Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
Impact Factor (GIF): 0.615
Impact Factor (SJIF): 2.092
June-August 2015; 3(2):156-168
Available online at http://www.jpcbs.info

Original Research Article

Phytochemical Analysis, Purification and Identification of *Hibiscus* Anthocyanins

Abba Pacôme Obouayeba[1*], Moussa Diarrassouba[2], Eric Francis Soumahin[3], Tanoh Hilaire Kouakou[4]

[1]Laboratory of Biochemical Pharmacodynamy, Department of Biosciences, Félix Houphouët- Boigny University of Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire
[2]Section of Life and Earth Sciences, Department of Sciences and Technology, Normal Superior School, 25 BP 663 Abidjan 25, Côte d'Ivoire
[3]Research Station of Bimbresso, National Agricultural Research Center, 01 BP 1536 Abidjan 01, Côte d'Ivoire
[4]Laboratory of Biology and Crop Improvement, Department of Natural Sciences, Nangui Abrogoua University of Abidjan, 02 BP 801 Abidjan 01, Côte d'Ivoire

***Corresponding Author:** Obouayeba Abba Pacôme, Laboratory of Biochemical Pharmacodynamy, Department of Biosciences, Félix Houphouët- Boigny University of Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire

Received: 23 June 2015 Revised: 30 June 2015 Accepted: 09 July 2015

ABSTRACT

Hibiscus sabdariffa is a medicinal and food plant rich in phytochemical compounds which are the source of its biological properties. The present work aim to study the phytochemical screening of *H. sabdariffa* for various medicinally important compounds and their quantification. The results showed that alkaloids, anthocyanins, flavonoids, saponins and tannins are present in calyces of the *Hibiscus sabdariffa*. Anthocyanin content was highest while the contents of other flavonoids and phenolic acids were lowest. Two *Hibiscus* anthocyanins such as cyanidin 3-O-glucoside and delphinidin 3-O-glucoside were purified and identified by CPC and HPLC in calyces of *Hibiscus sabdariffa*. The presence of phytochemical compounds constituting mainly calyces of *H. sabdariffa*, such as anthocyanins, flavonoids and phenolic acids justify its uses in folkloric medicines.

Keyword: *Hibiscus sabdariffa*; Phytochemical; Anthocyanins; Cyanidin 3-O-glucoside; Delphinidin 3-O-glucoside

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied using modern scientific

approaches. Ethnobotany and ethno-pharmacognosy, the basis of useful knowledge on plants in their relationship with traditional or popular therapeutic uses, constitute a guide for chemical, pharmacological and physiological studies that allow the establishment of a scientific foundation for supposed therapeutic properties. The results from these plants have revealed the potentials of medicinal plants in the area of pharmacology [1-3]. *Hibiscus sabdariffa* L., a member of the Malvaceae family, is an annual shrub that grows in regions where dry tropical weather prevails. It is known as roselle (English), l'oiselle (French), karkade (Arabic) and bissap (Wolof). In some countries, its shrubbery is used to decorative purposes, in others, the seeds and petals are used for human consumption. Nevertheless, in most cases it is cultivated with the purpose of using the calyces to produce infusions that are consumed like tea [4] or petals to produce infusions that are used for sauces and jams or preparation of Bissap: infusion and syrup producing a red drink, drunk fresh and very sweet (sometimes prepared with mint) in West Africa.

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 calyces (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 dried calyces are used in the preparation of local nonalcoholic cold beverage and as a hot drink highly appreciated in Côte d'Ivoire. This nonalcoholic drink called bissap prepared from the red calyces is popular and highly appreciated by population in most of the West African countries [5].

Anthocyanins are a group of plant pigments that are widely distributed in nature, which are responsible for the attractive colors of many flowers, fruits, grains and related products derived from them [6]. Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins, and they are found in the form of polyhydroxylated and or methoxylated heterosides which derive from the flavylium ion or 2-phenylbenzopyrilium in nature [7]. Six anthocyanidins are widespread in fruits and vegetables, which are cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin [8]. Anthocyanins are valuable as kinds of important quality indicators in foods and chemotaxonomic indicators in plants. Recent research has shown that anthocyanins have numerous health beneficial properties, which include antioxidant [5, 9], anticarcinogenic [10], antimicrobial [11], anti-inflammatory [12, 13], cardioprotective [12, 14] and hepatoprotective [2, 15] properties.

