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NUTRITIONAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *RAPHIONACME SPLENDENS* (SCHL.) TUBERS

Doka IG[1,2], El Tigani S[1], and S Yagi[1*]

Ibtisam Doka

*Corresponding author's email: sakinayagi@yahoo.com

[1]Botany Department, Faculty of Science, University of Khartoum, P.O. Box 321, Khartoum, Sudan

[2]Botany Department, Faculty of Science, University of Kordofan, Kordofan, Sudan

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ABSTRACT

People of Western Kordofan (Sudan) are endowed with a deep knowledge concerning the use of wild plants. Tubers of *Raphionacme splendens* Schl. subspecies *splendens* Flickr (family Apocynaceae), locally known as Elfayo, are used as a food reserve during times of famine or poor harvest. The aim of this study was to analyze the nutritional composition and antioxidant capacity of root tubers of *R. splendens*. Samples were collected from South-West Kordofan. Analyses included determination of moisture, carbohydrate, crude protein, fat, fibre, ash, minerals, vitamin C, amino acids and fatty acids composition. Antioxidant activity was determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2.2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) assays. The total phenolic content was also assessed. The results, which are referred to as (%) dry weight, showed that the tubers contained 3.2% protein, 18% carbohydrate, 0.5% lipid, 2.4% crude fibre, 3.5% ash, 79.2% moisture and gross energy 101.7 kJ/g. The total amino acids were 10776 mg/100g where the essential amino acids represented 28.2%. The more abundant essential amino acids were leucine (792mg/100g), isoleucine (712mg/100g) and threonine (536mg/100g). Methionine and lysine were the limiting amino acids. Minerals were potassium (259mg/100g), calcium (183mg/100g), magnesium (64mg/100g), phosphorus (37mg/100g), copper (3.6mg/100g), manganese (2.4mg/100g), zinc (1.8mg/100g) and iron (1.2mg/100g). Total saturated fatty acids were 45.6% whereas total unsaturated fatty acids were 54.4%. Oleic acid (32.56%) and palmitic acid (30.23%) were the most abundant fatty acids. Tubers displayed good antioxidant activity with IC_{50} values 0.987 and 1.559mg/mL against DPPH and ABTS radicals respectively. Vitamin C was 31.5mg/100g and total phenolic content was 60mg gallic acid equivalent (GAE) per 100g dry sample and they could be the main contributor to the antioxidant capacity of the tubers. In conclusion, the results of this study suggested that tubers of *R. splendens* could have beneficial effect for food and/or nutraceutical application for normal growth and adequate protection against diseases associated with reactions of free radicals.

Key words: *Raphionacme splendens*, proximate analysis, amino acids, fatty acids, antioxidant activity

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INTRODUCTION

The potential of many different wild edible plants as food for nutritional and health benefits is well recognized by many studies [1, 2]. In many parts of Africa, especially in rural communities, the use of wild edible plants as food source is an integral part of the culture of indigenous people [3, 4, 5].

Tuber crops along with other staples are commonly consumed in most countries in the world. They have great potential for ensuring food security and have ability to withstand drought. Cassava, Irish potato and sweet potato provide 93% of the root and tuber crops used for direct human consumption in the world. They rank among the top 10 food crops that are consumed as the main supply of food energy or carbohydrates [6, 7, 8].

