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Original Research Article

Hibiscus sabdariffa: Food and Medicinal Plant of the Ivorian Pharmacopeia- an Important Source of Bioactive Substances

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ABSTRACT

The petals of *Hibiscus sabdariffa*, is widely used to manufacture the « Bissap » beverage consumed in West Africa during various ceremonies, the present work has the objective to determine its phenolic compounds and to assess the pharmacological properties of extract of these petals. The phytochemical study was carried out by HPLC and this of pharmacological activities was made 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. After injection of doxorubicin, blood samples were collected for the carrying out of biochemical analyses of markers of hepatotoxicity (albumin, total and direct bilirubin, alanine transaminase, aspartate aminotransferase, and lactate dehydrogenase) and oxidative stress (thiobarbituric acid reactive substances, ferric reduction antioxidant parameter, and 2,2-diphenyl-1-picrylhydrazyl).

The results obtained led to the identification of two phenolic acids, four anthocyanins and 16 flavonoids. The results also showed that anthocyanins (delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside) and flavonoids (gossypetin, hibiscetin, quercetin and sabdaretin) are the major compounds of the petals extract of *Hibiscus sabdariffa* (PEHS). In addition, the results helped to highlight the antioxidant and hepatoprotective properties 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 hepatotoxicity and oxidative stress markers. However, PEHS 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 hepatotoxicity and oxidative stress. These results show that the consumption of soft drink commonly known as « Bissap » could help strengthen the antioxidant and hepatoprotective capacities of the organism.

Keyword: *Hibiscus sabdariffa*; anthocyanins; flavonoids; doxorubicin

INTRODUCTION

Anticancer drugs are widely used against variety of human tumors. However, while they generate acceptable out come in chemotherapy of some cancers, they also exhibit severe toxicity and undesirable side effects [1]. Doxorubicin (DOX) is an anthracycline glycoside antibiotic clinically known as adriamycin isolated from cultures of *Streptomyces peucetius* var *caesius*. The mechanisms of DOX-mediated cytotoxicity in cancer cells and normal tissues include mechanisms related to alterations of DNA and the production of free radicals [2]. However its use in chemotherapy has been limited largely due to its diverse toxicities on the body's tissues, including heart, liver, kidneys and nervous system [3, 4]. Several studies have shown that the combination of the inflammatory process, free radical oxidative stress, and lipid peroxidation is frequently associated with liver damage, induced by toxic agents such as DOX [5]. Persistent and irreversible liver damage has been as a side effect of DOX therapy. It has been observed that there is an increase in the apoptotic processes in liver tissue after a single dose of DOX [6]. The liver is, indeed, a very important vital organ, due to the vital role it plays in various biochemical and physiological processes, notably the metabolic and detoxification functions [7]. Before the crucial role of the liver in the body, it would be necessary to consider some protection of this organ against the toxic effect of doxorubicin exerted on it.

Many medicinal plants are used today in therapy of different diseases. *Hibiscus sabdariffa* L., a member of Malvaceae family, is example in the treatment and prevention of liver diseases such as confirmed and justified by very recent and important works which showed that generally, medicinal plants play a crucial role in protecting the liver [8]. In addition to that, the availability of this species and especially the use of its petals as food fall has become a habit for African populations. Since the juice obtained from the petals of this plant is used extensively in various ceremonies in West Africa in general and in Côte d'Ivoire in particular. The purpose of this study is to identify the major bioactive phytochemicals and to assess the antioxidant and hepatoprotective activities of *H. sabdariffa* petal extracts in Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals

All reagents, solvents and chemical compounds used for analysis met the quality criteria in accordance with international standards. It were 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, malvidin 3-O-glucoside, peonidin 3-O-glucoside, petunidin 3-O-glucoside, cyanidin 3-O-sambubioside, delphinidin 3-O-sambubioside), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) and 1,1,3,3-tetramethoxypropane purchased from Sigma-Aldrich (Steinheim, Germany). The acetonitrile, trichloroacetic acid (TCA), trifluoroacetic acid (TFA), 2-thiobarbituric acid (TBA), ferric chloride ($FeCl_3$, $6H_2O$), ferrous sulfate (FeSO4, $7H_2O$) and methanol (MeOH) were obtained from Merck (Darmstadt, Germany). The doxorubicin originated from SC Sindan-Pharma (Bucharest, Romania).

