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Full Length Research Paper

Antioxidant and antibacterial activities of *Hibiscus sabdariffa* L. extracts

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The present study investigated the total content of phenolic compounds, antioxidant activity, reducing power and chelating of ferrous ion in aqueous and alcoholic extracts of *Hibiscus sabdariffa* L. The total phenolic content was 77.2 mg/g and 87.7 mg/g for the aqueous and alcoholic extracts, respectively. The antioxidant activity was equal in rates for both the roselle alcoholic extract and artificial antioxidant BHT (75.67%), alcoholic extract showed the highest reducing ability with rate 222.60%, the chelating of ferrous ion in both aqueous and alcoholic extracts were 73.97 and 32.29% at 5 mg/ml concentration. The antibacterial activity of roselle extracts against *Escherichia coli, Staphylococcus aureus*, *Streptococcus mutans* and *Pseudomonas aeruginosa*, showed varying degrees of inhibition on the tested organisms.

Key words: *Hibiscus sabdariffa*, antioxidant, antimicrobial.

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 tea. Roselle flower and calyces is also known for its antiseptic, diuretic, antioxidant and antimutagenic properties (Salleh et al., 2002). The dried flowers of this plant contain gossipetine and hibiscin (anthocyanins); the petals yield glucoside hibiscritin (flavanol); and the calyces are rich in riboflavin, ascorbic acid, niacin, carotene, calcium and iron (Duke, 1983).

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 (Ali-Bradeldin et al., 2005). Roselle calyx extract is a good source of antioxidants from its anthocyanins (Ajiboye et al., 2011).

Anthocyanin is one type of flavonoid component that can be in Roselle calyces (Wang et al., 2002; Tsai et al., 2002). Tsai et al. (2002) suggest that anthocyanin is the major source of antioxidant capacity in roselle petale extract. Previous study reported that the aqueous extract of this plant could inhibit several nosocomial infectious bacteria such as methicillin-resistant *S. aureus* and *Klebsiella pneumoniae* (Liu et al., 2005). However, it is uncertain whether roselle calyx aqueous extract could inhibit the growth of *Salmonella typhimurium* DT104, *E. coli* O157:H7, *Listeria monocytogenes, Staphylococcus aureus* and *Bacillus cereus*. On the other hand, the protocatechuic acid, a polyphenol which is containing a 3,4-dihydroxy substructure, is a compound that naturally occurs in roselle calyx. Several *in vitro* studies have indicated that this compound can inhibit the growth of *E. coli* and fungi (Fernandez et al., 1996; Aziz et al., 1998).

Aqueous-methanolic extract of *H. sabdariffa* L. calyces have been found to exhibit antibacterial activities against *S. aureus, Bacillus stearothemophilus, Micrococcus luteus, Serratia mascences, Clostridium sporogenes, E. coli, K. pneumonae, B. cereus* and *Pseudomonas fluorescence* (Olaleye, 2007). Antibacterial effects of this plant extract against *Escherichia coli*, *P. aeruginosa* and *S. aureus* suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal

infection and diarrhea in man and skin diseases (Rogger et al., 1990).

The aim of this study was to evaluate the antioxidative and antimicrobial activity of aqueous and alcoholic extracts of calyx, and to evaluate the relationship between the antioxidative activity and total phenolic content of the roselle.

MATERIALS AND METHODS

Plant

The dried red roselle (Hibiscus sabdariffa L.) calyces were purchased from Basrah local market. 2 kg of calyx were grinded by electric grinder, and then samples were sieved and kept in polyethylene bags at 5°C in refrigerator.

Preparation of extracts

Alcoholic extraction

Prepared according to Harbone (1973), 5 g of powdered material along with 100 ml of alcohol was shaken well occasionally for the first 6 h and kept undisturbed for 18 h. The liquefied extract thus obtained was concentrated in a vacuum pump and the percentage was calculated with the weight of the roselle powder taken.

Aqueous extraction

For preparation of water extraction, 25 g sample of roselle was added to 250 ml distilled water and the mixture was boiled for 10 min while stirring with a magnetic stirrer. Then, the extract was filtered from filter paper (Gülçin et al., 2004).

