



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
Journals Inc.

www.academicjournals.com

Polyphenolic Content and Antioxidant Activity of *Hibiscus sabdariffa* Calyx

¹Chinedu Prosper Anokwuru, ¹Ijeoma Esiaba, ²Olusola Ajibaye and ¹Ayobami O. Adesuyi

¹Department of Chemical and Environmental Sciences, School of Science and Technology, Babcock University, Ilisan Remo, Nigeria

²Mataria Research Laboratory, Biochemistry Division, Nigerian Institute of Medical Research (NIMR), Yaba Lagos, Nigeria

Corresponding Author: Chinedu Prosper Anokwuru, Department of Chemical and Environmental Sciences, School of Science and Technology, Babcock University, Ilisan Remo, Nigeria

ABSTRACT

The polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx in methanol, ethanol, acetone and water extract were studied. The Total Phenolic Content (TPC) was determined using folin Ciocalteu method while the Total Flavonoid Content (TFC) was determined using aluminum chloride method. Antioxidant activities were determined using 2,2-diphenyl-1-picryl hydrazine (DPPH) inhibition and lipid peroxidation inhibition. Methanol extracted the highest total phenolic content (29.2 mg GAE/g DW) and was significantly different ($p < 0.05$) from the water and acetone extracts only. Acetone extracted the highest flavonoid content (53.6 mg QE/g DW) and was significantly different from the other solvents used. Methanol extract gave the highest inhibition to DPPH (78%) and was only significantly different ($p < 0.05$) from acetone and water extracts. Ethanol gave the highest inhibition to lipid peroxidation (26%) but was not significantly different ($p < 0.05$) from the other solvent extracts. There was a stronger correlation obtained between TPC and inhibition of DPPH ($r = 0.969$) compared to TFC and DPPH ($r = 0.742$). The study showed that methanol and ethanol were better solvents for the extraction of phenols of *Hibiscus sabdariffa* calyx compared to water and acetone. It also showed that phenols contributed more to the antioxidant activity of *Hibiscus sabdariffa* calyx compared to flavonoids.

Key words: *Hibiscus sabdariffa* calyx, phenols, flavonoids, antioxidant activity, solvents

INTRODUCTION

Antioxidants help to neutralise free radicals which are unstable molecules that are linked to the development of a number of degenerative diseases and conditions including cancer, cardiovascular disease, cognitive impairment, immune dysfunction, cataracts and macular degeneration (D'Abrosca *et al.*, 2007).

They exhibit their antioxidant activity by inhibiting lipid peroxidation (by inactivating lipoxygenase), scavenging free radicals and active oxygen species, preventing the decomposition of hydrogen peroxides into free radicals and chelating heavy metal ions (Ghimeray *et al.*, 2009; Veeru *et al.*, 2009). Mammalian cells possess elaborate defense mechanism for radical detoxification through the action of dietary antioxidants (α -tocopherol, β -carotene, ascorbic acid, glutathione, uric acid), some hormones (estrogen, angiotensin) and endogenous enzymes (superoxide dismutase,

glutathione peroxidase, catalase) which are able to remove oxygen free radicals formed in cells and thus protect against oxidative damage (Tawaha *et al.*, 2007; Subhasree *et al.*, 2009). Besides playing an important role in physiological systems, antioxidants have been used in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in foods are readily oxidized by molecular oxygen and are a major cause of quality deterioration, nutritional losses, off-flavour development and discolouration. The addition of synthetic antioxidants, such as propyl gallate, Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT) and tertiary butylhydroquinone has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity (Kahl and Kappus, 1993). The search for antioxidants from natural sources has received much attention and efforts have been put into identifying compounds that can act as suitable antioxidants to replace synthetic ones. In addition, these naturally-occurring antioxidants can be formulated to give nutraceuticals that can help to prevent oxidative damage from occurring in the body (Wong *et al.*, 2006).

Plant phenolics are commonly found in both edible and non-edible plants and their antioxidant activities mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quencher (Huda-Faujan *et al.*, 2009; Tawaha *et al.*, 2007). The antioxidant activity of polyphenols depends mainly on different structural features such as O-H bonds, dissociation energy, resonance delocalization of the phenol radical and steric hindrance derived from hydrogen substitution in the aromatic ring (Es-Safi *et al.*, 2007).

