Compositional Quality and Antioxidant Activity of *Hibiscus Sabdariffa*

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ABSTRACT

Compositional quality and antioxidant activity of Egyptian *Hibiscus sabdariffa* (roselle) seeds and calyces were investigated. Proximate composition revealed that Egyptian roselle seeds contained high amount of protein, lipid and fiber. All essential amino acids except threonine were present in higher levels than those recommended by FAO. Calcium, potassium and phosphorus were the most abundant minerals in both seeds and sepals, the levels were higher in sepals than seeds. Palmitic acid was the most predominant saturated fatty acid, while oleic and linoleic were the major unsaturated fatty acids in roselle seed oil. Moreover, six sterol compounds were found, sitosterol, brassicasterol and stigmasterol were in highest levels.Total phenolic compounds and antioxidant activity of water extract of roselle calyces were investigated. Different polyphenolic compounds (quercitin, kaemferol, catechins, protocatechuic acid, proanthocyanidin, gossypetine, hibiscetine, and sabdaretine) which play a key role as natural antioxidants were found. Anthocyanin pigments (delphinidin-3-glucoside and cyaniding-3-glucoside) which are responsible for red colour of roselle calyces were found in high amounts. The calyces extract of *Hibiscus sabdariffa* showed marked antioxidative activity, not only in linoleic acid but also in scavenging of the DPPH radical.

INTRODUCTION

Hibiscus sabdraiffa L. (roselle) belongs to the family Malvacea. It is an annual herb cultivated for its leaves, stem, seed and calyces (Babalola *et al*., 2001 and Fasoyiro *et al*., 2005 a,b). The origin of *Hibiscus sabdariffa* is not fully known, but it is belived to be native of tropical Africa (El-Meleigy, 1989). It is known by different synonyms and vernacular names such as roselle (Chenowarin *et al*., 1999, Tsai *et al*., 2002), karkadeh (Abu-Tarbouch *et al*., 1997) and mesta (Rao, 1996). There are different forms, botanical varieties and cultivars of roselle cultivated in many countries. One of the biggest world producers of roselle sepals is Egypt (Kosakowska *et al*., 2005).

In Egypt, "Karkadeh" is considered a very popular beverage and valuable medicinal plant due to its effect on lowering and/or adjusting the blood pressure without producing any side effect (Faraji and Tarkhani, 1999). Also, it has a favourable effect on the functions of stomach. It possesses a high intestinal antiseptic action and can be used to resist various infections of intestinal diseases (Owolabi *et al*., 1995).

Hibiscus sabdraiffa L. is rich in riboflavin, niacin, calcium and iron (Babalola *et al*., 2001, Qi *et al*., 2005 and Raifa *et al*., 2005). The *Hibiscus sabdariffa* petals are potentially a good source of antioxidant agents as anthocyanins and ascorbic acid (Prenesti *et al*., 2007). It was reported that the pigments (anthocyanins) which are responsible primarily for red colour were delphinidin-3-glucoside and cyaniding -3- glucoside (Kalt *et al*., 1992). Recently, the biological activities of anthocynin, such as antioxidant activity, protection from atherosclerosis and anticarcinogenic activity have been investigated, and shown to have some beneficial effects in the treatment of diseases (Tsai *et al*., 2002). *Hibiscus sabdraiffa* is also contains antioxidants including flavonoids, gossypetine, hibiscetine and sadderetine (Fasoyiro *et al*., 2005 a,b). Moreover, the extract of sepals also proved experimentally to have highly antibacterial properties (Abu-Tarboush and Ahmed, 1996 and Omobuwajo *et al*., 2000 and and Olaleye, 2007).

However most of the studies were focused on the benefits of the calyces rather than the seeds of the plant. There are published reports indicating that the seed are eaten in some parts of Africa, and also have been roasted as a substitute for coffee (Duke, 1983, Morton, 1987 and Bengaly *et al*., 2006).

The nutritional usefulness of the roselle seeds has been rarely studied as compared with calyces. Information from the literature indicated that Roselle whole seeds powder from other countries contained high amounts of protein, oil, carbohydrate (El-Adawy & Khalil, 1994, Rao, 1996 and Parkouda *et al*., 2008). Moreover, studies on chemical composition of roselle seeds indicated the presence of fatty acids (palmitic, oleic and linoleic acids) and sterols (Kosakowska *et al.,* 2005). However, nutritional compositions of seeds vary depending on the variety, location and environmental conditions where the seeds were grown (Mostafa *et al*., 2005).

The objectives of the present study were to evaluate the compositional quality of Egyptian roselle seeds and to investigate active compounds and antioxidative activity of dark red calyces extract.

