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Some anthocyanins isolated and identified from petals and calyces of *Hibiscus sabdariffa* (Malvaceae)

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Abstract

Hibiscus sabdariffa is medicinal and food plant rich in phytochemical compounds which are the source of its biological properties. The present work was conducted in objective to identify the anthocyanins which are medicinally important compounds by the methods such as CPC and HPLC. The results showed that four *Hibiscus* anthocyanins were isolated and identified from petals and calyces of *Hibiscus sabdariffa*. It is cyanidin 3-O-sambubioside, delphinidin 3-O-sambubioside cyanidin 3-O-glucoside and delphinidin 3-O-glucoside with cyanidin 3-O-sambubioside and delphinidin 3-O-sambubioside as the major compounds of the petal of this plant. The presence of these phytochemical compounds justifies its uses in folkloric medicines.

Keywords: *Hibiscus* anthocyanins, cyanidin 3-O-glucoside, cyanidin 3-O-sambubioside, delphinidin 3-O-glucoside, delphinidin 3-O-sambubioside

1. 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 ethnopharmacognosy, 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 [1-4]. *Hibiscus sabdariffa* L., a member of the Malvaceae family, is an annual dicotyledonous herbaceous shrub popularly known as roselle (English), l'oiselle (French), karkade (Arabic) and bissap (Wolof). This plant is well known in Asia and Africa and is commonly used to make jellies, jams and beverages. 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 dried petals 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 petals is popular and highly appreciated by population in most of the West African countries [5]. In folk medicine, it has been used to treat hypertension [6], inflammatory disease [7] and cancer [8]. The flowers of *Hibiscus sabdariffa* contain anthocyanins, flavonoids and polyphenols [9]. Studies have highlighted the role of polyphenolic acid, flavonoids and anthocyanins that may act as antioxidants or have other mechanisms contributing to the cardio protective actions [10]. Anthocyanins are members of the flavonoid group of phytochemicals that are widely distributed in nature, which are responsible for the attractive colors of many flowers, fruits, grains and related products derived from them [11]. 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-phenylbenzopyrylium in nature [12]. Six anthocyanidins are widespread in fruits and vegetables, which are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin [13]. These compounds are based on the same 2-phenylbenzopyrylium (flavylium) skeleton hydroxylated in 3, 5, and 7 positions and different in the number and position of hydroxyl and methoxyl groups in the B-ring [14]. Anthocyanins are valuable as kinds of important quality indicators in foods and chemotaxonomic indicators in plants. The roles of anthocyanin pigments as medicinal agents have been well-accepted dogma in folk medicine throughout the world, and, in fact, these pigments are linked to an amazingly broad-based range of health benefits. Recent research has shown that anthocyanins have numerous health beneficial properties, which include antioxidant [5, 15], anticarcinogenic [16, 17], antimicrobial [18], anti-inflammatory [19],

cardioprotective [19, 20] and hepatoprotective [4] properties. The regular and intensive use of the juice obtained from the flours 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. The aim of this work was to carry out the phytochemical study of the petals and calyces extract of *Hibiscus sabdariffa*. This will generate more knowledgeable informations on their potentiality for a wider utilization.

2. Materials and Methods

2.1 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, malvidin 3-O-glucoside, delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside) were purchased from Sigma-Aldrich (Steinheim, Germany). The trifluoroacetic acid (TFA), methanol (MeOH), n-butanol (n-BuOH), acetic acid and ethyl acetate (EtOAc) were obtained from Merck (Darmstadt, Germany).

2.2 Plant material

The petals and 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 petals and 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.

2.3 Preparation of extracts

The extracts were prepared according to the method of Kouakou *et al.* (2009) [21]. One hundred grams (100 g) petals or 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 petals extract of *H. sabdariffa* (PEHS) and the calyces' extract of *H. sabdariffa* (CEHS) which polyphenols content and compounds were previously determined by Obouayeba *et al.* (2014a) [5] as presented in Fig 1.

