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Phytochemical Analysis of Polyphenols from Petals Extract of *Hibiscus sabdariffa* (Malvaceae)

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Abstract

Hibiscus sabdariffa L. (Malvaceae) is food and medicinal plant rich in secondary metabolites, the source of its biological properties. The petals of *Hibiscus sabdariffa*, is widely used to manufacture the « Bissap » beverage consumed in West Africa during various ceremonies. The present work aims 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 *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 presence of these compounds whose pharmacological properties are known is an advantage for this plant and justifies their use in traditional medicine.Likewise, this study makes it possible to say that the non-alcoholic drink commonly called "Bissap", obtained from the aqueous extraction of petals of *H. sabdariffa*, would contribute to the protection of the organism of people who consume it.

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Keywords

Hibiscus sabdariffa, Phytochemical, Anthocyanins, Flavonoids, Polyphenols.

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 provide thousands of novel compounds, such as fragrance, flavorings, dyes, fibers, foods, beverages, etc. *Hibiscus sabdariffa* 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 (El-Saidy *et al.,* 1992). 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 *Hibiscus sabdariffa* is equally significant in alternative system of medicine as well as in conventional system of medicine. *Hibiscus 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 (Lin*et al.*, 2007; Mahadevan and Pradeep, 2009).

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. The fresh petals(the outer whorl of the flower) areeaten 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 petalsarerich in citric acid and pectin and so is useful for making jams, jellies (Lépengué *et al.*, 2009).

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, coronary heart disease, Alzheimer's disease (Birt, 2006), neurodegenerative disorders, atherosclerosis cataracts, and inflammation (Aruoma, 1998). Both epidemiological and clinical studies provided evidence that most of these phytochemicals exhibit their protective and disease-preventing functions through their antioxidant activities (Usoh *et al.*, 2005). Typical phytochemicals compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, flavonoids, phytic acid and many sterols (Mahadevan and Pradeep, 2009). As antioxidants, these species are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Oboh, 2006).

Hibiscus sabdariffa 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 (Dlaziel, 1973). 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 (Mahadevan and Pradeep, 2009, 2009). 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 (Olaleye, 2007). Its decoction is useful for high blood pressure and cough. The infusion of the red petals is used as a refrigerant drink in fevers and the alcoholic extract of petals inhibited Angiotensin-I converting enzyme (Jonadet*et al.*, 1990). The petals have also many medicinal applications to cure liver damage, hypertension and leukemia (Akanbi*et al.*, 2009). Many authors have also reported the antioxidant activity (Azevedo *et al.*, 2010), anti-inflammatory activity (Dafallah and Al-Mustafa, 1996), cardioprotective activity (Jonadet *et al.*, 1990), hepatoprotective activity (Wang *et al.*, 2000) and antibacterial activity (Garcia *et al.*, 2006) of *Hibiscus sabdariffa* petals.

The regular and intensive use of the juice obtained from the petals of *Hibiscus sabdariffa*as beverage in various ceremonies in West Africa in general and particularly in Côte d'Ivoire led us to initiate this study. Hence, the aim of this investigation was to study the preliminary phytochemicals screening, qualitative and quantitative analysis of polyphenols from the red petals extract of *Hibiscus sabdariffa*. 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, petunidin3-O-glucoside, delphinidin 3-O-sambubioside andcyanidin 3-O-sambubioside) 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 *Hibiscus sabdariffa* 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 hrs. 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.

Preparation of extract

The extract was prepared according to the method of Kouakou *et al.*, (2009). One hundred grams (100 g) petals of *Hibiscus sabdariffa* were extracted from 200 mL of acidified methanol with trifluoroacetic acid 0.1 % (v/v) for 24 hrs 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 *Hibiscus sabdariffa* which was used to achieve the different analyses.

Qualitative phytochemical screening

The preliminary phytochemicals screening of the plant was performed following the standard procedures adapted by various workers (Harborne, 1998; Phillipson, 2001).

Test for alkaloids

The characterization of alkaloids was performed using the Dragendorff's reagent and that of Bouchardat. In a capsule, dry evaporated 6 mL of the plant extract. The residue is taken up in 6 mL of ethanol at 60 ° and the alcoholic solution thus obtained is distributed in two test tubes. In the first tube, two drops Dragendorff's reagent (Potassium Bismuth Iodide) was added. The appearance of a precipitate or orange color indicates the presence of alkaloids. In the second tube, two drops of Bouchardat's reagent (dilute Iodine solution) has been added. The appearance of a reddish-brown color indicates a positive reaction.

Test for polyphenols

The total polyphenols were highlighted by the reaction with ferric chloride. A 2 mL portion of the plant extract was added two drops of alcoholic solution of 2 % ferric chloride. The appearance of a more or less dark blackish-blue or green color indicates the presence of polyphenolic compounds.