Determination of total phenolic

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). Extract solution (0.1 ml) containing 1000 µg extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0 to 100 mg, 0.1 ml^{-1}) and standard curve was obtained.

Antioxidant activity assay

The antioxidant activity analysis using ferric thiocyanate was performed according to the method reported by Osawa and Namiki (1981). 0.6 g of Roselle sample was dissolved in 0.12 ml of 98% ethanol, and 2.88 ml of a 2.51% linoleic acid solution in ethanol and 9 ml of a 40 mM phosphate buffer (pH 7.0) were added. The mixture was incubated at 40°C in a stoppered test tube in the dark for 3 days (72 h). During the incubation, a 0.1 ml aliquot was taken from the mixture, and diluted with 9.7 ml of 75% ethanol, followed by the addition of 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after adding the 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid, the absorbance of the red color was measured at 500 nm. The level of lipid peroxidation inhibition by each fraction was calculated from the absorbance ratio to that of a blank without any sample.

Determination of reducing power

The reducing power of extracts from Roselle sample was determined according to the method of Oyaizu (1986). Extracts solution in methanol and water at different amounts (0.2 to 1 mg) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After 2.5 ml of TCA (10%) was added, the mixture was centrifuged at 3000 rpm for 10 min. Supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1%) and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

Chelating of ferrous Ion

The published method by Decker and Welch (1990) was used to investigate the ferrous ion chelating ability of different Roselle sample fractions. A 5 ml amount of each Roselle sample fraction was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine solutions. The absorbance at 562 nm was determined after reaction for 10 min. A complex of Fe_2%/ferrozine showed strong absorbance at 562 nm

Antibacterial activity assay

The antibacterial activity of the extracts was determined using the agar cup diffusion as described by Adeniyi et al. (1996). A 1 ml of an overnight culture of each bacterial isolate (equivalent to 107 to 108 cfu ml^{-1}) was used to seed sensitivity test agar plates maintained at 45°C. The seeded plates were allowed to set, and a sterile cork borer of 8 mm diameter was used to cut equidistant wells on the surface of the agar. The wells were filled with 0.1 ml solution of each extract reconstituted with methanol at a concentration of 10 mg ml^{-1}. Gentamycin at 5 µg ml^{-1} was included as positive control. The plates were incubated at 37°C for 24 h after which the diameter of zones of inhibition were measured.

Statistical analysis

All analysis were conducted in triplicate (n = 3), and an ANOVA test (using SPSS statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by using the Duncan test ($p < 0.05$).

RESULTS

Over the years, the study on medicinal plants to reveal the mechanism of action and to justify their claims by traditional healers has been increased. An angle of this research has been the study of bioactive components and antioxidant properties of the H. sabdariffa L. The total phenolic compounds found in alcoholic and aqueous extracts of H. sabdariffa L. calyx were shown in Figure 1. The figure illustrates that there is a plain rise in the amount of phenolic compound extracted by ethanol,

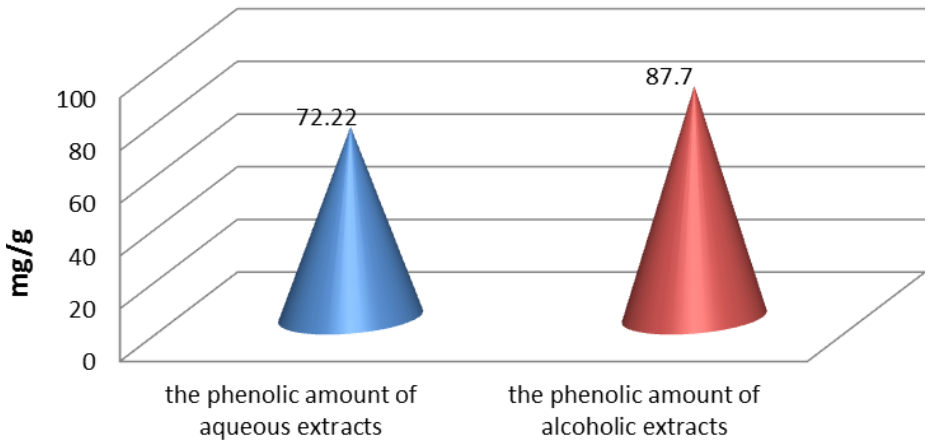

Figure 1. Total phenolic content of roselle extracts.

