




A COMPARATIVE STUDY ON SOME MAJOR CONSTITUENTS OF KARKADE (*HIBISCUS SABDARIFFA* L. – ROSELLE PLANT)

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ABSTRACT

Hibiscus sabdariffa plant {Botanical name: *Hibiscus sabdariffa* Linne (Malvaceae)}, commonly known as "rossell" in the English language, and "karkade" in Arabic. It is a plant widely cultivated in both tropical and subtropical regions including Sudan, (Kordofan and Darfur regions). Karkade calyces are used as a cold or hot drink when soaked in water, having refreshing, nutritional and medicinal characteristics. This study aimed at investigating the levels of some major constituents of the rossell calyces and compares their levels within the two varieties (Red and white calyces). The phytochemical, proximate and the specific analyses showed that the calcium levels in Sudanese karkade (25 mg/100g and 25.5 mg/100g in red calyces and white calyces, respectively) were lower than the levels reported by the previous studies, but the iron levels (55.5 mg/100g and 30.5 mg/100g) were higher than levels reported by the previous studies. More investigations may be required to interpret this low level of calcium in some Sudanese karkade varieties. The anthocyanin levels (1.8% and 1.05% in red calyces and white calyces, respectively), and the vitamin C levels (16.5 mg/100g and 16.9 mg/100g) were approximately similar in both red and white karkade calyces, but the level of hibiscus acid of the red calyces was lower than its level in the white karkade calyces. The notable variations between results reported in literature about karkade (*Hibiscus*) constituents may be due to some factors include the variations of the genotypes, environmental and botanical nature of the tested samples, besides the use of different methodologies by researchers concerning laboratory techniques and the sampling collection methods.

KEYWORDS: *comparative; constituents; Hibiscus sabdariffa; karkade; Roselle; red and white calyces.*

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INTRODUCTION

The plant

Karkade, or Roselle {Botanical name: *Hibiscus sabdariffa* Linne (Malvaceae)}, is a plant widely cultivated in both tropical and subtropical regions¹, including México, India, Saudi Arabia, China, Egypt, and Sudan²⁻⁵. *Hibiscus sabdariffa* L. is the scientific name of Roselle (in Arabic called “karkade”) ⁶. Roselle (*Hibiscus sabdariffa* L.) (karkade) had different common names including azelle, sorrel, Jamaican sorrel, Indian sorrel, Guinea sorrel, sour-sour, and Queensland jelly plant^{7,1}. It is a plant of increasing interest for its applications in health, nutrition, and cosmetic products. In Sudan, karkade is grown in western parts of the country (Kordofan and Darfur)⁸. May be carried later to the new world and cultivated there as in Central America⁹. Karkade (Roselle) is grown for its calyces, it is a cash crop cultivated by traditional farmers under rain-fed conditions¹⁰. The world's best karkade (roselle) comes from Sudan, but the quantity is low, and poor processing hampers quality^{11,12-15}.

This annual herb grows to 1.5 m or higher and produces elegant red flowers. The flowers (calyces and bract portions) are collected when slightly immature¹⁶⁻¹⁸. The two botanical types of roselle are *Hibiscus sabdariffa* var. *sabdariffa*, grown for thick fleshy, shiny-red calyces, and *Hibiscus sabdariffa* var. *altissima*¹⁹. The flowers or calyces are most popular for uses in beverages and natural pigments and used as ‘vegetables’²⁰ consumed either hot like tea or cold as a refreshing beverage²¹⁻²³, and are used in the preparation of jams and jellies. The juice from the calyces is claimed to be a health-enhancing drink due to its high content of vitamin C, anthocyanins and other antioxidants^{14,17-18,24-26}.

Phytochemical contents

Karkade flowers (calyces) contain various compounds such as polyphenols, including anthocyanins, flavonols, in addition to some other pigments^{17,27-29}, and acids such as oxalic, malic, citric, stearic, and tartaric acids, in addition to hibiscic or hibiscus acid (lactone of hydroxycitric acid) which composes 15% to 28% of the total acid content^{17,25}. The roselle oil is rich in gamma tocopherol³⁰, mucilage (62 %) ³¹. Amino acids such as arginine, aspartic acid, and glutamic acid were found in the protein isolated from the seed^{25,30}. Early studies reported that karkade (*Hibiscus sabdariffa*) contains protein (1.9 g/100g), fat (0.1

g/100g), carbohydrates (12.3 g/100g) and fibre (2.3 g/100g). The studies also reported that karkade is rich in vitamin C (14 mg/100 g), β -carotene (300 μ g/100g), calcium (1.72 mg/100g) and iron (57 mg/100g)³. Ascorbic acid content in karkade (*Hibiscus sabdariffa*) varies dramatically between fresh (6.7–14 mg/100g)^{1,3} and dried calyces (260–280 mg/100g)³. Some studies found that the anthocyanin content reached 1.7% to 2.5% of the dry weight in all strains. Polysaccharides are another key group of compounds present in large quantities in the cHs WE (karkade) may reach 10% level³⁰. Karkade (Roselle) is rich in anthocyanins, this major pigment, formerly reported as hibiscine, has been identified as daphniphylline^{30,32}.

Red and white, hot and cold extracts of karkade calyces

In some literature, it was cited that, the red and white karkade calyces contained 11 and 9.3% moisture respectively. Also, the following levels were found for red and white karkade respectively, 0.16 and 0.12% (fat), 13.2 and 12% (fiber), 7.88 and 7.35% (protein), 10.6 and 9.5% (ash), and 57.16 and 61.55% (carbohydrates). The red and white karkade calyces were also contained 11 and 15.5 mg/100 g of vitamin C, 60 and 50 mg/100g of calcium, 25 and 20 mg/100g of iron and 9 and 11 mg/100g titrable acidity, respectively. The sensory evaluation of cold and hot drinks made from both kinds of red and white karkade revealed that there was no significant difference in most phytochemical parameters^{33,34}. Hassab El Rassol (2015)³⁵ found similar TSS, for both hot and cold extracts of different *Hibiscus sabdariffa* (karkade) genotypes, and a significant difference in the pH values of the cold extracts of the genotypes³⁵. He reported that Ascorbic acid content was reduced from 72.87 mg/100g in the cold to 19.4 mg/100g in the hot extract for one of the hibiscus genotypes. The color intensity of different *Hibiscus* genotypes ranged from 0.014 to 0.298. He found that the hot extraction method was more effective for all measured parameters, except ascorbic acid content^{35,36}.