Test for flavonoids

Flavonoids were characterized by said reaction to cyanidin. A volume of 2 mL of the plant extract was evaporated to dryness. After cooling, the residue was taken up in 5 mL twice diluted hydrochloric alcohol in a test tube. Then, two to three magnesium turnings were added. The addition of three drops of isoamyl alcohol intensifies a pink-orange or violet, which shows the presence of flavonoids.

Test for anthocyanins

The presence of anthocyanins has been demonstrated by adding 2 mL of the plant extract with 2 mL of 2 N HCl. The appearance of a pink-red color that turns purplish blue after addition of ammonia indicates the presence anthocyanins.

Test for tannins

Tannins have been highlighted by Stisany's reagent. A volume of 5 mL of plant extract was evaporated to dryness. Then, 15 mL of Stisany's reagent (formalin 30 % concentrated HCl (2/1, v/v)) are added. Then the mixture was kept in a water bath at 80 °C for 30 min. After cooling under a stream of water, observation of large flake precipitate characterizes catechin tannins.

Test for quinones

Characterization quinones were carried out according the reaction Borntraeger. In a capsule, 2 mL of the plant extract are evaporated to dryness. The residue was triturated in 5 mL of HCl diluted 1/5 and then brought the solution to the boiling water bath for 30 min in a test

tube. After cooling under a stream of cold water, the hydrolyzate was extracted with 20 mL of chloroform in a test tube. The chloroform layer was then collected in another test tube and then, 0.5 mL of ammonia diluted twice was added thereto. The appearance of a color ranging from red to purple characterizes the presence of quinones.

Test for saponins

The saponins have been identified by adding to a test tube 10 mL of the plant extract. After stirring vertically for about 15 sec and left to stand for about 15 min, the formed foam height was measured. A greater than 10 mm foam height indicates the presence of saponins.

Test for polyterpenes and sterols

Polyterpenes and sterols were characterized by the Libermann-Buchard reaction. A volume of 5 mL of the plant extract was evaporated to dryness. The residue was dissolved hot in 1 mL of acetic anhydride and collected in a test tube. Then, 0.5 mL of concentrated sulfuric acid is poured along the wall of the tube. The appearance at the interphase of a purple or purple ring, turning blue then green, indicates a positive reaction.

Quantitative phytochemical screening

Quantitative chemical analysis of polyphenols, flavonoids and anthocyanins compounds were done by employing spectrophotometric technique.

Determination of total phenolic content

Total phenolic content (TPC) of freeze-dried extract was determined using Folin-Ciocalteu essay (Siriwoharn *et al.,* 2004). 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 Hariri *et al*.(1991). Fifty milligrams (50 mg) of freeze-dried extract was mixed in 5 mL of methanol 70 % (v/v). After 24 hrs, 0.5 mL of filtrate was 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 Lima *et al*., (2012). Ten milligrams (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) [21] and was expressed as mg cyanidin 3-O-glucoside (mg Cya3G)/g of freeze-dried extract (g FDE).All measures were performed in triplicate.

Centrifugal partition chromatography analysis

Analysis by centrifugal partition chromatography (CPC) was performed according to the method described by Bouat-Cottards and Burgaud (2005). The apparatus used to carry out the CPC is the FCPC 200® provided by

Kromaton Technologies (Angers, France). Quaternary biphasic solvent systems were prepared by mixture of ethyl acetate/n-butanol/water/ trifluoroacetic acid (50/50/900/1, v/v) for the stationary phase and (400/460/140/1, v/v) for the mobile phase at 25 °C. Two phases were obtained in each case, an aqueous phase and an organic phase. The solvents were pumped by a Gilson 321 binary pump-H1, two-way high-pressure gradient. The FCPC 200® column was filled with the stationary phase (aqueous phase) to 300 rpm in ascending mode. Two grams (2 g) of the calyces' extract of *Hibiscus sabdariffa* were dissolved in 8 mL of a mixture of stationary phase and mobile phase (1/1, v/v) and were then introduced into the column CPC through a high-pressure injection valve (3725 (i) 038 Rheodyne) equipped with a sampling loop 10 mL. The effluent was monitored with a UV-1010 detector Lambda equipped with a preparative flow cell. The rotor speed was increased to 1000 rpm. The organic phase from the mobile phase was then pumped into the column in ascending mode at a flow rate of 3 mL/min. Fractions of 9 mL were collected every minute by a fraction collector Gilson FC 204.The back pressure was 25 bars. The stationary phase retention at the end of the separation represented 75 % of the column volume.The experiments were conducted at room temperature.

Thin Layer Chromatography analysis

All the fractions were checked by thin layer chromatography (TLC) cellulose plates (Merck) and developed with n- butanol/acetic acid/water (4/1/5, v/v) upper phase.

