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Phytochemical Characterisation and Antioxidant Activity of *Hibiscus sabdariffa* (Malvaceae) Calyx Extracts

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ABSTRACT

The calyces of *Hibiscus sabdariffa*, is widely used to manufacture the « Bissap » beverage consumed in West Africa during various ceremonies, this study has the objective to determine its phenolic compounds and evaluate antioxidant activity of extract of these calyces. The phytochemical study was carried out by HPLC and this of antioxidant activity was performed using Wistar rats divided into groups. The treatments were administered via oral route and at single dose for seven days, followed by injection of doxorubicin. Blood samples were collected for the carrying out of markers of oxidative stress. The results obtained led to the identification of two phenolic acids, three anthocyanins and eight flavonoids. The results also showed that anthocyanins (delphinidin 3-O-glucoside and cyanidin 3-O-glucoside), flavonoids (eugenol, gossypetin and quercetin) and protocatechuic acid are the major compounds of the calyx extract of *Hibiscus sabdariffa* (CEHS). In addition, the results helped to highlight the antioxidant property of this plant. Indeed, the toxicity of doxorubicin expressed by the rats of group 2 were significantly different ($p<0.05$) from those of the other groups (control, 3 and 4) for both oxidative stress markers. However, CEHS had attenuated the side effect of doxorubicin through the rats of groups 3 and 4 were statistically identical ($p<0.05$) to the control group for markers of oxidative stress. These results show that the consumption of soft drink commonly known as « Bissap » could help strengthen the antioxidant capacity of the organism

Keywords: *Hibiscus sabdariffa, calyces extract, phenolic compounds, antioxidant activity, Wistar rats.*

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. The results from these plants have revealed the potentials of medicinal plants in the area of

pharmacology (Fatehi , 2003; Somova , 2003; Obouayeba , 2014b). *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 calyxes to produce infusions that are consumed like tea (Domınguez-Lopez , 2008) 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 calyx (the outer whorl of the flower) is eaten raw in salads, or cooked and used as a flavoring in cakes and is also used in making jellies, soups, sauces, pickles, puddings etc. The calyx is rich in citric acid and pectin and so is useful for making jams, jellies (Lepengue , 2009). 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 petals is popular and highly appreciated by population in most of the West African countries.

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 (Burns , 2001), 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 , 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).

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 main aim of this investigation was to identify the major phenolic compounds and to evaluate the antioxidant activity of the calyx 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. 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tris (2-pyridyl)-S-triazine (TPTZ), 1, 1, 3, 3-tetramethoxypropane, acetonitrile, phenolic acids standards (cafeic, chlorogenic, ferulic, protocatechuic and sinapic acids), flavonoids standards (astragalin, catechin, eugenol, gossypetin, gossypetrin, kaempferol, luteolin, myricetin, naringenin, quercetin, isoquercetin, quercitrin and rutin) and 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). Ascorbic acid, methanol, trifluoroacetic acid, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), ferric chloride ($FeCl_3$, $6H_2O$) and ferrous sulfate ($FeSO_4$, $7H_2O$) were obtained from Merck (Darmstadt, Germany). The doxorubicin originated from SC Sindan-Pharma (Bucharest, Romania).

Plant material

The calyces of *H. 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.

Animals

The animals used in this study were Wistar rats which average weight was 185±15 g. These animals which came from the animal house of the Pasteur Institute of Côte d'Ivoire (Adiopodoumé, Abidjan) were housed in cages in the animal house of the Biosciences Training and Research Unit, at room temperature. They had free access to food (pellets from Faci, Côte d'Ivoire) and water. All the experimental procedures were approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

Extract preparation

The extract was prepared according to the method of Kouakou (2009). One hundred grams (100 g) of *Hibiscus sabdariffa* calyces previously freeze dried were extracted in 200 mL of methanol acidified with trifluoroacetic acid 0.1 % (v/v) for 24 h at 4°C. The macerate was filtered successively on cotton wool and Whatman paper 3 mm. 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. One hundred milliliters (100 mL) of methanol 100 % were poured over the gel XAD7 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 calyx extract of *Hibiscus sabdariffa* (CEHS) which was used to achieve the different analyses.