Table 1. Antioxidant activity of aqueous and alcoholic *H. sabdariffa* L. extracts.

Concentrate mg/ml	Antioxidant activity (%)			
	Aqueous extract	Alcoholic extract	BHT	α-Tocopherol
20	14.02±0.00	19.5±0.00	11.89±0.01	29.72±0.01
40	27.44±0.01	31.87±0.01	40.01±0.00	32.43±0.01
60	35.85±0.01	56.87±0.01	52.43±0.01	42.70±0.00
80	46.83±0.01	72.78±0.00	67.56±0.01	62.16±0.01
100	62.55±0.00	75.67±0.01	75.67±0.01	69.72±0.02

Table 2. Reducing power of aqueous and alcoholic *H. sabdariffa* L. extracts.

Concentrate mg/ml	Reducing power (%)					
	Aqueous extract	Alcoholic extract	BHT	α-Tocopherol	Ascorbic acid	Citric acid
20	49.65±0.01	71.30±0.00	55.22±0.00	27.67±0.00	59.32±0.02	23.02±0.00
40	65.73±0.001	110.34±0.01	81.25±0.02	36.36±0.00	148.37±0.00	67.76±0.01
60	118.18±0.00	187.82±0.01	85.84±0.01	44.6±0.01	173.75±0.00	140.78±0.00
80	141.95±0.00	195.65±0.00	144.64±0.00	83.03±0.00	180.8±0.00	172.36±0.01
100	160.83±0.03	222.60±0.00	156.25±0.00	92.11±0.00	218.22±0.01	188.81±0.00

which was (87.7 mg/g) than the amount of phenolic compound extracted by water which was (72.22 mg/g). The antioxidative activities of H. sabdariffa L. calyx extracted by ethanol and water comparing with artificial antioxidant BHT, α-tocopherol is presented in Table 1. The antioxidative activities of ethanolic extraction and BHT was equal in rates (75.67%) followed by α-tocopherol (69.72%) and roselle aqueous extraction (62.55%) at the concentrate 5 mg/ml.

The reducing power of ethanolic and water *H. sabdariffa* L. calyx extracts are summarized in Table 2. The reducing power increased significantly ($P < 0.05$) with increasing of extracts concentration. The data shows that the reducing ability of all samples increased when the concentration of extracts increased. Alcoholic extract showed that the highest reducing ability was 222.60% at the concentration 5 mg/ml, which approaches to the rate of reducing power of ascorbic acid (218.22%) followed by citric acid (188.81%), the reducing ability of aqueous extract was (160.83%) which was higher than α-tocopherol (92.11%) and BHT (156.25%) at the same concentration.

The ferrous ion chelating ability of *H. sabdariffa* L. calyx alcoholic and aqueous extracts is shown in Table 3; EDTA hardly carried the ferrous ion chelating ability due to its chemical structure properties which was (95.74%)

Table 3. Chelating ferrous ion of aqueous and alcoholic *H. sabdariffa* L.

Concentrate mg/ml	Chelating ferrous ion (%)			
	Aqueous extract	Alcoholic extract	EDTA	Citric acid
20	27.85±0.00	25.75±0.001	23.18±0.01	9,12±0.00
40	48.13±0.01	26.16±0.00	34.90±0.00	17,02±0.01
60	51.70±0.01	28.75±0.00	94.83±0.03	26.57±0.01
80	71.03±0.00	31.75±0.001	95.13±0.01	34.83±0.00
100	73.97±0.01	32.29±0.001	95.74±0.00	52.92±0.00

Table 4. Antibacterial activity of water and ethanolic extracts of *H. sabdariffa* exhibited.

