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Nutritional Evaluation of Calyces and Epi-Calyces from Two Variants of *Hibiscus sabdariffa* L.: A Comparison

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ABSTRACT

Hibiscus sabdariffa L. (Roselle; Malvaceae) has been used traditionally as food, in herbal drinks, in hot and cold beverages, as flavouring agent in the food industry and as herbal medicine. The calyx of the roselle plant has long been recognised as a source of antioxidants. The objective of this study was to evaluate antioxidant activity, free radical-scavenging and total phenolic content in calyces and epi-calyces of the roselle plant. Methanol extracts showed a positive correlation between phenolic content and antioxidant activity, as with the increase of their phenolic content ($134.32 \pm 1.82 \mu\text{g GAE mg}^{-1}$ for the red variant and $154.60 \pm 1.54 \mu\text{g GAE mg}^{-1}$ for the white variant), the antioxidant activity of the of the two variants also increases (118.66 ± 0.96 and $102.54 \pm 1.08 \mu\text{g mL}^{-1}$, respectively). So have the potential to be used as food antioxidants. Moreover, nutritionally both the variants are rich in protein, crude fibre, ascorbic acid.

Key words: Antioxidant activity, *Hibiscus sabdariffa*, nutritional value, total phenolic compound

INTRODUCTION

The genus *Hibiscus* (family Malvaceae) includes more than 300 species of annual or perennial herbs or shrubs and commonly known as roselle or red sorrel. Among them *Hibiscus sabdariffa* L., which has many medicinal uses (Alarcon-Aguilar *et al.*, 2007). This plant is native to West Africa and widely grown in the Brahmaputra Valley region of the North Eastern part of India. This plant is widely used in traditional medicines in India, Africa and Mexico. Infusions of the leaves or calyces are traditionally used for their diuretic, choleric, febrifugal and hypotensive effects, decreasing the viscosity of the blood and stimulating intestinal peristalsis, recommended as a hypotensive (Morton, 1987). It is also useful in the treatment for cardiac, nerve diseases, cancer and liver toxicity. Besides its importance as a food or traditional medicine in the countries of its geographic origin, it is used worldwide as an important ingredient in industrially produced teas and beverages (Plotto, 2004). Young leaves and stems are eaten raw or cooked in salads and as a seasoning in curries. Fresh calyx (the outer whorl of the flower) is eaten raw in salads, or cooked and used as a flavoring in cakes and is also used in making jellies, soups, sauces, pickles, puddings, etc. (Lepengue *et al.*, 2009).

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses, which counteract the harmful effects of free radicals and other oxidants. Protection against free radicals can be enhanced by simply intakes of dietary antioxidants. It has been reported that the antioxidant activity of plant materials are well correlated with the content of their phenolic compounds (Halliwell and Gutteridge, 1999; Mau *et al.*, 2004; Mondal *et al.*, 2006;

Cakir *et al.*, 2003). Phenolic compounds, especially phenolic acids and flavonoids, are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices; thus, they are an integral part of the human diet. They have played an important role in the health care industry (Lopez *et al.*, 2007). To provide support to our body defense mechanism, our diet must contain sufficient amount of nutrients and antioxidants. Incorporation of antioxidant rich foods in our daily diet will be boosted our immune system and it is safer and cheaper than commercially available antioxidants. *Hibiscus* is considered as a great source of natural antioxidants (Hertog and Feskens, 1993). Studies demonstrated that the calyx of *Hibiscus* was rich in phenolic compounds and anthocyanins (Mourtzinis *et al.*, 2008). The present study was designed to study the nutraceutical properties and antioxidant activity of floral parts of two different variants of *H. sabdariffa* (Red and White) found in the Brahmaputra Valley agro climatic condition.

MATERIALS AND METHODS

The floral buds of *Hibiscus sabdariffa* were collected from the foot hill of Nilachal, Guwahati, Assam. [Location: 26°11'0" N 91°44'0" E with annual average rainfall 1.717 mm, average temperature ranging from 18-38°C, humidity 76.6%]. Collected floral buds were washed thoroughly, manually separated calyx and epicalyxes, sliced and oven dried at 60°C until we get constant weight. The dried slices were powdered and kept at 4°C for further analysis.