High performance liquid chromatography (HPLC) analysis

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 used was of HPLC grade (Sigma/Aldrich) and was degassed in an ultrasonic bath before using. The water was distilled using a Milli-Q system (Millipore). 50 mg of freeze-dried extract were dissolved overnight with 5 mL of methanol at 4°C in a blender. Sample was centrifuged at 3000 rpm for 10 min. Supernatant was collected and filtered through a Millipore membrane (0.45 µm). The filtrate was twice diluted with purified distilled water. 100 µL of filtrate were injected by an Agilent 1100 series autosampler and chromatograms were simultaneous monitored at 280 nm (phenol acids and flavonoids) and 521 nm (anthocyanins). The elution program was 5-15 % B (0-5 min), 15-25 % B (5-15 min), 25-100 % B (15-30 min) and 100 % B (30-40 min). A reference library of compounds was performed previously with purified compounds in laboratory and also with commercially available compounds such as phenol acids (cafeic, chlorogenic, ferulic, protocatechuic and sinapic acids), flavonoids (astragalin, catechin, epicatechin, eugenol, gossypetin, gossypetrin, gossypin, kaempferol, luteolin, myricetin, naringenin, quercetin, isoquercetin, quercitrin and rutin), anthocyanins (cyanidin, delphinidin, malvidin, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside and 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.

Assessment of antioxidant activity in vivo

Experimental protocol

The assessment of the antioxidant activity *in vivo* of the ECHS was carried out with 20 Wistar rats. The animals were divided into five groups of five rats according to the method described by Zanwar (2011) as follows:

1. Control group: 0.5 mL of 0.9 % NaCl
2. Group 2: 0.5 mL of 0.9 % NaCl + 15 mg/kg body weight (BW) of doxorubicin (DOX)
3. Group 3: 100 mg/kg of ECHS + 15 mg/kg BW of DOX
4. Group 4: 200 mg/kg of ECHS + 15 mg/kg BW of DOX

The rats of the control group and group 2 were treated with 0.5 mL of a solution of 0.9 % NaCl for 1 week. The animals of groups 3 and 4 were treated with the ACHS at different concentrations (respectively, 100 and 200 mg/kg BW) dissolved in NaCl 0.9 % for 1 week. The different administrations were made via oral route at single dose. 1 hr after the last treatments, the rats of groups 2-4 received the DOX via intraperitoneal route (15 mg/kg BW) dissolved in 0.9 % NaCl solution. 24 hrs after injection of doxorubicin, blood samples were taken at the carotid artery of each animal separately in tubes without anticoagulant (dry tubes). The serum was then separated by centrifugation at 2500 rpm for 10 min before being used for determination of the parameters of oxidative stress.

Estimation of lipid peroxidation

The estimation of lipid peroxidation was made in accordance with the method of Satoh, (1978). Lipid peroxidation, a major indicator of oxidative stress, was estimated by TBA reactive substances (TBARS) assay. Thus, 2.5 mL of TCA 20% (m/v) was added to 0.5 mL of serum to precipitate serum proteins. After centrifugation at 3000 rpm for 10 min, 2.5 mL of sulfuric acid (0.05 mol/L) and 2 mL of TBA 0.2% were added to the sediment. This mixture was then stirred and incubated afterwards in a boiling water bath for 30 min. After adding 4 mL of n-butanol, the reaction mixture was centrifuged again at the same speed, and then cooled to room temperature. The supernatant was then collected, and absorbance was read in a spectrophotometer (Spectronic Genesys 5, USA) at 532 nm. The calibration curve was obtained using different concentrations of 1, 1, 3, 3-tetramethoxypropane (1.9-30.5 µmol/L) as a standard to determine the concentration of TBA-malondialdéhydes (MDA) adducts in the sample.

