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Phytochemical and Antioxidant Activity of Roselle (*Hibiscus Sabdariffa* L.) Petal Extracts.

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ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is known for delicacy and also for medicinal properties. The present work aim to study the phytochemical screening of *Hibiscus sabdariffa* for various medicinally important compounds and their quantification. The results showed that alkaloids, anthocyanins, flavonoids, saponins, steroids, sterols and tannins are present in petals of the *H. sabdariffa*. Anthocyanin content was highest while the contents of phenols and flavonoids were lowest. HPLC analysis revealed two phenolic acids, 16 flavonoids and four anthocyanins in petal of *H. sabdariffa*. The major compounds were gossypetin, hibiscetin, quercetin and sabdaretin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside were the major anthocyanins. The antioxidant activity of the investigated extract has a scavenging ability of DPPH radical scavenging activity (around 97 %). The IC_{50} values of *H. sabdariffa* extract was 0.24 mg/ml while that of ascorbic acid used as the reference control was 0.35 mg/ml. This indicated that the compounds constituting mainly petals of *H. sabdariffa*, such as anthocyanins, flavonoids and phenolic acid contribute to the antioxidative activity. Our findings provide evidence that the petal extract of *H. sabdariffa* is a potential source of natural antioxidants, and this justify its uses in folkloric medicines.
Keywords: *Hibiscus sabdariffa*, phytochemicals, anthocyanins, flavonoids, phenols, antioxidant activity.

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INTRODUCTION

Progress in medicinal plant research has undergone a phenomenal growth during last decade. Worldwide trend towards the use of natural plant remedies has created an enormous need for information about the properties and uses of medicinal plant as antitumor, antianalgesic, insecticides. Besides medicines, plants provides thousand of novel compounds, such as fragrance, flavorings, dyes, fibers, foods, beverages, etc. Roselle (*Hibiscus sabdariffa* L.) is known for delicacy and also for medicinal properties. It is a plant which is widely grown in Central and West Africa and South East Asia [1]. This plant is used by people in Africa and particularly in Côte d'Ivoire via direct or indirect pathways in the treatment of several diseases. The approach of *H. sabdariffa* is equally significant in alternative system of medicine as well as in conventional system of medicine. *H. sabdariffa* is an aromatic, astringent, cooling herb that is currently used Tropical areas. It is known to have diuretic effects, to help lower fevers and is an antiscorbutic. The leaves are antiscorbutic, emollient, diuretic, refrigerant, and sedative. The plant is also reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, purgative and resolvent. It is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, and neurosis [2-4].

In Côte d'Ivoire, it is a highly source of vegetable food. Indeed, 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. The calyx is rich in citric acid and pectin and so is useful for making jams, jellies [5].

Phytochemicals are a group of non-nutrient bioactive compounds naturally found in plant parts such as flowers, leaves, fruits, roots, barks, spices and medicinal plants. In humans, numerous phytochemicals have been found to be protective and preventive against many degenerative diseases and pathological processes such as in ageing [6], coronary heart disease, Alzheimer's disease [7], neurodegenerative disorders, atherosclerosis cataracts, and inflammation [8]. Both epidemiological and clinical studies provided evidence that most of these phytochemicals exhibit their protective and disease-preventing functions through their antioxidant activities [9]. Typical phytochemicals compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, flavonoids, phytic acid and many sterols [10]. As antioxidants, these species are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases [11].

Hibiscus sabdariffa L., commonly known as Bissap (Senegal), Roselle (English), Oseille de Guinée (French) and Karkadeh (Arabic) is an erect annual herb cultivated for its seeds, petals and leaf [12]. It is used in preparation of local non-alcoholic cold beverage and as a hot drink. In Côte d'Ivoire, the production of a non-alcoholic drink called Bissap that is prepared from the red petals is popular. There are various polyherbal formulations present in the market which contains hibiscus as major constituents [13]. Roots orally used as a stomachic and externally as an emollient and leaves are eaten as vegetable after cooking. They are mainly used as diuretic, digestive, antiseptic, sedative, purgative, demulcent,

astringent and tonic [14]. Its decoction is useful for high blood pressure and cough. The infusion of the red petals is used as a refrigerant drink in fevers [15] and the alcoholic extract of petals inhibited Angiotensin-I converting enzyme [16]. The petals have also many medicinal applications to cure liver damage, hypertension and leukemia [17, 18]. Many authors have also reported the antioxidant activity [9, 19], anti-inflammatory activity [20], cardioprotective activity [16], hepatoprotective activity [21] and antibacterial activity [22] of Roselle petals.