Methods of analysis: Chemical analysis is done on moisture free basis. Analysis is carried out to estimate the macro nutrient components viz., total protein, total carbohydrates and ascorbic acid and phenolic compounds of the samples.

Total protein estimation: The total protein content of the samples was estimated by following the method developed by Lowry *et al.* (1951). Extraction was carried out with Tris-EDTA buffer (pH 7.5) used for the enzyme assay. For extraction, 500 mg of the sample is grinded well with a mortar and pestle in 5 mL of the buffer and after centrifuging, the supernatant is used for protein estimation. The reading is taken in a UV-Vis Spectrophotometer at 660 nm and the amount of protein present is calculated by plotting the value in a standard curve of Bovine Serum Albumin (BSA).

Total carbohydrate estimation: The total carbohydrate content of the samples was estimated by Anthrone method (Hodge and Hofreiter, 1962). To estimate the carbohydrate, 100 mg dried samples were hydrolyzed with 2.5 M HCl for about 3 h in a boiling water bath. Sodium carbonate was added to neutralize the extracts. Subsequently the extracts were centrifuged and supernatant were collected. The residue was washed thrice with distilled water and all the supernatants were pooled and final volume was adjusted to 100 mL. From this, 0.5 mL of the extracts was taken and volume made up to 1 mL distilled water and 4 mL of Anthrone reagent was added to the above solution. Absorbance was taken in a UV-Vis spectrophotometer at 630 nm and the amount of carbohydrate present was calculated by plotting the value in a standard curve of standard glucose solution.

Crude fibre content: Crude fibre in the samples was determined by the method described by Joslyn (1970). Defatted sample (2 g) was placed in a glass crucible and attached to the extraction unit where 150 mL boiling sulphuric acid (1.25%) solution was added. The sample was digested for 30 min and then the acid was drained out and the sample was washed with boiling distilled water.

Then, 150 mL sodium hydroxide solution (1.25%) was added. The sample was digested for 30 min, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in desiccators and Weighed (W1). The sample was then ash at 600°C in a muffle furnace (for 2 h, cooled in desiccators and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula:

$$\text{Crude fiber in ground sample (\%)} = \frac{\text{Loss in weight on ignition (W2-W1)}}{\text{Weight of the sample}} \times 100$$

Ascorbic acid content estimation: The amount of ascorbic acid present in the sample was calculated by extracting the sample in 4% oxalic acid and titrating the extract against the 2,6-dichloro phenol indophenols dye until the end point where pink colour appears that persist for a few minutes (Sadasivam and Theymoli, 1987). The amount of dye consumed is equivalent to the amount of ascorbic acid present in the samples. Standard ascorbic acid solution is used as the reference and the calculation is done by the following formula:

$$\text{Amount of ascorbic acid (mg/100 mL)} = \frac{0.5 \text{ mg} \times V_2 \text{ mL} \times 100 \text{ mL}}{V_1 \times 5 \text{ mL} \times \text{Wt. of the sample}} \times 100$$

Where:

V_1 = Volume of oxalic acid

V_2 = Volume of the sample

Total phenol content estimation: The total phenol content was determined by the Folin-Ciocalteu's method (Singleton and Rossi Jr., 1965). Powdered plant samples were extracted with 70% methanol and condensed. This extracts were diluted (1 mg mL⁻¹ concentration) with 70% methanol. From this, 200 µL of extracts (1 mg mL⁻¹) was taken and volume made up to 2 mL. The 0.3 mL of Folin-Ciocalteu reagent was added. After 5mins, 0.8 mL of 20% Na₂CO₃ was added and the final volume was made 5 mL. Absorbance was taken by UV-Vis spectrophotometer at 765 nm after 30 min incubation. The amount of phenol content was determined using gallic acid as standard. Results were expressed as µg mg⁻¹ (Gallic acid equivalent/dry weight) and the calculations were done by using the following formula:

$$\text{TPC} = \frac{C \times V}{m}$$

Where:

TPC = Total phenol content

C = Concentration of gallic acid (mg mL⁻¹)

V = Volume of plant extract (mL)

m = Weight of pure plant extract (g)

Antioxidant activity estimation: The antioxidant activities of the herb extract along with standard were assessed on the basis of the radical scavenging effect of stable DPPH

(Khalaf *et al.*, 2008). A solution of DPPH (0.2 mM) was prepared in 70% methanol and kept overnight. Stock solution (1 mg mL⁻¹) of the extract was prepared in 70% methanol. Various concentration of the extracts viz., 10, 20, 50, 100, 150, 200, 300, 400 and 500 µL were taken in different test tubes and the volume was made up to 1000 µL. One milliliter DPPH was added to each solution and kept at dark for 30 min. Ascorbic acid and Gallic acid were taken as standards. Optical density of these samples was measured at 517 nm along with blank where 1 mL methanol with 1 mL DPPH solution was taken. The activities of the samples are measured in terms of percent inhibition (IC₅₀) and calculated by the following formulae:

$$\text{Inhibition of DPPH activity (\%)} = \frac{A - B}{A} \times 100$$

Where:

A = Optical density of the blank

B = Optical density of the sample

Statistical analysis: The data were subjected to statistical analysis. All the assays were recorded in triplicates and the values were expressed as Mean ±SD. The IC₅₀ value was calculated by plotting a graph with percent inhibition on y-axis and concentration on x-axis.

RESULT AND DISCUSSION

Medicinal plants are a major source of bioactive compounds of therapeutic values. Herbal medicines are quite inexpensive, better compatibility with the human body with minimal side effects. The therapeutic values of a plant are mainly dependent upon its anti-oxidative property. The most common antioxidants present in herbs and fruits are vitamins C and E, carotenoids, flavonoids and thiol (SH) compounds, etc. There were several reports that the contribution of phenolic compounds to antioxidant activity was much greater than those of vitamin C and carotenoids (Sarma *et al.*, 2014; Prior *et al.*, 1998; Wang *et al.*, 1996; Luximon-Ramma *et al.*, 2003). Yang *et al.* (2012) reported that total phenol contents of plant extract played major roles in the antioxidant activity.

In the present investigation, the phyto-chemical analysis was done for total carbohydrate, protein, crude fibre, ascorbic acid, total phenolic content in extracts of both the red and white variants of *H. sabdariffa*. In white variant, the total phenol content was found to be higher (154.60±1.54 µg GAE mg⁻¹) as compared to red one (134.32±1.82 µg GAE mg⁻¹) and also the ascorbic acid content was higher in white variant (81.39±0.84 mg/100 g) as compared to red variant (72.55±0.83 mg/100 g). The carbohydrate content was quite similar in both the variants and was found to be 22.83±1.02 and 19.89±0.56 µg mg⁻¹, respectively. The protein content was found to be 0.22±0.007 and 0.17±0.012 µg mg⁻¹, respectively for white and red variants respectively. The crude fibre content of white and red variants was found to be 15.09±0.72 and 12.95±0.24%, respectively (Table 1). The antioxidant activity of white variety (IC₅₀ = 102.54±1.08 µg mL⁻¹) was also higher as compared to red variety (IC₅₀ = 118.66±0.96 µg mL⁻¹) as shown in Fig. 1.

The investigation suggests that the major source of antioxidant capacity of both the variants of *Hibiscus sabdariffa* (white and red) may be not from vitamin C but rather from phenolic