MATERIALS AND METHODS

2.1. Materials

Dark red sepals and seeds of *Hibiscus sabdariffa* were obtained from Medicinal and Aromatic Plants Research Station, Agricultural Research Centre, Ministry of Agriculture, Egypt.

2.2. Chemicals

All chemicals used for the study were of analytical grade from Merck (Darmstadt, Germany) unless stated otherwise. Folin–Ciocalteu reagent, gallic acid (98%) and l,l-Dipheny-2-picrylhydrazyl (DPPH) were obtained from Fluka Chemie AG (Switzerland). Linoleic acid and catechin were purchased from Sigma Co. (St. Louis, MO).

2.3. Preparation of samples

The seeds were removed manually from their capsules, and cleaned to get rid of foreign matters. Whole seeds were sun dried and ground using an electric mill, to a fine powder. The obtained powder was used for the determination of chemical composition (El-Adawy and Khalil, 1994).

The dried *Hibiscus sabdariffa* (20g) was extracted with boiling water (500ml) for 10 min for two times, and the collected filtrate was evaporated in a vacuum at 70°C on a rotary evaporator. This extract was weighted and dissolved in distilled water and used for the assessment of antioxidant activity (Duh and Yen, 1997).

2.4. Proximate analysis

Moisture, total nitrogen, total lipids, crude fiber, crud ash and starch were determined according to AOAC (1990). Moisture content of seed powders was determined according to an air-oven method. Total nitrogen and the protein content were determined based on the Kjeldahl method using the conversion factor of 6.25. Lipids were determined by using Soxhlet method. Ash content was determined by incinerating at 550 °C until the constant weight was achieved.

2.5 Amino acids Analysis

The amino acids content of the samples were analysed and quantitatively determined using the HPLC (Waters System Interface module 501, Hewlett Packard, California, USA). About 0.10 g of seeds powder was weighed into a medium wall pyrex test tube, and 15 ml of 6 M hydrochloric acid was added. The tube was quickly sealed and hydrolyzed under nitrogen environment in an oven at 110 °C for 24 hours. After hydrolysis, the mixture was allowed to cool at room temperature, transferred to 50 ml volumetric flask and 10 ml aminobutyric acid (AABA) was added as an internal standard. The volume was topped to 50 ml with deionised water. The mixture was filtered through a Whatman filter paper

No. 1, then through a Whatman filter paper No. 42 before the derivatization process. About 10 ml of filtered sample were transferred into derivatization tube, and evaporated to dryness under vacumm (100-200 mililitor) for 30 min. Subsequently, a redrying reagent (a mixture of methanol-triethylamine and deionised water (2:1:2, w/w/w)) was added and redried for another 30 min. After the final drying, a derivatization reagent (methanol-phenyltiocyanate [PITC] – triethylamine and deionised water [7:1:1:1, v/v/v/v]) was added. To ensure that the coupling reaction with PITC was completed, the derivatization process was et for 20 min. It was then evaporated to dryness for another 20 min. About 100 ml of sample diluent (a mixture of disodium hydrogen phosphate, deionised water, 10% orthophosphoric acid and acetonitrile) were added to the sample. Of the prepared sample, 20 ml of aliquot and 8 ml of blank solution were injected into Picotag column (C18, 3.9 x 150 mm, Waters, Medford, MA). All determinations were performed in duplicates. The quantification of each amino acid was determined from a standard calibration. Tryptophan was destroyed by acid hydrolysis (White and Hart, 1992) and thus, was not determined.

2.6. Minerals

Minerals were analyzed (Kosakowska *et al*., 2005) by ashing 1 g of sample at 550 °C in a Prolabo furnace (Paris France) and then dissolving it in HCl. Calcium (Ca), iron (Fe), magnesium (Mg), potassium (K), and sodium (Na) were determined from the resulting solution using Perkin-Elmer (Norwalk, CT, USA. Phosphorus was determined colorimetrically forming a phosphovanadomolybdate complex detected at 470 nm (AOAC 986.24) [8] with a Bausch and Lomb Spectronic 1001 spectrophotometer (Rochester, NY, USA).

2.7. Fatty acid composition and sterols

Total lipid was extracted from seed powder as described by Abdalla (2007). Fatty acid composition of *Hibiscus sabdariffa* seed oil samples was determined as follows:

50 mg of lipid was transferred into screw-cap vial, then 2 ml benzene and 10 ml 1% H2SO4 in absolute methanol were added and the vial was covered under stream nitrogen before heating, then saponification and methylation were carried out for 60 min. Methyl esters in each vial were extracted with 5 ml of petroleum ether.