The regular and intensive use of the juice obtained from the petals of Roselle as beverage in various ceremonies in West Africa in general and particularly in Côte d'Ivoire led us to initiate this study. Hence, the main aim of this investigation was to study the preliminary phytochemicals screening, qualitative and quantitative analysis of some secondary metabolites, and to evaluate the *in vitro* antioxidant activity of the red petals extract of Roselle. This will generate more knowledgeable informations on their potentiality for a wider utilization.

MATERIALS AND METHODS

Chemicals

All chemicals used were at least analytical grade. 1,1-diphenyl-1-picrylhydrazyl (DPPH), methanol, trifluoroacetic acid, phenolic acids standards (gallic, gentisic, cafeic, chlorogenic, ellagic, ferulic, *p*-coumaric, salicylic, sinapic and veratric acids), flavonoids standards (catechin, epicatechin, genistein, gossypin, naringenin, quercetin, isoquercetin, quercitrin, rutin and vanillin) and anthocyanins standards (cyanidin, delphinidin, malvidin, peonidin, petunidin, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, malvidin3-O-glucoside, peonidin 3-O-glucoside and petunidin3-O-glucoside) were purchased from Sigma-Aldrich (Steinheim, Germany). Ascorbic acid, Folin-Ciocalteu reagent, Neu reagent and sodium carbonate were obtained from Merck (Darmstadt, Germany).

Plant material

The dried petals of Roselle were used as source of plant materiel investigated in the present study. This materiel was purchased from a local market in Adjamé (Abidjan, Côte d'Ivoire). The petals were cut, cleaned, washed thoroughly under running tap water, drained and oven-dried at 55 °C for 12 h. They were packed in polythene bags and stored in air-tight containers for laboratory analysis. The dried Roselle petals were immediately packed in polythene bags and kept at low temperature (4 °C) till used.

Preliminary phytochemicals analysis

The Preliminary phytochemicals screening of the plant was performed following the standard procedures adapted by various workers [23, 24].

Preparation of the extract

100 g of Roselle petals previously freeze dried were extracted in 200 ml of methanol acidified with trifluoroacetic acid 0.1 % (v/v) for 24 h at 4 °C. The macerate was filtered successively on cotton wool and Whatman paper. After vacuum evaporation of the methanol in BÜCHI Rotavapor R-114 at 38 °C, we obtained a dry extract. Two hundred milliliters (200 ml) of distilled water were added to the dry extract and the aqueous extract was submitted to a filtration on gel XAD7, in order to eliminate sugars and chlorophyll pigments. The water obtained after filtration was discarded. 100 mL of methanol 100 % were poured over the gel X-AD7 and the methanolic filtrate obtained was evaporated to dryness with Rotavapor R-114 at 38 °C and dissolved again in a 100 mL of water. This filtrate was lyophilized with the freeze dryer CHRIST ALPHA 1-2. The dried extract obtained represents the petals crude extract of Roselle which was used to achieve the different analyses.

Qualitative and quantitative phytochemical screening

The presence of some phytoconstituents was highlighted by standards phytochemical methods. Phytochemical analysis of alkaloids, anthocyanins, flavonoids, polyphenols, quinones, saponins, tannins, terpenes and sterols were performed according to the methods described by [23, 24]. Quantitative and qualitative chemical analysis of phenols, flavonoids and anthocyanins compounds were done by employing spectrophotometric and high performance liquid chromatographic techniques.

Determination of total phenolic content

Total phenolic content (TPC) of freeze-dried extract was determined using Folin-Ciocalteu essay [25]. 0.2 mL of sample extract (1 mg of freeze-dried extract was dissolved in 1 mL of methanol) was mixed with 0.8 mL of distilled water, 0.5 ml of Folin-Ciocalteu's reagent (1:9 with water) and 1.5 ml of sodium carbonate (17 %, w/v). The tubes were incubated for 30 min in the dark at room temperature before absorbance was measured at 765 nm using a Jenway 6705 UV/Vis spectrophotometer against the blank sample contained the same mixture solution without the sample extract. A standard calibration plot was generated at 404 nm using known concentrations of gallic acid (20-120 µg/mL).TPC was calculated from the calibration splot and expressed as mg gallic acid equivalents (mg GAE) of phenol/g of freeze-dried extract (g FDE). The calibration equation for gallic acid was $y = 0.004x + 0.124$, $R^2 = 0.998$, where y is absorbance and x is concentration of gallic acid in µg/mL. All measures were performed in triplicate.

