Gezira Journal Of Health Sciences 2014 vol.10(1)

EDITORIAL

Determination of Total Phenolic Content and Antioxidant Activity of Roselle (*Hibiscus sabdariffa* L.) Calyx and Epicalyx Ethanolic (Alcoholic) Extract

Nizar Sirag[1], Elhadi MM[1], Algaili M Algaili[2] and Mohamed Ohaj[3]

1- Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan.
2- Department of Pathology, Faculty of Medicine, University of Gezira, Sudan.
3- Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, University of Gezira, Sudan.

Corresponding author: Nizar Sirag[1] E-mail: nizarsirag@gmail.com

Abstract:

Background: Medicinal plants contain physiologically active ingredients that over the years have been exploited in traditional medicine for the treatment of various ailments.

Objectives: This study was undertaken to investigate the total phenolic content and antioxidant capacity of Roselle (*Hibiscus sabdariffa*) calyx and epicalyx ethanolic extract.

Methods: The total phenolic content was estimated by Folin Ciocalteau method using gallic acid as standard while the antioxidant capacity was determined based on the plant extract to scavenge DPPH radical.

Results: The total phenolic content was found to be 41.07 mg gallic acid equivalent /g .The extract exhibited a significant measurable dose dependent inhibition of the DPPH activity. At a concentration of 250µg/ml, *sabdariffa* calyx epicalyx ethanolic extract scavenged 86% of DPPH radical whereas 125 and 50 µg/ml caused 53% and 23% DPPH inhibition respectively and a very mild inhibition was produced at a concentration of 5 µg/ml.

Conclusion: It can be concluded that *sabdariffa* calyx epicalyx ethanolic could be a potential source of antioxidant principles.

Key words: Phenolic compounds; Antioxidant activity; Reactive Oxygen Species; *sabdariffa* calyx and epicalyx.

Introduction:

Roselle (*Hibiscus sabdariffa*) is an edible plant used in various applications including foods. Among them, the most popular are the fleshy red calyces used for making wine, juice, jam, syrup, pudding, cakes, ice cream or herbal tea. Roselle flower and calyces is also known for its antiseptic, diuretic, antioxidant and antimutagenic properties [(5)]. Roselle is an important source of vitamins, minerals, and bioactive compounds, such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like β-sitoesterol and ergoesterol [(6)]. Roselle calyx extract is a good source of antioxidants especially anthocyanins. [(7)]

EDITORIAL

Oxygen free radicals, classified under the more general term of Reactive Oxygen Species (ROS) which includes non-radical species such as hydrogen peroxide, are highly reactive transient chemical species formed in all tissues during normal aerobic cellular metabolism, with the potential to initiate damage to the various intracellular components (nucleic acids, lipids, proteins) on which normal cell functioning depends. Free radicals provoked by various environmental chemicals as well as endogenous metabolism are involved in a number of diseases like tumors, inflammation, shock, atherosclerosis, diabetes, infertility, gastric mucosal injury, brain dysfunction, cancer and ischemia due to the oxidative damage to DNA, lipids, and proteins and which can result in failure of cellular functions. Free radicals and ROS are controlled in biological systems by some enzymes possessing antioxidant activities such as superoxide dismutase and peroxidase. [1, 2]

The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds. Phenolic antioxidants are reported to quench oxygenderived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical and the antioxidant activity of phenolics in several systems has indicated that they were as active as Buthylated hydroxyaniscle (BHA) or Buthylated hudroxytoluane (BHT) of the same functional group. [3]

An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 2, 2 diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant,

DPPH radical obtains one more electron and the absorbance decreases. [4]

Materials and Methods:

Materials

Chemicals and reagents:

2, 2 diphenyl-2-picryl hydrazyl (DPPH), gallic acid and quercetin were purchased from Sigma –Aldrich company (UK). Folin Ciocalteau reagent was purchased from Merck Company (Germany).

Plant material:

The dried calyces of *Hibiscus sabdariffa* were purchased from the local market in Wad-Medani, Sudan. The plant material was authenticated by the Department of Pharmacognosy, Faculty of Pharmacy, University of Gezira, Sudan.