Effects of storing temperature and time on the color of karkade

Beatrice *et al.* (2014)³⁷ reported that no color change was observed in the roselle-fruit blends during the 6 months of storage at 4°C. These results are similar to the findings of Saeed and Ahmed (1977)³⁸ about visual color change. Anthocyanins are responsible for the red color in karkade-mango juice blends, and color of anthocyanin is pH

dependent (the red flavylum is stable at low pH)³⁷. Vitamin C content of all karkade-fruit blends decreased during storage probably due to the sensitivity of vitamin C to oxygen, light, and heat³⁹. Many studies have been conducted on physicochemical and antioxidant properties of karkadeextract⁴⁰⁻⁴². During storage, the total phenolic content TPC decreased at 28°C more than at 4°C, irrespective of storage intervals, and some monomeric anthocyanins might have been transformed into polymeric compounds^{43,44}. The antioxidant capacity of fruits and vegetables, which benefits human health, is highly correlated with their anthocyanin and TPC⁴⁵; therefore, storage at 4°C should be encouraged when the products need to be stored for long time³⁷.

Medicinal applications of karkade

Many medicinal applications of the karkade plant have been developed around the world. There are reports in the scientific literature about the effects of karkade on decreasing the risk of hypertension, diabetes mellitus, and fever⁴⁶, besides, pyrexia, liver damage⁴⁷, leukemia⁴⁸. It can treat respiratory tract infections and used in treating wounds, and sores⁵¹. Extracts had an antioxidant potential⁵⁰. The fresh calyces are rich in riboflavin, niacin and iron⁵². Hatil Hashim, *et al.* (2006)⁴⁹ evaluated the antibiotic effect of *Hibiscus sabdariffa* (karkade), *E. coli* showed higher resistance to the plant extracts whereas *Pseudomonas aeruginosa* is more sensitive. An odd study of Amos (2003)⁵³ reported that aqueous extract of *Hibiscus sabdariffa* (karkade) calyces may contain psychoactive substances, which are sedative in nature with possible neuroleptic properties. The present study was at comparing the levels of some major constituents of karkade (*Hibiscus sabdariffa* L. - Roselle plant) within two varieties, the red and white dry calyces, aiming at evaluating the nutritional value of this Sudanese plant fruits.

MATERIAL AND METHODS

General

In this study, some chemical characteristics and constituents of dry red and white karkade (Roselle) (*Hibiscus sabdariffa*) calyces (Karkade calyces from Sudan) were measured. Reviewing some previous studies on karkade, particularly of

chemical analysis, and comparing results of this recent study with that previous studies. A series of laboratory experiments were undertaken at the College of Agricultural Studies, Sudan University of Science and Technology, at Shambat, Sudan.

Sampling

The plant material (Red karkade and white karkade) dry calyces were purchased from the local market of Omdurman, Sudan, the production of the last year (2017) rainfall season. Those are the two widely used cultivated varieties of *Hibiscus sabdariffa* L. (originally from *Elrahad* and *Elfashir* – Kordofan). The Identification and authentication of *Hibiscus sabdariffa* var were done by a collective agreement of the doctors and specialized technicians of the Central laboratory of College of Agricultural Studies, Sudan University of Science and Technology. The dry calyces were then crushed using a grinding machine.

Extraction

100 grams of each sample was weighted using sensitive balance, shaken with 500ml of distilled water at 200rpm for 24 h at room temperature. And subsequently filtered under suction (reduce pressure using vacuum pump), extract samples were transferred to ice form and dried using freeze dryer machine (-50 °C for 48 h). Dry samples were collected and kept in vials till used. Triplicate results were obtained for each sample and the mean value was reported.

The chemical composition of karkade (*Hibiscus*) flower (calyces)

The moisture content was determined according to the Association of Official Analytical Chemists (AOAC 2008)⁵⁴. Moisture content (%) was calculated by subtracting sample weight after drying from the sample weight before drying, oven (No.03-822, FN 400, Turkey) at 105 °C ± 1. The difference divided by the initial sample weight, then multiplied by 100%. The ash content was determined according to the method described by Pearson (1981)⁵⁵, five grams were put in a porcelain crucible and placed into a Muffle furnace (No.20. 301870, Carbolite, England) at 550 to 600 °C until a white-gray ash was obtained. The ash content was calculated as a percentage based on the initial weight of the sample, as follows:

$$\text{Ash \%} = \frac{[(\text{Wt of crucible} + \text{Ash}) - (\text{Wt of empty crucible})] \times 100}{\text{Initial weight (Wt)}}$$

The Fat content was determined according to the official method of AOAC 2008⁵⁴. The sample added to about 100 ml hexane into the extraction unit (Electrothermal, England) for 16 hr., then the

solvent was redistilled, and put in an oven at 105 °C., cooled, reweighed and the dried extract was registered as fat content and calculated as follows:

$$(W2 - W1) \div W3 \times 100 \quad (\text{where: } W3 = \text{initial weight of the sample})$$

Crude fiber content was determined according to the official method of the AOAC 2008⁵⁴. Defatted sample added to 20 ml of H₂SO₄ (0.26 N). After digestion, filtration, the precipitate boiled in 20 ml NaOH (0.23 N) solution for 30 min under a reflux condenser, filtered and rinsed, then the crucible was

dried at 105 °C (overnight) to a constant weight, cooled (in a desiccators), weighed, ashed in a Muffle furnace (No.20. 301870, Carbolite, England) at 550-600 °C until a constant weight was obtained and the difference in weight was considered as crude fiber:

$$\text{Crude fiber \%} = \frac{[\text{Dry residue crucible Cal}] - [\text{Ignited residue + crucible (g)}] \times 100}{\text{Sample weight}}$$