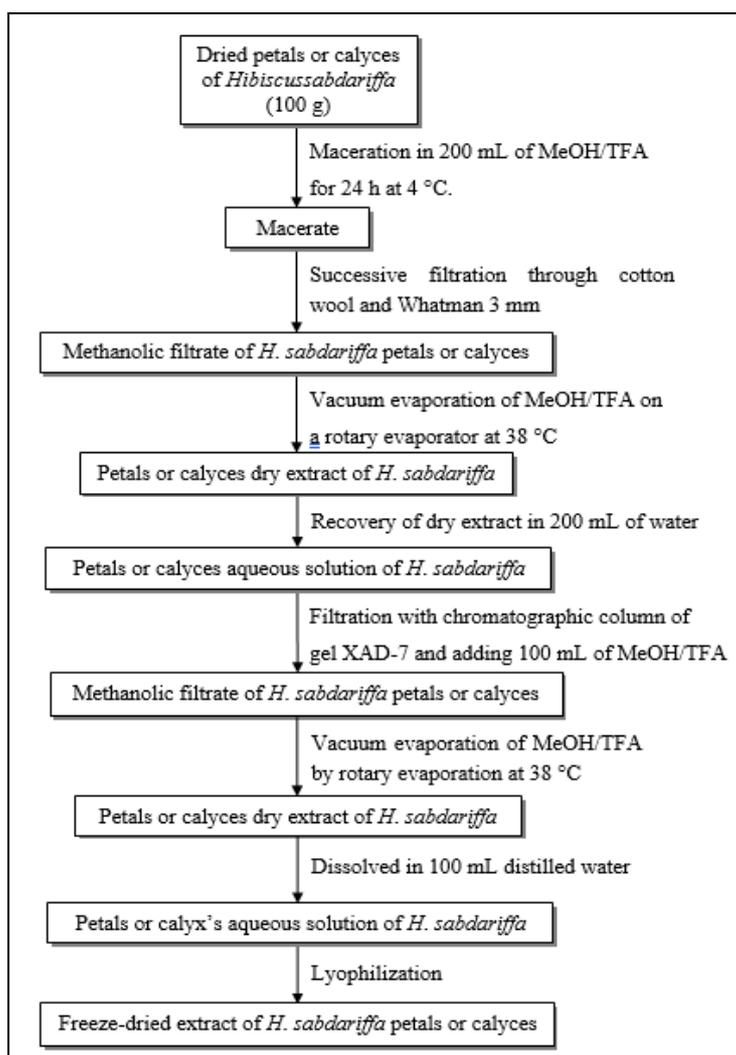


Fig 1: Diagram of obtaining from the petals and calyces extract of *Hibiscus sabdariffa*.

2.4 Centrifugal Partition Chromatography Analysis

Analysis by centrifugal partition chromatography (CPC) was performed according to the method described by Bouat-Cottards and Burgaud (2005) [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.

2.5 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.

2.6 High Performance Liquid Chromatography Analysis

High performance liquid chromatography (HPLC) analysis was conducted using the method described by Drust and Wrolstad (2001) [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) with a flow rate of 0.8 mL/min. The anthocyanins identification and peak assignments are based on their retention times, UV-VIS spectra comparing with standards and published data.

3. Results

3.1 Purification and Identification of *Hibiscus* Anthocyanins from petals

The results of HPLC chromatogram detected at 521 nm showed two major peaks (1 and 2) and two minor peaks (3 and 4) corresponding to the anthocyanins were isolated from the petals extract of *Hibiscus sabdariffa* (Fig 2). Peak assignments are based on matching UV-vis and identical HPLC retention time (Table 1) with known anthocyanins from a reference library of compounds previously purified and identified by anthocyanins identified in petals of *Hibiscus sabdariffa*. The majority compounds of the petals of *Hibiscus sabdariffa* that are Delphinidin 3-O-sambubioside and Cyanidin 3-O-sambubioside have been purified as shown in the figures 3 and 4.