High Performance Liquid Chromatography analysis

High performance liquid chromatography (HPLC)analysis was conducted using the method described by Drust and Wrolstad (2001). 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 acid in water; solvent B, 0.1 % trifluoroacetic acid in acetonitrile). Acetonitrile (MeCN) 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). Fifty milligrams (50 mg) of freeze-dried extract were dissolved overnight with 5 mL of 0.1 % trifluoroacetic acid in 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. One hundred microliters (100 µL) of filtrate were injected by an Agilent 1100 series autosampler and chromatograms were monitored at 521 nm. 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, petunidin 3-O-glucoside, delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside).

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.

Statistical analysis

Data were processed using Statistica software package version 7.1 (StatSoft Inc., Tulsa, USA). Analysis of variance (One-way ANOVA) was performed and means were separated by Newman-Keuls range test at $P<0.05$. Data are expressed as mean ± standard deviation (SD), n = 3.

Results and Discussions

Qualitative phytochemical screening

For this investigation, different phytochemicals from calyces were extracted and highlighted by different

methods; their presence (+) or absence (-) is shown in table 1. The results indicated that *Hibiscus sabdariffa* calyces contained alkaloids, anthocyanins, flavonoids, polyphenol, saponins and tannins which are the main phytochemical groups.

Quantitative phytochemical screening

The results of this study are presented in table 2 shows the content of anthocyanins, flavonoids, other flavonoids and phenolic acids of calyx extracts of *Hibiscus sabdariffa* determined by dosage or calculation. We noted that anthocyanins are the majority compound with 12.34 mg/g and represents 53.19 % of the phenolic compounds (polyphenols), followed by the other flavonoids with 8.36 mg/g (36.03 %) and finally phenolic acids with 2.50 mg/g (10.77 %).

Chromatographic profiles

Peak assignments are based on matching UV-vis and identical HPLC retention time (Tables 3 and 4) with known phenol acids, flavonoids and anthocyanins from a reference library of compounds previously purified and identified.

Centrifugal partition chromatography profile of freeze-dried of petals extract of *Hibiscus sabdariffa*

The freeze-dried petals extract of *Hibiscus sabdariffa* was analyzed by centrifugal partition chromatography (CPC) at 521 nm. The chromatogram obtained (Figure 1) shows several minority compounds (1) and two majority compounds (2 and 3).

Table.1 Phytochemical constituents of petals extract of *Hibiscus sabdariffa*

N°	Test	Petals extract
1	Alkaloids	+
2	Anthocyanins	+
3	Flavonoids	+
4	Polyphenols	+
5	Quinones	–
6	Saponins	+
7	Sterols	–
8	Tannins	+
9	Terpenoids	–

The sign (+) indicates the presence of the chemical group in the extract while the sign (-) indicates the absence of the chemical group.

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

Compound	Content (mg/g)	Ratio (%)
Anthocyanins	16.53 ± 1.10 [a]	60.26 [d]
Flavonoids	3.50 ± 0.85 [c]	12.72 [f]
Polyphenols	7.40 ± 0.50 [b]	26.98 [e]

Values are expressed as means ± standard deviation, n = 3.
The means followed by the same letter are not significantly different ($p<0.05$).
Ratio: content of a compound relative to the total content of compounds.

Table.3 Retention time in HPLC at 280 nm of the phenolic compound standards

N°	Phenolic Compounds	Retention time de (min)
1	Chlorogenic acid	14.719
2	Gallique acid	04.469
3	Protocatechicacid	15.471
4	Salicylic acid	16.770
5	Veratricacid	14.578
6	Catechin	09.885
7	Epicatechin	10.746
8	Eugenol	21.570
9	Genistein	11.322
10	Gossypetin	12.543
11	Gossypetrin	10.707
12	Gossypin	06.127
13	Gossytrin	15.790
14	Isoquercetin	15.995
15	Kaempferol	24.460
16	Kaempferol 3-O-glucoside: astragalin	25.396
17	Kaempferol 3-O-rutinoside: nicotiflorin	22.083
18	Luteolin	15.340
19	Luteolin 7-glucoside: cyranoside	27.601
20	Naringenin	20.309
21	Quercetin	23.870
22	Quercitrin	22.621
23	Rutin	17.110
24	*Trans*-resveratrol	17.049
25	Vanillin	14.412

HPLC: High performance liquid chromatography.

Table.4 Retention time in HPLC at 521 nm of the anthocyanin standards

N°	Anthocyanins	Retention time (min)
1	Cyanidin	23.998
2	Delphinidin	18.875
3	Malvidin	29.231
4	Peonidin	28.198
5	Petunidin	27.874
6	Cyanidin 3-O-glucoside	14.478
7	Delphinidin3-O-glucoside	15.143
8	Malvidin 3-O-glucoside	26.235
9	Peonidin 3-O-glucoside	20.976
10	Petunidin 3-O-glucoside	17.006
11	Cyanidin 3-O-sambubioside	13.712
12	Delphinidin 3-O-sambubioside	12.309

HPLC: High performance liquid chromatography.