Analysis of fatty acid methyl esters was carried out using Gas chromatography GC-4C Shimadzu CM (PFE) equipped with flame

ionization detector (FID) and glass column (3 m X 3 mm i.d) packed with 5% DEGC on 80/100 chromosorb. Column temperature was 180°C isothermal and detector temp was 270°C. Gas flow rates were 20 ml/min for N2, 75 ml/min for H_2 and 0.5 ml/min for air. Standard mixture of FAME was analyzed under identical conditions prior to running the sample. The concentration of FAME was calculated by triangulation method.

Sterols were separated and identified as described by Kosakowska *et al*., (2005) using the HPLC (Waters System Interface module 501, Hewlett Packard, California, USA).

2.8 Total phenolic content and active compounds

Total phenolic content was determined using the Folin-Ciocalteu's phenol reagent and expressed as gallic acid equivalents (mg gallic acid per gram of dried material) as described by Singleton *et al.* (1999).

Different active compounds were determined as described by Puckhaber *et al*. (2002) using high pressure liquid chromatography (HPLC). Various suppliers provided flavonoid and standard compounds with purity levels of 99% (St.Louis, Missouri, Milwaukee, Wisconson and New Brunswick, New Jersey). The LC system employed is a Hewlett-Packard (HP) 1050 Series modular system equipped with an 1100 Series diode array detector at Dundee University, UK.

2.9. Antioxidant activity of calyces extract

2.9.1. Determination of antioxidant activity in linoleic acid

Antioxidant activity was carried out by using the linoleic acid system (Osawa & Namiki, 1981). The extract (5 mg) was added to a solution mixture of linoleic acid (0.13 ml), 99.8% ethanol (10 ml), and 0.2 M phosphate buffer (pH 7.0, 10ml). The total volume was adjusted to 25ml with distilled water. The solution was incubated at 40°C and the degree of oxidation was measured according to the thiocyanate method (Mitsuda *et al.,* 1966), with lOm1 of ethanol (75%), 0.2ml of an aqueous solution of ammonium thiocyanate (30%), a 0.2ml sample solution and a 0.2ml ferrous chloride solution (20m~ in 3.5% HCl) being added sequentially. After stirring for 3 min, the absorption values of the mixtures measured at 500 nm were taken as the peroxide contents. The percent inhibition of linoleic acid peroxidation, 100- [(Abs increase of sample/Abs increase of control) x 100] was calculated to express antioxidative activity. All test data are the averages of triplicate analyses.

2.9. 2. Scavenging effect on DPPH radical

The effect of *Hibiscus sabdariffa* calyces water extracts on DPPH radical was estimated according to the method of Hatano *et al.*(1988). The herbal water extracts were passed through the cartridge (Sep-Pak Cis, Waters) with a syringe; the eluates were repeated twice under the same condition. The extracts decolorized were added to a methanolic solution (1 ml) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand at room temperature for 30min; the absorbance of the resulting solution was measured spectrophotometrically at 5 17 nm.

RESULTS AND DISCUSSION

3.1. Proximate analysis:

Proximate composition of *Hibiscus sabdariffa* are presented in Table (1). The average values of the major contents of the whole mature seeds of *Hibiscus sabdariffa* are showed that the seeds contain protein (26.20%), total lipids (20.40%), crude fiber (15.85%), crude ash (3.05%), starch (2.15%) and total carbohydrate (24.55%).

The values of all proximate components are in agreement with earlier studies (Al-Wandawi *et al*., 1984, El-Adawy and Khalil, 1994, Abou-Tarboush *et al*., 1997, Yagoub *et al*., 2004, Bengaly *et al*., 2006 and Parkouda *et al*., 2008).

The protein content of the seeds was higher when compared with other common seeds and legume beans such as black seeds (Saleh, 1992), sunflower seeds, melon seeds, chickpeas, cowpeas, pigeon peas, soybeans, and groundnuts (FAO, 2001).

The roselle seeds have been shown to be a good source of dietary fiber that contained a balance proportion of soluble and insoluble fractions.

Table (1). Proximate composition of *Hibiscus sabdariffa* seeds

Component	%
Moisture	7.80
Protein (N× 6.25)	26.20
Total lipids	20.40
Crude fiber	15.85
Crude ash	3.05
Starch[a]	2.15
Total carbohydrate[b]	24.55

Values are mean of three determinations.
[a]Starch percent in defatted and sugar-freed seed flour.
[b]Total carbohydrate is determined by difference.

3.2. Amino acids

Amino acid composition of *Hibiscus sabdariffa* seeds are presented in Table (2). The percentage composition of amino acids in the analyzed samples revealed that a total of 17 amino acids excluding tryptophan were recorded in the roselle seeds investigated in this study. Essential amino acids accounted for about 36.54 g/100 g protein and non-essential amino acids represented about 60.52 g/ 100 g protein. All essential amino acids except threonine occurred at higher levels in roselle seeds than those of FAO reference protein. Methionine and cystine were the main limiting amino acids present.