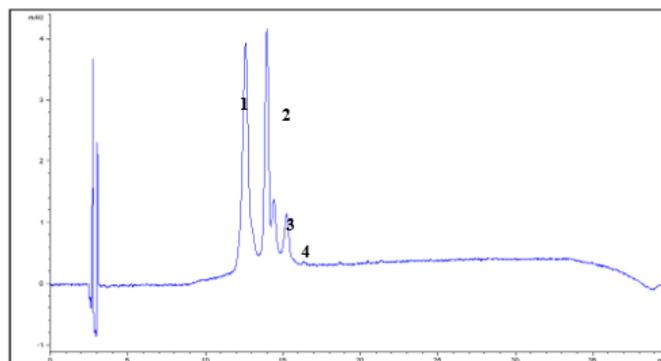


Fig 2: HPLC chromatogram of some *Hibiscus* anthocyanins from petals detected at 521 nm.

Peaks were identified by comparison with reference standards when available or by ¹H NMR data (retention time). 1. Delphinidin 3-O-sambubioside (12.523 min); 2. Cyanidin 3-O-sambubioside (13.910 min); 3. Cyanidin 3-O-glucoside (14.496 min); 4. Delphinidin 3-O-glucoside (15.323 min).

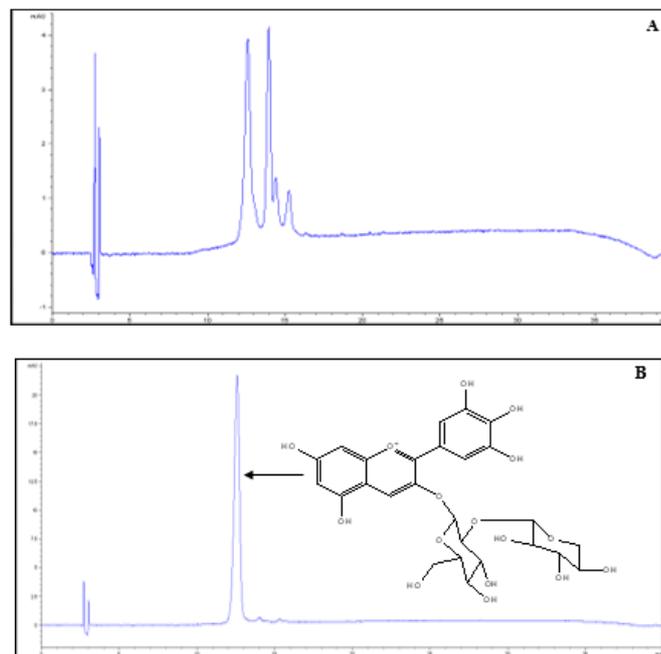


Fig 3: HPLC Chromatograms. A) *Hibiscus* Anthocyanins from petals extract; B) Delphinidin 3-O-sambubioside.

Chromatograms were obtained at 521 nm. Delphinidin 3-O-sambubioside was identified by comparison with the retention time.

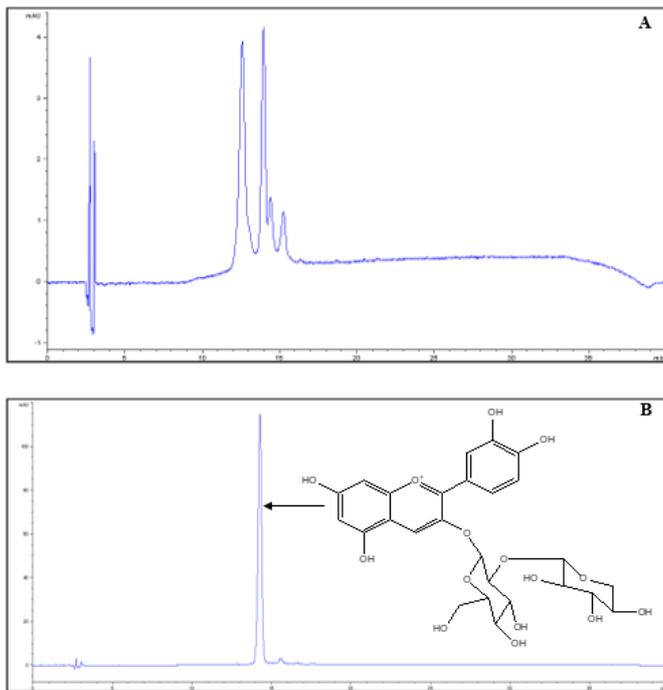


Fig 4: HPLC Chromatograms. A) *Hibiscus* Anthocyanins from petals extract; B) Cyanidin 3-O-sambubioside.