Figure 1. Diagram of obtaining from the petals extract of *Hibiscus sabdariffa*.

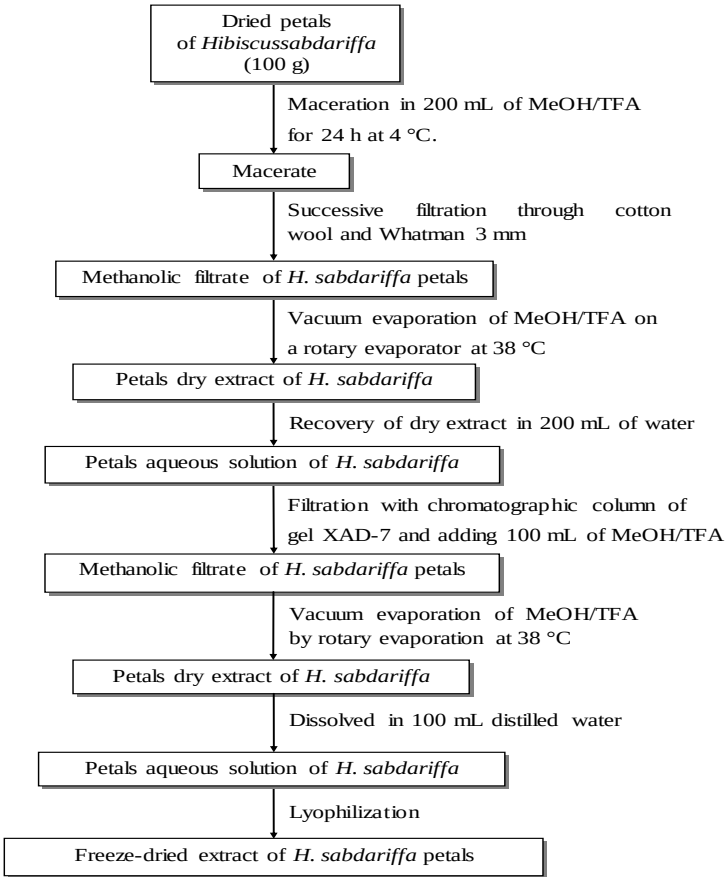


Figure 2.CPC profile of freeze-dried of petals extract of *Hibiscus sabdariffa*.
The flow rate is 3 mL/min at 1000 rpm and 9 mL fractions are collected every 3 min; CPC: centrifugal partition chromatography; 1: minority compounds; 2 and 3: majority compounds.

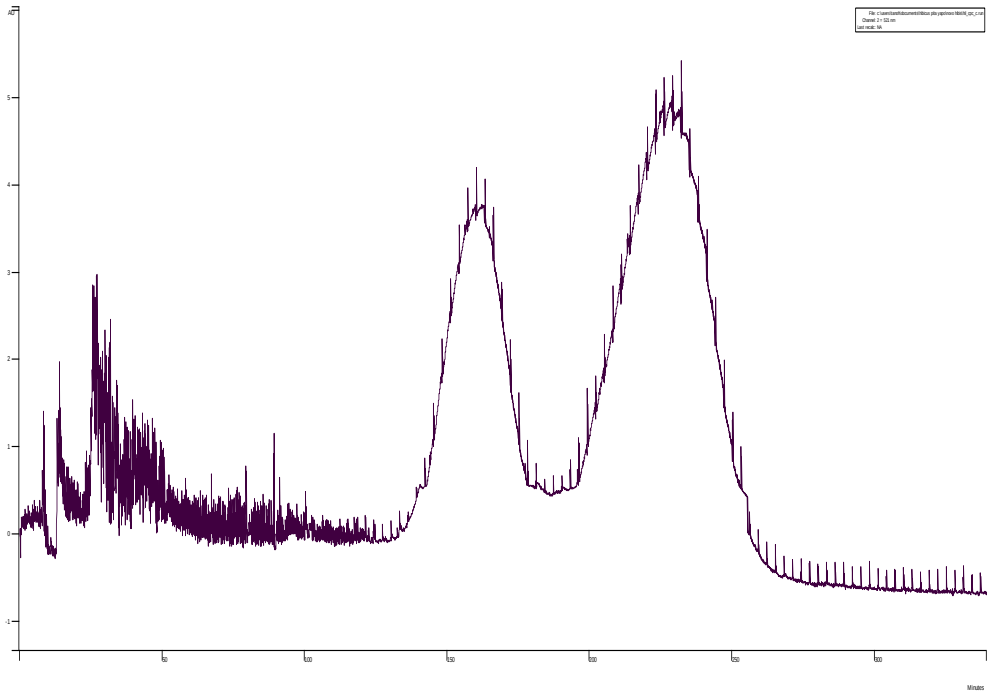


Figure 3.HPLC profile of phenolic compounds from petals extract of *Hibiscus sabdariffa*.
Detection is shown at 280 nm. Peaks were identified by comparison with reference standards when available or by ^{1}H-NMR data (retention time). 1. gossypetrin (10.671 min); 2. sabdaretin (11.919 min); 3. gossypetin (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).
HPLC: High performance liquid chromatography.