Plant material

The petals 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 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 Adiopodoumé (Abidjan, Côte d'Ivoire) 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 Ivograins, 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 *et al*. (2009) [9]. 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 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 pure methanol 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) which polyphenols content and compounds were previously determined by Obouayeba *et al*. (2014a) [10] as presented in Figure 1.

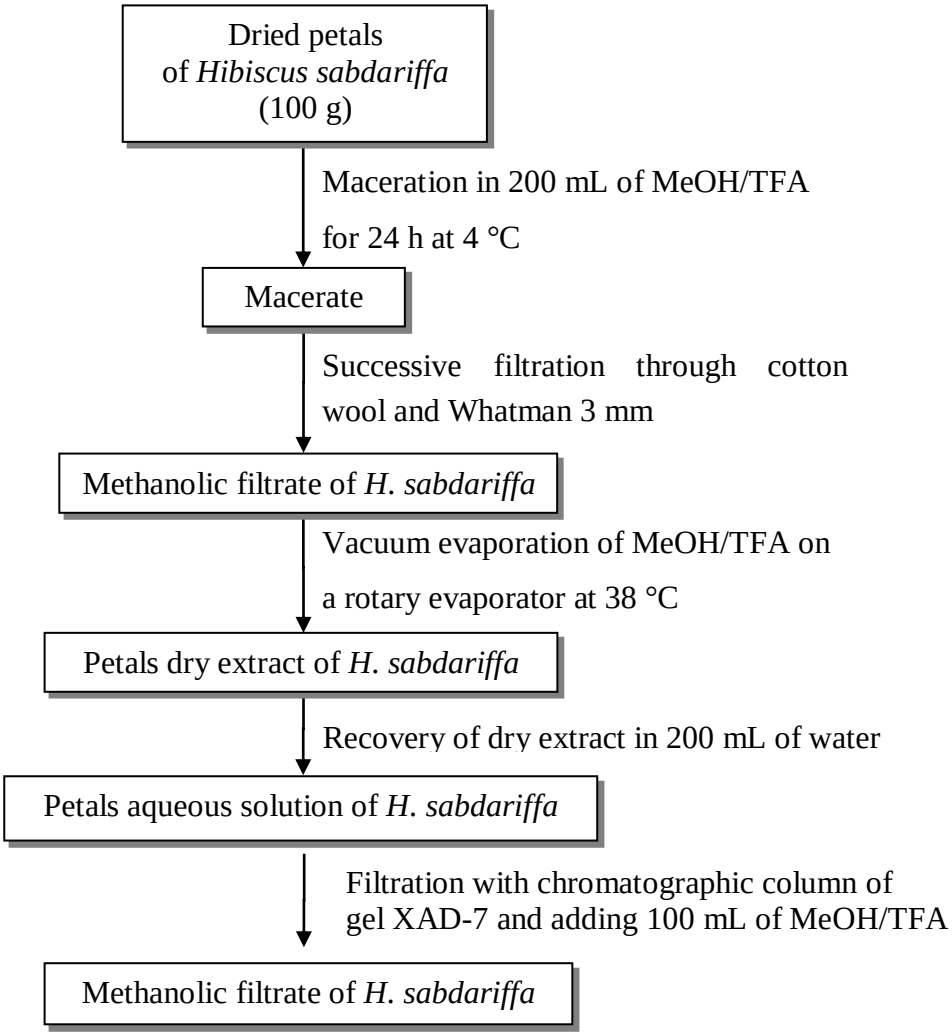
High performance liquid chromatography analysis

High performance liquid chromatography (HPLC) analysis was conducted using the method described by Drust and Wrolstad (2001) [11].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. 100 µL of filtrate were injected by an Agilent 1100 series autosampler and chromatograms were simultaneous monitored at 280 nm (phenol compounds i.e. phenol acids and flavonoids) and 521 nm (anthocyanins). The elution program was 5-15 % B (0-5 min), 15-25 % B (5-15 min), 25-100 % B (15-30 min) and 100 % B (30-40 min).

NMR spectra were recorded on a LC-NMR Agilent 1200 series HPLC/Bruker Avance III spectrometer operating at 600 MHz) for proton. A reference library of compounds was performed previously with purified compounds and identified by ^{1}H NMR in laboratory and also with commercially available compounds such as phenol acids standards, flavonoids standards and anthocyanins standards. 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.