Total soluble solids (TSS) was measured with a hand refractometer (Mettler Toledo, Schwerzenbach, Switzerland) and expressed as Brix. The pH was measured using a portable pH meter (HANNA HI9125, Cluj-Napoca, Romania). The Crude protein content was determined by micro-Kjeldahl method using a copper sulphate-sodium sulphate catalyst according to the official method of the AOAC 2008⁵⁴. Two grams sample transferred together with 4g Na₂SO₄ of Kjeldahl catalysts (No. 0665,

Scharlauchemie, Spain) and 25 ml of concentrated sulphuric acid into a Kjeldahl digestion flask. After digestion, the distillation of ammonia was carried out into 25 ml boric acid (2%) and sodium hydroxide solution (45%). Finally, the distillate was titrated with standard solution of HCl (0.1N) with bromocresol green and methyl red as an indicator until a brown reddish colour was observed, then calculated as follows:

$$\text{Nitrogen \%} = \frac{\text{Titre volume} \times \text{HCl (N)} \times \text{Nitrogen equivalent weight} \times 100}{\text{Sample weight} \times 1000}$$

$$\text{Crude protein \%} = \text{Nitrogen \%} \times \text{Protein conversion factor (6.25)}$$

Total and available carbohydrates were calculated by difference according to the following equations:⁵⁶

$$\begin{aligned} \text{Total carbohydrates} &= 100 - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash}) \\ \text{Available carbohydrates} &= \text{Total carbohydrates} - \text{Crude fiber.} \end{aligned}$$

Vitamin C content for the karkade solution had been determined according to the method used by Dashman(1996)⁵⁷ with some modifications using Folin-Ciocalteu reagent (FCR). Twenty milliliters of the sample with 2 mL of 10% tetrachloroacetic acid solution diluted to 100 mL with distilled water. One milliliter of the sample or standard solution (3 mg ascorbic acid in 1 mL distilled water) followed by the addition of 3 mL distilled water and 0.4 mL of FCR and incubated at room temperature for 10 min. The absorbance was read at

760 nm using a UV-VIS spectrophotometer (Perkin-Elmer). Titratable acids were determined using 0.1 N sodium hydroxide and phenolphthalein as an indicator and were expressed as % malic acid, and hibiscus acid, as hibiscus acid comprises a citric acid moiety with an additional hydroxyl group. To determine anthocyanin content in karkade, we followed the method used by Lee(2005)⁵⁸ who determined the total monomeric anthocyanin (TMA) content for roselle-fruit juice blends using the pH differential method:

$$A = (A_{250} - A_{700})_{\text{pH}1.0} - (A_{250} - A_{700})_{\text{pH}4.5}$$

$$AC = \frac{A \times MW \times DF \times 1000}{\epsilon L}$$

where A is the difference of sample absorbance between pH 1.0 and 4.5, ϵ is the molar extinction coefficient for cyanidin-3-glucoside (26,900 L/mol/cm), L is the path length of the spectrophotometer cell (1.0 cm), DL is the dilution factor and molecular weight (MW) of cyanidin-3-glucoside (449.2 g). The results were expressed as mg cyanidin-3-glucoside equivalent/100 g extract (mg/100 g). To determine the selected Minerals content (calcium and iron), five grams were placed into a muffle furnace (No.20. 301870, Carbolite, England) at 550 to 600 °C until a white-grey ash was obtained. Then, 10 ml of HCl (2.0N) was added in a hot sand bath for about 10-15 min., the ash was filtered, added to hot distilled water, and the concentrations of calcium and iron were measured using Atomic Absorption Spectrophotometer (3110-Perkin Elmer. USA).

STATISTICAL ANALYSIS

The data obtained were analyzed using Excel version 2016. And were presented as the mean standard deviation (SD) of triplicate trials of samples testing.

RESULTS AND DISCUSSION

General

The common name of Roselle (*Hibiscus sabdariffa*) in Sudan is "Karkade" and may be written "karkade"³⁵ and "karkadi"³⁴, rarely it is written as "Carcade", "karkadè", "Karkadeh", or "Kerkade". However, we prefer to use the term "karkade" as it is commonly pronounced (car – ka – day). Both white and red karkade types are grown from the same tree type in the same climatic and cultivation conditions, but genetic factors may cause colors differences. (Figure1)

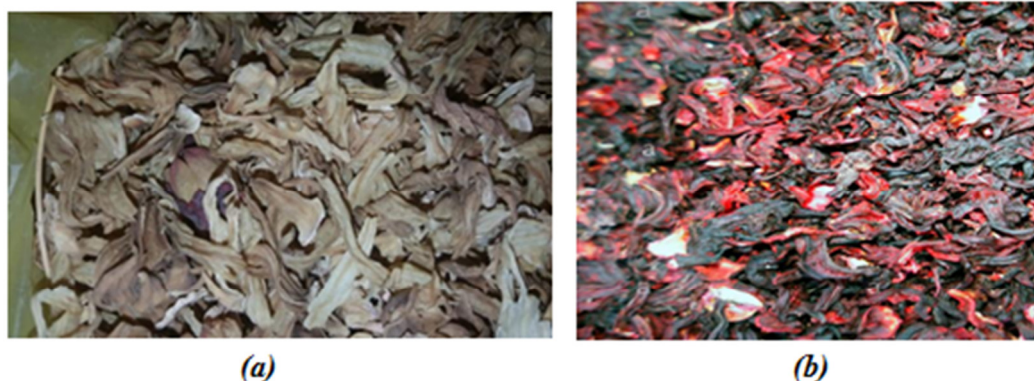


Figure 1
White karkade (a), and Red karkade (b) dry calyces from Sudan

The red karkade is the mostly used rather than white karkade in Sudan; it is used as a cold or hot drink (karkade tea) which is commonly used for releasing colds and respiratory infections symptoms. This medicinal use of karkade is almost due to its high content of Vitamin C.