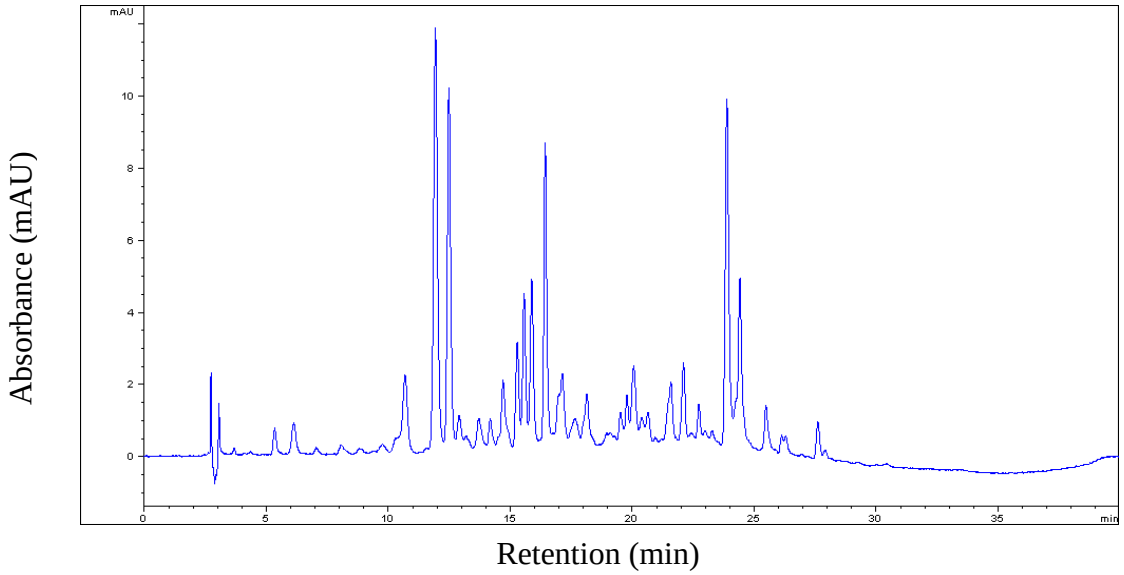
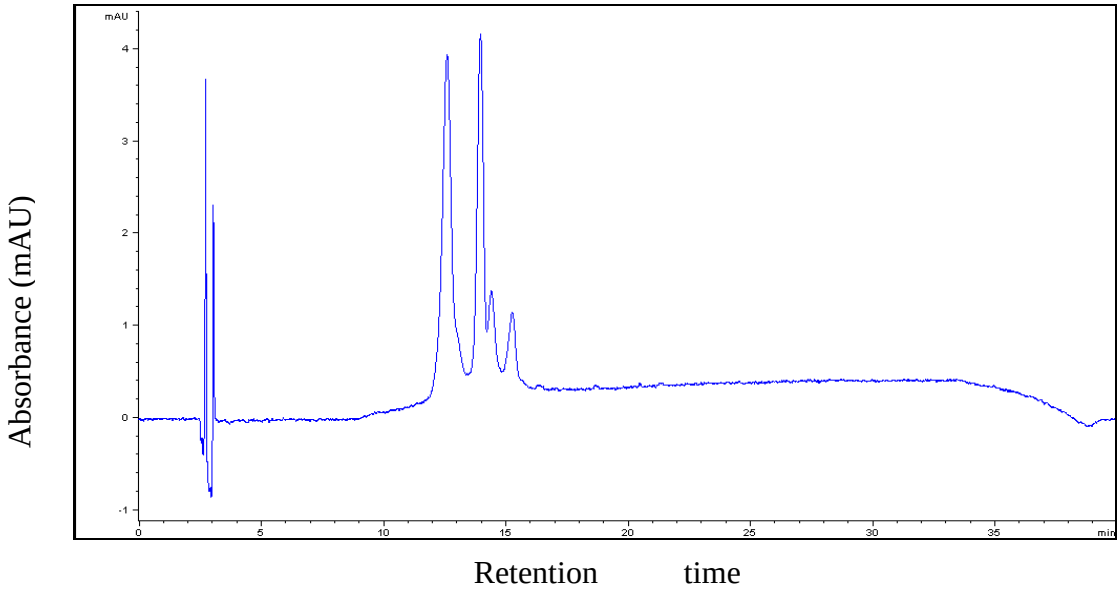