The amino acids content of the seeds were higher than previous studies (El-Adawy & Khalil, 1994; Rao, 1996; Abu-Tarboush *et al*. 1997). This may be attributed to higher protein content observed in the current study. In addition, all the essential amino acids were higher than wheat grain and rice (Samy, 1980).

Lysine content of raw and processed seeds was found to be higher and adequate for human requirement. The high Lysine may be used as a complementary food mixture for poor or low lysine sources.

Table (2). Amino acid composition of *Hibiscus sabdariffa* seeds

Amino acid	g/100g protein	FAO (1991)
Leucine	7.15	7.0
Isoleucine	4.24	4.0
Lysine	6.46	5.5
Methionine	0.78	-
Cystine	0.57	-
Phenylalanine	6.48	6.0
Tyrosine	4.30	4.0
Threonine	2.90	5.0
Valine	3.66	3.5
Total essential amino acid	36.54	
Alanine	3.16	
Arginine	6.50	
Aspartic acid	10.12	
Glutamic acid	19.20	
Glycine	15.10	
Histidine	2.10	
Praline	2.18	
Serine	2.16	
Total non essential amino acid	60.52	

3.3. Mineral contents

Mineral composition of the roselle seed and sepals are shown in Table (3). Calcium followed by potassium and phosphorus were the most abundant minerals in both seeds and sepals. Sepals contained higher amounts of minerals than seeds. Since some cereals flours are deficient in calcium e.g. pearl millet (46 mg/100g) and sorghum (15 mg/100g) (FAO, 2001), the fortification of flours with roselle seed flour might improve their dietary properties as was suggested by Bengaly *et al*. (2007). The results in this study were in agreement with El-Adawy and Khalil (1994) and Rao (1996). However, the previous values reported in the literature were higher than the current values. Mineral elements were reported to be significantly influenced by variety, location and environmental conditions (Koehler *et al*.1987; Barampama & Simard, 1993; Rao, 1996). These factors may be responsible for different variations exhibited from the current and previous values. Magenisum, sodium and iron of the seeds were present in low amounts, and another source may be necessary for supplementing some of these elements. Some cereal powders in the baking industry are very deficient in some elements, particularly calcium. Fortification of these powders with roselle seeds powder may improve their dietary properties.

Table (3). Minerals of *Hibiscus sabdariffa* seeds and sepals

Minerals (mg/100 g dry weight)	Seeds	Sepals
Calcium	335	1968
Magnesium	365	575
Sodium	472	105
Potassium	1215	1850
Iron	10	42
Phosphorus	618	1315

3.4. Fatty acid composition

The results of fatty acid composition of roselle seed oil (Table 4) revealed that palmitic acid was the most predominant saturated fatty acid (19.8% of total fatty acids), while oleic acid (29.5%) and linoleic acid (34.5%) were the major unsaturated fatty acids. Myristic (1.1%), linolenic (0.8%), arachidic (1.5%), erucic (1.1%) and lignoceric (1.4%) were present as minor fatty acids. Eicosadienic acid (2.5%) and arachidonic acid (2.3%) were found in low levels.

In this respect, Ahmad *et al* (1979) found that crude karkade seed oil contained 34.6 % oleic acid, 37.4% linoleic acid and 20.5% palmitic acid. Abou-Tarboush *et al*. (1997) reported that the fatty acid composition of *Hibiscus sabdariffa* seed oil contained 19.9% palmitic, 36.8% oleic and 33.4% linoleic acids in higher amounts than other fatty acids. Mostafa *et al*. (2005) and Kosakowska *et al*. (2005) published that roselle seed oil is a valuable source of saturated and unsaturated fatty acids. They concluded that palmitic, oleic and linoleic acids were the major fatty acid constituents.

Table (4). Fatty acid composition of *Hibiscus sabdariffa* seed oil

Fatty acid		% (Total fatty acids)
Myristic	$C_{14:0}$	1.1
Palmitic	$C_{16:0}$	19.8
Stearic	$C_{18:0}$	4.2
Oleic	$C_{18:1}$	29.5
Linoleic	$C_{18:2}$	34.5
Linolenic	$C_{18:3}$	0.8
Arachidic	$C_{20:0}$	1.5
Eicosadienic	$C_{20:2}$	2.5
Arachidonic	$C_{20:4}$	2.3
Erucic	$C_{22:1}$	1.1
Lignoceric	$C_{24:0}$	1.4
Unknown		1.3

Values are mean of three determinations.