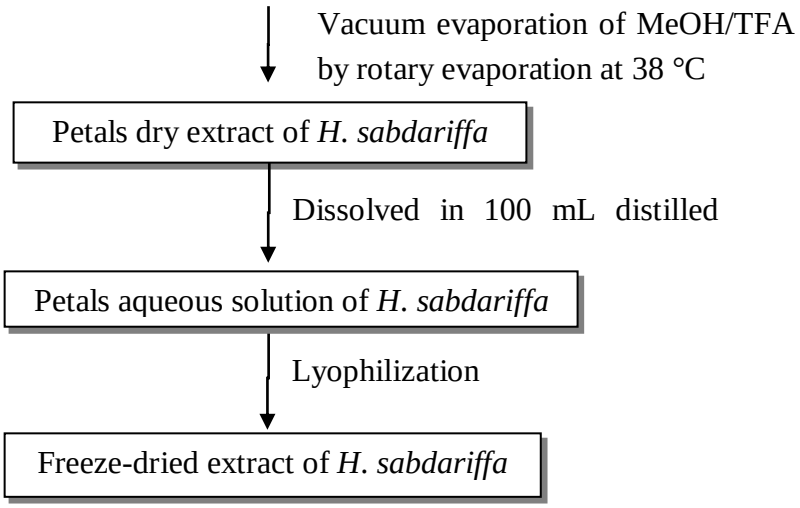


Fig. 1: Diagram of obtaining from the petals extract of *Hibiscus sabdariffa*

Assessment of hepatoprotective and antioxidant activities *in vivo*

Experimental protocol

The assessment of cardioprotective activity of the petals extract of *Hibiscus sabdariffa* (PEHS) was carried out with 20 rats using the method described by Mohan *et al*. (2011) [3]. The animals were divided into four groups of five rats as follows:

Control group: 0.5 mL of 0.9 % NaCl

Group 2: 0.5 mL of 0.9 % NaCl + 15 mg/kg body weight (BW) of Doxorubicin

Group 3: 100 mg/kg body weight of PEHS + 15 mg/kg body weight of Doxorubicin

Group 4: 200 mg/kg body weight of PEHS + 15 mg/kg body weight of Doxorubicin

The rats of the control group and group 2 were treated with 0.5 mL of a solution of 0.9 % NaCl for 7 days via oral route. The rats of groups 3 and 4 were treated with the petals extract of *Hibiscus sabdariffa* respectively at doses of 100 and 200 mg/kg BW for 7 days via oral route. The different treatments were made at single dose. The rats of the groups (2-4) received 0.5 mL of doxorubicin (15 mg/kg BW) via intraperitoneal route, one hour (1 hr) after the last treatment. Twenty-four hours (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 mins before being used for determination of the biochemical parameters of hepatotoxicity and oxidative stress. Similarly, liver samples of the sacrificed animals were collected, rinsed with distilled water, weighed and kept in 10 % formaldehyde (binding agent) for the histopathological study. The relative weight of the rats was determined by the following formula:

RLW (%) = (LW/BW D8) × 100

RLW: Relative liver weight

LW: Liver weight

BW D8: BW at the 8th day

Biochemical parameters of hepatotoxicity

Serum biochemical parameters of hepatotoxicity used in this study are of two types: biochemical substrates (albumin [ALB], total bilirubin [T-BIL] and direct bilirubin [D-BIL]) and enzymatic parameters (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and lactate dehydrogenase [LDH]. These hepatotoxicity markers were measured out with an automatic analyzer (Roche/Integra) using experimental kits (Cobas Integra) following the methods described by the manufacturers.

Antioxidant parameters

Estimation of lipid peroxidation

The estimation of lipid peroxidation was made in accordance with the method of Satoh (1978) [12]. 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 mins, 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 mins. 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) [13]. 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 mins, 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 et al. (1998) [14], 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 mins at room temperature and then centrifuged at 4000 rpm for 10 mins. 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 mins, 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) × 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 $p<0.05$. Data are expressed as mean ± standard deviation (SD), n = 5.