Figure.4 HPLC profile of anthocyanins from petals extract of *Hibiscus sabdariffa*.
Detection is shown at 521 nm. Peaks were identified by comparison with reference standards when available or by ^{1}H-NMR data (retention time). 1. delphinidin 3-O-sambubioside (12.681 min); 2. cyanidin 3-Osambubioside (13.389 min); 3. cyanidin 3-O-glucoside (14.389 min); 4. delphinidin 3-O-glucoside (15.238 min). HPLC: High performance liquid chromatography



High performance liquid chromatography profile of anthocyanins

The chromatogram showed that delphinidin 3-O-sambubioside (1) and cyanidin 3-O-sambubioside (2) are the major anthocyanins. Indeed, the absorbance of these two anthocyanins is four-fold lower than that of cyanidin 3-O-glucoside (3) and delphinidin 3-O-glucoside (4).

The screening of plants for medicinal value has been carried out by numerous researchers with the help of preliminary phytochemical analysis (Ram, 2001; Mungole and Chaturvedi, 2011). 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 (Lopes-Lutz *et al.*, 2008; Ni *et al.*, 2012).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 *Hibiscus sabdariffa* 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 *Hibiscus sabdariffa* petals was similar to referenced data, with some differences that may be due to genetic variability, type of soil and extractive solvent (Olaleye, 2007; Mungole and Chaturvedi, 2011). Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Ocho-Anin *et al.*, 2010). Anthocyanins were present in petals of *Hibiscus sabdariffa*. These compounds have the healing properties. The anthocyanins have been found to be cardioprotective, hypocholesterolemic; antioxidative and hepatoprotective (Jonadet *et al.*, 1990; Wang *et al.*, 2000). They also have an antioxidant activity (Siriwoharn *et al.*, 2004; Zhang *et al.*, 2011) and inhibit low density lipoprotein (LDL) oxidation (Rio *et al.*, 2013). 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 (Usoh *et al.*, 2005; Garcia *et al.*, 2006; Olaleye, 2007; Akanbi *et al.*, 2009). *Hibiscus sabdariffa* 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 (Azevedo *et al.,* 2010; Zhang *et al.,* 2013). It is a well-documented fact that most medicinal plants are enriched with polyphenol compounds that have excellent antioxidant properties (Zhang *et al.,* 2013). 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 (Chen *et al.,* 2003). The result of the phytochemical screening revealed that quinones, steroids and terpenoids were absent in petals of *Hibiscus sabdariffa*. Saponins were found to be present in petals of *Hibiscus sabdariffa*. Saponins having hypertensive and cardiac depressant properties (Birt, 2006). Dietary source of saponins offer preferential chemical preventive strategy in lowering the risk of human cancer (Halliwell *et al.,* 1992). Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism. Tannins play important role such as potent antioxidant (Lépengué *et al.,* 2009). The results of the quantitative phytochemical that showed that the petals extract of *H. sabdariffa* contains anthocyanins, polyphenols and flavonoids are similar to those of Lin *et al.,* (2007). They have a similarity in the relative amounts of these phytochemical constituents in the extracts of this plant; the discrepancies are probably due to the difference in the geographical area and climatic conditions. Similarly, these results corroborate those of Du and Francis (1973) that showed that anthocyanins are pigments majority of *H. sabdariffa* with determining a grade of 1.5 g/100 g of dried calyx. The significant presence of anthocyanins in flowers of *H. sabdariffa* indicates that this plant can play an important role in industries (food, textile, pharmaceutical and cosmetic). Indeed, several authors (Okonkwo, 2010; Salem*et al.,* 2014)have shown that anthocyanins were potential natural dyes for these industries. Nevertheless, they are beneficial to the health of consumers. Indeed, they are a potential source of natural antioxidant (Obouayeba *et al.,* 2014; Salem*et al.,* 2014).

The HPLC profiles of the phenolic compounds and anthocyanins of the *H. sabdariffa* petals extract obtained respectively at UV 280 and 521 nm demonstrate the presence of phenolic acids, flavonoids and anthocyanins thus confirming the results obtained during qualitative and quantitative phytochemical study. From the HPLC profile of the phenolic compounds of the *H. sabdariffa* petals extract, 18phenolic compounds were identified, including 16 flavonoids and two phenolic acids. These results obtained from the chromatographic analyzes of the petal extract of this plant agree with those of Ali *et al.,* (2005), Mahadevan and Pradeep (2009) and Ozdogan *et al.,* (2011). These authors reported the presence of these same phytochemicals in the calyxextract of this plant from chromatographic analyzes.