Hibiscus sabdariffa	Zones of inhibition of organism (mm)			
extracts	*E. coli*	*S. aureus*	*Streptococcus mutans*	*P. aeruginosa*
Water	40	40	28	27
Ethanolic	46	20	30	17

at the concentration 5 mg/ml. The aqueous extract performed the best ferrous ion chelating ability (up to 73.97%) followed by citric acid (52.92%). However, a decrease in ferrous ion chelating ability was observed in the alcoholic extracts (32.29%). Antibacterial activity test showed that aqueous and alcoholic extract of *H. sabdariffa* L. had growth inhibitory effect on several tested microorganism. Inhibition zone was wide against *E. coli, S. aureus*, *Str. mutans, and P. aeruginosa* for both extracts as shown in Table 4. The aqueous extract of *H. sabdariffa* showed equal inhibition ability against *E. coli* and *S. aureus (*40 mm) which was highest than *Str. mutans* (28 mm) and *P. aeruginosa* (27 mm). Alcoholic extract exhibited higher inhibition ability against *E. coli* (47 mm) than against *S. aureus* (20 mm), *Str. mutans* (30 mm) and *P. aeruginosa* (17 mm).

DISCUSSION

The chemical components contained in the flowers of *H. sabdariffa* include anthocyanins, flavonoids and polyphenols (Tzu-Lilin et al., 2007). The petals are potentially a good source of antioxidant agents as anthocyanins and ascorbic acid (Prenesti et al., 2007). The results of the current study clearly demonstrated that alcoholic extracts of *H. sabdariffa* L. contain highest phenolic compounds than aqueous extract as shown in Figure 1. This result indicates that alcohol is a better solvent than water for extraction of phenol from *H. sabdariffa* L. calyx and this result agrees with Anokwuru et al. (2011). The solubility of phenolic compounds is governed by the chemical nature of the plant, as well as the polarity of the used solvents. Ethanol is a good solvent for polyphenol extraction and is safe for human consumption (Shi et al., 2005). The current study agrees with results found by Christian and Jackson (2009) and Anokwuru et al. (2011) which were 5.25 and 27.6 mg/g of ethanol extracts of *H. sabdariffa* L. calyx, respectively. However, a research conducted by Koffi et al. (2010) indicated that ethanol was the best solvent for the extraction of phenol in the Ivorian plant.

Antioxidants are significant in the prevention of human illness and may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quencher of singlet oxygen formation (Andlauer and Furst, 1998). The statistical analysis results presented in Table 1 shows significant differences ($P < 0.05$) between roselle extracts and the artificial antioxidant, as well as the rate of antioxidant activity increases as the concentrate of roselle extract increased. Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Lu and Foo, 1995).

Antioxidant activity of the Roselle extract correlated strongly to its anthocyanin content (Tsai et al., 2002). According to Duh and Yen (1997), the roselle extract is an electron donor and can react with free radicals to convert them into more stable products and terminate radical chain reactions. Falade et al. (2005) indicated 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 (Buettner and Jurkiewicz, 1996). The reducing power has been used as one of the antioxidant capability indicators of medicinal herbs (Duh and Yen, 1997). Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe(III) to Fe(II) in the presence of the sample (Gülçin et al., 2003).

The data presented in Table 2 shows that the reducing ability of all samples increased when the concentration of

extracts was increased, and this may be because of the increment of the phenolic amount at the high concentration. This result is similar to that reported by Gülçin et al. (2003). Among all the reducing power tests, alcoholic extract of *H. sabdariffa* L. had the highest reducing ability which may be because of the use of an alcoholic solution which provides satisfactory result for the extraction process (Koffi et al., 2010). The results are in agreement with those reported by Shil et al. (2005), that the ethanol is a good solvent for polyphenol extraction and is safe for human.