In general, the Sudanese food diet is essentially composed of cereals, milk, eggs, fruits, and vegetables [9]. Cereal foods comprise about 49.8% of the total dietary energy supply while milk, eggs, and fish are about 16.9%, followed by sugar and sweets (10.1%) and roots and tubers (1.0%) [9]. Abdalla and Leonhäuser [10] reported that the farm households consume rarely vegetables and their consumption is mainly related to its availability in the village market and the income level of the household. The consumption of wild food is seasonal and usually gathered by children. *Raphionacmesplendens* Schl. subspecies *splendens* Flickr, locally known as Elfayo, is a wild root tuber belonging to the family Apocynaceae. *R. splendens* is an important water tuber used as a source of drinking water in the western part of Sudan especially in Kordofan region. The tubers are also used as a food reserve during times of famine or poor harvest. They are consumed raw as a staple and sometimes prepared as salad with onion. Sudan's flora is rich in wild plants which have good nutritional values. However, dietary utilization of non-domesticated plants has received very little attention in Sudan. To date, there is, to our knowledge, no available data on the food value of *R. splendens* tubers. Therefore, the present study aimed to analyze the nutritional composition and radical scavenging capacity of *R. splendens* tubers.

MATERIALS AND METHODS

Plant materials

Plants were collected from Southern-West Kordofan in July 2009, were identified and voucher specimens No. 1109KR6 was deposited in the Herbarium of Botany Department, Faculty of Science, University of Khartoum.

Preparation of samples and extracts

Tubers were washed with tap water after manually removing inedible parts, peeled, sliced and dried under shade at 30°C for two weeks to avoid direct loss of phytoconstituents from sunlight. All calculations were made on dry matter basis.
Ethanolic extract was also prepared for total phenolic and antioxidant capacity determination. The ethanol extract was prepared by soaking 20g of ground sample in

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200mL ethanol at ambient temperature for 6 hours. The extract was decanted, filtered and concentrated in a rotary evaporator to yield 1.8g.

Proximate analysis

Proximate analysis of the sample's moisture content, ash, ether extract and fibre content was done using the method reported by AOAC [11]. Nitrogen was determined by the micro-Kjeldahl method reported by Pearson [12]. Crude protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25. Carbohydrate content was estimated by subtracting the sum of the weights of protein, fibre, ether extract and ash from the total dry matter. Gross energy was calculated based on the formula by Eknayake *et al.* [13]: Gross energy (kJ/100g dry matter) = (crude protein x 16.7) + (crude lipid x 37.7) + (crude carbohydrates x 17.7).

Amino acids analysis

Amino acids composition of sample was measured as hydrolysate using an Amino Acid Analyzer (Sykam- S7130) based on high performance liquid chromatography technique [14]. Sample hydrolysis was prepared following the method of Moore and Stein [15]. Two hundred milligram of sample was taken into a hydrolysis tube. Five milliliters of 6 N HCl were added to the sample. The tube was tightly closed and incubated at 110°C for 24 hours. After incubation period, the solution was filtered and 200µL of the filtrate were evaporated to dryness at 140°C for an hour. The hydrolysate was diluted with 1 mL of buffer (citrate buffer pH 2.2). Aliquot of 150µL of sample hydrolysate was injected in cation separation column at 130°C. Ninhydrin solution and an eluent buffer (the buffer system composed of solvent A of pH 3.45 and solvent B of pH 10.85) were delivered simultaneously into a high temperature reactor coil (16m length) at a flow rate of 0.7mL/min. The buffer/ninhydrin mixture was heated at 130°C for 2min to accelerate chemical reaction of amino acid with ninhydrin. The products of the reaction mixture were detected at wavelength of 570nm (440nm for proline) on a dual channel photometer. The amino acids were identified by their retention time and wavelength ratio calculated from the areas of standards obtained from the integrator and expressed as mg/100g.

Mineral analysis

Minerals were analysed by dry-ashing 1g of the sample at 550°C in a furnace. The ash obtained was dissolved in 10% HCl, filtered through an acid-washed filter paper and made up to standard volume with de-ionised water. Sodium, potassium, calcium, magnesium, manganese, zinc, copper and iron contents were determined using atomic absorption spectrophotometry (Perkin Elmer A100, Tokyo, Japan). Phosphorus content was determined by employing the method reported using VanadoMolybdate and read on CECIL CE 3041 colorimeter [11].

