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

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ABSTRACT

Hibiscus sabdariffaplant{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 methodologiesbyresearchers 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 Rossell{Botanical name*: Hibiscus sabdariffa* Linne (Malvaceae)}, is a plant widely cultivated in both tropical and subtropical regions[1],includingMéxico, India, Saudi Arabia, China,Egypt,and Sudan[2-5]. *Hibiscus sabdariffa*L.is the scientific name of Roselle (in Arabic called "karkade")[6].Roselle (*Hibiscus sabdariffa*L.) (karkade) had different common names includerazelle, 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 America[9]. Karkade (Roselle) is grown for its calyces, it is a cash crop cultivated by traditional farmers under rain-fed conditions[10]. 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 immature[16-18].The two botanical types of roselle are *Hibiscus sabdariffa*var. *sabdariffa*, grown for thierfleshy, 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 beverage[21-23], andare 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 roselleoil is rich in gamma tocopherol[30], 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 µg/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% level[30]. 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 genotypes[35]. He reported that Ascorbic acid content was reduced from 72.87mg/100g in the cold to 19.4mg/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 flavylium 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 heat [39].Many studies have been conducted on physicochemical and antioxidant properties of karkadeextract[40-42].During storage,the total phenolic content TPC decreased at 28°C more than at 4°C, irrespective of storage intervals, and some monomericanthocyanins 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 TPC[45]; therefore, storage at 4°C should be encouraged when the products need to be stored for long time[37].

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 fever[46], besides, pyrexia, liver damage[47],leukemia[48]. It cantreat respiratory tract infectionsand used in treating wounds, and sores[51]. Extracts had an antioxidant potential[50].The fresh calyces are rich in riboflavin, niacin and iron[52].HatilHashim, *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 ofAmos(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 karkadeand 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 authentification of *Hibiscus sabdariffa*varwere 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, shacked 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 collectedand 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 2008[54]. The sample added to about 100 ml hexane into the extraction unit (Electrothermal, England) for 16 hr., thenthe 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:

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

Crudefibercontent was determined according to the official method of the AOAC 2008[54]. Defatted sample added to 20m1 of H_2SO_4 (0.26 N). After digestion, filtration, the precipitate boiled in 20 ml NaOH (0.23 N) solution for 30 min under a reflux condenser, filteredand 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 2008[54]. Two grams sample transferred together with 4g Na_2SO_4 of Kjeldahl catalysts (No. 0665, Scharlauchemie, Spain) and 25m1 of concentrated sulphuric acid into a Kjeldahl digestion flask. After digestion, the distillation of ammonia was carried out into 25m1 boric acid (2%) and sodium hydroxide solution (45%). Finally, the distillate was titrated with standard solution of HCI (0.1N) with bromocresol green and methyl red as an indicator until a brown reddish colour was observed, then calculated as follows:

$$\text{Nitrogen\%} = \frac{\text{Titre volume x HC1 (N) x Nitrogen equivalent weight x 100}}{\text{Sample weight x 1000}}$$

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

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

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

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 compromises 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:

$$A = (A_{250} - A_{700})_{pH1.0} - (A_{250} - A_{700})_{pH4.5}$$

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

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 determinethe selectedMinerals 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 HCI (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 ^{0}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 karkade[34]. 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 FAO[60]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 Gabb[59] which is 12%, and Kamal Edin (2016)[62], and the results reported by FAO[60] which are 13.2 and 12%. Both Duke[61] and Ismail[36] 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,Abdallah[34]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 fruitsmay be used to determine the correct maturity stage of fruits[63]. 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 Rassol[35] 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 Edin [62].(Table 1)

Table 1
The results of KarkadePhysico-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* triplicate*trials (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 Ismail[3], and that of Duke and Atchley[61]. 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 agriculture[16]. 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 Gabb[59], which is 5%. Ismail found a protein content in a range of 3.3 % - 7.88% [3]. Kamal[62] had analyzed some Sudanese karkade and found some differences between (cold extracted) and (hot extracted) calyces. Carbohydrateslevelswere 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 carbohydrates[60].In this study, Vitamin C level was found to be16.5mg/100g for red calyces, while the white calyces contain 16.9 mg/100g of the vitamin. These resultswere 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 plant[64]. 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 (%)*	Avaialable 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* triplicate*trials (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 calyces [12]. 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 harvesting [21]. 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 worldwide [11]. Some later results pointed out that rossell (Karkade) had a higher content of vitamin C than guava and orange [66].

Acids & Anthocyanin content

Titratable 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 reports[60] 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 relatedto 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 quality[67].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 acidof the red calyces was 8.5%, while the Mean of the whitecalyces was 10.05%.Results were somewhat lower than those found byAli[17] and Abu-Tarboush[25] which are 15% to 28% of hibiscus acid (lactone of hydroxycitric acid). But our results were in accordance with HassabEl Rassol[35]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 carbon[64].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 mass[60].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-sambubioside[27], 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 pigments[69].Sometimes the classification of the karkade varieties has been carried out based on the anthocyanidin content [68].The color of the calyces plays an important role in determining the quality of karkade[21].Cisse (2011) found a significant association ($q = 0.957$, $p < 0.01$) between the total monomeric anthocyanin content and the colour density of tested beverages[42]. The red color of the calyces may be due to the excessanthocyanins pigment in the Sudanese red type of karkade[30].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	Titratable 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 MoneimElhadiSulieman who found calcium content of spray-dried karkade powder ranged from 55mg/100g to 220mg/100g in different varieties he studied[71],and Abdallah[60] who found 60 and 50mg/100g of calcium. Actually, the calcium level had been mostly stated above 200 mg/100g in some international literature[65].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 FAO [33]which were 25 and 20mg/100g for red and white calyces, respectively. But our results were higher than the 11% value reported by USDA[65]. Ismail[3] 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* **triplicate***trials (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.

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