3.5. Sterol content

Sterols are one of the most important group of biologically active compounds in the roselle seeds. Six sterol compounds were found in the seed oil of investigated *Hibiscus sabdriffa* (Table 5). The seed oil was characterized by relatively high content of sitosterol followed by brassicasterol and stigmasterol. Campesterol and Δ^5-avenasterol were present in distinctly lower concentration.

Salama and Ibrahim (1979) found six different free sterols in the roselle seed oil; cholesterol, ergasterol, campesterol, stigmasterol, sitosterol and spinasterol. The studies of Holser *et al*. (2004) indicated the presence of campesterol, stigmasterol, sitosterol and ∆-avenasterol. Kosakowska *et al*. (2005) determined sterols in roselle seed oil by the HPLC analysis. Four free sterols were found, brassicasterol, campesterol, stigmasterol and sitosterol. They concluded that sitosterol, the most interesting sterol from the pharmacological point of view was found in highest content.

Table (5). Sterols of *Hibiscus sabdariffa* seed oil

Sterols	mg/kg oil
Cholesterol	0.25
Campesterol	0.80
Stigmasterol	1.22
Brassicasterol	1.32
Sitosterol	2.24
Δ^5- avenasterol	0.55

Values are mean of three determinations.

2.6. Phenolic compounds and antioxidant activity

The total phenolic content of roselle calyces extract was determined using the Folin-Ciocalteu's phenol reagent. Total phenol content was found to be 29.3 as gallic acid equivalent.

Different active compounds were found in roselle calyces extract (Table 6). Anthocyanin pigments (delphinidin-3-glucoside and cyaniding-3-glucoside) which are responsible for red colour were found in high amounts.

Bricage (1985) found that the calices of roselle contained high content of anthocyanic pigments; cyaniding (29%) and delphinidin (71%). Kalt *et al*. (1992) reported that the pigments (anthocyanins) which are responsible primarily for red colour were delphinidin-3-glucoside and cyaniding -3- glucoside. A number of active compounds obtained from roselle include anthocyanins, flavonols and protocatechuic acid have been found to have antioxidant activity, and to offer protection against cancer and LDL (Seca *et al*., 2001, Carton, 2001 and Essa *et al*., 2006).

Wang *et al*. (2000) and Hirunpanich *et al*. (2006) found that hibiscus anthocyanin, a pigment extract from the calyx of roselle, significantly reduced oxidative stress and lowering and/or adjusting the blood pressure.

In this study, different polphenolic compounds which play a key role as natural antioxidants were found in roselle calyces extract. Quercitin, kaemferol, catechins, protocatechuic acid, gossypetine, hibiscetine, and sabdaretine were identified.

The antioxidative effectiveness in natural sources was reported to be mostly due to phenolic compounds. Gutifinger (1981) indicated that phenolic compounds play an important role in inhibitory autoxidation of fatty foods. Yen and Chen (1995) and Abdalla (2007) noted that polyphenols are the most abundant group of compounds tea leaf and different medicinal plants and seem to be responsible for antioxidative activity. These investigations implied that the total phenolic compounds are closely related to antioxidative activity. Abdalla (2007) found a good correlation between polyphenol content of different medicinal plant and antioxidant activities.

Hibiscus sabdiriffa calyx exhibited strong antioxidant activity during the oxidation of linoleic acid in an aqueous dispersion (Table 6). This result indicated that the extracts of *Hibiscus sabdiriffa* contain high levels of total phenolic compounds and may contribute to inhibition of lipid peroxidation.

It is well known that free radicals play an important role in autoxidation of unsaturated lipids in foodstuff (Kaur and Perkins, 1991). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was used as a free radical to

evaluate antioxidant activity of some natural sources (Yen and Chen, 1995).

In the present work, the scavenging activity of the roselle calyces water extracts on inhibition of the DPPH radical was related to the amounts of phenolic compounds. The result demonstrated that the water extract of *Hibiscus sabdiriffa* have effective activities as hydrogen donors as primary antioxidant by reacting with the lipid radical.

Table (6). Total phenol content, major phenolic constituents and antioxidant activity of *Hibiscus sabdariffa* calyces extract

Constituent	Value
Total phenol content	29.3
Major phenolic constituents (mg/100 g dry weight)	
Delphindin-3-glucoside	39.2
Cyanidin-3-glucoside	25.6
Quercitin	25.4
Gossypetin	4.1
Hibiscetine	3.1
Sabdaretine	2.1
Catechins	2.5
Pro-catechuic acid	2.8
Kaempherol	1.5
Antioxidant activity	
Inhibition of linoleic acid peroxidation	
Calyces extract	22.5
BHT	21.8
Scavenging effect on DPPH radical	
Calyces extract	91.5
BHT	92.5

CONCLUSION

Compositional quality of Egyptian *Hibiscus sabdariffa* (roselle) seeds was investigated. Proximate composition, amino acids, minerals, fatty acids and sterols were determined. The roselle seeds have been shown to be a good source of protein, lipid and dietary fiber. All essential amino acids except threonine were present in higher levels than those recommended by FAO. Calcium, potassium and phosphorus were the most abundant minerals in both seeds and sepals, the levels were higher in sepals than seeds. Palmitic acid was the most predominant saturated fatty acid, while oleic and linoleic were the major unsaturated fatty acids in roselle seed oil. Moreover, sitosterol, brassicasterol and stigmasterol were found in higher concentrations than campesterol and Δ^5-avenasterol.