The regular and intensive use of the juice obtained from the calyces 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 the present work was to carry out the phytochemical study of the calyces' extract of *Hibiscus sabdariffa*. This will generate more knowledgeable informations on their potentiality for a wider utilization.

MATERIALS AND METHODS

Plant Material

The calyces of *Hibiscus sabdariffa* were used as plant material in the present study. The material was purchased from a local market in Adjamé (Abidjan, Côte d'Ivoire). The calyces were cut, cleaned, washed thoroughly under running tap water, drained and oven-dried at 55 °C for 12 hrs. The samples were packed in polyethylene bags and stored at 4 °C for laboratory analysis.

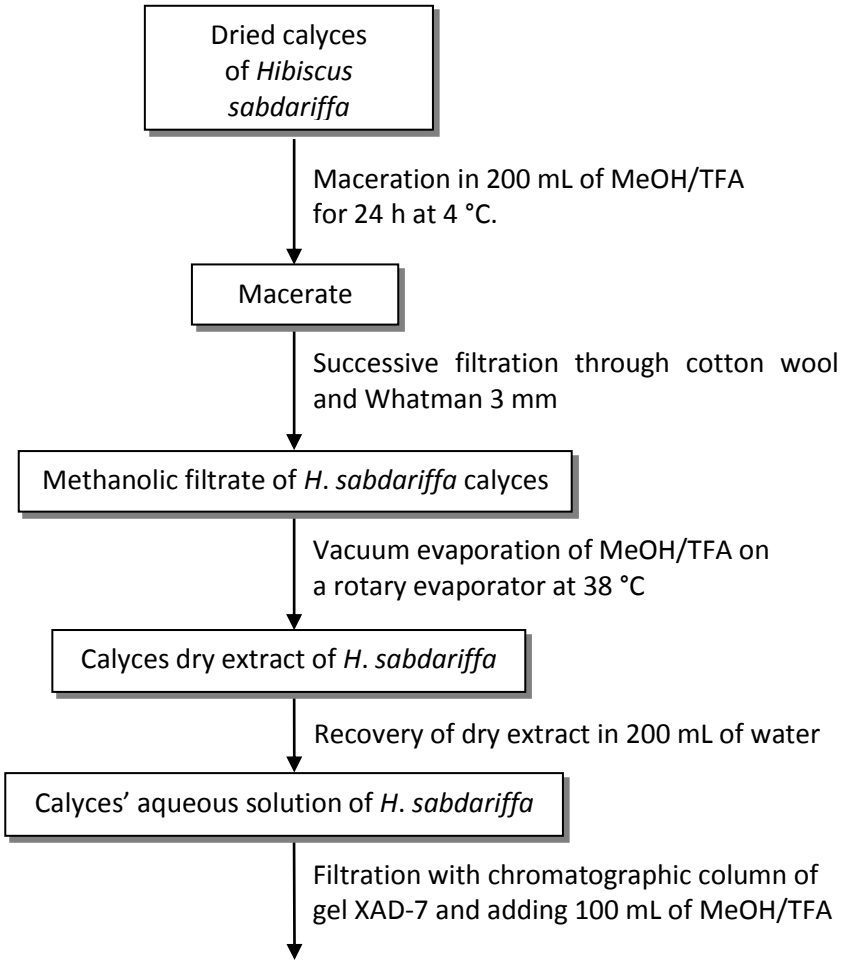
Drugs and Chemicals

All reagents, solvents and chemical compounds used for analysis met the quality criteria in

accordance with international standards. Anthocyanins standards (cyanidin, delphinidin, malvidin, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside and malvidin 3-O-glucoside) were purchased from Sigma-Aldrich (Steinheim, Germany). The trifluoroacetic acid (TFA), methanol (MeOH), n-butanol (n-BuOH), acetic acid, ethyl acetate (EtOAc), Folin-Ciocalteu reagent, Neu reagent and sodium carbonate were obtained from Merck (Darmstadt, Germany).