Physico-chemical parameters

The Phytochemical investigations and proximate analysis of Karkade calyces samples showed that the moisture is almost equal for all samples of dry red and white karkade (mean = 5.37% and 5.22%). The moisture is affected by the long storing period of the karkade calyces, which cause the dryness, particularly in the hot climate of Sudan (up to 50 °C in summer). This result is less than previous studies conducted on Sudanese karkade calyces, which is 11% moisture for red and 9.3% for white karkade³⁴. The ash content was 9.00% for red calyces and 10.01% for white calyces, this result is higher than

the result reported by Gabb (1997)⁵⁹ at 7%, and It is almost nearer to the results reported by FAO⁶⁰ which are 10.6% and 9.5%, but less than the results found by Duke and Atchley (1984)⁶¹. The fiber content was 9.15% for red calyces and 9.26% for white calyces. This result is lower than the result reported for some Sudanese karkade, by Gabb⁵⁹ which is 12%, and Kamal Edin (2016)⁶², and the results reported by FAO⁶⁰ which are 13.2 and 12%. Both Duke⁶¹ and Ismail³⁶ had reported fibre content of karkade, as 2.3g/100g. Total soluble solids (TSS) were 6.5% for the red and 7.0% for the white Karkade calyces. These results were almost similar to results reported in the literature, Abdallah³⁴ had reported 5% TSS for red karkade calyces and 5.5% for white karkade, indicating a little higher value for the white calyces. Solid contents and colour intensity of full spectrum images of fruits may be used to determine the correct maturity stage of fruits⁶³. The pH of the

red karkade and white karkadecalyces solution in water was 2.24 and 2.12, respectively. These values were not far from the values reported by HassabEl Rassol³⁵ who reported pH value (2.64) for hot

extract and (2.04) for the cold extract of some hibiscus types. pH results were also in line with results reported by Kamal Edin⁶². (Table 1)

Table 1
The results of Karkade Physico-chemical parameters

Parameters samples	Moisture content (%)*	Total ash (%)*	Crude fiber (%)*	TSS (%)*	pH*
Red roselle	5.37±0.02	9.00±0.40	9.15±3.48	6.50±0.17	2.24±0.01
White roselle	5.22±0.01	10.01±0.07	9.26±0.03	7.00±0.10	2.12±0.07

* Mean ±SD of triplicatetrials (n=3) on dry weight (DW) basis.

Biochemical parameters

Fat contents for red and white calyces were 0.78% and 0.87%, respectively. This result is higher than the value found at 0.1 - 0.3 g/100g by Ismail³, and that of Duke and Atchley⁶¹. Studies conducted in Sudan had almost reported a low fat level compared with the international records which stated fat levels as 0.16 for red and 0.12% for white karkade (rossell)³³, in addition to a result of 0.6% fat content reported by the US Department of agriculture¹⁶. In this study the crude protein content found to be 4.07% for the red; and 5.52% for the white dry calyces of karkade. These results of crude protein agree with the study on Sudanese karkade by Gabb⁵⁹, which is 5%. Ismail found a protein content in a range of 3.3 % - 7.88%³. Kamal⁶² had analyzed some Sudanese karkade and found some differences between (cold extracted) and (hot extracted) calyces. Carbohydrates levels were found at 69.39%; and 69.11% for the red and white

calyces, respectively. These carbohydrate levels were slightly higher than what reported by Abdallah *et al.* (57.16 and 61.55%)³⁴. The karkade plant as a whole is rich in carbohydrates⁶⁰. In this study, Vitamin C level was found to be 16.5mg/100g for red calyces, while the white calyces contain 16.9 mg/100g of the vitamin. These results were somewhat differed from some previous results reported by Abdallah *et al.*³⁴ (11 and 15.5 mg/100 g, respectively). Earlier studies had reported as low as 0.02–0.05% of ascorbic acid in rossell plant⁶⁴. Other Sudanese studies stated that karkade is rich in ascorbic acid (14 mg/100g, and 54 mg/100g)³. However, the present results are closer to levels reported by some international records which stated the value of vitamin C in rossell at 14%¹⁶. Hassab El Rassol (2015)³⁵ found a higher vitamin C content in cold extracts of some karkade types (72.87mg/100g), than that of hot extracts (19.4mg/100g). (Table 2)

Table 2
The results of Karkade (roselle) calyces Biochemical parameters

	Fat (%)*	Crude protein (%)*	Avaiailable CHO (%)*	Vitamin C mg /100g *
Red roselle	0.78±0.78	4.07±0.01	69.39±0.06	16.50±0.3
White roselle	0.87±0.015	5.52±0.02	69.11±0.01	16.90±0.45

* Mean ± SD of triplicatetrials (n=3) on dry weight (DW) basis.

These variations in results of vitamin C content between white and red karkade calyces may be due to the variations in the storing conditions including time, temperature, humidity, and packing of the karkade calyces¹². However, in Sudan, drying is the traditional method for preserving foods, Karkade dried or dehydrated either by leaving the fruit to partially dry on the plants, then harvested or by sun-drying post harvesting²¹. The bad storing and preserving conditions and the primitive traditional techniques of drying and processing the karkade calyces in Sudan decreases the quality of

Sudanese Karkade, although some reports had described the Sudanese karkade as the best worldwide¹¹. Some later results pointed out that rossell (Karkade) had a higher content of vitamin C than guava and orange⁶⁶.

