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Phytochemical and Chemical Composition of Water Extract of *Hibiscus Sabdariffa* (Red Karkade Calyces) in North Kordofan State-Sudan

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Abstract: *This study was carried out to investigate the phytochemical composition of the water extract of karkade calyces drink. karkade drink is a popular drink in Sudan, especially in the western part of Sudan. The results of the analysis showed that the best time for soaking is 24 hours, the drink is high in Polyuronides, reducing sugar and mineral nutrients required for the healthy growth by humans. The values for the elements calcium, iron, potassium, sodium, magnesium, manganese and copper in the water extract were found to be 0.55, 0.22, 0.46, 0.33, 0.21, 0.001 and 0.03mg/g respectively.*

Keywords: *phytochemical, red karkade, water extract.*

1. INTRODUCTION

Roselle is an annual herbaceous shrub belonging to the family malvacea. The plant cultivated mainly for its flowers. Seeds and leaves have some uses in traditional medicine. The plant is 1.5-2.5m tall. Its habit is variable and the leaves also in vary shape and size. The flowers are usually yellowish some times occurring with dark red pigmentation at the center. Two botanical types of roselle are recognized, *Hibiscus sabdariffa* var *altissma* and *Hibiscus sabdariffa* var *sabdariffa* [1]. The important part of the plant in western Sudan is calyces (sepals) which surrounding the fruit. Roselle (Hibiscus sabdariffa L.) is known in Sudan as Karkade, in Nigeria the common popular drink of roselle is known as zobo and the herb is used in folk medicine in the treatment of hypertension [2, 3, 4.]

Karkade is an important cash crop and source of income for small farmers of western Sudan, the Kordofan and Darfur areas. The crop is growing mainly in traditional farming systems exclusively under rain fed conditions[5, 6]. Roselle has many industrial and domestic uses. Karkade drink is a non alcoholic local beverage made from dried petals, it is gaining wide acceptance in Sudan, and it is used as a beverage, where the dried calyces is immersed in water to prepare a colorful drink or boiled in water and taken as hot drink. Traditionally the product has been used for medicinal purposes for relief of sour throat and for healing wounds as an anti-septic. The crop has been greatly renovated and gaining importance in the manufacture of many small industries, e.g. jams, jellies,sweet, sauces,cosmetic and tablets and also used as a coloring material for food and beverages. Also has been to be folk treatment for cancer, obesity, diabetes and hypertension[7]. The methanolic extract of *H. Sabdariffa* calyces shrinks guinea-pig tracheal chain, rat uterus, rat diaphragm and inhibit rat ileal[8]. The juice from the calyces was claimed to be a health-enhancing drink due to its high content of vitamin C, anthocyanins and other antioxdants[9].

The aim of this research is to determine the suitable time for extraction, biochemical and minerals constituents in water extract of karkade calyces.

2. MATERIALS AND METHODS

2.1. Plant Materials

The red karkade calyces were obtained from Khour Tagat area about 12 Km east of Elobeid city.

2.2. Extraction Method

Because karkade pigments will be used as a beverage, color for some foods and drugs, so it is important to use aqueous extraction methods. 10 grams of karkade calyces were extracted using cold water (200ml) for 1, 2, 4, 16 and 24 hours.

2.3. Methods

The methods used in this work were described and recommended by the Association of official Analytical Chemists (AOAC. (1980). Association of official Analytical Chemists. Official methods of Analysis 13th edn. Washington. DC.)

2.4. Determination of pH

The pH of the extracted karkade (10g soaked in 200ml distilled water) was measured using a pH meter (Jenway, model 3020).

2.5. Minerals Contents

Minerals contents were determined by atomic absorption spectrometry (Agilent, perkin Elmer AAS Device Model GBC 932, U.S.A. 1996).

Metal content (ppm) = dilution factor x (AAS) reading.