Extract Preparation

The extract was prepared according to the method of Kouakou et al [16]. One hundred grams (100 g) calyces 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 low-pressure 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 solution was submitted to a filtration on gel XAD-7, in order to eliminate sugars and chlorophyll pigments. One hundred milliliters (100 mL) of acidified methanol with trifluoroacetic acid 0.1 % (v/v) were poured over the gel XAD-7 and the methanolic filtrate obtained was resubmitted to low-pressure vacuum evaporation in BÜCHI Rotavapor R-114 at 38 °C. The dry extract obtained was dissolved in 100 mL of distilled water. The aqueous solution was lyophilized with the freeze dryer CHRIST ALPHA 1-2. The dried extract obtained represented the calyces' extract of *H. sabdariffa* (CEHS) which polyphenols content and compounds were previously determined by Obouayeba et al [5] as presented in Fig. 1.



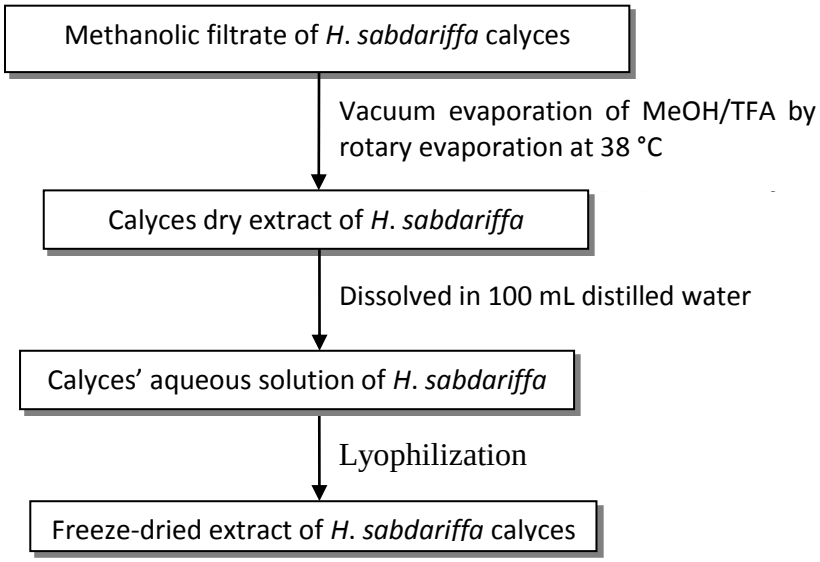


Fig. 1: Diagram of obtaining the calyces extract of *Hibiscus sabdariffa*.

Qualitative Phytochemical Screening

The presence of some phytoconstituents in the extract was highlighted by standards phytochemical methods. Phytochemical analysis of alkaloids, anthocyanins, flavonoids, polyphenols, quinones, saponins and tannins were performed according to the methods described by Phillipson [17].

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.

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 [18]. 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 765 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 [19]. Fifty milligrams (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 Lima et al [20]. 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 a 10 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).

Centrifugal Partition Chromatography Analysis

Analysis by centrifugal partition chromatography (CPC) was performed according to the method described by Bouat-Cottards and Burgaud [22]. 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 (TLC) Analysis

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

High Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was conducted using the method described by Drust and Wrolstad [23].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). A reference library of compounds was performed previously with commercially available compounds such as anthocyanin standards

(cyanidin, delphinidin, malvidin, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, malvidin 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.

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

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.

Table 1: Qualitative Analysis of the Phytochemical of Calyces Extract in Hibiscus sabdariffa.

Sl. No.	Chemical Test	Extract
1	Alkaloids	+
2	Polyphenols	+
3	Flavonoides	+
4	Anthocyanins	+
5	Tannins	+
6	Quinones	–
7	Saponins	+

(+) Present ; (-) Absent.

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 %).

Table 2: Quantitative Data of Various Phytochemicals in the Calyces Extract of *Hibiscus sabdariffa*.