Ferrous ion, commonly found in food systems, is well known as an effective pro-oxidant (Hsu et al., 2003). Polyphenols can chelate pro-oxidant metal ions, such as iron and copper, thus preventing free radical formation from these pro-oxidants (Kris-Etherton et al., 2002). The ferrous ion chelating effect of *H. sabdariffa* L. extract is presented in Table 3. Aqueous extract had high ferrous ion chelating capability comparing with EDTA. An effective ferrous ion chelator affords protection against oxidative damage by removing iron that may otherwise participate in $HO^{\cdot}$ generation through the fenton type reactions. Ferric ions (Fe^{3+}) also produce radicals from peroxides, although the rate is ten-fold less than that of ferrous ion. Ferrous ions (Fe^{2+}) are the most powerful pro-oxidant among the various species of metal ions (Gülçin et al., 2006).

In the present study, a variety of gram positive (*S. aureus and S. mutans)* and gram negative (*E. coli* and *P. aeruginosa)* bacteria were used in screening antimicrobial activity of aqueous and alcoholic extracts of *Hibiscus sabdariffa* L.

The results of the current study clearly indicated that aqueous and alcoholic extracts of *Hibiscus sabdariffa* L. inhibit the growth of tested microorganism, however, the effectiveness varied against the different tested microorganisms. Study results are in agreement with Olaleye (2007) who reported that aqueous-methanolic extract of *H. sabdariffa* calyces have been found to exhibit antibacterial activities against *S. aureus*, *B. stearothemophilus*, *Micrococcus luteus*, *Serratia mascences*, *Clostridium sporogenes*, *E. coli*, *Klebsiella pneumonae*, *B. cereus and P. fluorescence*.

The antibacterial activity of the cycle extracts of *H. sabdariffa* can be attributed to the action of the phytochemical compounds it contains (Babayi et al., 2004). These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Marjorie, 1999). These results agree with Garcia**-**Alonso et al. (2006) who found that plant polyphenols have been demonstrated as potential antibacterial. Polyphenolic compounds and/or volatile oils are known to inhibit a wide range of organisms (Cheesbrough, 1984). Antibacterial activity of gossypetin isolated from *H. sabdariffa* was investigated and the activity may be due to polyphenolic nature of the flavonoid gossyypetin (Mounnissamy et al., 2002).

Conclusion

The present study indicated that both aqueous and ethanol extracts from the calyx *H. sabdariffa* L. have significant natural phenols content and antioxidant activity. As compared to the artificial antioxidant BHT and α-tocophero, the aqueous and alcoholic extracts possess strong reducing power and high ferrous ion chelating ability. Roselle calyx aqueous and alcoholic extracts of *H. sabdariffa* L. effectively and dose-dependently inhibited the growth of *E. coli*, *S. aureus*, *Str. Mutans* and *and P. aeruginosa*.

REFERENCES

Adeniyi B, Odelola A, Oso A (1996). Antimicrobial potentials of Diospyros mespiliformis (Ebenaceae). Afr. J. Med. Sci. 25:179-184.

Ajiboye TO, Salawu NA, Yakubu MY, Oladiji AT, Akanji MA, Okogun JI (2011). Antioxidant and drug detoxification potentials of *Hibiscus sabdariffa* anthocyanin extract. Drug Chem. Toxicol. 34:109-115.

Ali-Bradeldin H, Al-Wabel N, Gerald B (2005). Phytochemical pharmacological and toxicological aspects of *Hibiscus sabdariffa*: A review. Phytother. Res. 19:369-375.

Andlauer W, Furst P (1998). Antioxidative power of phytochemicals with special reference to cereals. CFW 43:356-359.

Anokwuru P, Esiaba I, Ajibaye O, Adesuyi O (2011). Polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx. Res. J. Med. Plant. 5:557-566.

Aziz NH, Farag SE, Mousa LA (1998). Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios 93:43-54.

Babayi H, Kolo I, Okogun I, Ijah J (2004). The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. Biokemistri 6:106-111.

Buettner GR, 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.

Cheesbrough M (1984). Tropical Health Technology. Cambridge University Press, U.K. p. 479.

Christian R, Jackson C (2009). Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varities of sorrel (*Hibiscus sabdariffa*) during maturity. J. Food compos. anal. 22:663-667.

Duke A (1983). *Hibiscus sabdariffa* L. Handbook of Energy Corps. Available at.http://www.hort.purdue.edu/newcrop/duke_energy/Hibiscus_sabdariffa.html [Last accessed 15 December 2010].