Acids & Anthocyanin content

Titrateable acids (TA) level of the red calyces was 11.5mg/100g, and of the white calyces was 12.5 mg/100g., this result is slightly higher than what reported by Abdallah (2011)³⁴ and FAO reports⁶⁰ which are 9mg/100g for red, and 11mg/100g for

white karkade dry calyces. A significant difference of titrable acidity was observed between red and white karkade samples when simple statistical analysis conducted by the assistance of some professional technicians. That difference had been reported previously by Abdallah (2011)³⁴. Usually, the titrable acidity (TA) of the plant is related to TSS levels, to calculate the ratio TSS/TA, which may be used as a nutritional indicator of the plant, and important indexes for consideration of quality⁶⁷. Abeytilakarathna (2013)⁶³ had reported the ratio (TSS/TTA) of some hibiscus types as ranged between 8.98 and 16.27. However, the TSS/TA ratio which we found in our study of red and white karkade, could not be used as a taxonomic key to differentiating between the two types, i.e. there were no detectable differences between TSS and TA values of the two karkade types. Acidity, as hibiscus acid (Citric acid derivative), was in the range of 8% to 13% for all extracts. The Mean level of hibiscus acid of the red calyces was 8.5%, while the Mean of the white calyces was 10.05%. Results were somewhat lower than those found by Ali¹⁷ and Abu-Tarboush²⁵ which are 15% to 28% of hibiscus acid (lactone of hydroxycitric acid). But our results were in accordance with Hassab El Rassol³⁵ who reported a range of 9.32% to 14.70% for all extracts he studied. A range of 13–24% was reported by Eggensperger (1996)⁶⁴. Hibiscus acid compromises

a citric acid moiety with an additional hydroxyl group at the second carbon⁶⁴. Hibiscus acid is one of the major organic acids in karkade, which include (oxalic acid, tartaric acid, malic acid, and succinic acid), those organic acids compose (15-30%) of the karkade calyces mass⁶⁰. In this study, the Anthocyanin (anthocyanidin) content of the red calyces was 1.8% and of white calyces was 1.05% of the dry weight. A nearly similar anthocyanin content was observed in some other studies which amount for about 1.5 g per 100g of dry weight of karkade, in terms of delphinidin-3-sambubioside²⁷, and 1.7% to 2.5%⁶⁸. The anthocyanins are a group of flavonoid derivatives and natural pigments present in the dried flowers of karkade and their colour varies with pH. Anthocyanin (also known as “hibiscin”, later named delphinidin-3-sambubioside) are common plant pigments⁶⁹. Sometimes the classification of the karkade varieties has been carried out based on the anthocyanidin content⁶⁸. The color of the calyces plays an important role in determining the quality of karkade²¹. Cisse (2011) found a significant association ($q = 0.957$, $p < 0.01$) between the total monomeric anthocyanin content and the colour density of tested beverages⁴². The red color of the calyces may be due to the excess anthocyanins pigment in the Sudanese red type of karkade³⁰. Juliani (2009)⁷⁰ had found that Roselle is rich in anthocyanins. (Table 3)

Table 3
Acidity & Anthocyanin content of red and white karkade (roselle) calyces

Parameter	Titrateable acids (TA) (mg/100g) *	Hibiscus acid (g/100g) *	Anthocyanin content (g/100g) *
Red calyces	11.50±0.10	8.50±0.30	1.80±0.20
White calyces	12.50±0.15	10.05±0.42	1.05±0.04

* Mean ± SD of triplicate trials (n=3) on dry weight (DW) basis.

Minerals (Calcium and Iron)

The calcium level we found in the red karkade calyces was 25mg/100g and 25.5mg/100g in the white calyces. These are low levels of calcium in this plant compared with the level reported by Abdel Moneim Elhadi Sulieman who found calcium content of spray-dried karkade powder ranged from 55mg/100g to 220mg/100g in different varieties he studied⁷¹, and Abdallah⁶⁰ who found 60 and 50mg/100g of calcium. Actually, the calcium level had been mostly stated above 200 mg/100g in some international literature⁶⁵. The low calcium level found by this study may be due to the chemical nature of the element, as calcium may chelate with some compounds forming complexes, which may cause interference during analysis. More

investigations may be required to interpret this low level of calcium in some Sudanese karkade varieties. The red calyces contain 55.5mg/100g of iron, while the white calyces contain 30.5mg/100g. These results are not far from the values reported by Agriculture administration of FAO³³ which were 25 and 20mg/100g for red and white calyces, respectively. But our results were higher than the 11% value reported by USDA⁶⁵. Ismail³ study on Sudanese karkade had reported a level of (57 mg/100 g) of iron. The high Iron level we found in this study is not astonishing, probably due to the chemical nature of the Iron element which enables it to exist in many ionic forms such as ferric or ferrous states, all of them were detectable and give results on analysis. (Table 4)

Table 4
Minerals content of red and white karkade calyces

Parameter	calcium content (mg/100g) *	iron content (mg/100g) *
Red calyces	25.00 ± 0.21	55.50 ± 0.20
White calyces	25.50 ± 0.29	30.50 ± 0.10

* Mean ± SD of triplicatetrials (n=3) on dry weight (DW) basis.

CONCLUSION

A similar, or slightly different levels of most tested parameters had been observed between the two karkade varieties (Red and White calyces). The crude protein content, the mean content of acidity as hibiscus acid (Citric acid derivative), and the Titratable acids (TA) level of red calyces were lower than that of the white calyces. Calcium levels in Sudanese karkade were lower than the levels reported by the previous studies, but the iron levels were higher. Our results disagree with some Sudanese studies concerning, carbohydrates, vitamin C, calcium content, and iron content. The variations may be due to one, or more, of the following factors: 1- The variation in the genotypes, environmental and botanical nature of the tested samples, including the geographical origin of the plant. 2- Analyzing mixtures of red and white karkade calyces. 3- Use of different extraction methods of the analysis. The usage of the local Arabic term "karkade" instead of, or beside the term "Roselle" in published scientific articles may facilitate the internet searching process for researchers. However, further studies are required

to correlate the therapeutic uses of karkade and the chemical profile of the extracts being used.