Determination total flavonoids

Total flavonoids content (TFC) of freeze-dried extract was determined using the method described by [26]. 50 mg of freeze-dried extract was mixed in 5 mL of methanol 70 % (v/v). After 24 h, 0.5 mL of filtrate were mixed with 50 µL of Neu reagent. The absorption was determined at 404 nm using a Jenway 6705 UV/Vis spectrophotometer against the blank sample containing the same mixture solution without the sample extract and compared to the one of standard quercetin (0.05 mg/ml) treated with the Neu reagent. A

standard calibration plot was generated at 404 nm using known concentrations of quercetin (10-100 µg/mL). TFC was calculated from the calibration splot and expressed as mg quercetin equivalents (mg QE)/g of freeze-dried extract (g FDE). The calibration equation for quercetin was y = 0.0156x + 0.07, R^2 = 0.987, where y is absorbance and x is concentration of quercetin in µg/ml. All measures were performed in triplicate.

Determination of total anthocyanin

Total anthocyanin content (TAC) of freeze-dried extract was determined using the method described by [27]. 10 mg of freeze-dried extract was mixed in 5 mL of methanol acidified with trifluoroacetic acid 0.1 % (v/v). Aliquots of the extracts were taken in a10 mL glass tube and adjust to a volume of 3 mL with methanol acidified with trifluoroacetic acid (TFA) and the absorbance was measured at 530 nm using a Jenway 6705 UV/Vis spectrophotometer against the blank sample containing the mixture methanol/TFA 0.1 % without the sample extract, TAC was estimated as cyanidin 3-O-glucoside at 530 nm using a molar extinction coefficient of 26,900 L/mol/cm) and molar mass (449 g/mol) [28] and was expressed as mg cyanidin-3-glucoside (mg Cya3G)/g of freeze-dried extract (g FDE).

High performance liquid chromatography (HPLC) analysis

HPLC analysis was conducted using the method described by [29].The analyses were carried out on a HPLC (Agilent), model-LC 1100 series, equipped with a degasser, an autosampler automatic injector, a high pressure pump and a UV/Visible detector at multiple wavelengths wave, and running on Windows XP Workstation. HPLC experiments were conducted using a Prontosil C-18 column (5 µm particle size, 250 x 4 mm I.D.) with a flow rate of 1 mL/min at room temperature. The mobile phase used was a binary gradient eluent (solvent A, 0.1 % trifluoroacetic acidin water; solvent B, 0.1 % trifluoroacetic acid in acetonitrile). Acetonitrile used was of HPLC grade (Sigma/Aldrich) and was degassed in an ultrasonic bath before using. The water was distilled using a Milli-Q system (Millipore). 50 mg of freeze-dried extract were dissolved overnight with 5 ml of methanol at 4 °C in a blender. Sample was centrifuged at 3000 rpm for 10 min. Supernatant was collected and filtered through a Millipore membrane (0.45 µm). The filtrate was twice diluted with purified distilled water. 100 µL of filtrate were injected by an Agilent 1100 series autosampler and chromatograms were simultaneous monitored at 280 nm (phenol compounds i.e. phenol acids and flavonoids) and 521 nm (anthocyanins). The elution program was 5-15 % B (0-5 min), 15-25 % B (5-15 min), 25-100 % B (15-30 min) and 100 % B (30-40 min). NMR spectra were recorded on a LC-NMR Agilent 1200 series HPLC/Bruker Avance III spectrometer operating at 600 MHz) for proton.A reference library of compounds was performed previously with purified compounds and identified by NMR in laboratory and also with commercially available compounds such as phenol acids (gallic, gentisic, cafeic, chlorogenic, ellagic, ferulic, *p*-coumaric, salicylic, sinapic and veratric acids), flavonoids (catechin, epicatechin, genistein, gossypin, naringenin, quercetin, isoquercetin, quercitrin, rutin and vanillin), anthocyanins (cyanidin, delphinidin, malvidin, peonidin, petunidin, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, malvidin3-O-glucoside, peonidin 3-O-glucoside and petunidin 3-O-glucoside). This database contains the retention time of these compounds which can be compared with those obtained from unknown samples and proceeds to the identification of the component molecules.