RESULTS

Phytochemical analysis of PEHS

Chromatogram HPLC of phenolic acids and flavonoids

The Figure 2 shows the HPLC profile of the petals extract of *Hibiscus sabdariffa* obtained at 280 nm. It identified 18 phenolic compounds from the petals of this plant. It's about:

- Phenolic acids: chlorogenic acid (4) and protocatechuic acid (6);
- Flavonoids: gossypétrine (1), sabdaretin (2), gossypetin (3), luteolin (5), gossytrin (7), hibiscetin (8), rutin (9), hibiscetrin (10), myricetin (11), eugenol (12), nicotiflorine (13), quercitrin (14), quercetin (15), kaempferol (16), astragalin (17) and the cyranoside (18).

The major compounds present in the petals of *H. sabdariffa* are sabdaretin (2), gossypetin (3), hibiscetin (8) and quercetin (15).

Chromatogram HPLC of anthocyanins

The HPLC profile of anthocyanins present in the petals extract of *Hibiscus sabdariffa* obtained at 521 nm is shown in Figure 3. Four anthocyanins have been identified. There are two diglucidiques anthocyanins (delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside) and two monoglucidiques anthocyanins (cyanidin 3-O-glucoside and delphinidin 3-O-glucoside). Delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside are the major anthocyanins while cyanidin 3-O-glucoside and delphinidin 3-O-glucoside are the minor anthocyanins from the petals of *Hibiscus sabdariffa*.

Assessment of hepatoprotective activity

Effects of the PEHS on the BW, the weight and the relative liver weight of rats after injection of DOX

The results of this study are shown in Table 1. The analysis of this Table 1 shows that the action of DOX has significantly affected the liver (target organ) of rats. Indeed, these results showed that the weight and the relative weight of the liver of the rats of group 2 were statistically superior ($p<0.05$) to those of animals of the other groups (control, 3 and 4). However, the treatments with PEHS inhibit the action of DOX. Thus, the weight and the relative weight of the rats in the control group and groups 3-4 were statistically identical ($p<0.05$). On the other hand, the DOX had no effect on the BW of animals. The BW of rats was statistically the same before and after injection of DOX.

Table 1: **Effect of the PEHS on body weight, the weight and relative liver weight of rats after injection of doxorubicin**

Groups	Treatments	BW D7 (g)	BW D8 (g)	LW (g)	RLW (%)
Control group	0.5 mL of NaCl 0.9 %	200.6 ± 8.5 [ab]	197.5 ± 09.2 [a]	4.40 ± 0.43 [b]	2.25 ± 0.35 [b]
Group 2	0.5 mL of NaCl 0,9 % + 15 mg/kg of BW of DOX	220.6 ± 9.2 [a]	208.0 ± 12.5 [a]	7.70 ± 0.37 [a]	3.70 ± 0.52 [a]
Group 3	100 mg/kg of BW of PEHS + 15 mg/kg of BW of DOX	206.7 ± 9.8 [a]	199.8 ± 11.2 [a]	5.70 ± 0.25 [b]	2.85 ± 0.45 [b]
Group 4	200 mg/kg of BW of PEHS + 15 mg/kg of BW of DOX	210.4 ± 7.2 [a]	206.1 ± 08.6 [a]	5.20 ± 0.52 [b]	2.52 ± 0.25 [b]

The values of the parameters studied are expressed as mean ± SD, n = 5. In the same column the means followed by the same letter are not significantly different ($p<0.05$).BW: Body weight; BW D7: Body weight 7th day; BW D8: body weight 8th day; LW: Liver weight; RLW: relative liver weight. PEHS: Petals extract of *Hibiscus sabdariffa*; DOX: Doxorubicin; SD: standard deviation.

Effects of the PEHS on biochemical substrates after injection of DOX in rats

The results of this study are presented in Table 2. These results show that after injection of the DOX, the rats of group 2 were significantly different ($p<0.05$) from those of the other groups (control, 3 and 4) which were identical for all the analyzed parameters (ALB, T-BIL and D-BIL). The value of the ALB of rats in group 2 was statistically inferior ($p<0.05$) to those of rats in the other groups (control, 3 and 4) which values were of the same order of magnitude. Meanwhile, the values of T-BIL and D-BIL of the animals in group 2 were statistically superior to those of animals in the other groups (control, 3 and 4) which showed values of the same importance. Our results show that the values of the three parameters studied in rats of groups 3 and 4 were statistically identical ($p<0.05$) to those of the control group in each case. The PEHS inhibited the toxicity of DOX.