Plants are thought to manufacture phenols for a variety of reasons but primarily as a part of their response to stress. Phenolic compounds have anti-bacterial, anti-viral and anti-fungi actions in the plant itself and by virtue of being hydrogen-donating antioxidants, they protect against oxidative stress-which can adversely affect the health of plants as well as humans. In humans, cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions (Mudgal *et al.*, 2010). Phenols contribute to wound healing by providing materials for lignification of cell walls as they are oxidized (Miller and Ruiz-Larrea, 2002). They also possess anticancer, anti-inflammatory, anti-allergic, estrogenic and immune-stimulating agent (Tawaha *et al.*, 2007).

The extraction of phenolic compounds in plant material is influenced by their chemical nature, the extraction method, the sample size, time and storage conditions as well as the presence of interfering substances. The phenolic extracts of plants are always a mixture of different classes of phenols which are selectively soluble in the solvents. The use of an alcoholic solution provides satisfactory results for the extraction process (Koffi *et al.*, 2010).

For extraction of polyphenols from plant matter, different solvent systems have been used and their efficiency varies. Extraction yield depends on the solvent and method of extraction. Commonly used solvents for extracting various substances from plant material are water, aqueous mixtures of ethanol, methanol and acetone (Jakopic *et al.*, 2009).

DPPH assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. The assay is based on the measurements of the antioxidants' ability to scavenge the stable radical DPPH. DPPH is a stable nitrogen-centered free radical which produces violet colour in methanol solution. DPPH radicals react with suitable reducing agents during which the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up (Blois, 1958). It is a stable free radical with a characteristic absorption at 517 nm and was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases (Kubola and Siriamornpurn, 2008).

Lipid peroxidation is the oxidative degradation of Polyunsaturated Fatty Acids (PUFA) and involves free radicals. This is a basic membrane damage process and results in deleterious effects. Initiation of lipid peroxidation was carried out by the addition of ferrous sulphate. This occurs by the formation of hydroxyl radicals by Fenton's reaction (Braugher *et al.*, 1986). These produce Malondialdehyde (MDA) which reacts with Thiobarbituric Acid (TBA) to form a pink chromogen. This inhibition could be caused by the absence of ferryl-perferryl complex or by scavenging the hydroxyl radical or the superoxide radicals or by changing the Fe^{3+}/Fe^{2+} ratio or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself (Baskar *et al.*, 2007).

Hibiscus sabdariffa L., is a shrub belonging to the family Malvaceae. It is thought to be of Asia (India to Malaysia) or Tropical Africa origin. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Florida, Hawaii and Philippines. It is commonly known as Roselle or red sorrel in English, is native of India and Malaysia where it is commonly cultivated. It is known as mesta/meshta in India subcontinent, bissap in Senegal, the Congo and France, wanjo in the Gambia, zobo in Nigeria, Karkade in Egypt, Saudi Arabia and Sudan, omutete in Namibia and sorrel in the Caribbean and Jamaican. Botanically, it is described as thick red fleshy and cup shaped calyxes plant. The calyxes are rich in phenolic compounds with marked physiological activities. *Hibiscus* flowers contains gossypetin, glucoside, hibiscin, hibiscus anthocyanin and protocatechuic acid which may have diuretic and choleric effects decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis (Josiah *et al.*, 2010; Mahadevan and Pradeep, 2009).

The dried calyx is used as a diuretic, sedative, refrigerant and for making a drink by boiling in water and adding a little salt, pepper, asa-foetida and molasses to treat mental fatigue due to excessive heating by the Gondas tribal of Karnataka. *H. sabdariffa* is reported to possess antihypertensive, antioxidant, anti-cancer, anticlastrogenic, hypolipidaemic, hepatoprotective, anti-stress, antispasmodic, diuretic and antidiarrheal activities (Joshi and Parle, 2006). The aim of this research is to determine the solvent that best extracts the phenolics and also give the highest antioxidant activity.

MATERIALS AND METHODS

Chemicals: Methanol, Ethanol, Acetone, Gallic acid, Thiobarbituric Acid (TBA), 2,2-diphenyl-2-picrylhydrazyl (DPPH) and Quercetin, Sodium Dodecyl Sulphate (SDS) and Trichloro Acetic Acid (TCA) were all analytical grade.