Duh PD, Yen GC (1997). Antioxidative activity of three herbal water extracts. J. Food Chem. 60:639-645.

Falade OS, Otemuyiwa IO, Oladipo A, Oyedapo OO, Akinpelu BA, Adewusi SRA (2005). The chemical composition and membrane stability activity of some herbs used in local therapy for anemia. J. Ethnopharmacol. 102:15-22.

Fernandez MA, Garcia MD, Saenz MT (1996). Antibacterial activity of the phenolic acids fractions of *Scrophularia frutescens* and *Scrophularia sambucifolia*. J. Ethnopharmacol. 26:11-14.

Garcia-Alonso J, Ros G, Vidal-Guevara L, Periago J (2006). Acute intake of phenolic-rich juice improves antioxidant status in healthy

subjects. Nutr. Res. 26:330-339.
Gulcin I, Oktay M, Kırecci E, Kufrevioglu OL (2003). Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. fch. 83:371-382.
Gülçin V, Mshvildadze A, Gepdiremen, Elias R (2006). Antioxidant activity of a triterpenoid glycoside isolated from the berries of *Hedera colchica*: 3-O-(_-D-glucopyranosyl)-hederagenin, Phytother. Res. 20:130-134.
Harbone B (1973). Phytochemical methods, Chapman and Hall Limited, London. pp. 49-189.
Hsu C, Chen W, Weng Y, Tseng C (2003). Chemical composition, physical properties, and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 83:85-92.
Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med. 113(Suppl.9B):71S–88S.
Lu F, Foo LY (1995). Toxicological aspects of food antioxidants. In: Madhavi DL, Deshpande SS, Salunkhe DK, editors. Food antioxidants. New York: Marcel Dekker.
Mounnissamy VM, Kavimaini S, Gunasegaran R (2002). Antibacterial activity of gossypetin isolated from *Hibiscus sabdariffa*, Antiseptic 99(3):81-82.
Koffi E, Sea T, Dodehe Y, Soro S (2010). Effect of solvent type on extraction of polyphenols from twenty three Ivorian plants. J. Anim Plant Sci. 5(3):550-558.
Liu KS, Tsao SM, Yin MC (2005). *In vitro* antibacterial activity of roselle calyx and protocatechuic acid. Phytother Res. 19:942-945.
Marjorie C (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-582. PMID: 10515903
Olaleye T (2007). Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. J. Med. Plants Res. 1(1):9-13.
Osawa T, Namiki M (1981). A novel type of antioxidant isolated from leaf wax of eucalyptus leaves. Agric. Biol. Chem. 45:735-739.
Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44:307-315.
Prenesti E, Berto S, Daniele P and Toso S. (2007). Antioxidant power quantification of decoction and cold infusions of *Hibiscus sabdariffa* flowers. Food Chem. 100:433-438.
Rogger YS, John LI, Mark LW (1990). General Microbiology. 5th ed. Macmillan education Ltd London. pp. 626-642.
Salleh N, Runnie I, Roach D, Mohamed S, Abeywardena Y (2002). Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts. JAFC 50:3693-3697.
Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y (2005). Extraction of polyphenolics from plant material for functional foods-engineering and technology. Food Rev. Int. 21:139-166.
Shil J, Nawaz H,Pohorly J, Mittal G, Kakuda Y, Jiang Y (2005). Extraction of polyphenolics from plant material for functional foods-engineering and technology. Food Rev. Int. 21:139-166.
Slinkard K, Singleton VL (1977). Total phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticult. 28:49-55. SPSS (2009). SPSS Statistical package for window ver .17. O. Chicago :SPSS.Inc .
Tsai J, McIntosh J, Pearce P, Camden B, Jordan B (2002). Anthocyanin and antioxidant capacity in roselle (*Hibiscus Sabdariffa* L.) extract. JCIFST, 35:351-356.
Tzu-Lilin, Lin HH, Chen CC, Lin MC, Chou MC, Wang CJ (2007). Hibiscus Sabdariffa extract reduces serum cholestero in men and women. Nutr. Res. 27:140-145.