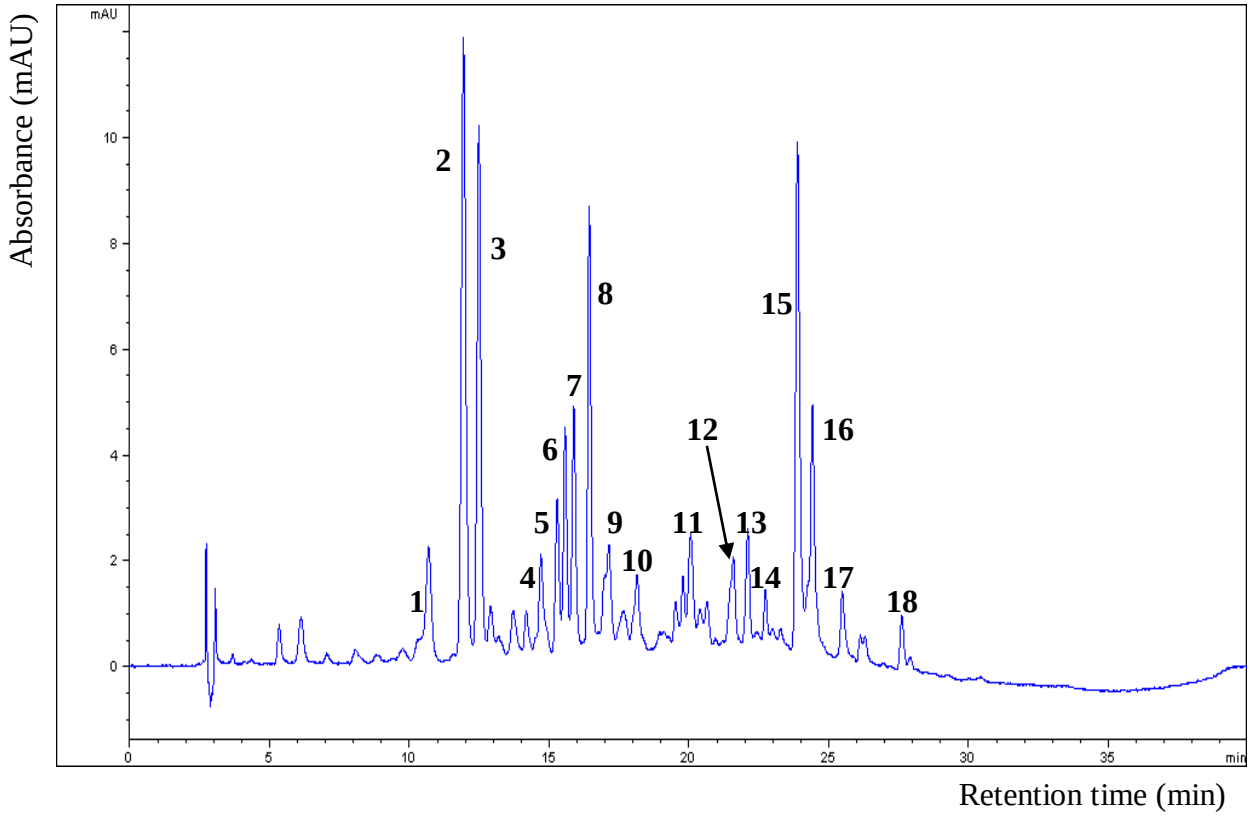


Fig. 2: HPLC profile of phenolic acids and flavonoids from petals extract of *Hibiscus sabdariffa*

Chromatogram was obtained 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).