Sl. No.	Compounds	Contents (mg/g)	Ratio (%)
1	Anthocyanins	12.34 ± 2.30^{c}	53.19^{c}
2	Flavonoids	20.70 ± 1.50^{b}	89.20^{b}
3	Other Flavonoids*	8.36 ± 0.72^{d}	36.03^{d}
4	Polyphenols	23.21 ± 2.70^{a}	99.98^{a}
5	Phenolic Acids*	2.50 ± 0.12^{e}	10.77^{e}

Values are expressed as mean ± standard deviation (SD), n = 3.
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.
*: values determined by calculation.

Purification and Identification of *Hibiscus* Anthocyanins

The results of chromatograms shows that two anthocyanins such as cyanidin 3-O-glucoside (Fig. 2) and delphinidin 3-O-glucoside (Fig. 3) were purified and identified from the calyces extract of *Hibiscus sabdariffa*. Peak assignments are based on matching UV-vis and identical

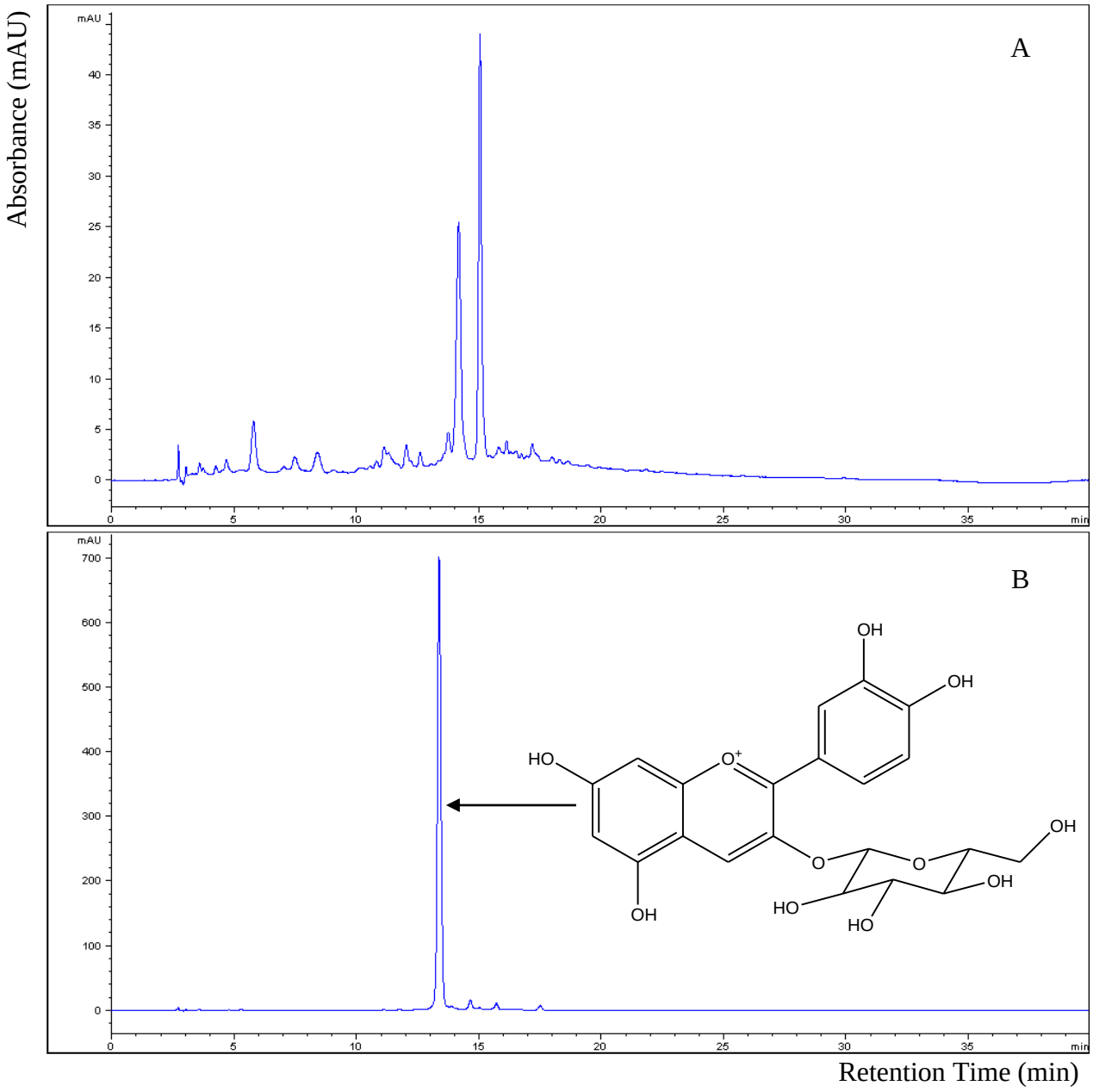


Fig. 2: HPLC Chromatogram Profiles of A: *Hibiscus* Anthocyanins from calyces extract and B: Cyanidin 3-O-glucoside.