Total antioxidant capacity (TAC) assay

The TAC assay was made using the method described by Benzie and Strain (1996). The serum TAC was determined by measuring its ability to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by the ferric reduction antioxidant parameter (FRAP) method. This method enables to read at 593 nm, the change in absorbance of a blue compound (Fe (II)-tripyridyltriazine) resulting from the reducing action of antioxidants. The FRAP reagent was a mixture consisting 300 mmol/L acetate buffer (pH=3.6), 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L of $FeCl_3$, $6H_2O$ according to the ratio 10/1/1.

On that respect, 20 µL of serum was added to 300 µL of freshly prepared FRAP reagent and preheated at 37°C. After incubation of the reaction medium at 37°C for 10 min, the absorbance of the blue complex was read in a spectrophotometer (Spectronic Genesys 5, USA) at 593 nm against a blank (300 µL FRAP reagent + 10 mL distilled water). Standard Fe^{2+} solutions were prepared at concentrations ranging from 1.56 to 100 mmol/L from ferrous sulfate ($FeSO_4$, $7H_2O$) in distilled water. The results were expressed in µmol ferric ions reduced to the form of ferrous ion per liter (FRAP value).

DPPH radical scavenging activity

The antiradical activity of the serum was carried out according to the method of Yokozawa (1998) with some modifications. It is a method that enables to measure the ability of the serum to inhibit the free radicals produced by the DPPH. A volume of 200 µL of acetonitrile (60% in distilled water) was added to 200 µL of serum in order to deproteinize the samples. The mixture was then incubated for 2 min at room temperature and then centrifuged at 4000 rpm for 10 min. 200 µL of supernatant was then added to 200 µL of a methanolic DPPH solution (100 mmol/L), and the reaction mixture was supplemented with 1 mL of methanol and stirred vigorously. After incubation at room temperature for 10 min, the absorbance was read in a spectrophotometer (Spectronic Genesys 5, USA) at 517 nm. The serum-free acetonitrile solutions were used as control. The ability of the serum to inhibit the free radicals produced by the DPPH was calculated using the following formula:

DPPH inhibition (%) = ([absorbance of blank – absorbance of sample]/absorbance of blank) x 100

Where absorbance of blank is the absorbance of the serum-free, DPPH solution and absorbance of the sample, the absorbance of the reaction mixture containing DPPH and deproteinized serum.

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 $P < 0.05$. All values are expressed as mean ± standard deviation (SD), n = 5..

RESULTS AND DISCUSSION

Identification of phenolic compounds of Hibiscus sabdariffa calyx

The screening of plants for medicinal value has been carried out by numerous researchers with the help of phytochemical analysis (Ram, 2001; Mungole , 2011). 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 (Ongoka , 2006; Lopes-Lutz , 2008; Ni , 2012; Obouayeba , 2014a). The selection of plant parts such as calyx which yields maximum secondary metabolites is the prime or prerequisite step in this investigation.

We identified 10 compounds in Hibiscus sabdariffa calyces (Figure 1): phenolic acids i.e. protocatechuic acid (peak 1) and chlorogenic acid (peak 3); flavonoids i.e. eugenol (peak 2), gossypetin (peak 4), kaempferol (peak 5), quercetin (peak 6), myricetin (peak 7), luteolin (peak 8), rutin (peak 9) and astragalin (peak 10). These compounds were identified by comparison with reference standards whose retention times are presented in table 1. The phenolic acids represent 20 % of total phenolic compounds while flavonoids constitute 80 %. This suggests that flavonoids are predominating. The major phenolic compounds in calyces of Hibiscus sabdariffa are protocatechuic acid (peak 1), eugenol (peak 2), gossypetin (peak 4) and quercetin (peak 6). The presence of these compounds confirms the earlier reports (Ali , 2005; Mahadevan and Pradeep, 2009; Ozdogan , 2011; Carvajal-Zarrabal , 2012). In addition, they reported the presence of astragalin (kaempferol 3-O-glucoside), kaempferol, myricetin, luteolin, rutin and chlorogenic acid. All phenolic compounds isolated in petals of Roselle have pharmacological properties reported by several authors (Dafallah and Al-Mustafa, 1996; Wang , 2000; Cissé , 2009; Rio , 2013). This clearly shows the use of this plant as herbal medicine.