Total antioxidant activity

Antioxidant activity of extracts measured included radical-scavenging activity**.** The free radical scavenging capacity of the extracts was determined using the 1,1-diphenyl-1-picrylhydrazyl (DPPH) assay [30].The freeze-dried extract was mixed with methanol to prepare the stock solution (1 mg/mL). Freshly prepared DPPH solution (5.9 mg/100 mL methanol) was taken in test tubes and extracts were added followed by serial dilutions to every test tube such that the final volume was 2 mL, and discoloration was measured at 517 nm after incubation for 30 min in the dark at ambient temperature. Measurements were performed at least in triplicate. Ascorbic acid, a well-known antioxidant, was used as a reference standard and dissolved in methanol to bring the stock solution to the same concentration (1 mg/mL). The control sample was prepared, which contained the same volume without any extract and methanol was used as the blank. The inhibition ratio (percent) was calculated according to the following equation:

%inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] X 100

The actual decrease in absorption induced by the tested compounds was compared with the positive control. The IC_{50} value was calculated using the dose inhibition curve.

Statistical Analysis

Data were processed using Statistica SPSS package version 7.5. Analysis of variance (ANOVA) was performed and means were separated by Newman-Keuls range test at $P \leq 0.05$. Data are the means of three replicates. The Kruskal-Wallis test is performed to determine significant differences ($P<0.05$) between the inhibition percentages of antioxidants.

RESULTS AND DISCUSSION

Phytochemical screening

The screening of plants for medicinal value has been carried out by numerous researchers with the help of preliminary phytochemical analysis [31, 32]. Phytochemical screening test is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated by several researchers [30, 33, 34].The selection of plant parts such as petals which yields maximum secondary metabolites is the prime or prerequisite step in this investigation. For this, different phytochemicals from petals were extracted and highlighted by different methods; their presence (+) or absence (-) is shown in table 1. The results indicated that Roselle petals contained alkaloids, anthocyanins, flavonoids, saponins, steroid, sterols and tannins which are the main phytochemical groups with biological activities. The composition of the aqueous extract of Roselle petals was similar to referenced data, with some differences that may be due to genetic variability, type of soil and extractive solvent [14, 32, 33]. Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents [35]. Anthocyanins were

present in petals of Roselle. These compounds have the healing properties. The anthocyanins have been found to be cardioprotective, hypocholesterolemic; antioxidative and hepatoprotective [16, 21]. They also have an antioxidant activity [25, 36] and inhibit low density lipoprotein (LDL) oxidation [37]. Alkaloids also interfere with cell division; hence the presence of alkaloids in the plant makes it a possible remedy in the treatment of cancer. Flavonoids are well known for their anti-viral, anti-inflammatory, antioxidant activity, cytotoxic and also used in the treatment of hypertension, diabetes, rheumatic fever [9, 14, 18, 22]. Roselle shows the presence of flavonoids in the petals of flowers, it can be of use to cure above mentioned disorders and as antioxidant agent. In the present study, polyphenols were detected. Polyphenols have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of polyphenols compounds, especially their anti-inflammatory activity, antiviral and cytotoxic activity [19, 36]. It is a well documented fact that most medicinal plants are enriched with polyphenol compounds that have excellent antioxidant properties [2, 36]. Polyphenols are active in curing kidney and stomach problems and have been found to be helpful in protection and prevention against many degenerative diseases and pathological processes such as in ageing degenerative diseases, coronary heart disease, Alzheimer's disease, neurodegenerative disorders and atherosclerosis cataracts [6, 38]. The result of the phytochemical screening revealed that quinones, steroids and terpenoids were absent in petals of Roselle. Saponins were found to be present in petals of Roselle. Saponins having hypertensive and cardiac depressant properties [7]. Dietary source of saponins offer preferential chemical preventive strategy in lowering the risk of human cancer [39]. Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism. Tannins play important role such as potent antioxidant [4]. Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [40]. It is clear that petal of Roselle possess good phytoconstituents that will be helpful in future for the cure of different types of diseases.