Methods:

Extraction of plant material:

One hundred grams of coarsely powdered calyces of *Hibiscus sabdariffa* were extracted by maceration using ethanol (70%) in a conical flask for 72 hours in dark, filtered and evaporated by a rotary evaporator at 60 ^{o}C. The resulting solution was freeze dried and kept in a refrigerator until use.

Antioxidant activity of *Hibiscus sabdariffa* calyx ethanolic extract:

Sample stock solution (1 mg/ml) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in ethanol. One ml of a 0.3 mM 2, 2 diphenyl-2-picryl hydrazyl (DPPH) in ethanol solution was added to a 2.5 ml solution of the different concentrations of the extract and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below:

$$AA\% = \frac{\text{(Absorbance of control – Absorbance of sample)}}{\text{Absorbance of control}} \times 100$$

EDITORIAL

Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as control. Stock solution (1 mg/ml) of quercetin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in ethanol used as positive control (8).All experiments were done triplicate recorded as means± standard deviation.

Determination of total phenolic content in *Hibiscus sabdariffa* calyx and epicalyx ethanolic extract:

The total phenol content in the ethanolic extract of *sabdariffa* calyx and epicalyx was determined with Folin Ciocalteau reagent by the method described by Chinedu *et al*., (2011) (9).The crude extract (50 mg) was mixed with Folin Ciocalteau reagent (1ml) and deionized water (7.5 ml).The mixture was kept at room temperature for 5 minutes and then 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 minutes at room temperature. After incubation the absorbencies against the reagent blank were determined at 760 nm using UV/visible spectrophotometer .The total phenolic content of the plant was expressed as mg/g Gallic acid equivalent. All samples were analyzed in triplicates recorded as means± standard deviation.

Results and Discussion:

The total phenolic content of calyx and epicalyx was found to be 41.07 mg gallic acid /g (Table 1) similar to those reported by other researchers [9-11] who also described the antioxidant activity to these compounds.

The capacity of scavenging diphenyl picryl hydrazine (DPPH) by the ethanolic extract of *sabdariffa* calyx is presented in Table 2 and Figure 1. The antioxidant molecules that can quench DPPH free radicals and convert them to colourless product; resulting in a decrease in UV absorbance [8, 9].In this quantitative assay the extract exhibited a notable dose dependent inhibition of the DPPH activity. At a concentration of 250 µg/ml. *sabdariffa* calyx extract scavenged 86% of DPPH radicals whereas it produced a very mild inhibition at a concentration of 5

µg/ml compared to those produced by the standard antioxidant, quercetin (Table 3 & Figure 2).

Table 1: Total phenolic content of *Hibiscus sabdariffa* calyx and epicalyx ethanolic extract.

Type of analysis	Concentration in mg gallic acid/gram sample
Total phenolic content	41.07 mg gallic acid /gram.

Table 2: DPPH scavenging activity of *Hibiscus sabdariffa* calyx and epicalyx ethanolic extract.

Concentration	Scavenging activity
5 µg/ml	2 %
10 µg/ml	11 %
50 µg/ml	23%
125 µg/ml	53 %
250 µg/ml	86 %

Table 3: DPPH scavenging activity of quercetin.

EDITORIAL

Concentration	Scavenging activity
5 µg/ml	25%
10 µg/ml	37.5%
50 µg/ml	62.1%
125 µg/ml	85.8%
250 µg/ml	89.7%