All identified phenolic compounds in *H.sabdariffa* petals have pharmacological properties demonstrated by several authors (Garcia *et al.,* 2006; Rio *et al.,* 2013). Four anthocyanins were identified in the petals of *H. sabdariffa* from the HPLC profile at 521nm of the extract of this plant. These are delphinidin 3-O-sambubioside, cyanidine 3-Osambubioside,cyanidine 3-O-glucoside and delphinidin 3-O-glucoside. These results agree with those of Ali *et al.,* (2005), Segura-Carretero *et al.,* (2008) and Salazar et Gonzalez *et al.,* (2012). The results of this study are also in agreement with those of Du and Francis (1973), who identified these same anthocyanins in the calyces of *H. sabdariffa* with delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside, as majority compounds. Moreover, the results of Palé *et al.,* (2004), on the characterization and measurement ofantiradical activities of anthocyanins from plants in Burkina Faso, showed the presence of delphinidin 3-O-sambubioside, cyanidin 3-O-sambubioside and delphinidine 3-O-glucoside inthe calyces of *Hibiscus sabdariffa* flowers. The same is true for Khafaga and Koch (1980), who found anthocyanins from samples of *H. sabdariffa* from Egypt, India, Thailand and Latin America.

In conclusion, *Hibiscus sabdariffa* is medicinal and food plant rich in phytochemical compounds such as anthocyanins, flavonoids and phenolic acids of interest responsible for its pharmacological properties. The juice of flowers of *H. sabdariffa*, commonly known as Bissap is used in the preparation of local nonalcoholic cold beverage and as a hot drink.

In Côte d'Ivoire, this production of a nonalcoholic drink called Bissap that is prepared from the red petals is popular. The use of *H. sabdariffa*petals as natural antioxidants, natural colorants, and an ingredient of functional foods seems to be promising.

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References

Akanbi WB, Olaniyan AB, Togun AO, Ilupeju AEO, Olaniran OA (2009). The effect of organic and inorganic fertilizer on growth, calyx yield and quality of Roselle (*Hibiscus Sabdariffa* L.) Am-Eur. J. Sust. Agr. 3(4): 652-657.

Ali BH, Wabel NA, Blunden G (2005). Phytochemical, Pharmacological and Toxicological Aspects of *Hibiscus sabdariffa* L.: A Review. Phytother. Res. 19:369-375.

Azevedo J, Fernandes I, Faria A, Oliveira J, Fernandes A, Freitas V, Mateus N (2010). Antioxidant properties of anthocyanidins, anthocyanidin-3-glucosides and respective portisins. Food Chemistry 119:518-523.

Birt DA (2006). Phytochemicals and cancer prevention: from epidemiology to mechanism of action. J. Am. Diet. Assoc. 106:20-24.

Chaovanalikit R, Thompson MM, Wrolstad RE. (2004). Characterization and Quantification of Anthocyanins and Polyphenolics in Blue Honeysuckle (*Lonicera caerulea* L.). J. Agr. Food Chem. 52:848-852.

Chen CC, Hsu JD, Wang SF, Chrang HC, Yang MY, Kao ES, Ho YO, Wang CJ (2003). *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterol-fed rabbits.J. Agr. Food Chem. 51(18):5472-5477.

Dafallah AA, Al-Mustafa Z (1996). Investigation of the anti-inflammatory activity of *Acacianilotica* and *Hibiscus sabdariffa*. Am. J. Chin. Med. 24:263-269.

Dlaziel JM. The Useful Plants of West Tropical Africa. Crown Agent for Overseas Government and Administrations: London, 1973, pp. 129-130.

Drust RW, Wrolstad RE (2001). Separation and characterization of Anthocyanins by HPLC. In current Protocols in Foods Analytical Chemistry, Wrolstad R.E., Ed. John Wiley and Sons, New York, pp 1-13.

Du CT, Francis, FJ (1973). Anthocyanins of roselle (*Hibiscus sabdariffa* L.) J. Food Sci., 38:810-812.

El-Saidy SM, Ismail I. El-Zoghbi MZ (1992). A study on roselle extraction asa beverage or as a source for anthocyanins. J.Agr.Res.19:831-839.

Garcia VMN, Rojas G, Zepeda LG, Aviles M, Fuentes M, Herrera A, Jimenez EE (2006). Antifungal and antibacterial activity of four selected Mexican medicinal plants. Pharm. Biol. 44:297-300.

Halliwell B, Gutteridge JM, Cross CE (1992). Free radicals, antioxidants, and human disease: where are we now? J. Lab. Clin. Med. 119:598-620.

Harborne JB (1998). Phytochemical methods: A Guide to Modern techniques of plants Analysis. Chapman and Hall London, United Kingdom, pp. 40-137.

Hariri EB, Sallé G, Andary C (1991). Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (*Viscum album* L.). Protopl. 162:20-26.

Jonadet M, Bastide J, Boyer B, Carnat AP, Lamaison JL (1990). *In vitro* enzyme inhibitory and *in vivo* cardio-protective activities of *Hibiscus sabdariffa* L. J. Pharmacol. Belgium, 45:120-124.

Khafaga ER, Koch H (1980). Reifgrad und qualität von karkadeh (*Hibiscus sabdariffa* L. var *sabdariffa*)-I-organische saüren. Angew. Bota. 54:287-293.