Total phenolic compounds and antioxidant activity of water extract of dark red roselle calyces were investigated. Total phenol content was found to be 29.3 as gallic acid equivalent. Different polyphenolic compounds (quercitin, kaemferol, catechins, protocatechuic acid, gossypetine, hibiscetine, and sabdaretine) which play a key role as natural antioxidants were found. Anthocyanin pigments (delphinidin-3-glucoside and cyaniding-3-glucoside) which are responsible for red colour of roselle calyces were found in high amounts. The calyces extract of *Hibiscus sabdariffa* showed marked antioxidative activity, not only in linoleic acid but also in scavenging of the DPPH radical. These investigations implied that the total phenolic compounds are closely related to antioxidant activity. Further investigation of the roselle nutritional composition and antioxidant effects may be needed to confirm its health benefits to humans.

REFERENCES

Abdalla, A.E., Darwish, S., Ayad, E.H. and El-Hamahmy, R. (2007). Egyptian mango by-product 1. Compositional quality of mango seed kernel. Food Chemistry 103, 1134–1140**.**

Abu-Tarboush, H.M. and Ahmed, S.B. (1996). Studies on Karkade (*Hibiscus sabdariffa*): protease inhibitors, phytate, in *vitro* protein digestibility and gossypol content. Food Chemistry, 56, 15-19.

Abu-Tarboush, H. M., Ahmed, S. A. B., & Al Kahtani, H. A. (1997). Some nutritional properties of Karkade (*Hibiscus sabdariffa)* seed products. *Cereal Chemistry, 74,* 352-35.

Ahmad, M., Husain, S. And Osman, S. (1979). *Hibiscus sabdariffa* seed oil. J. Sci. Food Agric., 30, 424-428.

Association of Official Analytical Chemists. (1990). *Official Methods of Analysis*. *14th ed*. *(Williams S, ed.)*. AOAC, Virginia.

Babalola, S.O., Babalola, A.O. and Aworth, O.C. (2001). Compositional attributes of roselle (*Hibiscus sabdariffa* L.). Journal of Food Technology in Africa, 6, 133-134.

Barampama, Z., & Simard, R.E. (1993). Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*Phaseolus vulgaris*) grown in Burundi, *Food Chemistry, 47,* 159-167.

Bengaly, M., Bere, A. and Traore, A. (2006). The chemical composition of bikalga, a traditional fermented roselle seed condiment. J. Food and Plant Chemistry, 1, 7-11.

Bricage P (1985). Etude des ompetitio pigmentaires du bissap, *Hibiscus sabdariffa* L., Malvacées. IV. Influence des ompetitio parentaux et des conditions stationnelles sur la germination et le développement des individus: ompetition entre individus. Bulletin de l'I.F.A.N. T46, série A, n° 1-2: 140-166.

Carton, E. (2001). Specific antioxidant activity of caffeoyl derivative and other natural phenolic compounds, LDL protection against oxidation and decrease in the proinflammatory lysophosphatidylcholine production. Journal of natural products, 64, 480-486.

Duh, P. and Yen, G. (1997). Antioxidative activity of three herbal water extracts. Food Chemistry, 60, 639-645.

Duke, J.A. (1983). Malvaceae Roselle. In: *Handbook of Energy Crops* (pp 345-369). El-Adawy, T. A., & Khalil, A. H. (1994). Characteristic of Roselle seeds as a new source of protein and lipid. *Journal of Agricultural and Food Chemistry, 42* (9), 1896-1900.

El-Adawy, T. And Khalil, A. (1994). Characteristics of roselle seeds as a new source of protein and lipid. J. Agric. Food Chem., 42, 1896-1900.

El-Meleigy, S. (1989). Physiological studies on roselle plant *Hibiscus sabdariffa* L. Ph.D. Thesis, Ain Shams Univ.

Essa, M.M., Subramanian, P., Manivasagam, T., Dakshayani, K. and Subash, S. (2006). Protective influence of *Hibiscus sabdariffa*, an edible medicinal plant, on tissue lipid peroxidation and antioxidant status in hyperammonemic rats. African Journal of Traditional, Complementary and Alternative Medicines, 3, 10-21.