Table 1: Phytochemical constituents of petals extract in *Hibiscus sabdariffa*

N°	Test	Aqueous extract
1	Alkaloids	+
2	Anthocyanins	+
3	Flavonoids	+
4	Phenols	+
5	Quinones	-
6	Saponins	+
7	Steroids and sterols	-
8	Tannins	+
9	Terpenoids	-

(+) Present ; (-) Absent

Table 2 shows the content of anthocyanins, flavonoids and phenols petal extracts of *Hibiscus sabdariffa*. We noted that anthocyanins are the majority compound with 16.53 mg/g and represents 60.26 % of the three compounds, followed by the phenols with 7.40 mg/g (26.98 %) and finally flavonoids with 3.50 mg/g (12.76 %). These results are closed to

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those of [3], because they have a similarity in the relative amounts of chemical constituents (anthocyanins, phenols, flavonoids) in the different extracts. The differences are probably due to the discrepancy between geographical areas and to climatic conditions. Anthocyanins are known to inhibit low density lipoprotein (LDL) oxidation, serving as a chemo-preventive agent and possess an antioxidant activity [25, 36, 37]. Roselle which has higher content of anthocyanin is also sourced as a good food colorant in wine and related product. Roselle's petals have low content in phenols and flavonoids. Nevertheless, they are beneficial to the health of consumers. Indeed, they are a potential source of natural antioxidant [2, 36].

Table 2. Quantitative data of various phytochemicals in the petals extract of *Hibiscus sabdariffa*

Compounds	Contents (mg/g)	Ratio
Anthocyanins	16.53 ± 1.10 [a]	0.6026 [d]
Flavonoids	03.50 ± 0.85 [b]	0.1272 [e]
Polyphenols	07.40 ± 0.50 [c]	0.2698 [f]

Values are means of triplicate determination (n = 3); ± standard deviation (SD);
Means followed by the same letter were not significantly different at 5 % (test of Newman-Keuls);
Ratio: content of a compound relative to the total content of compound.

The HPLC phenolic profile was monitored at 280 nm and is illustrated in Figure 1.

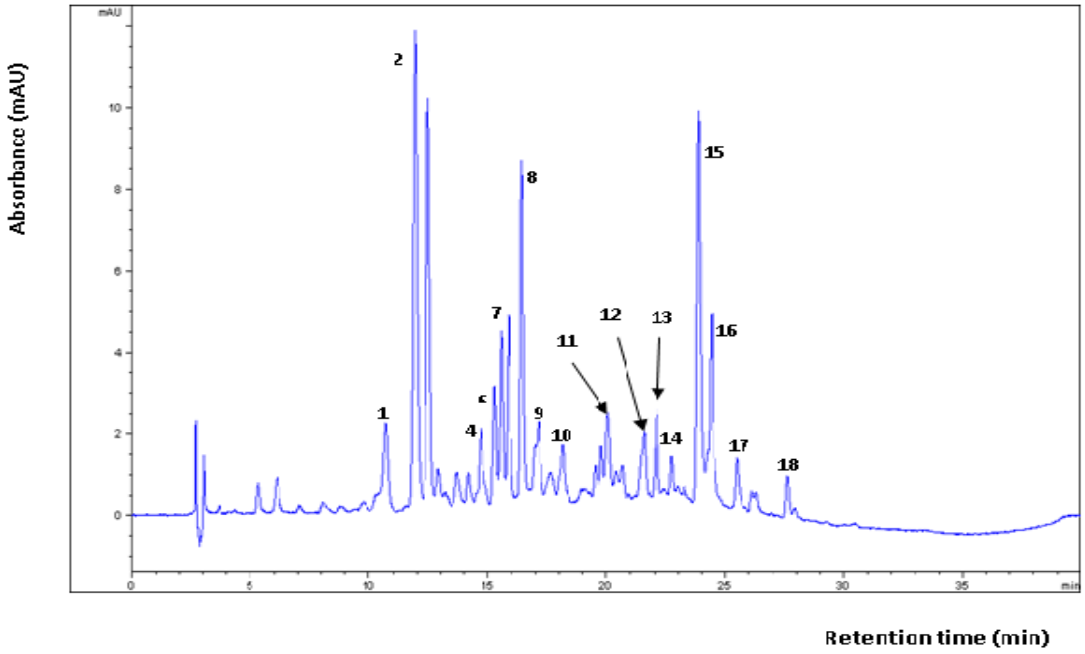

Figure 1. HPLC profile of phenolic compounds from petals extract of *Hibiscus sabdariffa*