Laima C, Remigijus D, Pranas V, Antanas S (2012). Determination of the total phenolic and anthocyanin contents and antimicrobial activity of *Viburnum opulus* fruit juice. Plant Foods Hum. Nutr. 67(3): 256-261.

Lépengué NA, Mouaragadja I, M'batchi B, Aké S (2009). Etude de quelques caractéristiques physicochimiques du filtrat de culture de *Phoma sabdariffae* Sacc., agent pathogène dela roselle. Sci. Nat. 6(2): 95-105.

Lin TL, Lin HH, Chen CC, Lin MC, Chou MC, Wang CJ (2007). *Hibiscus sabdariffa* extract reduces cholesterol in men and women. Nutr. Res. 27:140-145.

Lopes-Lutz D, Alviano SD, Alviano SC, Kolodziejczyk PP (2008). Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochem. 69:1732-1738.

Mahadevan NS, Pradeep K (2009). *Hibiscus sabdariffa* Linn. An overview. Nat. Prod. Rad. 8:77-83.

Mungole A, Chaturvedi A (2011). *Hibiscus sabdariffa* L. a rich source of secondary metabolites. Int. J. Pharm. Sci. Rew. Res. 6:83-87.

Ni Q, Xu G, Lu G, Gao Q, Zhou C, Zhang Y (2012). Investigation of the stability and antioxidant properties of anthocyanins-based purple potato

colorants after processing. Afr J. Biotech. 11(14): 3379-3387.

Oboh U (2006). Antioxidant properties of some commonlyconsumed and underutilized tropical legumes. Eur. Food Res. Technol. 224:61-65.

Ocho-Anin AL, Kouakou TH, Brou KD, Kouadio YJ, Gnakri D (2010). Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L. (Fabaceae) cultivated in Côte d'Ivoire. J. Appl. Biosci. 31:1928-1934.

Okonkwo TJN (2010). *Hibiscus sabdariffa* anthocyanins: a potential two-color end-point indicator in acid-base and complexometric titrations. Int. J. Pharma. Sci. Rev. Res. 4(3):123-128.

Olaleye MT (2007). Cytotoxicity and antibacterial of Methanolic extract of *Hibiscus sabdariffa*. J. Med. Plants Res. 1(1):9-13.

Ozdogan FP, Orhan N. Ergun F (2011). Studies on the Conformity of *Hibiscus sabdariffa* L. Samples from Turkish Market to European Pharmacopeia. FABAD J. Pharm. Sci. 36:25-32.

Palé E, Kouda-Bonafos M, Narco M (2004). Caractérisation et mesure des activités anti-radicalaires d'anthocyanes de plantes du Burkina Faso. Comptes Rendus Chimie 7: 973-980.

Phillipson JD (2000). Phytochemistry of medicinal plants. Phytochem. 56(3):237-248.

Ram RL (2001). Preliminary phytochemical analysis of medicinal plants of South Chotanagpur used against dysentery. Adv. Plant Sci. 14:525-530.

Rio DD, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Alan-Crozier A (2013). Dietary (Poly) phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases.Ant. Redox Sig. 18(14):1818-1892.

Salazar-Gonzalez C, Vergara-Balderas FT, Ortega-Regules AE, Guerrero-Beltran JA (2012). Antioxidant properties and color of *Hibiscus sabdariffa* extracts. Ciencia Investig. Agraria 39(1): 79-90.

Segura-Carretero A, Puertas-Mejia MA, Cortacero-Ramírez S, Beltran R, Alonso-Villaverde C, Joven J, Dinelli G, Fernandez-Gutierrez A (2008).Selective extraction, separation, and identification of anthocyanins from *Hibiscus sabdariffa* L. using solid phase extraction-capillary electrophoresis-mass spectrometry (time-of-flight /ion trap). Electroph. 29(13):2852-61.

Siriwoharn T, Wrolstad RE, Finn CE (2004). Influence of cultivar, maturity, and sampling on blackberry (Rubus L. hybrids) anthocyanins, polyphenolics and antioxidant properties. J. Agr. Food Chem. 52:8021-8030.

Usoh IF, Akpan EJ, Etim EO, Farombi EO (2005). Antioxidant Actions of Dried Flower Extracts of *Hibiscus sabdariffa* L. On Sodium Arsenite-Induced Oxidative Stress in Rats. Pak. J. Nutr. 2005; 4(3):135-141.

Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide induced hepatic toxicity in rats. Food Chem. Toxicol. 38:411-416.

Zhang M, Hettiarachchy NS, Horax R, Kannan A, Praisoody AMD, Muhundan A, Mallangi CR (2011). Phytochemicals, antioxidant and antimicrobial activity of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii* leaves. J. Med. Plants Res. 5(30): 6672-6680.

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