Faraji, M.H. and Tarkhani, A.H. (1999). The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. Journal of Ethnopharmacology, 7, 231-236.

FAO. (2001). Improving Nutrition through Home Gardening-A Training Package for preparing Field Workers in Africa. Rome, Italy: Food and Agriculture Organization of United Nation.

FAO/WHO. (1991). Protein quality evaluation. Report of Joint FAO/WHO expert consultation. Rome, Italy: Food and Agriculture Organization of United Nation.

Fasoyiro, S.B., Babalola, S.O. and Owosibo, T. (2005a). Chemical composition and sensory quality of fruit-flavoured roselle (*Hibiscus sabdariffa*) drinks. World Journal of Agricultural Sciences, 1, 161-164.

Fasoyiro, S.B., Ashaye, O.A., Adeola, A. and Samuel, F.O. (2005b). Chemical and storability of fruit-flavoured (*Hibiscus sabdariffa*) drinks. World Journal of Agricultural Sciences, 1, 165-168.

Gutfinger, T. (1981). Polyphenols in olive oils. JAOCS, 58, 966-968.

Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root. Chem. Pharm. Bull., 36, 2090-2097.

Hirunpanich, V., Utaipat, A., Morales, N., Sato, H. and Suthisisang, C. (2006). Hypocholesterolemic and antioxidant effect of aqueous extracts from dried calyx of *Hibiscus sabdariffa* L. in hypercholesterolemic rats. Journal of Ethnopharmacology, 103, 252- 260.

Holser, R. and Boven, V. (2004). Phytosterol composition of hybride *Hibiscus* seed oil. J. Agric. Food Chem., 52, 2546-2548.

Kalt, W., Prange, R.K., Lidster, P.D. (1992). Postharvest colour development of strawberries. Canadian Journal of Plant Science, 73, 541-548.

Koehler, H. H., lung-Hsia Chang, C. H., Scheier, G., & Burke, D. W. (1987). Nutrition composition, protein quality and sensory properties of thirty-six cultivars of dry beans (*Phaseolus vulgaris*). *Journal of Food Science*, *52,* 1335- 1340. Liquid chromatographic analyses of amino acids in feed and foods modification of the Pico-Tag method. 1984. Millipore Corp.

Kosakowska, O., Weglarz, Z., Sidky, M. and Geszprych, A. (2005). Sterols and fatty acids in the seed of roselle cultivated in Egypt. Herba Polonica, 51, 18-23.

Milsuda, H., Yasumoto, K. and Iwami, K. (1966). Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyo Skokuryo, 19, 210-214.

Morton, J. (1987). Roselle. In Morton, J.F., *Fruits of warm climates* (pp. 281-286).

Mostafa, H., Hala, B., Hemmat, K. and Merfat, S. (2005). Improving the characteristics of roselle seeds as a new source of protein and lipid. J. Applied Sci. Res., 1, 161-167.

Olaleye, M.T. (2007). Cytotoxicity and antibacterial activity of Methanolic extract of *Hibiscus sabdariffa*. Journal of Medicinal Plants Research, 1, 9-13.

Omobuwajo, T.O., Sanni, L.A. and Balami, Y.A. (2000). Physical properties of sorrel (*Hibiscus sabdariffa*) seeds. Journal of Food Engineering, 45, 37-41.

Osawa, T. and Namiki, M. (1981). Anovel type of antioxidant isolated from leaf wax of eucalypus leaves. J. Agric. Biol. Chem., 45, 735-739.

Owolabi, O.A., Adegunlye, B.J. and Obiefuna, P.C. (1995). Mechanism of relaxant effect mediated by an equeous extract of *Hibiscus sabdariffa* petal in isolated aorta. International Journal of Pharamacology, 33, 210-214.

Prenesti, E., Berto, S., Daniele, P. and Toso, S. (2007). Antioxidant power quantification of decoction and cold infusion of *Hibiscus sabdariffa* flowers. Food Chemistry, 100, 433-438.

Parkouda, C., Biawara, B. and Ouoba, L. (2008). Technological and physico-chemical characteristics of bikalya, alkaline fermented seeds of *Hibiscus sabdariffa*. African J. Biotech., 7, 916-922.

Puckhaber, L.S., Stipanovie, R.D. and Bost, G.A. (2002). Analyses for flavonoid aglycones in fresh and preserved *Hibiscus* flowers. In Trends in new crops and new uses. Edited by Janick, J. and Whipkey, A. ASHS Press, Alexandria, VA.