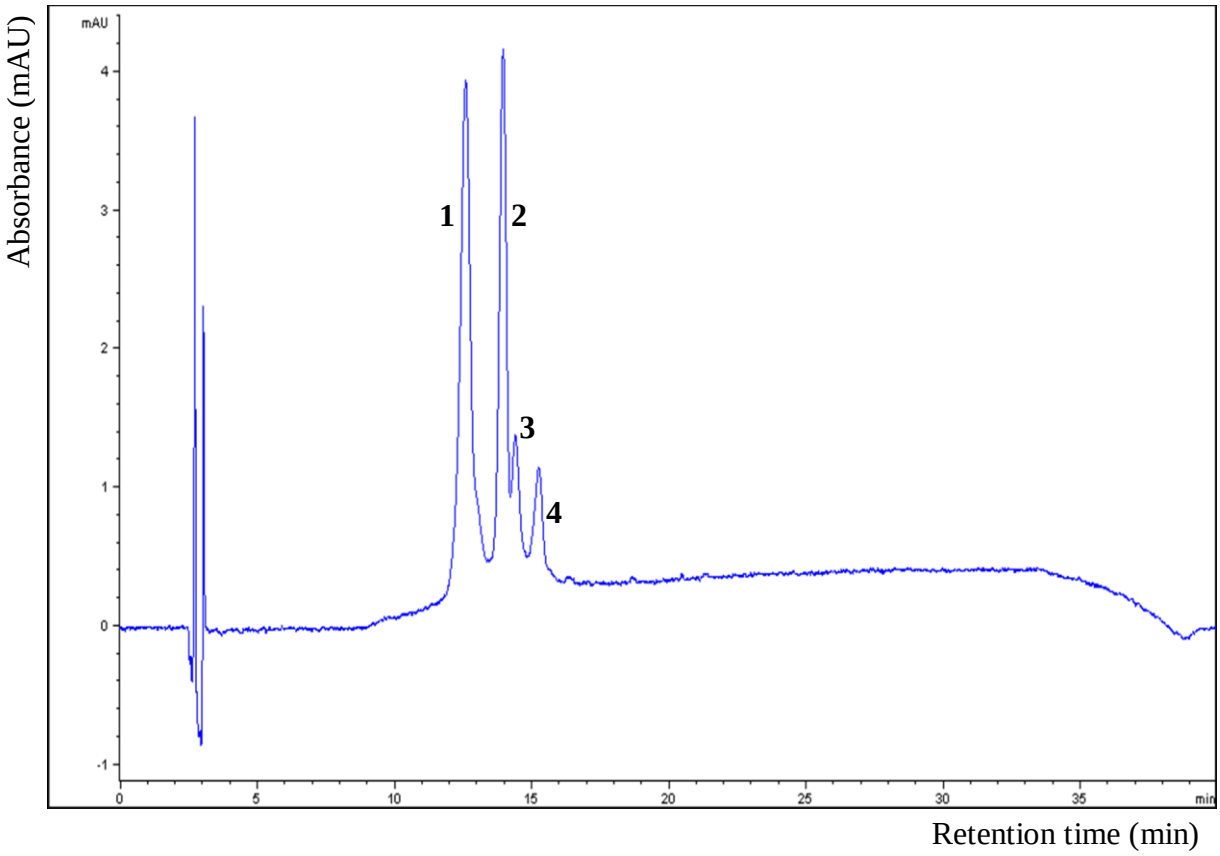


Figure 3: HPLC profile of anthocyanins from petals extract of *Hibiscus sabdariffa*
Chromatogram was obtained at 521 nm. Peaks were identified by comparison with reference standards when available or by ^{1}H NMR data (retention time). 1. delphinidin 3-O-sambubioside (12.681 min); 2. cyanidin 3-O-sambubioside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

Effects of the PEHS on enzymatic parameters after injection of DOX in rats

The results of this study are presented in Table 3. After injection of DOX, we noticed that the rats of group 2 differ significantly ($p<0.05$) from those of the other groups (control, 3 and 4) regardless of the enzymatic parameter analyzed (ALT, AST, and LDH). Indeed, the values of the parameters studied in group 2 were statistically superior, regardless the parameter analyzed, to those of rats in the other groups (Control, 3and 4). These results also show the inhibitory action of the PEHS (groups 3 and 4) on the toxicity induced by doxorubicin through the concentrations of the parameters studied (ALT, AST and LDH) of animals in groups 3 and 4 which were statistically identical ($p<0.05$) to control group.

Table 2: Effect of the PEHS on biochemical substrates after injection of doxorubicin in rats

Groups	Treatements	ALB(g/L)	T BILI (mg/L)	D BILI(mg/L)
Control group	0.5 mL of NaCl 0.9 %	43.30 ± 2.87 [a]	6.07 ± 0.50 [b]	2.12 ± 0.17 [b]
Group 1	0.5 mL of NaCl 0.9 % + 3 mg/kg of BW of DOX	25.50 ± 3.23 [b]	15.93 ± 1.63 [a]	4.80 ± 0.40 [a]
Group 3	100 mg/kg of BW of PEHS + 3 mg/kg of BW of DOX	38.52 ± 2.34 [a]	7.70 ± 0.48 [b]	2.35 ± 0.15 [b]
Group 4	200 mg/kg of BW of PEHS + 3 mg/kg of BW of DOX	41.02 ± 2.15 [a]	6.84 ± 0.44 [b]	2.61 ± 0.18 [b]

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). ALB: Albumin, T BILI: Total Bilirubin, D BILI: Direct Bilirubin. BW: body weight, PEHS: Petals extract of *Hibiscus sabdariffa*, DOX: Doxorubicin, SD: standard deviation.