Detection is shown at 280 nm. Peaks were identified by comparison with reference standards when available or by HNMR data (retention time). 1. gossypetrin (10.671 min); 2. sabdaretin (11.919 min); 3. gossypetin (12.466 min); 4. chlorogenic acid (14.690 min); 5. luteolin (15.270 min); 6. protocatechuic acid (15.548 min); 7. gossytrin (15.863 min); 8. hibiscetin (16.418 min); 9. rutin (17.120 min); 10. hibiscetrin (18.129 min); 11. myricetin (20.045 min); 12. eugenol (21.570 min); 13. nicotiflorine (22.082 min) 14. quercitrin (22.795 min); 15. quercetin (23.866 min) ; 16. kaempferol (24.399 min); 17. astragalin (25.465 min) ; 18. cyranoside (25.596 min).

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We identified 18 compounds in *Hibiscus sabdariffa* petals: phenolic acids i.e. chlorogenic acid (4) and protocatechuic acid (6); flavonoids i.e. gossypetrin (1); sabdaretin (2); gossypetin (3); luteolin (5); gossytrin (7); hibiscetin (8); rutin (9); hibiscetrin (10); myricetin (11); eugenol (12); nicotiflorine (13); quercitrin (14); quercetin (15) ; kaempferol (16); astragalin (17) ; cyranoside (18). The phenolic acids represent 11 % of total phenolic compounds while flavonoids constitute 89 %. This suggests that flavonoids are predominating. The major phenolic compounds in petals of Roselle are sabdaretin (2), gossypetin (3), hibiscetin (8) and quercetin (15). The presence of sabdaretin, gossypetin, hibiscetin, eugenol, quercetin and protocatechic acid confirms the earlier reports [10, 41, 42]. In addition, they reported the presence of astragalin (kaempferol 3-O-glucoside), nicotiflorine (kaempferol 3-O-rutinoside), luteolin, gossytrin and chlorogenic acid. All phenolic compounds isolated in petals of Rosellehave pharmacological properties reported by several authors [6, 21, 22, 18, 19, 37]. This clearly shows the use of this plant as herbal medicine.