Qi, Y., Chin, K.L., Malekian, F. and Gager, J. (2005). Biological characteristics, nutritional and medicinal value of roselle *Hibiscus sabdariffa*. Circular-Urban Forestry Natural Resources and Environment, No. 604, 1-2.

Raifa, A., Hemmat, K., Hala, B. and Mervat, S. (2005). Increasing the active constituents of sepals of roselle plant by applying gibberellic acid and bezyladenine. J. App. Sci. Res., 1, 137-146.

Rosamond, W.D. (2002). Dietary Fibre and prevention of cardiovascular disease. *Journal of American College of Cardiology, 39,* 57-59.

Rao, P.U. (1996). Nutrient composition and biological evaluation of mesta (*Hibiscus sabdariffa*) seeds. *Plant Food for Human Nutrition, 49,* 27-34.

Rosamond, W.D. (2002). Dietary Fibre and prevention of cardiovascular disease. *Journal of American College of Cardiology, 39,* 57-59.

Salama, R. and Ibrahim, S. (1979). Ergosterol in *Hibiscus sabdariffa* seed oil. Planta Medica, 36, 221.

Saleh Al-Jassir, M. (1992). Chemical composition and microflora of black cumin (*Nigella sativa L*.) seeds growing in Saudi Arabia. *Food Chemistry, 45,* 139-242.

Samy, M.S. (1980). Chemical and nutritional studies on Roselle seeds (*Hibiscus Sabdariffa* L.). *Journal of oleo science, 10,* 32-38.

Seca, A.M.L., Silva, A.M.S., Silvestre, A.J.D, Cavaleiro, J.A.S., Domingues, F. M. J and Neto, C. P. (2001). Phenolic constituents from the core of kenaf (*Hibiscus cannabinus*). Phytochemistry, 56, 759-767.

Seena, S., Sridhar, K. R. & Jung, K. (2004). Nutritional and antinutritional evaluation of raw and processed seeds of a wild legume, *Canavalia cathartica* of coastal sand dune of India. *Food Chemistry, 92(3),* 465-472

Singleton V. L., Orthofer R. and Lamuela-Raventos R. M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods in Enzymology, 29, 152 – 178.

Tasi, P.J., Chew, A.K. and Tseng, M.N. (2002). Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. Food Research International, 35, 351-356.

Tseng, T. H., Kao, E. S., Chu, C. Y., Chou, F. P., Lin Wu, H. W., & Wang, C. J. (1997). Protective effects of dried flower extracts of *Hibiscus sabdariffa L*. against oxidative stress in rat primary hepatocytes. *Food and Chemical Toxicology, 35(12),*1159-1164.

Wang, C.J., Wang, J.M., Chu, W.L. and Tseng, T.H. (2000). Protective effect of *Hibiscus* anthocyanins against tert-butyl hydroperoxyide induced hepatic toxicity in rats. Food and Chemical Toxicology, 38, 411-416.

Yagoup, A., Mohamed, B., Ahmed, A. and El Tinay, A. (2004). Study on a traditional Sudanese fermented roselle. J. Agric. Food Chem., 52, 6143-6150.

Yen, G. and Chen, H. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 43, 27-32.

الملخص العربى

الجودة التركيبية والنشاط المضاد للأكسدة للكركدية

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أوضحت نتائج دراسة الجودة التركيبية أن بذور الكركدية المصرى تحتوى على نسب مرتفعة من البروتين والزيت والألياف. وأن البروتين عالى الجودة لإحتواءة على جميع الأحماض الأمينية الضرورية بنسب أعلى من الموصى بها من منظمة الأغذية والزراعة. وأن الكالسيوم والبوتاسيوم والفوسفور كانت من أهم المعادن المتواجدة بنسب مرتفعة وقد إرتفع معدلها فى السبلات عن البذور. كما أوضحت النتائج أن الزيت المستخلص من بذور الكركدية المصرى غنى بالحامض الدهنى المشبع "البالمتيك" وبالأحماض الدهنية غير المشبعة "الأولييك واللينولييك" ويحتوى على العديد من مركبات الإستيرولات أهمها سيتوستيرول و براسكاستيرول و ستيجماستيرول. وقد تم تقدير محتوى الفينولات الكلية والمركبات الفينولية وكذا النشاط المضاد للأكسدة فى المستخلص المائى لسبلات الكركدية المصرى غامق اللون وأوضحت النتائج إحتواء المستخلص على نسبة مرتفعة من الفينولات الكلية وعلى العديد من المركبات الفينولية الفعالة بجانب وجود مركبات الأنسوسيانين المسئولة عن اللون الأحمر لسبلات الكركدية. أوضحت الإختبارات المختلفة أن لهذا المستخلص الطبيعى نشاط مضاد للأكسدة أقوى من مضادات الأكسدة الصناعية.