Fatty acids profile

Fatty acids profile of total lipids was determined after trans-esterification with 14% boron trifluoride in methanol (1:1 v/v). Fatty acid methyl esters were analyzed by GC–MS (QP 2010 Shimadzu GC-MS equipment, Shimadzu Corporation. Kyoto, Japan). Supelco equity 1 column with a film thickness of 30m x 0.25microns was used. The total flow rate was 24mL/min and column flow rate was 1mL/min. Ultra high purity Helium was used as the carrier gas with injector split ratio of 20: 1. The ion source and

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inter-phase temperatures were 200ºC and 250ºC respectively. The solvent cut time of 4min and detector gain was 0.70kv. A Wiley 229 library search was conducted on major peaks of the sample in order to identify the components of the sample. The relative percentage of each compound was also determined.

Determination of vitamin C

The modified method of Bahorun *et al*. [16] was used to determine the vitamin C content of tubers. Ten grams of sample was blended with 40mL of a solution of 3% metaphosphoric acid in 8% glacial acetic acid, pH 1.5, for 1min. The extract was then mechanically shaken for 15min in darkness and filtered through glass wool. After filtration the clear extract was stored at −40°C prior to analysis by the 2,6-dichloroindophenol titrimetric method [17].

Determination of total phenolics

Total phenolic content in the ethanol extract of tubers was determined using modified Folin–Ciocalteu method [18]. Ethanol extract was re-suspended in ethanol to make 50mg/mL stock solutions. An aliquot of the extract was mixed with 5mL Folin–Ciocalteu reagent (previously diluted with water at 1:10 v/v) and 4mL (75g/L) of sodium carbonate. The tube was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. Absorbance was then measured at 765nm using the SHIMADZU UV-2550 UV-VS spectrophotometer. Total phenolic content was expressed as gallic acid equivalents (mg/100g) using the following equation based on the calibration curve: $y = 0.0057x$, $R^2 = 0.9315$, where x was the absorbance.

Antioxidant activity studies

DPPH radical-scavenging test

Antioxidant activity of the ethanol extract of tubers was estimated using DPPH *in vitro* method [19]. Test sample was dissolved separately in methanol to get test solution of 1mg/mL and then different concentrations (1, 5, 10, 20, 40, 60, 80 and 100µg/mL) were prepared by diluting with methanol. Assays were performed in 96-well, microtiter plates. One hundred and fourty microlitres (µL) of 0.6×10^{-6}mol/L DPPH was added to each well containing 70µL of sample. The mixture was shaken gently and left to stand for 30min in the dark at room temperature. The absorbance was measured spectrophotometrically at 517nm using a microtiter plate reader (Synergy HT Biotek, logiciel GEN5). Blank was done in the same way using methanol and sample without DPPH and control was done in the same way but using DPPH and methanol without sample. Ascorbic acid was used as an antioxidant standard compound. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) =

$$1- [(Abs_{sample} - Abs_{blank})]/ (Abs_{control})] \times 100$$

Where;

Abs_{sample} is the absorbance of DPPH radical + sample;

Abs_{blank} is the absorbance of sample+ methanol;

$Abs_{control}$ is the absorbance of DPPH radical + methanol.

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The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. The IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science, Chicago, Il, USA) using Pearson's correlation coefficient had a good coefficient of correlation, (R^2=0.998) [14].

ABTS radical-scavenging test

A second *in vitro* method was performed to estimate antioxidant potential of the ethanol extract: ABTS assay, based on the method of Re *et al*. [20]. Test sample was dissolved in methanol to get test solution of 1 mg/mL. A series of extract solutions of different concentrations (1, 5, 10, 20, 40, 60, 80 and 100µg/mL) were prepared by diluting with methanol. The ABTS radical cation ($ABTS^{*+}$) was produced by reacting 7mM stock solution of ABTS with 2.45mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12h before use. The obtained $ABTS^{*+}$ solution was diluted with methanol to an absorbance of 0.700±0.02 at 734nm. One hundred and ninety microlitre of $ABTS^{*+}$ solution was added to each well containing 10µL of sample. The mixture was shaken gently and left to stand for 15min in the dark at room temperature. The absorbance was measured spectrophotometrically at 734nm using a microtiter plate reader (Synergy HT Biotek®, logiciel GEN5). The $ABTS^{*+}$ scavenging capacity of the extract was compared with that of ascorbic acid and the percentage inhibition calculated as:

ABTS radical scavenging activity (%) =

$$[(Abs_{control} - Abs_{sample})]/ (Abs_{control})] \times 100$$

Where;

$Abs_{control}$ is the absorbance of $ABTS^{*+}$ (=0.700 ± 0.02);

Abs_{sample} is the absorbance of sample + $ABTS^{*+}$.

The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity obtained from three replicate assays. The IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, (R^2=0.9926) [14].

Statistical analysis

All analyses were performed in triplicate and data reported as mean ± standard deviation (SD). Statistical analyses were performed for the analysis of the Pearson correlation coefficients with the Statistical Package for the Social Sciences (spssx/pc) software (SPSS, Chicago, IL).

RESULTS

Nutritional value

The proximate composition of raw *R. splendens* tubers is given in Table 1. Results, which referred to (%) dry weight, showed that the tubers contained 3.2% protein content, 18% carbohydrate, 0.5% lipid, 2.4% crude fibre, 3.5% ash, 79.2% moisture and gross energy 101.7kJ/g.

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Amino acids content

The composition and amount of amino acids in raw *R. splendens* tubers are presented in Table 2. The total amino acids of *R. splendens* tubers were 10776mg/100g and the total essential amino acids were 3040mg/100g. The most abundant essential amino acids were leucine (792mg/100g), isoleucine (712mg/100g) and threonine (536mg/100g). The roots contained an abundance of the non-essential amino acids, in decreasing order, aspartic acid (1960mg/100g), glutamic acid (1656mg/100g), alanine (1336mg/100g) and proline (1184mg/100g).

Minerals content

Mean values for mineral content of raw *R. splendens* tubers are presented in Table 3. Minerals were potassium (259mg/100g), calcium (183mg/100g), magnesium (64mg/100g), phosphorus (37mg/100g), copper (3.6mg/100g), manganese (2.4mg/100g), zinc (1.8mg/100g) and iron (1.2mg/100g).

Fatty acids composition

The fatty acids composition of raw *R. splendens* tubers was low, only 14 types are reported (Table 4). Total saturated fatty acids were 45.6% whereas, total unsaturated were 54.4%. Mono-unsaturated fatty acids and poly-unsaturated fatty acids accounted for 33.54% and 20.86%, respectively. Ratio of unsaturated fatty acid: saturated fatty acid (U:S) was 1.6. Oleic acid (32.56%) and palmitic acid (30.23%) represented the most abundant unsaturated and saturated fatty acids, respectively.

Antioxidant activity, vitamin C and total phenolic content

The antioxidant property of raw *R. Splendens* tubers was also investigated using the DPPH and ABTS assays (Table 5). The IC_{50} value of the tubers against DPPH radicals was 0.987mg/mL and was 1.559mg/mL against ABTS radicals. Vitamin C content of the tubers was found to be 31.5mg/100 g and their total phenolic content was 60mg gallic acid equivalent (GAE) per 100g dry sample (Table 5).