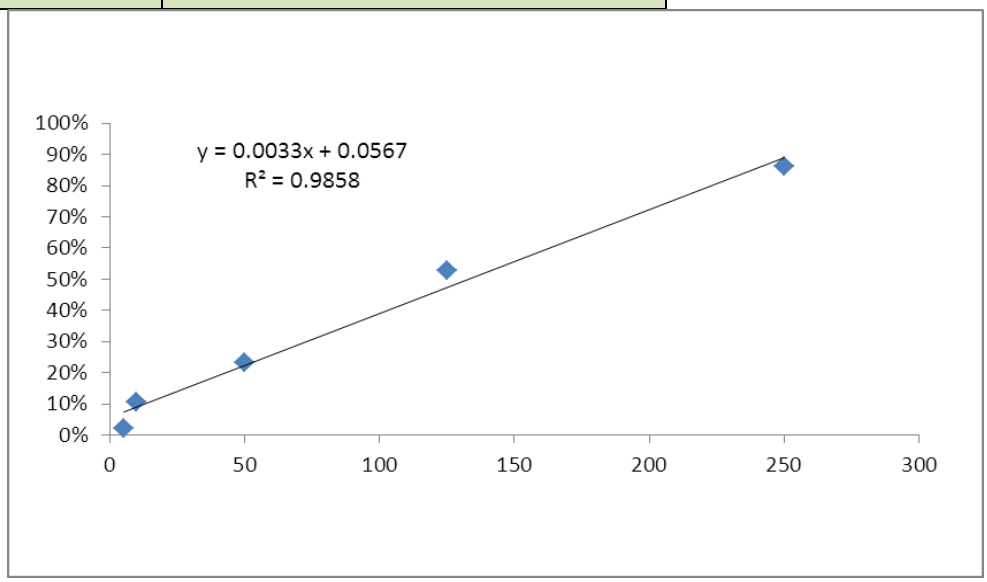

Figure 1: DPPH scavenging activity of *Hibiscus sabdariffa* calyx and epicalyx ethanolic extract.

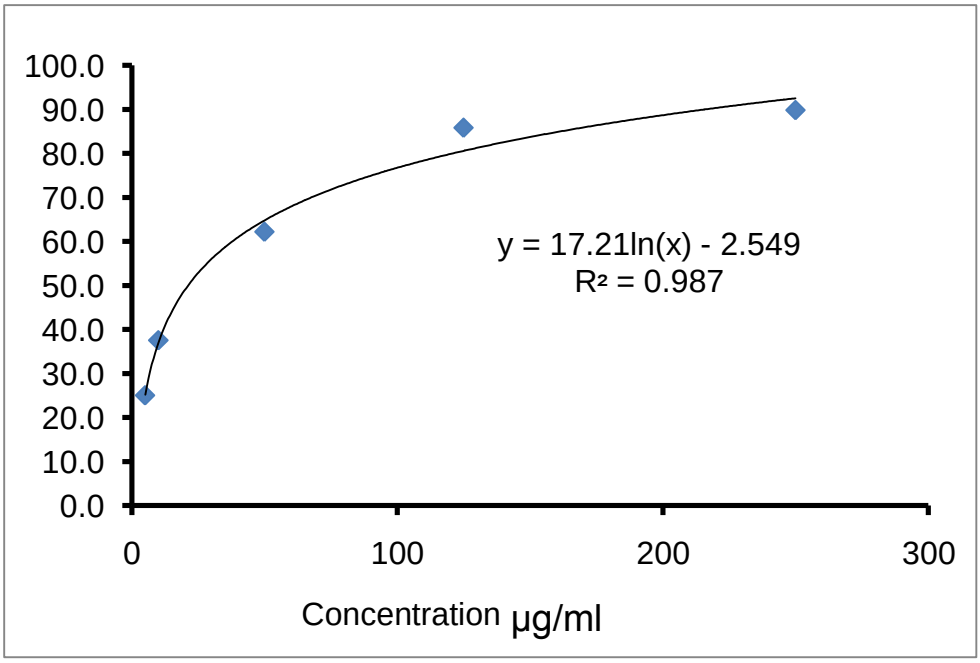

Figure 2: DPPH scavenging activity of Quercetin.

The effectiveness of *sabdariffa* calyx and epicalyx in termination and/or scavenging free radicals was reported by so many research workers [9, 12, 13] who documented that the antioxidant activity of *sabdariffa* calyx and epicalyx could be attributed to the presence of phenolic compounds.Moreover, it was reported

EDITORIAL

that the extract of roselle was found to be very high in ascorbic acid content or ascorbate, which is a well-known natural antioxidant and excellent reducing agent. (14-16)

The antioxidant activity of various *sabdariffa* extracts proved to protect induced hepatotoxicity (17), DNA damage (18) and cytotoxicity. (19)

It can be concluded that *sabdariffa* calyx could be a potential source of antioxidant principles.