Materials and extraction methods: Dried *Hibiscus sabdariffa* calyx was purchased from Ilisan Remo market located Ogun State, Nigeria, in January 2010 and ground to powder to obtain fine particles. Twenty gram of the sample was soaked with 250 mL of methanol, ethanol, acetone and water each for 72 h. The filtrate from each sample was concentrated using the rotary evaporator at 40°C. The dried extracts were weighed and stored frozen.

Determination of plant percentage yield: The percentage yield was obtained from this formula:

$$\frac{W_2 - W_1}{W_0} \times 100$$

where, W_2 is the weight of the extract and the container, W_1 is the weight of the container alone and W_0 is the weight of the initial dried sample.

Determination of total phenolic content: The total phenolic content was estimated by Folin-Ciocalteu colorimetric method, based on the procedure in Singleton and Rossi (1965). Procedure: Briefly, the crude extract (50 mg) was mixed with Folin-Ciocalteu reagent (0.5 mL) and deionized water (7.5 mL). The mixture was kept at room temperature for 5 min and then 10 mL of 7% sodium carbonate was added to the mixture and then incubated for 90 min at room temperature. After incubation the absorbance against the reagent blank was determined at 760 nm. The amount of phenolic compound in the extract was determined from the standard curve using gallic acid ($R^2 = 0.9986$). The total phenolic content of the plant was expressed as mg Gallic Acid Equivalent (GAE)/g dry weight. All samples were analyzed in triplicates.

Determination of total flavonoid content: The TFC were measured following a spectrophotometric method (Dewanto *et al.*, 2002). Briefly, extract of each plant material (1 mL containing $100 \mu\text{g mL}^{-1}$) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% NaNO_2 solution (0.3 mL) was added to each volumetric flask; at 5 min, 10% AlCl_3 (0.3 mL) was added and at 6 min 1.0 M NaOH (2 mL) was added. Water (2.4 mL) was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm. TFC were determined as quercetin equivalents (mg g^{-1} of dry weight). Three readings were taken for each sample and the result averaged.

Determination of DPPH radical scavenging activity: A solution of DPPH mixed with that of a substance that can donate a hydrogen atom gives rise to the reduced form with change in colour from deep violet to pale yellow colour. DPPH is a useful reagent for investigating free radical scavenging activities of phenolic compounds. The reduction of DPPH absorption is indicative of the capacity of the extract to scavenge free radicals, independently of any enzymatic activity.

Procedure: This was carried out according to the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay system (Mensor *et al.*, 2001). One mL of a 0.3 mM DPPH methanol solution was added to a 2.5 mL solution of the extract and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%), using the formula:

$$\text{AA \%} = \frac{(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{blank}}} \times 100$$

where, Abs is absorbance.

Determination of inhibition of lipid peroxidation: A modified Thiobarbituric Acid Reactive Substances (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid-rich media (Ruberto *et al.*, 2000).

Procedure: Egg homogenate (0.5 mL, 10% v/v) and 0.1 mL of each extract were added to a test tube and made up to 1 mL with distilled water. 0.05 mL of FeSO_4 (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium deodecyl sulphate and 20% TCA were added and the resulting mixtures were vortexed and then heated at 95°C for 60 min. After cooling, 5.0 mL of butan-1-ol was added to each tube and centrifuged at 300 rpm for 10 min. The absorbance of the organic layer was measured at 532 nm. The percentage inhibition of lipid peroxide formation by the extract was calculated:

$$\% \text{ Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

RESULTS

The total phenolic content of *Hibiscus sabdariffa* calyx was expressed in milligram Gallic Acid Equivalent per gram. The values represent the Mean of triplicates \pm SE of each extract.

The total flavonoid content of *Hibiscus sabdariffa* calyx was expressed as milligram Quercetin Equivalent per gram. The values represent the Mean of triplicates \pm SE of each extract.

Percentage yield: The percentage yield of *Hibiscus sabdariffa* calyx in different solvents is shown in Table 1. The result showed that methanol extract of *Hibiscus sabdariffa* calyx had the highest yield and significantly different ($p < 0.05$) from ethanol, acetone and water.

Total phenolic content: The result of the Total Phenolic Content (TPC) of *Hibiscus sabdariffa* calyx in different solvents is shown in Table 1. The result of the total phenolic content of *Hibiscus sabdariffa* calyx in different solvents showed that methanol extract yielded 29.2 mgGAE/g, ethanol extract yielded 27.6 mgGAE/g, acetone extract yielded 19.3 mgGAE/g and water extract yielded 20.2 mgGAE/g.