Chromatograms were obtained at 521 nm. Cyanidin 3-O-glucoside was identified by comparison with reference standards when available (retention time).

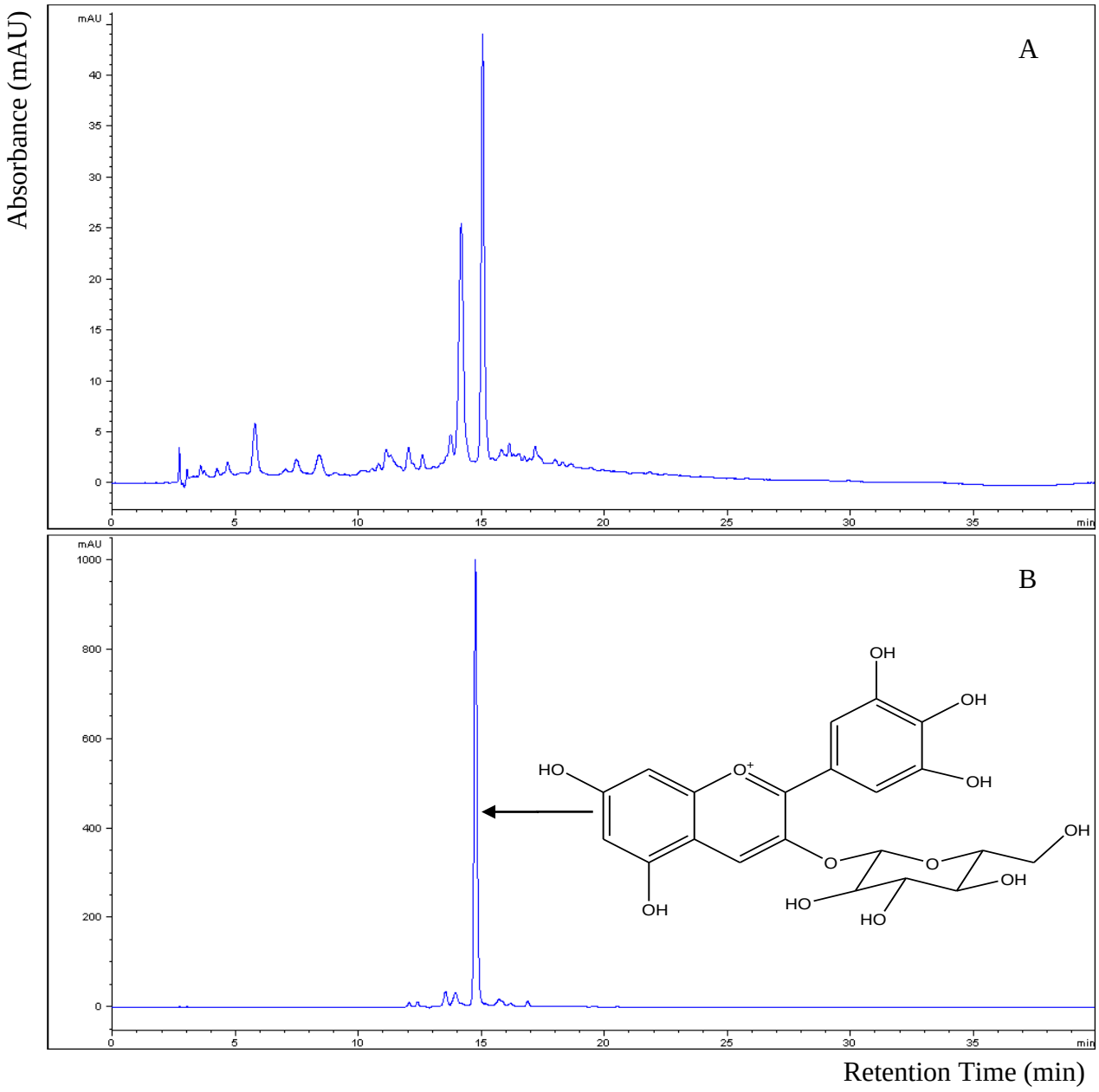


Fig. 3: HPLC Chromatogram Profiles of A: *Hibiscus* Anthocyanins from calyces extract and B: Delphinidin 3-O-glucoside.

Chromatograms were obtained at 521 nm. Delphinidin 3-O-glucoside was identified by comparison with reference standards when available (retention time).

HPLC retention time with known anthocyanins from a reference library of compounds previously purified and identified by anthocyanins identified in calyces of *Hibiscus sabdariffa* (table 3).

Table 3: HPLC retention time of anthocyanin standards at 521 nm.

Sl. No.	Anthocyanin	Retention time (min)
1	Cyanidin	09.435
2	Delphinidin	10.204
3	Malvidin	10.976
4	Cyanidin 3-O-glucoside	12.597
5	Delphinidin 3-O-glucoside	15.143
6	Malvidin 3-O-glucoside	15.720

DISCUSSION

The screening of plants for medicinal value has been carried out by numerous researchers with the help of preliminary phytochemical analysis [2, 24, 25]. 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 [26-28]. The selection of plant parts such as calyces which yields maximum secondary metabolites is the prime or prerequisite step in this investigation. The results indicated that *Hibiscus sabdariffa* calyces contained alkaloids, anthocyanins, flavonoids, saponins, steroid, sterols and tannins which are the main phytochemical groups with biological activities.

The results of the qualitative phytochemical study of the calyces extract of *H. sabdariffa* show the presence of alkaloids, anthocyanins, flavonoids, polyphenols, saponins and tannins in this extract. These results corroborate those of several authors on the phytochemical composition of aqueous extract of the leaves [29], the alcoholic extract of the leaves [29], aqueous and alcoholic extracts of the seeds [29], methanolic extract of the calyces [30], aqueous and hexane extracts of calyces [31] of *Hibiscus sabdariffa*. Furthermore, the qualitative and quantitative phytochemical analysis revealed the presence