Table 3: Effect of the PEHS on enzymatic parameters after injection of doxorubicin in rats

Groups	Treatments	LDH(UI/L)	ALAT(UI/L)	ASAT(UI/L)
Control group	0.5 mL of NaCl 0.9 %	103.4± 12.41 [c]	40± 2.77 [b]	60.22± 4.32 [b]
Group 2	0.5 mL of NaCl 0.9 % + 15 mg/kg of BW of DOX	241± 15.54 [a]	72.22± 5.49 [a]	190.04± 9.56 [a]
Group 3	100 mg/kg of BW of PEHS + 15 mg/kg of BW of DOX	137.6± 14 [bc]	45.5± 3.81 [b]	68.9± 3.31 [b]
Group 4	200 mg/kg of BW of PEHS + 15 mg/kg of BW of DOX	114.5± 9.30 [c]	42.94± 5.39 [b]	63.48± 4.06 [b]

The values of the parameters studied are expressed as mean ± SD, n = 5. In the same column, the means followed by the same letter are not significantly different (p<0.05). LDH: Lactate dehydrogenase; ALAT: alanine aminotransferase; ASAT: Aspartate aminotransferase. PAHS: Petals extract of *Hibiscus sabdariffa*; DOX: Doxorubicin; BW: Body weight; SD: standard deviation.

Antioxidant activity *in vivo*

The results of this study are shown in Table 4. After injection of DOX, 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, 2 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 3-4 were statistically identical (p<0.05).

Table 4: Effect of the PEHS on oxidative stress parameters after injection of doxorubicin in rats

Groups	Treatments	TBARS (µmol/L)	FRAP (µmol/L)	DPPH (%)
Control group	0.5 mL of NaCl 0.9 %	1.23 ± 0.10 [b]	137.55 ± 6.93 [a]	89.52 ± 4.47 [a]
Group 2	0.5 mL of NaCl 0.9 % + 15 mg/kg of BW of Doxo	7.59 ± 0.42 [a]	69.37 ± 7.44 [b]	17.00 ± 2.80 [b]
Group 3	100 mg/kg of BW of PEHS + 15 mg/kg of BW of Doxo	1.87 ± 0.27 [b]	127.22 ± 5.96 [a]	70.46 ± 5.20 [a]
Group 4	200 mg/kg of BW of PEHS + 15 mg/kg of BW of Doxo	1.41 ± 0.17 [b]	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 ($p<0.05$). PEHS: Petals 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; BW: body weight.

DISCUSSION

We identified 22 compounds in *Hibiscus sabdariffa* petals: two phenolic acids, four anthocyanins and 16 flavonoids. The major phytochemical compounds identified in petals of *H. sabdariffa* were gossypetin, hibiscetin, quercetin and sabdaretin (flavonoids) while delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside (anthocyanins). The presence of a large number of phytochemicals is in accordance with the results of Mahadevan and Pradeep (2009) [15]. These results show the richness of the natural plant substances as have several authors reported [16, 17]. In addition, these compounds possess several pharmacological properties. Indeed, according to Lin et al (2007) [18], the presence of anthocyanins, flavonoids and polyphenols in the petals extract of *H. sabdariffa* promotes cholesterol reduction in human serum. Similarly, McKay et al. (2010) [19] showed that the presence of these molecules in this plant is a therapeutic support in the treatment of hypertension.

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 [20, 21]. It is a well-documented fact that most medicinal plants are enriched with polyphenol compounds that have excellent antioxidant properties [21]. 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 [22].

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 [21, 23] have shown that anthocyanins were potential natural dyes for these industries. The identification of these properties in these natural compounds gives them the label of bioactive substances. It is clear that petal of *Hibiscus sabdariffa* possess good phytoconstituents that will be helpful in future for the cure of different types of diseases.