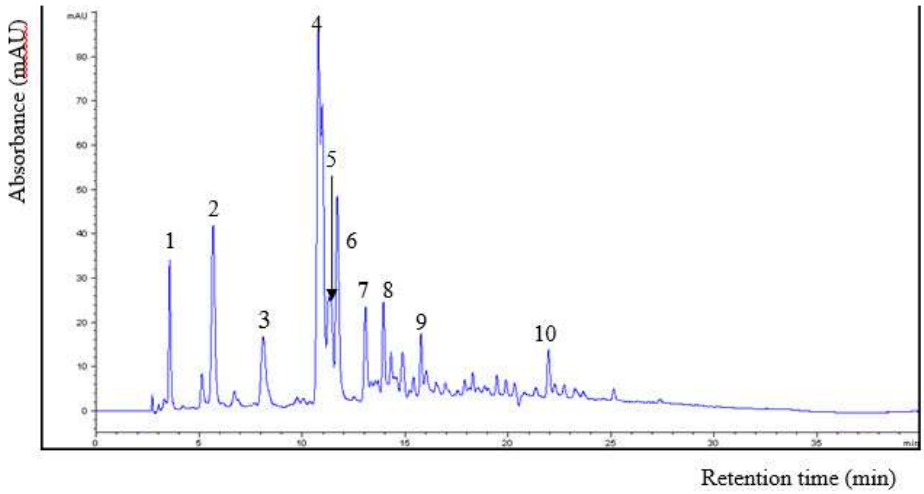

Figure 1. HPLC profile of phenolic compounds from calyx's extract of *Hibiscus sabdariffa*

Detection is shown at 280 nm. Peaks were identified by comparison with reference standards when available (retention time). 1. protocatechuic acid (3.542 min); 2. eugenol (5.655 min); 3. chlorogenic acid (8.084 min); 4. gossypetin (10.850 min); 5. kaempferol (11.371 min); 6. quercetin (11.684 min); 7. myricetin (13.050 min); 8. luteolin (13.922 min); 9. rutin (15.747 min); 10. astragalin (21.936 min).

Table 1. HPLC retention time of phenolic compounds standards at 280 nm

Peak number	Phenolic compound	Retention time (min)
1	Protocatechuic acid	04.469
2	Eugenol	06.125
3	Caffeic acid	06.517
4	Ferulic acid	06.883
5	Sinapic acid	07.792
6	Chlorogenic acid	08.570
7	Catechin	09.570
8	Gossypetin	10.707
9	Kaempferol	11.462
10	Quercetin	11.870
11	Myricetin	12.403
12	Isoquercetin	13.239
13	Luteolin	14.107
14	Gossypetrin	15.273
15	Rutin	15.540
16	Quercitrin	17.048
17	Astragalin	21.716
18	Naringenin	22.625

Three different Hibiscus sabdariffa anthocyanins were separated by reverse phase HPLC (Figure 2). These anthocyanins were identified by comparison with reference standards whose retention times are presented in table 2. Chromatograms showed that cyanidin 3-O-glucoside (peak 1) and delphinidin 3-O-gluoside (peak 2) are the major anthocyanins. Indeed, the absorbance of this one anthocyanin is four-fold lower than that of malvidin 3-O-glucoside (peak 3). The presence these anthocyanins in calyx of Roselle were mentioned by many authors (Ali , 2005; Segura-Carretero , 2008; Salazar-Gonzalez , 2012). However, the major anthocyanins vary depending on the varieties and also the culture country. This clearly shows the influence of soil and climatic conditions on the anthocyanins biosynthesis. Delphinidin, cyanidin and their glycoside derivatives have significant antioxidant activity (Azevedo , 2010). Others studies showed a protective effect of anthocyanin against coronary heart disease and cancers (Chen , 2006). Their regular consumption of the juice obtained from the calyces of Hibiscus sabdariffa is beneficial in prevention cardiovascular and neurodegenerative diseases (Wang , 2000; Kang , 2003; Rio , 2013). The anthocyanins have a recognized pharmacological effect and their red colour is an attractive source of natural food colorants (Yamakawa , 1997; Okonkwo , 2010; Villani , 2013).