of phenolic compounds, specifically flavonoids and anthocyanins. The presence of these phytochemicals probably explains the many pharmacological properties of the plant. Indeed, according to Lin et al [32], the presence of anthocyanins, flavonoids and polyphenols in the calyces' extract of *H. sabdariffa* promotes cholesterol reduction in human serum. Similarly, McKay et al [33] showed that the presence of these molecules in this plant is a therapeutic support in the treatment of hypertension. Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents [34] and are also known for their antiplasmodial activity and their absence of toxicity to immunity markers [1]. It is clear that calyces of *Hibiscus sabdariffa* possess good phytoconstituents that will be helpful in future for the cure of different types of diseases.

The results of the quantitative phytochemical that showed that the calyces' extract of *H. sabdariffa* contains anthocyanins, polyphenols and flavonoids are similar to those of Lin et al [32]. 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 [35] that showed that anthocyanins are pigments majority of *H. sabdariffa* with determining a grade of 1.5 g/100 g of dried calyces. 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 [36, 37] have shown that anthocyanins were potential natural dyes for these industries.

The *Hibiscus* anthocyanins were purified and identified in this work are cyanidin 3-O-glucoside and delphinidin 3-O-glucoside. The presence these anthocyanins in calyces of *Hibiscus sabdariffa* were mentioned by many authors [35, 38]. These phytochemical compounds have pharmacological properties. Indeed, Delphinidin and its glycoside derivatives have significant antioxidant activity [39]. Several epidemiological studies have shown a protective effect against coronary heart disease and the consumption of anthocyanins [40]. Regarding cyanidin and its glycoside derivatives, they have antioxidant properties [22, 39] and by scavenging free radicals, it protects cells from oxidative damage and reduces the risk of cardiovascular damage [40] and certain cancers.