AUTHORS CONTRIBUTION STATEMENT

Nuha Mohammed, Fatima Abdalla, and SharafEldin conceived of the presented idea. Fatima A. and Sharaf E. developed the theory and performed the computations. All the authors together verified the analytical methods. Fatima A. supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. JF. M. Fruits of Warm Climates. Miami, USA; 1987. 281-286 p. Available from: <https://www.cabdirect.org/cabdirect/abstract/19876763104>
2. Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U OY. Effects of roselle (*Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-ph. Food chem Toxicol .1999;37(6):591–601. DOI:10.1016/s0278-6915(99)00041-1
3. Ismail A, Ikram EHK NH. Roselle (*Hibiscus sabdariffa* L.) seeds nutritional composition protein quality and health benefits. food. 2008;2(1):1–16. Available from: www.academia.edu/download/45189886/Food_211-160.pdf
4. A. S. The pharmacological characteristics of *Hibiscus sabdariffa* L. Planta Med. 1962;10:48–52. DOI:10.1055/s-0028-1100273
5. Yagoub Ael G, Mohamed BE, Ahmed AH ETA. Study on furundu, a traditional Sudanese fermented roselle (*Hibiscus sabdariffa* L.) seed: Effect on in vitro protein digestibility, chemical composition, and functional properties of the total proteins. J Agric Food Chem. 2004;52(20):6143–50. DOI:10.1021/jf0496548
6. LS C. An introduction to the botany of

- tropical crops. U.K. London, UK.: Longman Group Limited; 1976. 371 p. Available from: <https://www.cabdirect.org/cabdirect/abstract/19800384880>
7. Mahadevan N SK. Hibiscus sabdariffa Linn: An overview. NISCAIR. 2009;8(1):77–83. Available from: <http://nopr.niscair.res.in/handle/123456789/3769>
 8. FB. P. Karkade investigation, progress report UNDP. Available from: <http://www.fao.org/sudan/en/>
 9. Wilson FD, Menzel MY. Kenaf (*Hibiscus cannabinus*), roselle (*Hibiscus sabdariffa*). Econ Bot. 1964;18(1):80–91. DOI:10.1007/bf02904005
 10. El Naim AM AS. Effect of weeding frequencies on growth and yield of two roselle (*Hibiscus sabdariffa*L) Varieties under rain fed. Aust J Basic Appl Sci. 2010;4(9):4250–5.
 11. Mohamad O, Nazir BM, Rahman MA HS. Roselle: A new crop in Malaysia. Bio Malaysia: A grand international biotechnology event. Bull PGM Kuala Lumpur. 2002; DOI:10.15282/ijame.12.2015.7.0242
 12. B. C. The delightful Hibiscus sabdariffa. Tea Coffee Trade J. 1993;165(1):100–2. DOI:10.1007/978-1-4020-4585-1_2474
 13. Caleb Mc R. Market survey Hibiscus sabdariffa. Agribusiness in sustainable natural African plant products. Herb Res Found. 2000; DOI:10.1007/978-1-4020-4585-1_2474
 14. Bahaelden Babiker Mohamed AAS and AAD. Roselle (*Hibiscus sabdariffa*L.) in Sudan, cultivation and their uses. Bull Environ Pharmacol Life Sci. 2012;1(6):48–54. Available from: <http://www.beppls.com/may2012/10.pdf>
 15. Food and agriculture organization of the United Nations (FAO). Yearbook of the United Nations 2002. UN; 2004. p. 1470–1. DOI:10.18356/877ef4ac-en
 16. Usda, nrcs. The plants Database. Hibiscus sabdariffa L. National Plant Data Center. Baton Rouge, LA.70874-4490 USA.; 2009. DOI:10.1007/978-1-4020-4585-1_2474
 17. Ali BH, Wabel N Al, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review. Phyther Res. 2005;19(5):369–75. DOI:10.1002/ptr.1628
 18. Campbell-Platt G. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. Food Control. 1997;8(3):167–8. DOI:10.1016/s0956-7135(97)88721-x
 19. Cyclotron Laboratory annual report, June 15, 1973--June 15, 1974. Office of Scientific and Technical Information (OSTI); 1974. DOI:10.2172/4283030
 20. Villani T, Juliani HR, Simon JE, Wu Q-L. Hibiscus sabdariffa: Phytochemistry, Quality Control, and Health Properties. ACS Symposium Series. American Chemical Society; 2013. p. 209–30. DOI:10.1021/bk-2013-1127.ch014
 21. Bahaelden Babiker Mohamed, Abdelatif Ahmed Sulaiman and Abdelhafiz Adam Dahab. Roselle (*Hibiscus sabdariffa*L.) in Sudan, cultivation and their uses. Bull. Environ. Pharmacol. Life Sci. 2012; Volume 1 [6]: 48 – 54. Online ISSN 2277 – 1808. India. Available from: <http://www.beppls.com/may2012/10.pdf>
 22. National Biodiversity Action Plan (N.B.A.P.). Biodiversity in Kordofan region.. El-Obeid Agricultural Research Station, Sudan. Report SUD/97/G31; 1999. 41-43 p. Available from: http://www.indiaenvironmentportal.org.in/files/National_Biodiversity_Action_Plan.pdf
 23. Tsai J and OM. Colour degradation of dried roselle during storage. J Food Sci. 1996;23(629–640).DOI: 10.1002/jsfa.4685
 24. Haruna AK. Cathartic activity of Soborodo: the aqueous extract of calyx of *Hibiscus sabdariffa* L. Phyther Res. 1997;11(4):307–8. DOI:10.1002/(sici)1099-1573(199706)11:4%3C307::aid-ptr42%3E3.0.co
 25. Abu-Tarboush HM, Ahmed SAB, Al Kahtani HA. Some Nutritional and Functional Properties of Karkade (*Hibiscus sabdariffa*) Seed Products. Cereal Chem J. 1997;74(3):352–5. DOI:10.1094/cchem.1997.74.3.352
 26. Haji Faraji M, Haji Tarkhani AH. The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. J Ethnopharmacol. 1999;65(3):231–6. DOI: 10.1016/s0378-8741(98)00157-3
 27. Du ct, francis fj. Anthocyanins of roselle (*Hibiscus sabdariffa*, L.). J Food Sci. 1973;38(5):810–2. DOI:10.1111/j.1365-2621.1973.tb02081.x
 28. Wilkinson M, Sweeny JG, Iacobucci GA. High-pressure liquid chromatography of anthocyanidins. J Chromatogr A. 1977;132(2):349–51.