Total Flavonoid Content (TFC): The result of the Total Phenolic Content (TPC) of *Hibiscus sabdariffa* calyx in different solvents is shown in Table 1. The result of the total flavonoid content of *Hibiscus sabdariffa* calyx in different solvents showed that methanolic extract yielded 36.7 mgQE/g, ethanol extract yielded 33.8 mgQE/g, acetone extract yielded 53.6 mgQE/g and water extract yielded 33.3 mgQE/g.

Inhibition of DPPH radical: The DPPH radical-scavenging activity of the methanol, ethanol, acetone and water extract of *Hibiscus sabdariffa* calyx is shown in Fig. 1. Methanol extract inhibited 78 \pm 0.25%, ethanol extract inhibited 69 \pm 0.46%, acetone extract inhibited 37 \pm 0.01% and water extract inhibited 63 \pm 9.97% of the DPPH free radicals.

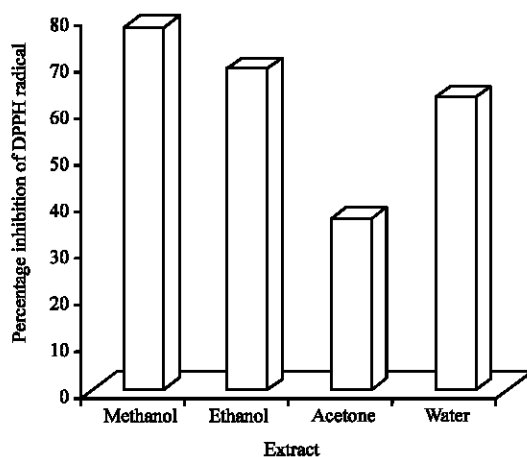


Fig. 1: Percentage inhibition of the DPPH radicals by methanol, ethanol, acetone and water extracts of *Hibiscus sabdariffa* calyx. The values represent the Mean of triplicates \pm SE of each extract

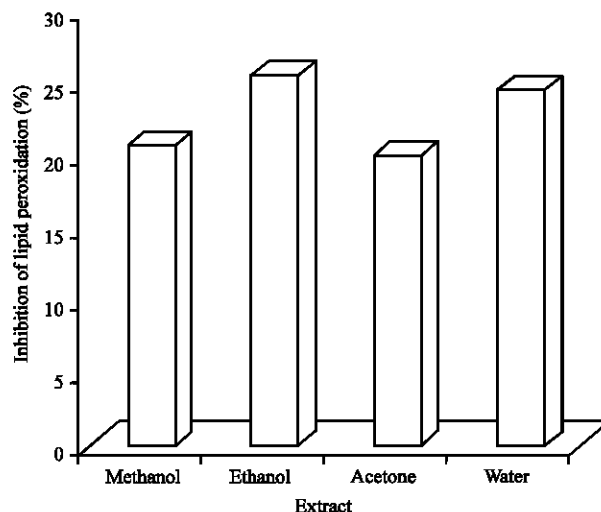


Fig. 2: Percentage inhibition of lipid peroxidation by methanol, ethanol, acetone and water extracts of *Hibiscus sabdariffa* calyx. The values represent the Mean of triplicates±SE of each extract

Table 1: Percentage extract yield, total phenolic content and total flavonoid content of *Hibiscus sabdariffa* calyx

Solvent	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Methanol	27.3±0.33	29.2±0.03	36.7±0.01
Ethanol	12.8±0.12	27.6±0.03	33.8±0.02
Acetone	5.9±0.09	19.3±0.02	53.6±0.03
Water	4.0±0.12	20.2±0.06	33.3±0.01

Inhibition of lipid peroxidation: The lipid peroxidation inhibition activity of the methanol, ethanol, acetone and water extract of *Hibiscus sabdariffa* calyx is shown in Fig. 2. Methanol extract inhibited 21±2.08%, ethanol extract inhibited 26±4.14%, acetone extract inhibited 20±0.01% and water extract inhibited 25±1.6% of the lipid peroxidation.

DISCUSSION

Phenols: Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups (Hatano *et al.*, 1989). Methanol has been reported by Yao *et al.* (2004) to be the most suitable solvent in the extraction of polyphenolic compounds from plant tissue due to its ability to inhibit the action of polyphenol oxidase that causes the oxidation of polyphenols.

