and wheat germ by atomic absorption spectroscopy. Cereal Chem., 49: 158-167.

Goli, A., M. Barzegar and M. Sahari, 2005. Antioxidant activity and total phenolic compounds of pistachio (*pistachia vera*) hull extracts. Food Chem., 92: 521-525.

Halliwell, B., J.M.C. Gutteridge and O.I. Aruoma, 1987. The deoxyribose method-a simple test-tube assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem., 165: 215-219.

Hirunpanich, V., A. Utaipat, N.P. Molales, N. Bunyapraphtsala, H. Sato, A. Herunsale *et al*., 2006. Hypocholesterolemic and antioxidant effects of aqueous extracts from the dried calyx of *Hibiscus sabdariffa L.* in hypercholesterolemic rats. J. Ethnopharmacol., 103: 252-260.

John, N.A., F. Zhong, K.J. Mothibe, M.L. Bangoura and C. Lagnika, 2012. Quantification of total polyphenolic content and antimicrobial activity of cocoa (*Theobroma cacao* L.) bean shells. Pak. J. Nutr., 11: 574-579.

Kappus, H., O.I. Aruoma and B. Halliwell, 1991. Lipid Peroxidation-mechanism and Biological Relevance. Free Radicals and Food Additives. Taylor and Francis Press, London, UK, pp: 59-75.

Matthäus, B., 2002. Antioxidant activity of extracts obtained from residues of different oilseeds. J. Agric. Food Chem., 50: 3444-3452.

Mohammed, R., J. Fernandez, M. Pineda and M. Aguilar, 2007. Roselle (*Hibiscus sabdariffa*) seed oil is a rich source of gamma-tocopherol. J. Food Sci., 72: 207-211.

Morton, J.F., 1987. Roselle. In: Morton, J.F. (Ed.), Fruits of Warm Climates. Miami, FL.

Muhammad, T.B. and A.B. Shakib, 1995. Jus *hibiscus*: bukan sekadar minuman biasa. Dewan Ekonomi, pp: 12-14.

Okawa, M., J. Kinjo, T. Nohara and M. Ono, 2001. Dpph (1, 1-diphenyl-2 -picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. Biol. Pharm. Bull., 24: 1202-1205.

Omobuwajo, T.O., L.A. Sanni and Y.A. Balami, 2000. Physical properties of sorrel (*Hibiscus sabdari* A) seeds. J. Food Eng., 45: 37-41.

Phanturat, P., S. Benjakul, W. Visessanguan and S. Roytrakul, 2010. Use of pyloric caeca extract from bigeye snapper (*Priacanthus macracanthus*) for the production of gelatin hydrolysate with antioxidative activity. LWT Food Sci. Technol., 43: 86-97.

Prasongwatana, V., S. Woottisin, P. Sriboonlue and V. Kukongviriyapan, 2008. Uricosuric effect of roselle (*Hibiscus sabdariffa*) in normal and renal-stone former subject. J. Ethnopharmacol., 117: 49-495.

Savage, G.P., 2001. Chemical composition of wallnuts (*Juglans regia L.*) grown in New Zealand. Plants Foods Hum. Nutr., 56: 75-82.

Shahidi, F., C.M. Liyana-Pathirana and D.S. Wall, 2006. Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chem., 99: 478-483.

Sleelig, G.F., 1980. Noncatalytic subunits of human blood plasma coagulation factor XIII. Preparation and partial characterization of modified forms. J. Biol. Chem., 255918: 8881-8886.

Sultana, B., F. Anwar and M. Ashraf, 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14: 2167-2180.

Tzeng, Y.M., L.L. Diosady and L.J. Rubin, 1990. Production of canola protein materials by alkaline extraction, precipitation and membrane processing. J. Food Sci., 55: 1147-1156.

Wu, H.C., H.M. Chen and C.Y. Shiau, 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int., 36: 949-957.

Zhang, H., F. Chen, X. Wang and H.Y. Yao, 2006. Evaluation of antioxidant activity of parsley (Petroselinum crispum) essential oil and identification of its antioxidant constituents. Food Res. Int., 39: 833-839.