- DOI:10.1016/s0021-9673(00)89311-5
29. Sáyago-Ayerdi SG, Arranz S, Serrano J, Goñi I. Dietary Fiber Content and Associated Antioxidant Compounds in Roselle Flower (*Hibiscus sabdariffa*L.) Beverage. *J Agric Food Chem.* 2007;55(19):7886–90. DOI:10.1021/jf070485b
 30. Mohamed R, Fernández J, Pineda M, Aguilar M. Roselle (*Hibiscus sabdariffa*) Seed Oil Is a Rich Source of α -Tocopherol. *J Food Sci.* 2007;72(3):S207–11. DOI:10.1111/j.1750-3841.2007.00285.x
 31. F M. Folk-remedy plants and esophageal cancer in Coro. Venezuela. *Morris Arbor. Bull, Univ of Penna.,* 1974;25:24–34. Available from: <http://agris.fao.org/agris-search/search.do?recordID=US201303165831>
 32. Salama R, Ibrahim S. Ergosterol in *Hibiscus sabdariffa* Seed Oil. *Planta Med.* 1979;36(07):221–2. DOI:10.1055/s-0028-1097275
 33. FAO *Hibiscus sabdariffa*, in traditional food plant. FAO food and nutrition paper. Rome, Italy: Food and agriculture organization,; 328-332 p. Available from: <http://www.fao.org/about/en/>
 34. Suliman AMA, Ali AO, A.A. Idris SE, Abdualrahm MAY. A Comparative Study on Red and White Karkade (*Hibiscus sabdariffa* L.) Calyces, Extracts and Their Products. *Pakistan J Nutr.* 2011;10(7):680–3. DOI:10.3923/pjn.2011.680.683
 35. Rassol H El. Hager Mohammed. Phytochemical properties of cold and hot calyces Extracts of three genotypes of Karkade (*Hibiscus sabdariffa* var. *sabdariffa*). PhD thesis, Khartoum Univ Sudan,. 2015; Available from: <http://khartoumspace.uofk.edu/handle/123456789/17421>
 36. Ismaiel SMO. Antimicrobial and Anti-inflammatory Activity of Plants Indigenous to Sudan *Hibiscus sabdariffa* also revealed a good antimicrobial activity. Thesis,. 2008; Available from: <http://khartoumspace.uofk.edu/123456789/23938>
 37. Mgaya-Kilima B, Remberg SF, Chove BE, Wicklund T. Influence of storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends in plastic bottle. *Food Sci Nutr.* 2014;2(2):181–91. DOI:10.1002/fsn.3.97
 38. Saeed AR AM. Storage stability of carbonated beverage from roselle calyces (*Hibiscus sabdariffa*). Sudan. *J Food Sci Technol.* 1977;9:78–81. Available from: <http://agris.fao.org/agris-search/search.do?recordID=US201301272895>
 39. Ziena HMS. Quality attributes of Bearss Seedless lime (*Citrus latifolia* Tan) juice during storage. *Food Chem.* 2000;71(2):167–72. DOI:10.1016/s0308-8146(00)00064-9
 40. Tsai P-J, Huang H-P. Effect of polymerization on the antioxidant capacity of anthocyanins in Roselle. *Food Res Int.* 2004;37(4):313–8. DOI:10.1016/j.foodres.2003.12.007
 41. Chumsri P, Sirichote A IA. Studies on the optimum conditions for the extraction and concentration of roselle (*Hibiscus sabdariffa* Linn.) extract. *J Sci Technol.* 2008;30:133–9. Available from: <http://www.thaiscience.info/Journals/Article/SONG/10617799.pdf>
 42. Cisse M, Vaillant F, Kane A, Ndiaye O, Dornier M. Impact of the extraction procedure on the kinetics of anthocyanin and colour degradation of roselle extracts during storage. *J Sci Food Agric.* 2011;92(6):1214–21. DOI:10.1002/jsfa.4685
 43. Iversen CK. Black Currant Nectar: Effect of Processing and Storage on Anthocyanin and Ascorbic Acid Content. *J Food Sci.* 1999;64(1):37–41. DOI:10.1111/j.1365-2621.1999.tb09856.x
 44. Ochoa MR, Kessler AG, Vullioud MB, Lozano JE. Physical and Chemical Characteristics of Raspberry Pulp: Storage Effect on Composition and Color. *LWT - Food Sci Technol.* 1999;32(3):149–53. DOI:10.1006/fstl.1998.0518
 45. Fang Z, Zhang M, Sun Y, Sun J. How To Improve Bayberry (*Myrica rubra*Sieb. et Zucc.) Juice Color Quality: Effect of Juice Processing on Bayberry Anthocyanins and Polyphenolics. *J Agric Food Chem.* 2006;54(1):99–106. DOI:10.1021/jf051943o
 46. Ageless The trusted Herbal Anti-aging. Herbal remedies using Roselle (*Hibiscus sabdariffa*);, 1999. Available from: <http://www.ageless.co.za/rosella.htm>. Viewed in Aug 2018.
 47. Odigie IP, Ettarh RR, Adigun SA. Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. *J Ethnopharmacol.* 2003;86(2–3):181–5. DOI:10.1016/s0378-8741(03)00078-3