2.6. Moisture Content

Two-grams of karkade calyces were placed in a weighed porcelain crucible. The sample was left in an oven at 105C^{o} for three hours and transferred to a desiccator for one hour. The moisture content was calculated as follows:

Moisture content% = [(difference in weight(g))/(sample weight(g))] x100%

2.7. Ash Content

Two-grams of karkade calyces were placed in porcelain crucible. The crucible was placed in a muffle furnace at 550C^{o} until light gray ash of a constant weight was obtained. Ash% was calculated as follows:

Ash% = [Ash weight (g)/ (sample weight (g))] x100%

3. PHYTOCHEMICAL SCREENING

The water extract was used for the following tests:

3.1. Alkaloids

30 ml of the water extract was evaporated to dryness. The residue was dissolved in 5ml of 2% HCl. 3 drops of Mayer`s reagent were added to the dissolved residue. Yellowish-white precipitate indicates the presence of alkaloids.

3.2. Polyuronides (pectins, mucilage, gums)

2 ml of the aqueous extract were added drop wise to a test tube containing 10ml of alcohol or acetone. If a thick precipitate was formed, it would be separated off by filtration or centrifugation, washed away with alcohol and then stained with specific stains (hematoxylin, toluidine blue or methylene blue). The occurrence of a violet or blue precipitate indicated a positive reaction.

3.3. Saponins

2ml of the water extract were placed in a test tube and 2ml of distilled water were added and the blue was corked and shaken vigorously. Formation of persistent foam, which remained stable for at least one hour, indicated the presence of saponins.

3.4. Tannins

1ml of water extract with ferric chloride solution was used. When the extract contained both types of tannins, a hydrochloric acid formaldehyde solution (Styassny`s reagent) was boiled with reflux. Under these conditions, the catechol tannins were condensed as a red precipitate, and then filtered. The solution thus obtained was neutralized with sodium acetate and some drops of ferric chloride were added. If gallic tannins were present, a deep blue color would develop.

3.5. Carbohydrates

5ml of water extract were put in a test tube and two drops of Molish`s reagent were added. 2 drops of concentrated sulfuric acid were added down the side of the tube, to form a separate lower layer of 2cm deep.

3.6. Barford`s test

0.5 ml of the aqueous extract was placed in a test tube and 0.3ml of Barford`s reagent was added and the tube was rolled and placed on a boiling water bath for 5minutes.The development of a reddish brown precipitate indicated the presence of a reducing sugar.

3.7. Benedict`s test

0.3ml of benedict`s reagent was added to 2.5 ml of the aqueous extract and the tube was rolled on boiling water bath and left for 5 minutes. An orange red precipitate indicated the presence of monosaccharides. The different classes of secondary metabolites present in the water extract of roselle calyces are tabulated in table 2.

4. RESULTS AND DISCUSSION

Table 1 shows the extraction of pigment with different time. The extraction was complete, as judged by the absence of color, after 24 hrs.

Table 1. *Effect of extraction time on the amount of pigment*

Time in hrs	Absorbance at 520nm
0.0	0.00
1	0.702
2	0.785
4	0.926
16	0.974
24	1.198

Table 2 shows the photochemical analysis of the water extract of the red karkade calyces. The results revealed the presence of very high amount of Polyuronides, high amount of Reducing sugar, medium amount of Saponins and low amount of Alkaloids.

Polyuronides have effect on congestive heart failure by increasing the force of myocardiac contraction and exert their hypotensive effect by inhibiting Na^{+}-K^{+} ATPase. The presence of polyuronides in karkade calyces gives credence to their traditional use in the treatment of hypertension[10]. A reducing sugar contains aldehyde or ketone in its molecular structure and plays a role in oxidation-reduction reaction that can create an energy source for the body. Saponins bind to cholesterol to form insoluble complexes and this prevents the cholesterol reabsorption and results in a reduction of serum cholesterol[11]. Sponins have found to be potentially useful for the treatment of hypercholesterolemia[12]. Alkaloids are widely used as cancer chemotherapeutic agents[13].