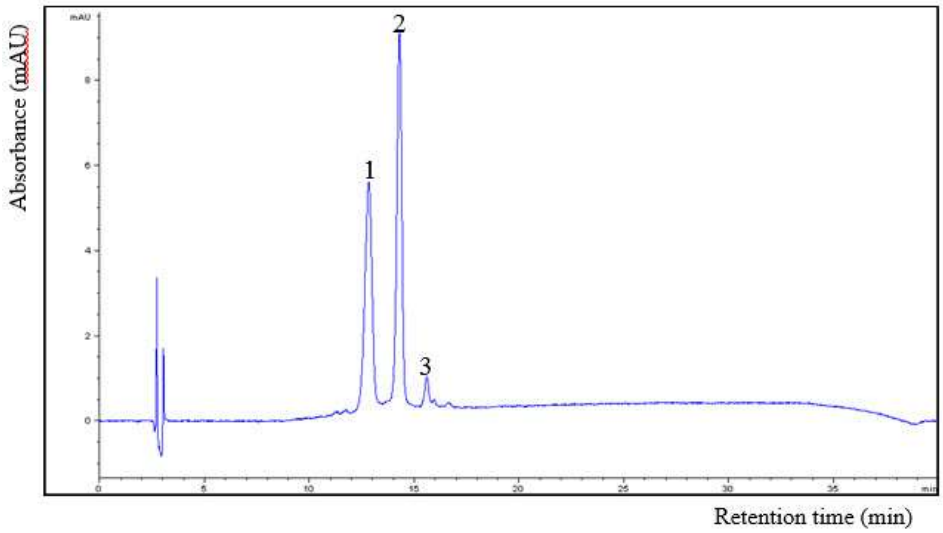

Figure 2. HPLC profile of anthocyanins from calyx's extract of Hibiscus sabdariffa

Detection is shown at 521 nm. Peaks were identified by comparison with reference standards when available (retention time). 1. cyanidin 3-O-glucoside (12.772 min); 2. delphinidin 3-O-glucoside (14.450 min); 3. Malvidin 3-O-glucoside (15.875 min).

Table 2. HPLC retention time of anthocyanin standards at 521 nm

Peak number	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

Antioxidant activity in vivo

The results of this study are shown in Table 3. After injection of doxorubicin, regardless of the oxidative stress parameter studied (TBARS, FRAP, and DPPH), these results enable to assert that the rats of group 2 were statistically different ($p<0.05$) from those of the other groups (control, 3 and 4). In the case of FRAP and DPPH tests, the value of group 2 for each parameter was significantly lower ($p<0.05$) than the control group. However, concerning the values of TBARS, we noticed that the value of group 2 was significantly superior ($p<0.05$) to that of the control group. The values of the parameters tested in rats of the control group and Groups 3 and 4 were statistically identical ($p<0.05$).

The mechanism of doxorubicin-mediated tissue damage suggests an underlying process of oxidation. Therefore, the hypothesis on which this investigation was based is that if the anthocyanin extract of dried flowers of H. sabdariffa possesses antioxidant properties, therefore, it would prevent lipid peroxidation and other metabolic side effects of doxorubicin caused by its oxidant action. Present results demonstrated reasonably well that treatment of rats with the CEHS prior to doxorubicin intoxication significantly inhibited its cytotoxic and other metabolic side effects in the heart. The injection of the doxorubicin caused a significant increase ($p<0.05$) of the TBARS value of rats in group 2 relative to those of rats in other groups (control, 3 and 4). It induces a decrease of the value of FRAP and the percentage inhibition of DPPH of rats in group 2 compared to those of rats in other groups (control, 3 and 4).