48. Tseng T-H, Hsu J-D, Lo M-H, Chu C-Y, Chou F-P, Huang C-L, et al. Inhibitory effect of Hibiscus protocatechuic acid on tumor promotion in mouse skin. *Cancer Lett.* 1998;126(2):199–207. DOI:10.1016/s0304-3835(98)00010-x
49. Kamali HHE, Mohammed MF. Antibacterial activity of Hibiscus sabdariffa, Acacia seyal var. seyal and Sphaeranthus suaveolens var. suaveolens against upper respiratory tract pathogens. *Sudan J Med Sci.* 2007;1(2). DOI:10.4314/sjms.v1i2.38451
50. Agbafor KN, Akubugwo EI, Ogbashi ME, Ajah PM, Ukwandu CC. Chemical and Antimicrobial Properties of Leaf Extracts of Zapoteca portoricensis. *Res J Med Plant.* 2011;5(5):605–12. DOI:10.3923/rjmp.2011.605.612
51. Yadong Q, Chin K, Malekian F, Behane M and GJ. Biological characteristics, nutritional and medicinal value of Roselle, Hibiscus sabdariffa. *Urban For Nat Resour Environ Circ.* 2005;(604):2.
52. Ahmed M El Naim and SEA. Effect of Weeding Frequencies on Growth and Yield of Two Roselle (Hibiscus sabdariffaL) Varieties under Rain Fed. Department of Crop Science, Faculty of Natural Resources and Environmental Studies, University of Kordofan, Elobied, Sudan. *Aust J Basic Appl Sci.* 2010;4(9):4250–5.
53. Amos S, Binda L, Chindo BA, Tseja A, Odutola AA, Wambebe C. Neuropharmacological Effects of Hibiscus sabdariffa Aqueous Extract. *Pharm Biol.* 2003;41(5):325–9. DOI:10.1076/phbi.41.5.325.15933
54. AOAC Association of Official Agricultural Chemists. Official Methods of Analysis. Gaithersburg. DW. Washington DC: Assoc. of analytical chemists; 2008. DOI:10.5962/bhl.title.44636
55. Harold Egan, Ronald S Kirk, Ronald Sawyer DP. Harold Egan, Ronald S Kirk, Ronald Sawyer, David Pearson. 8th ed. Pearson's chemical analysis of foods. Edinburgh; New York: Churchill Livingstone; 1981. DOI:10.1016/0300-483x(75)90104-3
56. Merrill AL & WB. Energy value of foods: basis and derivation. *Agriculture Handbook No. 74.* Washington, DC .ARS United States Department of Agriculture; 1973. Available from: [http://www.scirp.org/\(S\(vtj3fa45qm1ean45vffcz55\)\)/reference/ReferencesPapers.aspx?Re](http://www.scirp.org/(S(vtj3fa45qm1ean45vffcz55))/reference/ReferencesPapers.aspx?ReferenceID=1769288)
57. Dashman T, Blocker DE BN. Laboratory manual for human nutrition. 2nd ed. New York, NY: Harwood Academic Publishers; 1996. Available from: <https://www.bookdepository.com/Laboratory-Manual-Human-Nutr-2-Nora-Baker/9783718606085>
58. Lee J, Durst RW WR. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC.* 2005;88:1269–78. Available from: <https://www.ingentaconnect.com/content/aoac/jaoac/2005/00000088/00000005/art00002>
59. Karkadeh G. S. A brief introduction, economics. File No 12. The Sudan foundation. London, UK; 1997. Available from: <https://munazaki.com/2013/07/12/sudanesekarkadehhibiscus/>
60. FAO. Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper No. 66. Rome; Available from: <https://www.ncbi.nlm.nih.gov/pubmed/9743703>
61. Atchley D and. Proximate analysis. The handbook of plant science in agriculture. CRC Press Inc.; 1984. 227-234 p. Available from: <http://www.worldcat.org/title/crc-handbook-of-plant-science-in-agriculture/oclc/13643013>
62. Mohammed KEEY and HM. Comparison of Cold and Hot juice extraction from hibiscus (Karkadeh) and baobab (Tabaldi). *Int J Innov Res Sci Eng Technol.* 2016;3. Available from: https://www.researchgate.net/profile/Kamal_Eldin_Yassin/publication/301239618_article_3/links/570e887308aee328dd653dfc.pdf
63. Abeytilakaratna PD, Fonseka RM, Eswara JP, Wijethunga K. Relationship between total solid content and red, green and blue colour intensity of strawberry (Fragaria x ananassa Duch.) fruits. *J Agric Sci.* 2013;8(2):82. DOI:10.4038/jas.v8i2.5743
64. Eggensperger H WMH-E. Einhautvertraglicher Wirkstoffkomplex aus AHA's und Polysacchariden. *Parfumerie und Kosmetik: Agriculture Organization of the UN (FAO).*; 1996. pp 540-543. DOI:10.1007/978-3-662-02107-1_3
65. USDA: National Genetic Resources Program. Taxon: Hibiscus sabdariffa L. Germplasm Resources Information Network – (GRIN)

- [Online Database],. Beltsville, Maryland: National Germplasm Resources Laboratory; 2007.DOI:10.17660/actahortic.1995.413.16
66. Tee, Noor I. Nutrient composition of Malaysian foods. Institute of medical research. Koalalumpur, Malaysia.; 1997. 299 p. Available from: <http://agris.fao.org/agris-search/search.do?recordID=XF2015019876>
 67. Suktanarak S and TS. Quantitative analysis of total soluble solids and titratable acidity of sweet tamarind by SW-NIRS. Int J Biosci Biochem Bioinforma 2013;3(4). Available from:<http://www.ijbbb.org/papers/224-S024.pdf>
 68. Borrás-Linares I, Fernández-Arroyo S, Arráez-Roman D, Palmeros-Suárez PA, Del Val-Díaz R, Andrade-González I. Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). Ind Crops Prod. 2015;69:385–94. DOI:10.1016/j.indcrop.2015.02.053
 69. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. Food Chem. 2014;165:424–43. DOI:10.1016/j.foodchem.2014.05.002
 70. Juliani HR, Welch CR, Wu Q, Diouf B, Malainy D, Simon JE. Chemistry and Quality of *Hibiscus* (*Hibiscus sabdariffa*) for Developing the Natural-Product Industry in Senegal. J Food Sci . 2009;74(2):S113–21. DOI:10.1111/j.1750-3841.2009.01076.x
 71. Sulieman AM. Spray Drying of Karkade (*Hibiscus sabdariffa* L.) Calyces and Evaluation of the Product. Int J Food Eng. 2014;10(1). DOI:10.1515/ijfe-2013-0045