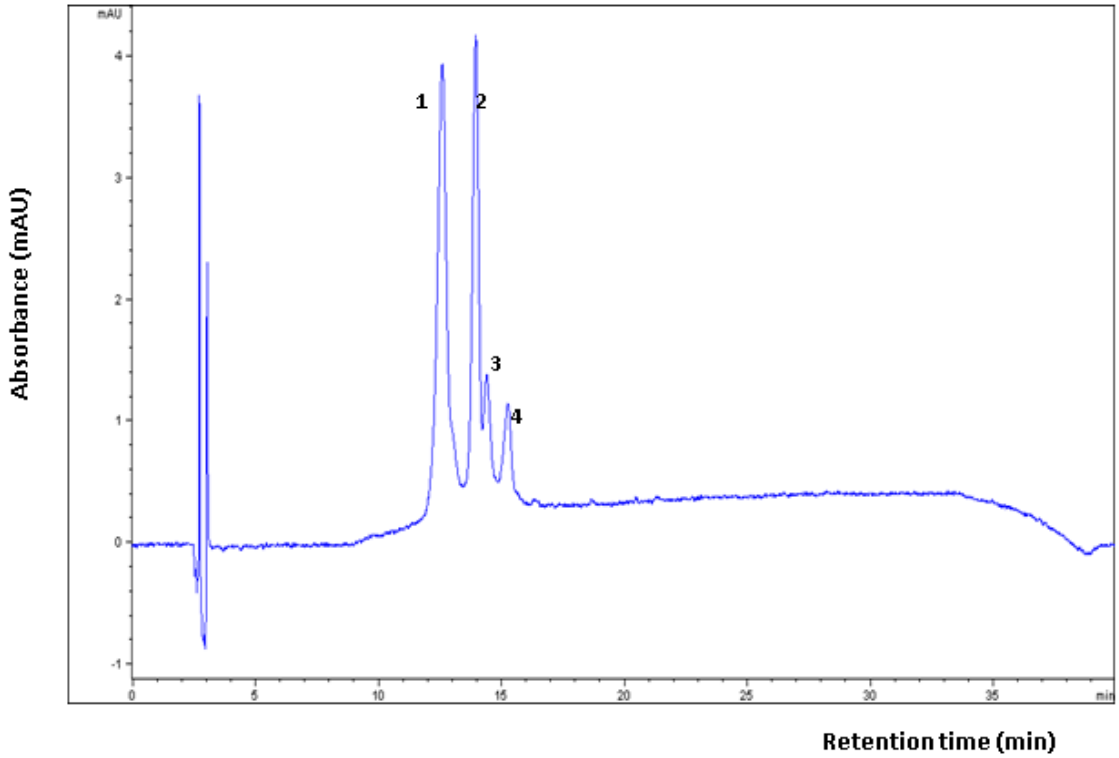

Figure 2. HPLC profile of anthocyanins from petals extract of *Hibiscus sabdariffa*

Detection is shown at 521 nm. Peaks were identified by comparison with reference standards when available or by HNMR data (retention time). 1. delphinidin 3-O-sambubioside (12.681 min); 2. cyanidin 3-O-sambubioside (13.389 min); 3. cyanidin 3-O-glucoside (14.389 min); 4. delphinidin 3-O-glucoside (15.238 min).

Four different *Hibiscus sabdariffa* anthocyanins were separated by reverse phase HPLC (Figure 2). Peak assignments are based on matching UV-vis and identical HPLC retention time with known anthocyanins from a reference library of compounds previously purified and identified by Anthocyanins identified in petals of Roselle have pharmacological properties reported by several authors [6, 22, 18, 19, 37]. Chromatograms showed that delphinidin 3-O-sambubioside (peak 1) and cyanidin 3-O-sambubioside (peak 2) are the major anthocyanins. Indeed, the absorbance of these two anthocyanins is four-fold lower than that of cyanidin 3-O-glucoside (peak 3) and delphinidin 3-O-glucoside (peak 4). The

presence these anthocyanins in petals of Roselle were mentioned by many authors [41, 43, 44]. However, the major anthocyanins vary depending on the varieties and also the culture country. This clearly shows the influence of soil and climatic conditions on the anthocyanins biosynthesis. Delphinidin and its glycoside derivatives have significant anti-oxidant activity [19, 36]. Several epidemiological studies have shown a protective effect against coronary heart disease and the consumption of anthocyanins [16, 21, 45]. Regarding cyanidin and its glycoside derivatives, they have antioxidant properties [19, 25, 36] and by scavenging free radicals, it protects cells from oxidative damage and reduces the risk of cardiovascular damage [45] and certain cancers. It is possible that the consumption of cyanidin inhibits the development of obesity and diabetes and may also reduce the inflammatory processes [46]. Other studies have shown that the glycoside derivative may play a role in the treatment of cancer [47].

DPPH radical-scavenging activity of the *Hibiscus sabdariffa* petals extract

In this study, the antioxidant activity of the dried petal extracts of *Hibiscus sabdariffa* was investigated using the DPPH scavenging assay by determining the total antioxidant capacity of the extract. All these have proven the effectiveness of the petal extract of *H. sabdariffa* compared with the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron that is responsible for the absorbance at 517 nm and also for the visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extract and ascorbic acid is shown in figure 3. Accordingly, as shown in figure 3, the DPPH radical-scavenging activity of Roselle petals was shown to occur in a dose-dependent manner. All extracts concentrations showed a good inhibitory activity against the DPPH radical. However, it is worth noting that all the extract concentrations used showed the strongest antioxidant activity than ascorbic acid, except the concentration 0.1 mg/mL. The concentration required to inhibit 50 % radical-scavenging effect (IC_{50}) was determined from the results of a series of concentrations tested. A lower IC_{50} value corresponds to a larger scavenging activity. The IC_{50} values of the Roselleextract was 0.24 mg/ml while that of ascorbic acid, a well-known antioxidant compound used as the reference control in this study, its IC_{50} value was 0.35 mg/mL. These results imply that there are abundant antioxidative phytochemicals present in the petal extracts of Roselle. During oxidative stress, excessive free radicals are produced that are known to cause damage to the biomolecules [48]. Antioxidant studies indicate that Roselle should possess the ability of either inhibiting free radical formation or itself be a free radical scavenger. Our *H*. *Sabdariffa* extract showed similar results as previously reported by [21]. In the dose-response experiment, it could be observed that total inhibition was never achieved. The maximum inhibition varied between 80 and 97 % in the presence of 0.8 mg/mL extract. According to [49], the antioxidant capacity is highly correlated with the polyphenolic compounds contents in plants. Thus, the high antioxidant activity observed with extracts of petals in Roselle would be linked to the presence of polyphenolic compounds such as phenolic acids, flavonoids and anthocyanins. The antioxidant activity of polyphenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides

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[50]. In this respect, polyphenolic compounds like flavonoids and phenolic acids as well as anthocyanins commonly found in plants have been reported to have multiple biological effects, including an antioxidant activity [25, 51]. Antioxidant activity increased with increasing extract concentrations for all the samples.

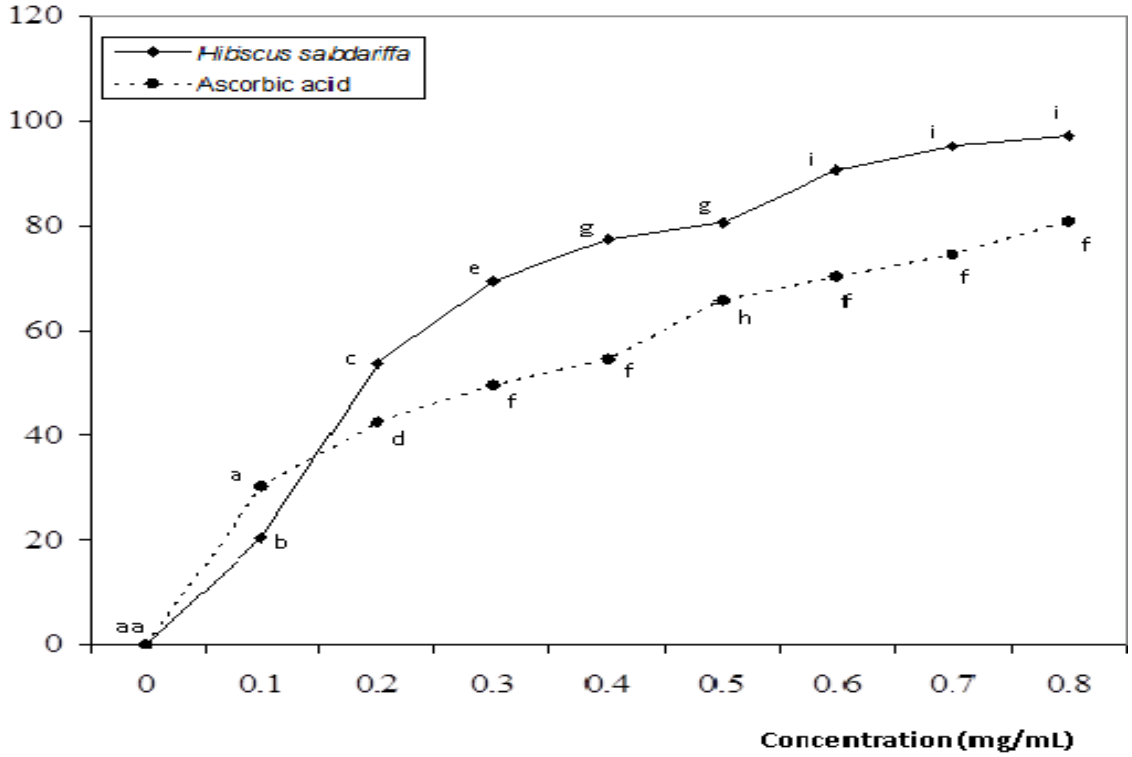

Figure 3. Free radical-scavenging activity of extracts from petals of *Hibiscus sabdariffa* measured by DPPH assay

Values are means of triplicate determination (n = 3); Means followed by the same letter were not significantly different at 5% (test of Kruskal-Wallis).

Hibiscus sabdariffa is an excellent source of dietary phytochemicals such as anthocyanins, flavonoids and phenolic acids. The use of Roselle petals as natural antioxidants, natural colorants, and an ingredient of functional foods seems to be promising.

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