Table 2. *phytochemical screen of water extract of roselle calyces. ++++ Very high amount. +++ High amount. ++ Medium. + Low. – Not detected*

Chemical compound	**Intensity**
Polyuronides(pectins, mucilage,gums)	++++
Reducing sugar	+++
Saponins	++
Alkaloids	+
Tannins	-

The physicochemical analysis of water extract of roselle calyces are shown in table 3. The calcium content was 0.55mg/g, iron content was 0.22mg/g, potassium content was 0.46mg/g, sodium content was 0.33mg/g, magnesium content was 0.21 mg/g, manganese content was 0.001mg/g and copper content was 0.03 mg/g. Every enzyme requires cofactors to function properly. Most of these cofactors are mineral elements. These results support its uses as a nutrition source for human body. The pH of the water extract was found to be 2.35this indicated

that the karkade extract has a reasonable taste for drinking. The moisture was found to be 10.8g/100g and the ash content was found to be 9.7g/100g. The variation between the amounts of chemical constituents of karkade extract in this study with other published papers may be due to many factors such as type of irrigation, soil, climate and fertilizers.

Table 3. *Physicochemical constituents of water extract of roselle calyces*

Constituents	Amount (mg/g)	Constituents	Amount
Ca	0.55	Mn	0.001mg/g
Fe	0.22	Cu	0.03mg/g
K	0.46	moisture	10.8g/100g
Na	0.33	Ash	9.7g/100g
Mg	0.21	pH	2.35

5. CONCLUSION

From the results of this study it can be concluded that the water extract of roselle calyces it contains Polyuronides, reducing sugar and minerals which are important for biological function and as a nutrition source. The karkade drink can stay for a long time without deterioration because its acidity can inhibit the growth of some unwanted microorganism.

REFERENCES

[1] Rice, R.P; Rice I. W and Tindal, H. D. (1990). Fruits and vegetables production in Warm Climates. Macmillan press limited.

[2] Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. Food chem. Toxicol 38(5): 411-416

[3] Odigie IP, Ettarh RR, Adigun SA. (2003). Chronic administration of aqueous extract of Hibscus sabdariffa attenuates hypertension and reverses cardiac hyperytrophy in 2K-1C hypertensive rats J. Ethnophamacol. 86 (2-3): 181-185.

[4] Olaleye MT, Akindahunsi AA (2005). Hypotensive activity of methanolic extract of the calyces of Hibiscus sabdariffa L on Normotensive rats. In recent progress in medicinal plants series. Plant Bioactives in Traditional Medicine. Stadium press LLC.U.S.A. 12(9):283-288.

[5] Osman,A.K; Sulaiman,A.A;and Mekki,M.a.2007.on farm evaluation of tools for karkade peeling. Rep. Agricultural Research station, P.o.Box. 429. Elobeid,Sudan pp.2.

[6] El Naim, A.M. and Ahmed, S.E. (2010). Effect of weeding frequencies on growth and yield of two roselle (Hibiscus sabdariffa L) varieties under rain fed. Australian journal of basic and applied sciences 4(9): 4250-4255, ISSN 1991-8178.

[7] Osuntgum, B and Aboaba, O.O. 2004. Microbiological and physio-chemical evaluation of some none-alcoholic beverags Pakistan. Nut. 3(3): 188-192.

[8] Salah, A.M., Gathumbi, J., Vierling, W. (2002). Inhibition of intestinal motility by methanolic extracts of Hibiscus sabdariffa L. (Malvaceae) in rats.Phytother Res. 16: 283–285.

[9] Mohamad, O., Mohd. Nazir, B., Abdul Rahman, M. and Herman, S. (2002). Roselle: A new crop in Malaysia. Bio Malaysia: A grand international biotechnology event. Bulletin PGM. Kuala Lumpur.

[10] Brian FH, Thomas-Bigger Jr. J, Godman G(1985). The pharmacol. Basis of Therapeutics. 7th edition, Macmillan publishing company, New York, pp. 716-718.

[11] Cheeke RR (1971). Nutritional and Physiological Implication of Saponins. A Review. Can. J. Anim. Sci. 51: 621 – 632.

[12] Malinow MR, McLaughlin P, Kohler GO, Livingstone AL. (1977). Alfalfa saponins: a family of substances potentially useful for treatment of hypercholesterolemia. Clin. Res. 25: 974-979.

[13] Chabner BA, Hortwitz TL (1990). Plant alkaloids. In: Pinedo HM, Chabner BA, Longo DL, eds: Cancer chemotherapy Boil. Responses 66:627-637.