References:

1. Fattman, C.L; Schaefer, L.M. and Oury, T.D (2003).Extracellular Superoxide Dismutase in Biology and Medicine. *Journal of Free Radical Biology and Medicine*, 35: 236 -238.
2. Kasai, H; Fukada, S and Yamaizumi, Z (2000). Action of Chlorogenic Acid in Vegetable and Fruits as an Inhibitor of 8-Hydroxydeoxyguanosine Formation *in vitro* and in a Rat Carcinogenesis Model. *Journal of Food and Chemical Toxicology*, 38(5):467-470.
3. Mammadov, A; Ramazan, L and Ertem, V (2011). Antioxidant Activity and Total Phenolic Content of *Gagea fibrosa* and *Romulea ramiflora*. *Iranian Journal of Chemistry and Chemical Engineering*, 30(3):57-62.
4. Koleva, II; Van Beek and, Evstatieva, LN (2002) Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, 13: 8-17.
5. Salleh, N; Runnie, I; Roach, D; Mohamed S and Abeywardena, Y (2002). Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts. *Journal of Agriculture and Food Chemistry*, 50:3693-3697.
6. Ali-Bradeldin, H; Al-Wabel, N and Gerald, B (2005). Phytochemical pharmacological and toxicological aspects of *Hibiscus sabdariffa*: A review. *Journal of Phytotheray Research*, 19:369-375.
7. Ajiboye, TO; Salawu, NA; Yakubu, MY and Oladiji, AT, (2011). Antioxidant and drug detoxification potentials of *Hibiscus sabdariffa* anthocyanin extract. *Journal of Drug and Chemical Toxicology*, 34:109-115.
8. Mensor, LL; Menezes, FS; Reis, TC and Leitao, SG (2001).Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Journal of Phytotherapy Research*, 15:127-130.
9. Chinedu, P; Esiaba, I; Olusola, A and Adesuyi, A (2011). Polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx. *Research Journal of Medicinal Plants*, 5(5):557-566.
10. Enrico, P; Silvia, B; Pier, G and Simona, T (2007). Antioxidant power quantification of decoction and cold infusions of *Hibiscus sabdariffa* flowers. *Journal of Food Chemistry*, 100: 433-438.
11. Azza, A; Ferial, M and Esmat, A (2011). Physicochemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus sabdariffa).Journal of American science*, 7(7):445-456.
12. Alaa, G (2012). Antioxidant and antibacterial activities of *Hibiscus sabdariffa* L. extracts. *African Journal of Food Science*, 6(21): 506-511.
13. Liuqing , Y;Ying G; Ting ,Z; Jiangli ,Z and Fang , L(2012). Antioxidant capacity of extracts from calyx fruits of roselle (*Hibiscus sabdariffa* L.) *African Journal of Biotechnology*, 11(17): 4063-4068.
14. Buettner , GR and Jurkiewicz BA (1996). Chemistry and biochemistry of ascorbic acid. In Handbook of Antioxidants, ed. Cadenas, E., and Packer, L., USA: Marcel Dekker, Inc. p. 94.
15. Falade, OS; Otemuyiwa; IO; Oladipo, A and Oyedapo ,OO (2005). The chemical composition and membrane stability activity of some herbs used in local therapy for anemia. *Journal of Ethnopharmacology*, 102:15-22.
16. Prenesti, E; Berto, S; Daniele, P and Toso, S. (2007). Antioxidant power quantification of decoction and cold infusions of *Hibiscus sabdariffa* flowers. *Journal of Food Chemistry*, 100:433-438.
17. Liu, C.L., Wang, J.M and Chu, C.Y. (2002). *In vivo* protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. *Journal of Chemical Toxicology*, 40: 635–641.
18. Lazze, M.C., Pizzala, R and Savio, M. (2003). Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells. *Journal of Mutation Research*, 535:103–115.
19. Tseng, T.H., Wang, C.J., Kao, E.S and Chu, C.Y. (1996). Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rat primary hepatocytes. *Journal of Chemico-Biological Interactions*, 101: 137–148.