The result of this study showed that there was no significant difference between the phenolic content of the methanol and ethanol extract ($p < 0.05$) but the two extracts were significantly different from water and acetone extract ($p < 0.05$). Compared with the percentage yield of all the extract, there was a decline from methanol to ethanol and acetone but an increase in the phenolic content of water extract (Table 1). This result showed that alcohols are better solvents than acetone and water for the extraction of phenols from *Hibiscus sabdariffa* calyx. The TPC of the aqueous extract of *Hibiscus sabdariffa* calyx reported by Oboh (2008), (13.3 mg g⁻¹) and Mohd-Esa *et al.* (2010) (1.85 to 2.9 mg g⁻¹ for aqueous methanol) were lower than the aqueous TPC in this study (20 mg g⁻¹). The values (5-24 mg g⁻¹) of ethanol extracts of *Hibiscus sabdariffa* calyx reported by Christian and Jackson (2009) were lower than the values reported for the ethanol

extract in this study. Furthermore, in the extraction of phenolic compounds from different cultivars of green walnuts, Jakopic *et al.* (2009) reported that the methanol extracted significantly higher amount of phenols compared to ethanol. However, a research conducted by Koffi *et al.* (2010) indicated that ethanol was the best solvent for the extraction of phenols in the Ivorian plants under study. There was no found literature that compared all the four solvents used in this study. It has been reported by Singleton *et al.* (1999) that the Folin-Ciocalteu assay has been shown not to be specific to just polyphenols but to any other substance that could be oxidised by the Folin reagent. In addition, phenolic compounds, depending on the number of phenolic groups they have, respond differently to the Folin-Ciocalteu reagent. Therefore, the total phenolic content of *Hibiscus sabdariffa* calyx in this study may not be the exact content of the phenols present.

The phenolic compounds in the extract are more often associated with other biomolecules (proteins, polysaccharides, terpenes, chlorophyll, lipids and inorganic compounds) and a solvent must be found that is suitable for extracting them (Koffi *et al.*, 2010). The extraction of other biomolecules probably could explain the very high percentage yield obtained in the methanol extract of this study (Table 1) which was significantly higher ($p < 0.05$) than ethanol, acetone and water. According to Sultana *et al.* (2009), the high percentage yield of the methanol and ethanol extract in this study could be due to the ability of these solvents to dissolve endogenous compounds together with the phenols.

The strong linear correlation ($r = 0.816$) between the percentage yield of the extracts and the total phenolic content suggest that high phenolics were extracted from *Hibiscus sabdariffa* calyx.

Flavonoids: Flavonoids are large compounds occurring ubiquitously in food plants. They occur as glycosides and contain several phenolic hydroxyl groups on their ring structure. Many flavonoids are found to be strong antioxidants capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups (Cao *et al.*, 1997). The result of the total flavonoid content showed that the acetone extract had the highest flavonoid content (53.6 mgQE/g) and significantly different ($p < 0.05$) compared to methanol, ethanol and water. This suggests that the flavonoids present in *Hibiscus sabdariffa* calyx were better extracted by acetone. The stronger linear correlation between the percentage yield and TFC ($r = 0.963$) compared to the relationship between percentage yield and TPC, also suggest that flavonoids were principal constituent of the crude extracts in the four solvents used in this study. The result also showed that more flavonoids were extracted from the plant since the values of the TFC were higher than the values of the TPC (Table 1).

DPPH: DPPH assay is a preliminary test to investigate the antioxidant potential of extracts (Kaur *et al.*, 2008). The result of the DPPH free radical scavenging activity (Fig. 1) showed that methanol extract of *Hibiscus sabdariffa* calyx inhibited more free radicals than ethanol, acetone and water. However, it was only significantly different from only water and acetone extract. Due to different antioxidant potentials of different compounds, the antioxidant activity of extract is strongly dependents on the extraction solvent (Jang *et al.*, 2007). The methanol extract recorded the highest phenolic content and also had the highest antioxidant activity; however, the acetone extract that gave the highest flavonoid content recorded the lowest antioxidant activity. This suggests that the flavonoid contents of the acetone extract may be glycosylated making the flavonoids not freely available for antioxidant activity. There was a positive linear correlation between the antioxidant activity and total phenolic content of *Hibiscus sabdariffa* calyx ($r = 0.969$).