Chromatograms were obtained at 521 nm. Cyanidin 3-O-sambubioside was identified by comparison with the retention time.

Table 1: Retention time in HPLC at 521 nm of the anthocyanin standards.

Nº	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	14.178
7	Delphinidin 3-O-glucoside	15.543
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.

3.2 Purification and Identification of *Hibiscus* Anthocyanins from calyces

The results of chromatogram show that two anthocyanins such as cyanidin 3-O-glucoside and delphinidin 3-O-glucoside (Fig 5) were purified and identified from the calyces' extract of *Hibiscus sabdariffa*. Peak assignments are based on matching UV-vis and identical HPLC retention time (Table 1) with known anthocyanins from a reference library of compounds previously purified and identified by anthocyanins identified in calyces of *Hibiscus sabdariffa*. The two compounds of the calyces of *Hibiscus sabdariffa* that are Cyanidin 3-O-glucoside and Delphinidin 3-O-glucoside have been purified as shown in the figures 6 and 7.

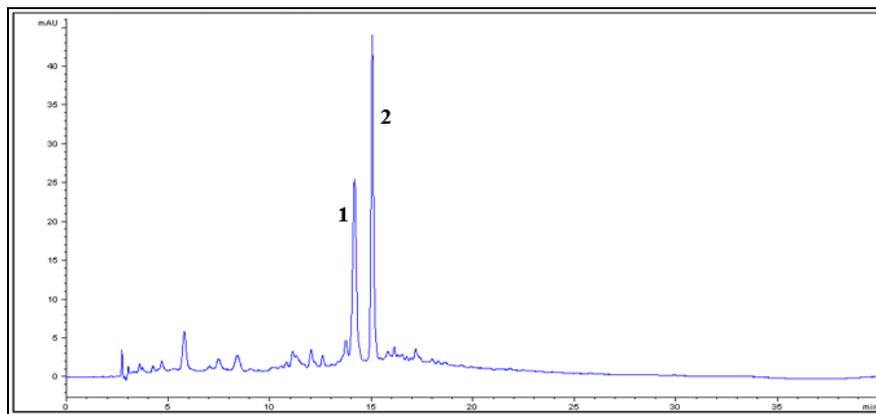


Fig 5: HPLC chromatogram of some *Hibiscus* anthocyanins from calyces detected at 521 nm.

Peaks were identified by comparison with reference standards when available or by ^1H NMR data (retention time). 1.

Cyanidin 3-O-glucoside (14.115); 2. Delphinidin 3-O-glucoside (15.323 min).