DISCUSSION

A comparison of the proximate composition of this tuber and other tubers consumed in Africa (Table 1) indicated that this tuber has a relatively higher protein content (3.2%) than wild cassava (1.3%), potato (2.0%), sweet potato (1.6%) and comparable to that of wild yams (3.2%). Total carbohydrate was high (18%) but lower than that reported for wild cassava and sweet potato (27%) but comparable to those of potato and wild yam (19%). Lipid content was relatively low (0.5%) but was relatively high compared to wild cassava (0.0%), potato (0.1%), sweet potato (0.2%) and wild yams (0.1%). The crude fibre content was 2.4%, whereas, the ash content was 3.5%. These values were also relatively higher than those of the other root tubers listed in Table 1. Moreover, these results were also found to be higher than those reported for the root vegetable carrot which contained 1.0% protein, 8.8% carbohydrate, 0.2% lipid and 0.8% ash [21]. The gross energy value was 101.7kj/g comparable to that reported for radish (94.0kj/g) [21].

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Essential amino acids represented 28.2% of the total amino acids of *R. splendens* tubers. The most abundant essential amino acids were leucine representing 20.3% of RDA, isoleucine representing 35.6% of RDA and threonine representing 35.7% of RDA. Methionine (7.2% of RDA) and lysine (6.7% of RDA) were the limiting amino acids.

Macro-minerals like calcium and phosphorus play major structural roles and others like sodium and potassium function as electrolytes. Micro-minerals often serve as catalysts in enzyme reactions [22]. Deficiency or excess of elements may cause a number of disorders. Raw *R. splendens* tubers appeared to be especially rich in calcium and magnesium representing 18.3% and 16% of RDI respectively. Copper and manganese were the predominant micro-nutrient elements and their levels exceeded the RDI. Other micro-nutrient elements were generally in low concentrations.

The fatty acids profile of raw *R. splendens* tubers resembles most edible oils where oleic acid and palmitic acid were the most predominant acids. The ratio of unsaturated fatty acid: saturated fatty acid (1.6) was similar to that of sweet potato and *Dioscorea dumetorum* but considerably lower than that of Irish potato [24, 25].

Results of the antioxidant property of *R. splendens* tubers showed that tubers have higher scavenging capacity than those reported for yam varieties (IC_{50} value $_{DPPH}$ ranged from 1.7 to 14.800mg/mL) from Philippine and for potato varieties (IC_{50} value $_{DPPH}$ ranged from 41.815 to 58.195mg/mL) from Iran [25, 26]. Typical compounds that possess antioxidant activity have been characterized as vitamin C and phenolic compounds. In this study, the content of vitamin C of tubers of *R. splendens* was relatively in the range of cassava (15–45 mg/100g) and potato (12.4-27.8mg/100g) [27, 28]. The total phenolic content of the tubers was relatively low. Thus, tubers of *R. splendens* might be considered as good sources of natural antioxidants that could help in the prevention of cancer, inflammation and other diseases related to radical mechanisms.

The nutritional importance of dietary diversity is now widely recognized. Growing a range of local crops supplemented by wild-harvested species helps provide such diversity in the diet [29]. Wild plants represent an important part of the diet for rural people in western Sudan. Their consumption provides them with most of their daily requirements of macro and micronutrients and in many cases they also have medicinal properties and form part of local health care systems. Results of nutritional composition of tubers of *R. splendens* suggest that, this plant could contribute greatly in alleviating malnutrition in Sudan.

CONCLUSION

Nutritionally, *R. splendens* tubers could successfully substitute and/or complement the domesticated tubers consumed in Sudan and could contribute greatly towards meeting human nutritional requirements for normal body growth. Moreover, the results of this

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study suggested that the tubers could have beneficial effect for food and/or nutraceutical application in the promotion of health.