CONCLUSION

Hibiscus sabdariffa is a 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* L., 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 calyces is popular. The use of *H. sabdariffa* calyces as natural antioxidants, natural colorants, and an ingredient of functional foods seems to be promising.

ACKNOWLEDGEMENTS

Authors are thankful to Technicians Goussebeo François, Mohi Donald and Yao Parfait (National Agricultural Research Center, Côte d'Ivoire) and the Research Group on biologically Active Plant Compounds, University of Bordeaux Segalen, France, for their contribution to the realization of this study. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

None Declared

REFERENCES

1. Obouayeba AP et al. Evaluation of the effect of alkaloids of *Mitragyna ciliata* on markers of immunity and some hematological parameters in rabbits. Int J Res Biosci 2015a; 4(2): 36-43.
2. Obouayeba AP et al. Hepatoprotective and antioxidant activities of *Hibiscus sabdariffa* petal extracts in Wistar rats. Int J Basic Clin Pharmacol 2014b; 3(5): 774-780.
3. Ahoua ARC et al. Antioxidant activity of eight plants consumed by great apes in Côte d'Ivoire. Afr J Biotechnol 2012; 11(54): 11732-11740.
4. Konan AG et al. Polyphenols content and antioxidant capacity of traditional juices consumed in Côte d'Ivoire. J Appl Biosci 2015; 87: 8015-8021.
5. Obouayeba AP et al. Phytochemical and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) petal extracts. Res J Pharm Biol Chem Sci 2014a; 5(2):1453-1465.
6. Wu XL and Prior RL. Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: Vegetable, nuts, and grains. J Agric Food Chem 2005; 53(8): 3101-3113.

7. Wu XL et al. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem 2004; 52(26): 7846-7856.
8. Kerio LC et al. Characterization of anthocyanins in Kenyan teas: Extraction and identification. Food Chem 2012; 131: 31-38.
9. Christian KR and Jackson JC. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. J Food Compos Anal 2009; 22: 663-667.
10. Lee S et al. Induction of apoptosis in Human leukaemia U937 cells by anthocyanins through downregulation of Bcl-2 and activation of caspases. Int J Oncol 2009; 34: 1077-1083.
11. Viskelis P et al. Anthocyanins, antioxidative and antimicrobial properties of American Cranberry (*Vaccinium macrocarpon* Ait) and their press cakes. J Food Sci 2009; 74: 157-161.
12. Obouayeba AP et al. Cardioprotective and anti-inflammatory activities of a polyphenols enriched extract of *Hibiscus sabdariffa* petal extracts in wistar rats. J Pharmacogn Phytochem 2015b; 4(1): 57-63.
13. Meraiyebu AB et al. Anti-inflammatory Activity of Methanolic Extract of *Hibiscus sabdariffa* on Carrageenan Induced Inflammation in Wistar Rat. Int J Pharma Sci Invent 2013; 2(3): 22-24.
14. Ojeda D et al. Inhibition of angiotensin converting enzyme (ACE) activity by anthocyanins delphinidin- and cyanidin-3-Osambubiosides from *Hibiscus sabdariffa*. J Ethnopharmacol 2010; 128: 7-10.
15. Ologundudu A et al. The effect of *Hibiscus* anthocyanins on 2, 4-dinitro-phenylhydrazine-induced hepatotoxicity in rabbits. Int J Phys Sci 2009; 4(4): 233-237.
16. Kouakou TH et al. Polyphenol levels in two cotton (*Gossypium hirsutum* L) callus cultures. Acta Bot Gallica 2009; 156(2): 223-231.
17. Phillipson JD. Phytochemistry of medicinal plants. Phytochem 2000; 56(3): 237-248.
18. Siriwoharn T et al. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics and antioxidant properties. J Agric Food Chem 2004; 52: 8021-8030.
19. Hariri EB et al. Involvement of flavonoids in the resistance of two poplar cultivars to mistletoe (*Viscum album* L.). Protoplasma 1991; 162(1): 20-26.
20. Laima C et al. Determination of the total phenolic and anthocyanin contents and antimicrobial activity of *Viburnum opulus* fruit juice. Plant Food Hum Nutr 2012; 67(3): 256-261.
21. Chaovanalikit R et al. Characterization and Quantification of Anthocyanins and Polyphenolics in Blue Honeysuckle (*Lonicera caerulea* L.). J Agric Food Chem 2004; 52: 848-852.
22. Bouat-Cottard S. and Burgaud H. Séparation et purification de colorants synthétiques par chromatographie de partage centrifuge à partir d'un brut réactionnel à l'échelle semi-préparative. Spectra Anal 2005; 244: 42-45.
23. Drust RW and Wrolstad RE. Separation and characterization of Anthocyanins by HPLC. In current Protocols in Foods Analytical Chemistry, Wrolstad R.E., eds. New York, John Wiley and Sons, 2001; p 1-13.
24. Obouayeba AP et al. Evaluation of immunostimulatory activity of alkaloids *Mitragyna ciliata* in rabbits (*Oryctolagus cuniculus*). Int J Biosci 2014c; 5(8): 200-206.
25. Koné MW and Kandé B. Qualitative analysis of the pyrrolizidine alkaloids from 11 Asteraceae and Boraginaceae use in traditional medicine in Côte d'Ivoire. J Res Phytochem 2012; 6(3): 75-83.