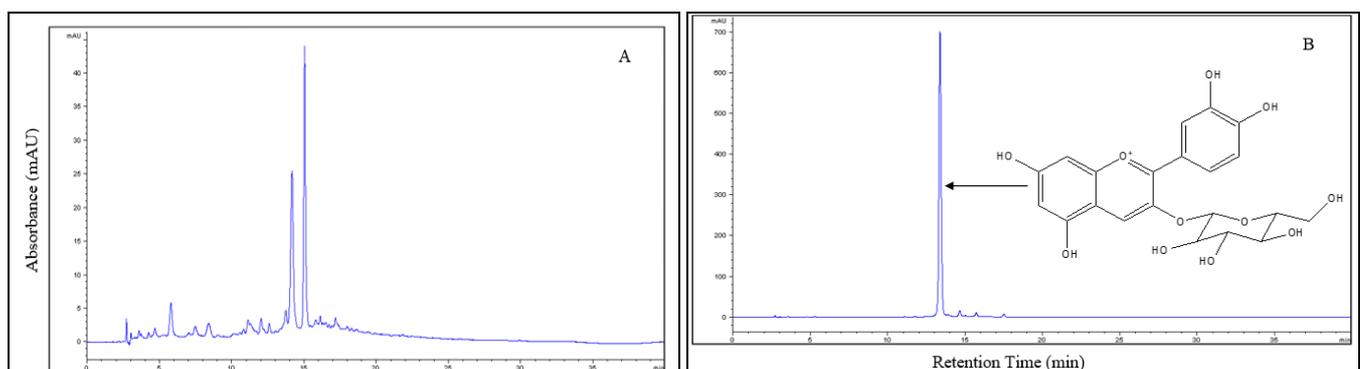


Fig 6: HPLC Chromatogram Profiles of A: *Hibiscus* Anthocyanins from calyces' extract and B: Cyanidin 3-O-glucoside.

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

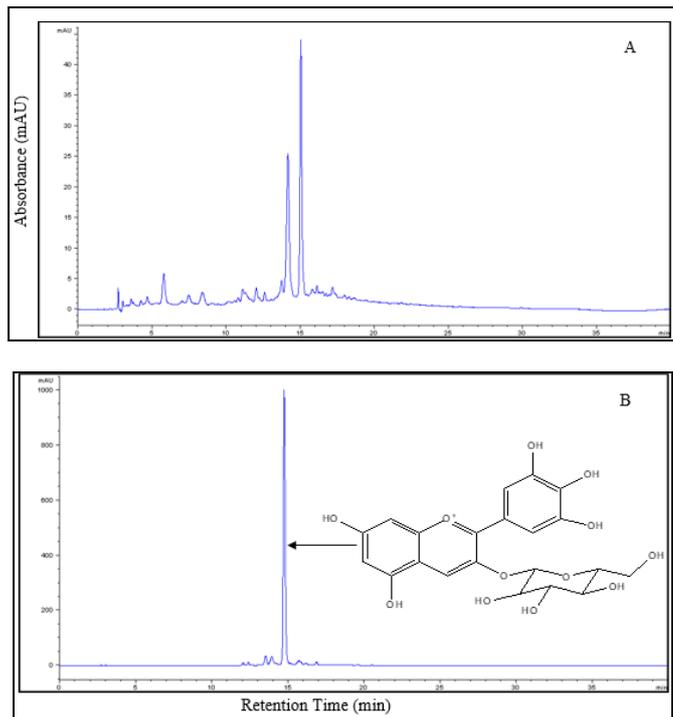


Fig 7: HPLC Chromatogram Profiles of A: *Hibiscus* Anthocyanins from calyces' extract and B: Delphinidin 3-O-glucoside

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

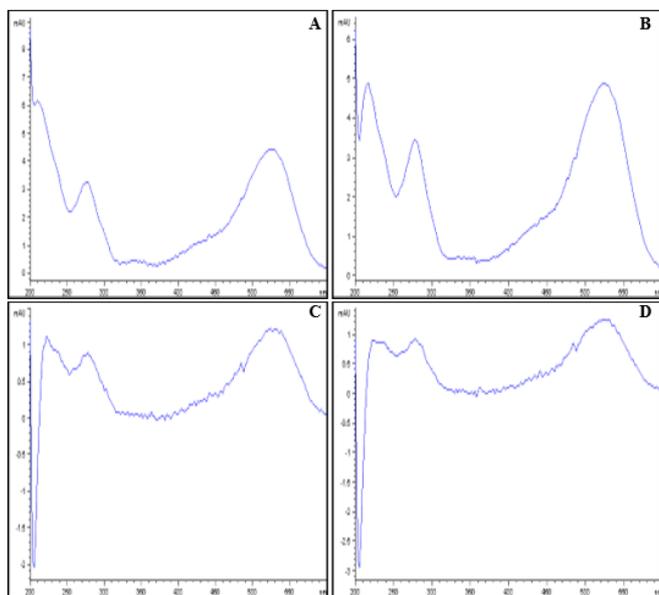


Fig 8: UV-visible spectra of four anthocyanin pigments isolated from *Hibiscus sabdariffa*. A) Cyanidin 3-O-sambubioside (λ_{\max} = 522 nm); B) Delphinidin 3-O-sambubioside (λ_{\max} = 525 nm); C) Cyanidin 3-O-glucoside (λ_{\max} = 530 nm); D) Delphinidin-3-O-glucoside (λ_{\max} = 527 nm). Maximum wavelength (λ_{\max}).