This result suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. This result was consistent with the findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity (Cai *et al.*, 2004; Tawaha *et al.*, 2007; Zheng and Wang, 2001).

The positive linear correlation ($r = 0.742$) between flavonoid content and antioxidant activity also suggest that the flavonoid contents of *Hibiscus sabdariffa* calyx contributed to the antioxidant activity. However, there was a stronger correlation with phenols indicating that more phenols were available for inhibition of free radicals compared to flavonoids.

Lipid peroxidation: The result in this study (Fig. 2) showed that ethanol extract had the highest inhibition of lipid peroxidation followed by water, methanol and acetone. However, these inhibitions recorded were lower than the inhibitions recorded with the DPPH free radical assay. The low percentage inhibition of lipid peroxidation could be due to the storage of the extract before use. There are reports (Jang *et al.*, 2007) that indicate that storage of extracts can decrease the phenolic content of the extract which affects the stability of the antioxidant activity of the extract due to chemical or enzymatic decomposition. The inhibitory activity of the ethanol extract in this study (26%) was far lower than 71.3% recorded by Ochani and D'Mello (2009). There was a very low positive correlation ($r = 0.137$) between TPC and inhibition of lipid peroxidation. This could suggest that inhibition of lipid peroxidation may not be a good assay to determine antioxidant activity of *Hibiscus sabdariffa* calyx. The result also showed a negative correlation ($r = -0.779$) between TFC and inhibition of lipid peroxidation. These poor correlations could be as a result of deterioration of the lipids during storage. It could also suggest that both phenolics and flavonoids did not contribute to the inhibition of lipid peroxidation of *Hibiscus sabdariffa* calyx in this study.

CONCLUSION

In the present study, methanolic extract of *Hibiscus sabdariffa* calyx gave the highest extraction yield, phenolic contents and antioxidant activity against DPPH free radicals. Acetone extract gave the highest flavonoid content but had the least inhibition to DPPH free radicals. The result also showed that a higher amount of flavonoids were extracted compared to phenols in all the solvents however; there was a stronger linear correlation between the phenolic contents and inhibition of DPPH free radicals than flavonoid contents. This suggests that phenolics contribute more to the antioxidant activity of *Hibiscus sabdariffa* calyx. All the extracts showed low inhibition to lipid peroxidation.

REFERENCES

- Baskar, R., V. Rajeswari and S.T. Kumar, 2007. *In vitro* antioxidant studies in leaves of *Annona* species. Indian J. Exp. Biol., 45: 480-485.
- Blois, M.S., 1958. Antioxidant determination by the use of stable free radicals. Nature, 181: 1199-2000.
- Braugher, J.M., L.A. Duncan and R.L. Chase, 1986. The involvement of iron in lipid peroxidation. Importance of ferric to ferrous iron in initiation. J. Biol. Chem., 261: 10282-10289.
- Cai, Y., Q. Luo, M. Sun and H. Corke, 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with cancer. Life Sci., 74: 2157-2184.
- Cao, G., E. Sofic and R.L. Prior, 1997. Antioxidant and pro-oxidant behaviour of flavonoids: Structure activity relationships. Free Radical Biol. Med., 22: 749-760.