26. Lopes-Lutz D et al. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. Phytochem 2008; 69: 1732-1738.
27. Ni Q et al. Investigation of the stability and antioxidant properties of anthocyanins-based purple potato colorants after processing. Afr J Biotechnol 2012; 11(14): 3379-3387.
28. Koné MW et al. Assessing Sub-saharain Erythrina for Efficacy: Traditional uses, Biological activities and Phytochemistry. Pak J Biol Sci 2011; 14(10): 560-571.
29. Mungole A. and Chaturvedi A. *Hibiscus sabdariffa* L. a rich source of secondary metabolites. Int J Pharma Sci Rev Res 2011; 6(1): 83-87.
30. Olaleye MT. Cytotoxicity and antibacterial of Methanolic extract of *Hibiscus sabdariffa*. J Med Plants Res 2007; 1(1): 9-13.
31. Ewansiha JU. Evaluation of the antimicrobial activity of roselle (*Hibiscus sabdariffa* L.) leaf extracts and its phytochemical properties. Peak J Med Plant Res 2014; 2(1): 1-5.
32. Lin TL et al. *Hibiscus sabdariffa* extract reduces cholesterol in men and women. Nutr Res 2007; 27: 140-145.
33. McKay DL et al. *Hibiscus sabdariffa* L. Tea (Tisane) Lowers Blood Pressure in Prehypertensive and Mildly Hypertensive Adults. J Nutr 2010; 140: 298-303.
34. Ocho-Anin AL, Kouakou TH, Brou KD, Kouadio YJ, Gnakri D. Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L. (fabaceae) cultivated in Côte d'Ivoire. J Appl Biosci 2010; 31: 1928-1934.
35. Du CT and Francis FJ. Anthocyanins of Roselle (*Hibiscus sabdariffa* L.). J Food Sci 1973; 38: 810-812.
36. Salem MZM et al. Studies on biological activities and phytochemicals composition of Hibiscus species- A review. Life Sci J 2014; 11(5): 1-8.
37. Okonkwo TJN. *Hibiscus sabdariffa* anthocyanins: a potential two-colour end-point indicator in acid-base and complexometric titrations. Int J Pharma Sci Rev Res 2010; 4(3): 123-128.
38. Salazar-Gonzalez C et al. Antioxidant properties and color of *Hibiscus sabdariffa* extracts. Cienc Investig Agrar 2012; 39(1): 79-90.
39. Azevedo J et al. Antioxidant properties of anthocyanidins, anthocyanidin-3glucosides and respective portisins. Food Chem 2010; 119: 518-523.
40. Mink JM, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs JDR. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am J Clin Nutr 2007; 85: 895-909.

Cite this article as:
Abba Pacôme Obouayeba, Moussa Diarrassouba, Eric Francis Soumahin, Tanoh Hilaire Kouakou. Phytochemical Analysis, Purification and Identification of Hibiscus Anthocyanins. J Pharm Chem Biol Sci 2015; 3(2):156-168