4. Discussion

The screening of plants for medicinal value has been carried out by numerous researchers with the help of phytochemical analysis [2, 24-26]. Phytochemical screening 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 [5, 27-29]. Thus, four anthocyanins were isolated and identified from the petals of *Hibiscus sabdariffa* with two major anthocyanins such as the cyanidin 3-O-sambubioside and the delphinidin 3-O-sambubioside. The two minor anthocyanins isolated and identified from the petals of this plant are the cyanidin 3-O-glucoside and the delphinidin 3-O-glucoside. However, on the four anthocyanins isolated and identified only two have been purified, it is the diglucosides namely cyanidin 3-O-sambubioside and delphinidin 3-O-sambubioside) as the show the Fig. 4 and 5. Our results are in accordance with those obtained by several authors [5, 30, 31]. These phytochemical compounds have pharmacological properties. Indeed, Delphinidin and its glycoside derivatives have significant antioxidant activity [15, 32]. Several epidemiological studies have shown a protective effect against coronary heart disease and the consumption of anthocyanins [20, 33]. Regarding cyanidin and its glycoside derivatives, they have antioxidant properties [32, 34] and by scavenging free radicals, it protects cells from oxidative damage and reduces the risk of cardiovascular damage [20, 33] and certain cancers. According to Lin *et al.* (2007) [9], the presence of anthocyanins in the calyces' extract of *H. sabdariffa* promotes cholesterol reduction in human serum. Similarly, McKay *et al.* (2010) [35] showed that the presence of these molecules in this plant is a therapeutic support in the treatment of hypertension. This clearly shows the use of this plant as herbal medicine. Thus, several works were showed that the anthocyanins are the major constituents in *Hibiscus* extract [9, 26, 36, 37] which natural water-soluble pigments are belonging to the class of flavonoids [38]. 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, Okonkwo, (2010) [39] have shown that anthocyanins were potential natural dyes for these industries. In addition, these results were further supported by the results of the UV visible spectral properties (Fig. 3) that were corroborated with those of Du and Francis (1973) [36]. 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 [34, 36]. These phytochemical compounds have pharmacological properties. Indeed, Delphinidin and its glycoside derivatives have significant antioxidant activity [32]. Several epidemiological studies have shown a protective effect against coronary heart disease and the consumption of anthocyanins [33]. Regarding cyanidin and its glycoside derivatives, they have antioxidant properties [22, 32] and by scavenging free radicals, it protects cells from oxidative damage and reduces the risk of cardiovascular damage [33] and certain cancers.

5. Conclusions

Hibiscus sabdariffa is medicinal and food plant rich in phytochemical compounds such as polyphenols in particular in anthocyanins, 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 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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6. References