- Christian, K.R. and J.C. Jackson, 2009. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. J. Food Composition Anal., 22: 663-667.
- D'Abrosca, B., S. Pacifico, G. Cefarelli, C. Mastellone and A. Fiorentino, 2007. *Limoncella* apple, an Italian apple cultivar: Phenolic and flavonoid contents and antioxidant activity. Food Chem., 104: 1333-1337.
- Dewanto, V., X. Wu, K.K. Adom and R.H. Liu, 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem., 50: 3010-3014.
- Es-Safi, N.E., J. Bauhaire, C.L. Guerneve and P.H. Ducrot, 2007. Synthesis and antioxidant activity of modified (+)-catechin derivatives. Structure-activity relationship. Am. J. Food Technol., 2: 618-629.
- Ghimeray, A.K., C. Jin, B.K. Ghimine and D.H. Cho, 2009. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in azadirachta indica A. Juss grown in foothills of Nepal. Afr. J. Biotechnol., 8: 3084-3091.
- Hatano, T., R. Edamatsu, M. Hiramatsu, A. Mori and Y. Fujita *et al.*, 1989. Effect of interaction of tannins with co-existing substances VI. Effect of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem. Pharmaceutical Bull., 37: 2016-2021.
- Huda-Faujan, N., A. Noriham, A.S. Norrakiah and A.S. Babji, 2009. Antioxidant activity of plant methanolic extracts containing phenolic compounds. Afr. J. Biotechnol., 8: 484-489.
- Jakopic, J., R. Veberic and F. Stampar, 2009. Extraction of phenolic compounds from green walnuts fruits in different solvents. Acta Agriculturae Slovenica, 93: 11-15.
- Jang, H.D., K.S. Chang, Y.S. Huang, C.L. Hsu, S.H. Lee and M.S. Su, 2007. Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. Food Chem., 103: 749-756.
- Joshi, H. and M. Parle, 2006. Nootropic activity of calyces of *Hibiscus sabdariffa* linn. Iranian J. Pharmacol. Ther., 5: 15-20.
- Josiah, S.J., O. Omotuyi, K.A. Oluyemi, U.I. Ezea and E.S. Uahunmwangho *et al.*, 2010. Protective role of aqueous extract of *Hibiscus sabdariffa* calyx against potassium bromated induced tissue damage in winster rats. Afr. J. Biotechnol., 9: 3218-3222.
- Kahl, R. and H. Kappus, 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. J. Food Control Res., 196: 329-338.
- Kaur, R., S. Arora and B. Singh, 2008. Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss. Bioresour. Technol., 99: 7692-7698.
- Koffi, E., T. Sea, Y. Dodehe and S. Soro, 2010. Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. J. Anim. Plant Sci., 5: 550-558.
- Kubola, J. and S. Siriamornpurn, 2008. Phenolic content and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*. Food Chem., 110: 881-890.
- Mahadevan, N.S. and K. Pradeep, 2009. *Hibiscus sabdariffa*: An overview. Nat. Prod. Radiance, 8: 77-83.
- Mensor, L.L., F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. Santos, C.S. Coube and S.G. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127-130.
- Miller, N.J. and M.B. Ruiz-Larrea, 2002. Flavonoid and other plant phenols in diet: Their significance as antioxidants. J. Nutr. Environ. Med., 12: 39-51.

- Mohd-Esa, N., F.S. Hern, A. Ismail and C.L. Yee, 2010. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa*) extracts and potential exploitation of their seeds. *Food Chem.*, 122: 1055-1060.
- Mudgal, V., N. Madaan, A. Mudgal and S. Mishra, 2010. Dietary polyphenol and human health. *Asian J. Biochem.*, 5: 154-162.
- Oboh, G., 2008. The neuroprotective potential of sour (*Hibiscus sabdariffa calyx*) and green (*Camellia sinensis*) teas on some pro-oxidant induced oxidation stress in brain. *Asian J. Clin. Nutr.*, 1: 40-49.
- Ochani, P.C. and P. D'Mello, 2009. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. Leaves and calyces extracts in rats. *Indian J. Exp. Biol.*, 47: 276-282.
- Ruberto, G., M.T. Baratta, S.G. Deans and H.J. Dorman, 2000. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essentials oils. *Planta Medica*, 66: 687-693.
- Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Viticult.*, 16: 144-158.
- Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol.*, 29: 152-178.
- Subhasree, B., R. Baskar, K.R. Laxmi, S.R. Lijina and P. Rajasekaran, 2009. Evaluation of antioxidant potential in selected green leafy vegetables. *Food Chem.*, 115: 1213-1220.
- Sultana, B., F. Anwar and M. Ashraff, 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14: 2167-2180.
- Tawaha, K., F.Q. Alali, M. Gharaibeh, M. Mohammad and T. El-Elimat, 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. *Food Chem.*, 104: 1372-1378.
- Veeru, P., M.P. Kishor and M. Meenakshi, 2009. Screening of medicinal plants for antioxidant activities. *J. Med. Plant Res.*, 3: 608-612.
- Wong, S.P., L.P. Leong and J.H.W. Koh, 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.*, 99: 775-783.
- Yao, L., Y. Jiang, N. Datta, R. Singanusong and X. Liu *et al.*, 2004. HPLC analyses of flavonols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. *Food Chem.*, 84: 253-263.
- Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.*, 49: 5165-5170.