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Table 1: Proximate composition and nutritional data of *Raphionacme splendens* tubers

Parameters	*R. splendens*	Wild Cassava[a]	Potato[a]	Sweet potato[a]	Wild Yam[a]
Protein (%)	3.2 ± 0.02	1.3[a]	2.0[a]	1.6[a]	3.2[a]
Lipid (%)	0.5 ± 0.01	0.0[a]	0.1[a]	0.2[a]	0.1[a]
Carbohydrate (%)	18 ± 0.07	27.6[a]	19.0[a]	27.5[a]	19.0[a]
Dietary fibre (%)	2.4 ± 0.02	1-2 [b]	0.4[a]	1.0[a]	0.8[a]
Ash (%)	3.5 ± 0.02	1.1[a]	1.0[a]	0.9[a]	1.1[a]
Moisture (%)	79.2 ± 0.6	58-81[b]	50-81 [b]	50-81 [b]	65-73 [b]
Gross energy value (kj/g)	101.7	149 [c]	110	121 [c]	119 [c]

Values are means (± SD) of triplicate analysis and are expressed on a dry weight basis.
Source: [a], Food Composition Tables For Use In Africa [30].[b], IITA [31]. [c], FAO [32]

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 16 No. 1 March 2016 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 2: Amino acids profile of *Raphionacme splendens* tubers (dry weight basis, mg/100g)

Amino acids	*R. splendens* tubers	Adult requirement/day*
Essential		
Thr	536	1500
Met	72	1000
Ile	712	2000
Leu	792	3900
Tyr + Phe	488	2500
Lys	200	3000
His	240	1000
Total	3040	
Non-essential		
Asp	1960	
Ser	432	
Glu	1656	
Gly	120	
Ala	1336	
Arg	1048	
Pro	1184	
Total	7736	
Total aminoacids	10776	

Values are means (± S. D.) of triplicate analysis. *Source: FAO/WHO/UNU [33]

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Table 3: Mineral composition of *Raphionacme splendens* tubers (dry weight basis, mg/100g)

Element	Concentration (mg/100g)
Potassium	259 ± 0.01
Calcium	183 ± 0.02
Phosphorus	37 ± 0.3
Copper	3.6 ± 0.01
Manganese	2.4 ± 0.02
Iron	1.2 ± 0.01
Zinc	1.8 ± 0.01

Values are means (± SD) of triplicate analysis

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 16 No. 1 March 2016 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 4: Composition of fatty acids of *Raphionacme splendens* tubers

Fatty acids		% fatty acid
Caproic acid	C6:0	0.34
Undecanoic acid	C11:0	ND
Lauric acid	C12:0	ND
Tridecanoic acid	C13:0	ND
cis-9 Myristoleic acid (n-5)	C14:1	ND
Myristi acid	C14:0	0.75
cis-10-Pentadecenoic acid	C15:1	ND
Pentadecanoic acid	C15:0	1.65
cis-9 Palmitoleic acid (n-7)	C16:1	0.98
Palmitic acid	C16:0	30.23
cis-6,9,12y-Linolenic acid (n-6)	C18:3	5.90
cis-9,12 Linoleic acid (n-6)	C18:2	2.44
trans 9,12 Linolelaidic acid	C18:2	11.16
Oleic acid (n-9)	C18:1	32.56
Stearic acid	C18:0	6.73
cis-3,8,11,14 Arachidonic acid (n-6)	C20:4	1.38
Heneicosanoic acid	C21:0	0.38
Behenic acid	C22:0	0.41
Tricosanoic acid	C23:0	5.11
Nervonic acid (n-9)	C24:1	ND
Total saturated fatty acids (TSFAs)		45.6
Total unsaturated fatty acids (TUSFAs)		54.4
Mono-unsaturated fatty acids (MUSFAs)		33.54
Poly-unsaturated fatty acids (PUSFAs)		20.86

Values are means(± S. D.) of triplicate analysis

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Table 5: Vitamin C, total phenolic content and antioxidant activity of *Raphionacme splendens* tubers

	Vitamin C (mg/10 g)	Total phenolic content (mg GAE/100g)	IC_{50} (mg/mL) DPPH	IC_{50} (mg/mL) ABTS
Tubers	31.5 ± 0.01	60 ± 0.02	0.987 ± 0.01	1.559 ± 0.01
Ascorbic acid			0.027 ± 0.03	0.025 ± 0.02

Values are means(± S. D.) of triplicate analysis

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