The injection of the DOX caused a significant increase ($p<0.05$) of weight and relative liver weight of rats of group 2 relative to those of the rats of the other groups (control, 3 and 4). It

also induces an increase in values of the T-BIL and D-BIL and decreases that of ALB. The enzymatic parameters (ALT, AST, and LDH) are also increased in each case in the rats of group 2 compared to those of rats in the other groups (control, 3 and 4). These effects of DOX are an indication that the action of DOX has significantly affected the liver (target organ) sparing the rest of the body of rats during the time of observation. The results of this study corroborate the works of several authors [24, 25] who have shown that following the injection of a substance inducing hepatotoxicity like DOX, increases of liver weight of rats, as well as the relative liver weight of rats were observed. The increase in value of T-BIL and D-BIL [7, 26] and decrease of ALB [27] are also associated to DOX-induced liver toxicity. Hepatic cytolysis, loss of biochemical functions generate the inhibition of ALB production, expressed by a decrease in ALB. ALB, the major serum protein, plays two main roles, maintenance of the oncotic pressure and transport of various substances such as iron, fatty acids, calcium, hormones, and bilirubin. The latter cannot, therefore, be transported to the hepatocytes to undergo different transformations explaining the accumulation the T-BIL and D-BIL are high. The hepatic cytolysis is accompanied by the alteration of the cell membrane with the loss of the functions thereof. These results are in line with those obtained by several authors [7, 25, 27]. These results enable to assert once again the hepatotoxicity of DOX [4, 28, 29] and highlight the important role of the liver in detoxification of toxic substances [7, 30].

Our results had shown that the treatments with the PEHS (Groups 3 and 4) inhibited the toxicity of DOX. Indeed the different doses of *H. sabdariffa* had attenuated the side effect of DOX like reductions of relative liver weight, ALB, and inhibition of serum liver biomarkers (ALT, AST, and LDH). *Hibiscus sabdariffa* had also normalized the concentration of the T-BIL and D-BIL. This action of *H. sabdariffa* is used to assert the hepatoprotective property of PEHS as already shown some authors [31-34].

The hepatoprotective properties of PEHS are probably due to the presence of major phytochemical compounds of this extract that are gossypetin, hibiscetin, quercetin, sabdaretin, delphinidin 3-O-sambubioside, and cyanidin 3-O-sambubioside. Indeed, some authors have been reported the hepatoprotective property of *H. sabdariffa* extracts rich in anthocyanins [31, 32, 34]. Similarly, other authors have shown the hepatoprotective property of flavonoids including quercetin [35, 36].

The mechanism of DOX-mediated tissue damage suggests an underlying process of oxidation. Therefore, the hypothesis on which this investigation was based is that if the petals extract of *H. sabdariffa* possesses antioxidant properties, therefore, it would prevent lipid peroxidation and other metabolic side effects of DOX caused by its oxidant action. Present results demonstrated reasonably well that treatment of rats with the PEHS prior to DOX intoxication significantly inhibited its cytotoxic and other metabolic side effects in the liver.

The injection of the DOX 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 [31-34]. 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 [31-34]. 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 [30, 31, 34]. The results that show the TAC (FRAP test) of the PEHS corroborate those of Ajuwon et al. (2012) [37]. They show a FRAP value in rats treated by DOX significantly inferior ($p<0.05$) to that in rats from the control group. These results would mean that the injection of DOX 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 DOX 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 DOX 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 DOX-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 aqueous 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 DOX. These results are in line with those of several authors [3, 4, 29]. They reflect the antioxidant properties of the extract of *H. sabdariffa* [31, 32, 34] in line with the conclusions of the works of some authors [38, 39]. These *in vivo* antioxidant activities of PEHS are in accordance with our previous study which had demonstrated the *in vitro* antioxidant activity of the petals extract of *H. sabdariffa* based on the presence of flavonoids and anthocyanins [10].

CONCLUSION

Hibiscus sabdariffa is an excellent source of dietary phytochemicals such as anthocyanins, flavonoids and phenolic acids. In Côte d'Ivoire, the juice of petals of this plant 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 *in vivo* hepatoprotective and antioxidant properties of *H. sabdariffa* can consequently offer a liver protection to the population who consume it and prevent liver injuries. The use of *Hibiscus sabdariffa* petals as natural antioxidants, natural colorants, and an ingredient of functional foods seems to be promising.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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