The results of lipid peroxidation test are in concordance with those obtained by some authors (Wang , 2000; Ologundudu , 2009; Ahmad-Raus , 2012). The high value of TABRS in group 2, significantly different ($p<0.05$) from that of the control group, indicates lipid peroxidation of polyunsaturated membrane leading to cell necrosis with accumulation of MDA in the serum of rats (Wang , 2000; Ologundudu , 2009; Ahmad-Raus , 2012). The production of MDA in biological tissues is mainly due to free radicals attacks during oxidative stress. There is an alteration of the cell membrane which is the basis of the loss of biochemical and physiological functions of the cell that occurs in cell necrosis (Wang , 2000; Ologundudu , 2009; Samaresh , 2010). The results that show the TAC (FRAP test) of the CEHS corroborate those of Obouayeba (2014b). They show a FRAP value in rats treated by doxorubicin significantly inferior ($p<0.05$) to that in rats from the control group. These results would mean that the injection of doxorubicin causes an oxidative stress with an excessive production of free radicals at the origin of the disequilibrium of the balance antioxidants/pro-oxidants in favor of the latter. They could also be explained by the fact that the injection of doxorubicin would lead to a failure of the antioxidant defense system through the inactivation of enzymes, biochemical

substrates, and trace elements. The results of the measurement of inhibition of DPPH radicals show that the rats from group 2 are significantly different ($p<0.05$) from the ones in the control group.

These results probably reflect the fact that the injection of doxorubicin has brought about an oxidative stress responsible for the failure of the natural antioxidant defense system due to inactivation of enzymes, biochemical substrates, and trace elements. The results of these tests (TBARS, FRAP, and DPPH) clearly show that the doxorubicin-induced oxidative stress in the liver is well correlated with the observed hepatotoxicity through the increase or decrease of the values of the various parameters studied. Nevertheless, treatments of the calyx extract to
H. sabdariffa (Groups 3 and 4) have identical values statistically ($p<0.05$) than the control group regardless either test (TBARS or FRAP or DPPH), indicate the inhibitory effect of these on the oxidative stress induced by the doxorubicin. These results are in line with those of several authors (Wang , 2000; Ologundudu , 2009; Subash , 2011; Hurkadale , 2012). They reflect the antioxidant properties of the extract of H. sabdariffa in line with the conclusions of the works of some authors (Subash , 2011; Hurkadale , 2012).

Table 3. Effect of CEHS on oxidative stress parametres after injection of doxorubicin in rats

Groups	Treatments	TBARS (µmol MDA/L)	FRAP (µmol Fe^{2+}/L)	DPPH (%)
Control group	0,5 mL of NaCl 0,9 %	1,23 ± 0,10 [a]	137,55 ± 6,93 [a]	89,52 ± 4,47 [a]
Group 2	0,5 mL of NaCl 0,9 % + 15 mg/kg BW of DOX	7,59 ± 0,42 [b]	69,37 ± 7,44 [b]	17,00 ± 2,80 [b]
Group 3	100 mg/kg BW of CEHS + 15 mg/kg BW of DOX	1,87 ± 0,27 [a]	127,22 ± 5,96 [a]	70,46 ± 5,20 [a]
Group 4	200 mg/kg BW of CEHS + 15 mg/kg de BW of DOX	1,41 ± 0,17 [a]	130,28 ± 8,13 [a]	78,17 ± 6,10 [a]

The values of the parameters studied are expressed as mean ± SD, n = 5.
In the same column values, studied parameter followed by the same letter are not significantly different ($p<0.05$).
CEHS: Calyces extract of *Hibiscus sabdariffa*, SD: Standard deviation, TBARS: Thiobarbituric acid reactive substances, FRAP: Ferric reduction antioxidant parameter, DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, DOX: Doxorubicin.

CONCLUSION

Hibiscus sabdariffa is an excellent source of dietary phytochemicals such as anthocyanins, flavonoids and phenolic acids. In Côte d'Ivoire, 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 is popular. These results show that the consumption of Bissap could help strengthen the antioxidant capacity of the organism. The use of Roselle calyces as natural antioxidants, natural colorants, and an ingredient of functional foods seems to be promising.

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