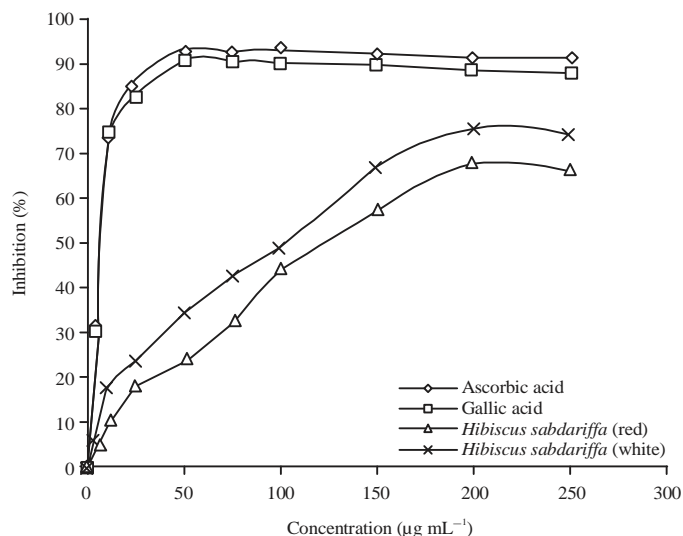


Fig. 1: DPPH free radical scavenging activity of methanolic herb extracts of *Hibiscus sabdariffa* at 517 nm

Table 1: Phytochemical analysis of *Hibiscus sabdariffa* (red and white)*

Sample	Carbohydrate content (µg mg ⁻¹)	Protein content (µg mg ⁻¹)	Crude fibre (%)	Ascorbic acid (mg/100 g)	Total phenol content (µg GAE mg ⁻¹)	Inhibition concentration (IC ₅₀) (µg mL ⁻¹)
<i>Hibiscus sabdariffa</i> (Red)	19.89±0.56	0.17±0.012	12.95±0.24	72.55±0.83	134.32±1.82	118.66±0.96
<i>Hibiscus sabdariffa</i> (White)	22.83±1.02	0.22±0.007	15.09±0.72	81.39±0.84	154.60±1.54	102.54±1.08
<i>Hibiscus sabdariffa</i> (Red)	19.89±0.56	0.17±0.012	12.95±0.24	72.55±0.83	134.32±1.82	118.66±0.96
<i>Hibiscus sabdariffa</i> (White)	22.83±1.02	0.22±0.007	15.09±0.72	81.39±0.84	154.60±1.54	102.54±1.08
<i>Hibiscus sabdariffa</i> (Red)	19.89±0.56	0.17±0.012	12.95±0.24	72.55±0.83	134.32±1.82	118.66±0.96
<i>Hibiscus sabdariffa</i> (White)	22.83±1.02	0.22±0.007	15.09±0.72	81.39±0.84	154.60±1.54	102.54±1.08

*Values represented in the table are Mean±SD of three replicates

compounds. The white variety of *Hibiscus sabdariffa* shows more anti-oxidative property than the red variant. Therefore, the supplementation of these natural antioxidants through a balanced diet containing adequate fruit could be much more effective than the supplementation of an individual antioxidant such as vitamin C or vitamin E. Obouayeba *et al.* (2014) revealed that *Hibiscus sabdariffa* is an excellent source of dietary phytochemicals such as anthocyanins, flavonoids and phenolic acids. Pharmacognostical studies of calyx and epicalyxes of *Hibiscus sabdariffa* will be helpful for proper value addition of the floral parts of the plant. It is clear that floral parts may have good phyto-constituents like carbohydrates, crude fibre, ascorbic acid and phenolic content and therefore may used as food supplementary to diet. Moreover, the floral parts of the plant may be used clinically better to explore other activities like antihyperlipidmic, antihypercholester olemic and antiviral activities for human society in future.

CONCLUSION

The findings of the work show that calyx and epi-calyx of both variants of *Hibiscus sabdariffa* contains potent antioxidant principles which are likely total phenolic content and ascorbic acid as well. Therefore, crude extract may be further purified to isolate the antioxidant constituents of the plant for pharmaceutical applications.