- Koné MW, Kakou-Ngazon ES, Dosso M. Assessing Sub-Saharan Erythrina for Efficacy: Traditional uses, Biological activities and Phytochemistry. *Pakistan Journal of Biological Sciences*. 2011; 14(10):560-571.
- Koné MW, Kandé B. Qualitative analysis of the pyrrolizidine alkaloids from 11 Asteraceae and Boraginaceae use in traditional medicine in Côte d'Ivoire. *Research Journal of Phytochemistry*. 2012; 6(3):75-83.
- Obouayeba AP, Soumahin, EF, Atsin GJO, Diabaté S, Latte T, Kouakou TH *et al.* Evaluation of the effect of alkaloids of *Mitragyna ciliata* on markers of immunity and some hematological parameters in rabbits. *International Journal of Research in Biosciences*. 2015a; 4(2):36-43.
- Obouayeba AP, Boyvin L, M'boh MG, Diabaté S, Kouakou TH, Djaman AJ *et al.* Hepatoprotective and antioxidant activities of *Hibiscus sabdariffa* petal extracts in Wistar rats. *International Journal of Basic and Clinical Pharmacology*. 2014b; 3(5):774-780.
- Obouayeba AP, Djyh NB, Diabaté S, Djaman AJ, N'guessan JD, Koné M *et al.* Phytochemical and Antioxidant Activity of Roselle (*Hibiscus Sabdariffa* L.) Petal Extracts, *Research Journal of Pharmaceutical Biological and Chemistry Sciences*. 2014a; 5(2):1453-1465.
- Haji FM, Haji TA. The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. *Journal of Ethnopharmacology*. 1999; 65:231-236.
- Dafallah AA, Al-Mustafa Z. Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. *American Journal of Chinese Medicine*. 1996; 24(3-4):263-269.
- Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U *et al.* Effects of roselle (*Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in F344 rats. *Food Chemistry and Toxicology*. 1999; 37(6):591-601.
- Lin TL, Lin HH, Chen CC, Lin MC, Chou MC, Wang CJ. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. *Nutrition Research*. 2007; 27:140-145.
- Rimm EB, Stamfer MJ. Antioxidants for vascular disease. *Medical Clinics of North America*. 2000; 84:239-249.
- Wu XL, 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. *Journal of Agricultural and Food Chemistry*. 2005; 53(8):3101-3113.
- Wu XL, Gu LW, Prior PL, McKay S. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. *Journal of Agricultural and Food Chemistry*. 2004; 52(26):7846-7856.
- Kerio LC, Wachira FN, Wanyoko JK, Rotich MK. Characterization of anthocyanins in Kenyan teas: Extraction and identification. *Food Chemistry*. 2012; 131:31-38.
- Escribano-Bailón MT, Santos-Buelga C, Rivas-Gonzalo JC. Anthocyanins in cereals. *Journal of Chromatography A*. 2004; 1054(1-2):129-141.
- Christian KR, Jackson JC. Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. *Journal of Food Composition and Analysis*. 2009; 22:663-667.
- Chang YC, Huang HP, Hsu JD, Yang SF, Wang CJ. *Hibiscus* anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells. *Toxicology and Applied Pharmacology*. 2005; 205:201-212.
- Hou DX, Tong X, Terahara N, Luo D, Fujii M. Delphinidin 3-sambubioside, a *Hibiscus* anthocyanin, induces apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathway. *Archives of Biochemistry and Biophysics*. 2005; 440:101-109.
- Viskeliš P, Rubiskienė M, Jasutiene I, Sarkinas A, Daubaras R, Cesoniene L. Anthocyanins, antioxidative and antimicrobial properties of American Cranberry (*Vaccinium macrocarpon* Ait) and their press cakes. *Journal of Food Sciences*. 2009; 74:157-161.
- Obouayeba AP, Méité S, Boyvin L, Yeo, D, Kouakou, TH, N'guessan JD. Cardioprotective and anti-inflammatory activities of a polyphenols enriched extract of *Hibiscus sabdariffa* petal extracts in wistar rats. *Journal of Pharmacognosy and Phytochemistry*. 2015b; 4(1):57-63.
- Ojeda D, Jimenez FE, Zamilpa A, Tortoriello J, Alvarez L. Inhibition of angiotensin converting enzyme (ACE) activity by anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa*. *Journal of Ethnopharmacology*. 2010; 128:7-10.
- Kouakou TH, Waffo-Teguo P, Kouadio YJ, Valls J, Badoc A, Mérillon JM. Polyphenol levels in two cotton (*Gossypium hirsutum* L) callus cultures. *Acta Botanica Gallica*. 2009; 156(2):223-231.
- Bouat-Cottard S, 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 Analyse*. 2005; 244:42-45.
- Drust RW, Wrolstad RE. Separation and characterization of Anthocyanins by HPLC. In *current Protocols in Foods Analytical Chemistry*, Wrolstad R.E., Ed. John Wiley and Sons, New York, 2001, 1-13.
- N'guessan K, Kadja B, Zirihhi GN, Traoré D, Aké-Assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte d'Ivoire). *Sciences and Nature*. 2009;