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REFERENCES

- Alarcon-Aguilar, F.J., A. Zamilpa, M.D. Perez-Garcia, J.C. Almanza-Perez and E. Romero-Nunez *et al.*, 2007. Effect of *Hibiscus sabdariffa* on obesity in MSG mice. *J. Ethnopharmacol.*, 114: 66-71.
- Cakir, A., A. Mavi, A. Yildirim, M.E. Duru, M. Harmandar and C. Kazaz, 2003. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. *J. Ethnopharmacol.*, 87: 73-83.
- Halliwell, B. and J.M.C. Gutteridge, 1999. *Free Radicals in Biology and Medicine*. 3rd Edn., Oxford University Press, Oxford, New York, pp: 105-245.
- Hertog, M.G., E.J. Feskens, P.C. Hollman, M.B. Katan and D. Kromhout, 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly study. *Lancet*, 342: 1007-1011.
- Hodge, J.E. and B.T. Hofreiter, 1962. *Methods in Carbohydrate Chemistry*. 17 Edn., Academic Press, New York.
- Joslyn, M.A., 1970. *Methods in Food Analysis*. Academic Press, New York pages: 176.
- Khalaf, N.A., A.K. Shakya, A. Al-Othman, Z. El-Agbar and H. Farah, 2008. Antioxidant activity of some common plants. *Turk. J. Biol.*, 32: 51-55.
- Lepengue, N.A., I. Mouraragadja, B.M. Batchi and S. Ake, 2009. Etude de quelques caracteristiques physico chimiques du filtrat toxique de *Phoma sabdariffae* Sacc., agent pathogene de la roselle. *Sci. Nat.*, 6: 95-105.
- Lopez, V., S. Akerreta, E. Casanova, J.M. Garcia-Mina, R.Y. Cavero and M.I. Calvo, 2007. *In vitro* antioxidant and anti-rhizopus activities lamiaceae herbal extracts. *Plant Food. Hum. Nutr.*, 62: 151-155.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Luximon-Ramma, A., T. Bahorun and A. Crozier, 2003. Antioxidant actions and phenolic and vitamin C contents of common *Mauritian exotic* fruits. *J. Sci. Food Agric.*, 83: 496-502.
- Mau, J.L., C.N. Chang, S.J. Huang and C.C. Chen, 2004. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem.*, 87: 111-118.
- Mondal, S.K., G. Chakraborty, M. Gupta and U.K. Mazumder, 2006. *In vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. *Ind. J. Exp. Biol.*, 44: 39-44.
- Morton, J.F., 1987. *Fruits of Warm Climates*. Florida Flair Books, Miami, ISBN: 10-0961018410, pp: 221-239.
- Mourtzinis, I., D.P. Makris, K. Yannakopoulou, N. Kalogeropoulos, I. Michali and V.T. Karathanos, 2008. Thermal stability of anthocyanin extract of *Hibiscus sabdariffa* L. in the presence of β -cyclodextrin. *J. Agric. Food Chem.*, 56: 10303-10310.
- Obouayeba, A.P., N.B. Djyh, S. Diabate, A.J. Djaman, J.D. N'Guessan, M. Kone and T.H. Kouakou, 2014. Phytochemical and antioxidant activity of roselle (*Hibiscus sabdariffa* L.) petal extracts. *Res. J. Pharm. Biol. Chem. Sci.*, 5: 1453-1465.

- Plotto, A., 2004. Hibiscus: Post-production management for improved market access. Food and Agriculture Organization of the UN, (FAO).
- Prior, R.L., G. Cao, A. Martin, E. Sofic and J. McEwen *et al.*, 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species. *J. Agric. Food Chem.*, 46: 2686-2693.
- Sadasivam, S. and B. Theymoli, 1987. Practical Manual in Biochemistry. Tamil Nadu Agricultural University, Coimbatore, India, pp: 14.
- Sarma, A., P. Sarmah, D. Kashyap and A. Kalita, 2014. Evaluation of nutraceutical properties and antioxidant activity of *Garcinia cowa* Roxb. Ex Choisy fruits found in Assam (India). *World J. Pharm. Pharmaceut. Sci.*, 3: 853-853.
- Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with 315 phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Viticulture*, 16: 144-158.
- Wang, H., G. Cao and R.L. Prior, 1996. Total antioxidant capacity of fruits. *J. Agric. Food Chem.*, 44: 701-705.
- Yang, L., Y. Gou, T. Zhao, J. Zhao, F. Li, B. Zhang and X. Wu, 2012. Antioxidant capacity of extracts from calyx fruits of roselle (*Hibiscus sabdariffa* L.). *Afr. J. Biotechnol.*, 11: 4063-4068.