- 6(1):1-15.
25. Obouayeba AP, Latte T, Okoma M, Diabaté S, Kouakou TH. Evaluation of immunostimulatory activity of alkaloids *Mitragyna ciliata* in rabbits (*Oryctolagus cuniculus*). International Journal of Biosciences. 2014c; 5(8):200-206.
 26. Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou TH. Phytochemical Analysis, Purification and Identification of *Hibiscus* Anthocyanins. Journal of Pharmaceutical Chemical and Biological Sciences. 2015d; 3(2):156-168.
 27. Koné WM, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in North Côte d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. Journal of Ethnopharmacology. 2004; 93:43-49.
 28. Zirihhi GN, Datté JY, Kra-Adou KM, Grelhier P. Phytochemical and pharmacological studies of the alcoholic extract (MFA) of *Fagara macrophylla* (Oliv.) Engl. (Rutaceae): the chemical structure of the active compound inducing antipaludic activity. Journal of Chinese Clinical Medicine. 2007; 2(4):205-210.
 29. Obouayeba AP, Okoma KM, Diarrassouba M, Diabaté S, Kouakou TH. Phytochemical Characterisation and Antioxidant Activity of *Hibiscus sabdariffa* (Malvaceae) Calyx Extracts. Journal of Agri-Food and Applied Sciences. 2015c; 3(2):39-46.
 30. Kouakou TH, Kouakou LK, Decendit A, Badoc A, Da-Costa G, Mérillon JM *et al.* Preparative Purification of Delphinidin 3-*O*-sambubioside from Roselle (*Hibiscus sabdariffa* L.) Petals by fast Centrifugation Partition Chromatography. Journal of Advances in Chemistry. 2014; 6(2):994-999.
 31. Borrás-Linares I, Herranz-López M, Barrajon-Catalán E, Arráez-Román D, González-Álvarez I, Bermejo M *et al.* permeability Study of Polyphenols Derived from a Phenolic-Enriched *Hibiscus sabdariffa* extract by UHPLC-ESI-UHR-Qq-TOF-MS. International Journal of Molecular Sciences. 2015; 16:18396-18411.
 32. Azevedo J, Fernandes I, Faria A, Oliveira J, Fernandes A, Freitas V *et al.* Antioxidant properties of anthocyanidins, anthocyanidin-3-glucosides and respective portisins. Food Chemistry. 2010; 119:518-523.
 33. Mink JM, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA *et al.* Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. American Journal of Clinical Nutrition. 2007; 85:895-909.
 34. Salazar-Gonzalez C, Vergara-Balderas FT, Ortega-Regules AE, Guerrero-Beltran JA. Antioxidant properties and color of *Hibiscus sabdariffa* extracts. Ciencia Investigacion Agraria. 2012; 39(1):9-90.
 35. McKay DL, Chen CY, Saltzman E, Blumberg JB. *Hibiscus sabdariffa* L. Tea (Tisane) Lowers Blood Pressure in Prehypertensive and Mildly Hypertensive Adults. Journal of Nutrition. 2010; 140:298-303.
 36. Du CT, Francis FJ. Anthocyanins of Roselle (*Hibiscus sabdariffa* L.). Journal of Food Science. 1973; 38:810-812.
 37. Kouakou TH, Konkon NG, Ayolié K, Obouayeba AP, Abeda ZH, Koné M. Anthocyanin production in calyx and callus of Roselle (*Hibiscus sabdariffa* L.) and its impact on antioxidant activity. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):9-15.
 38. Smith MAL, Marley KA, Seigler D, Singletary KW, Meline B. Bioactive Properties of Wild Blueberry Fruits. Journal of Food Sciences. 2000; 65(2):352-356.
 39. Okonkwo TJN. *Hibiscus sabdariffa* anthocyanins: A potential two-color end-point indicator in acid-base and complexometric titrations. International Journal of Pharmaceutical Sciences Review Research. 2010; 4(3):123-128.