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Section A: Natural Products & Metabolomics

Phytochemical Study and Evaluation of the Antioxidant Activity of Aqueous and Ethanolic Extracts of *Hua gabonii*

Clément Mutunda Mbadiko[1,2], Gédéon Ngiala Bongo[1,2]*, Koto-te-Nyiwa Ngbolua[1], Sylvie Ngwadi Kasongo[1,2], Adrien Masunda[3], Nadège Kabamba Ngombe[4], Paulin Mutwale Kapepula[4], Marie-Claire Dembo Yandju[1], Pius Tshimankinda Mpiana[5], Théophile Fundu Mbemba[1,2]

[1]*Department of Biology, Faculty of Sciences, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo.* [2]*Research Laboratory of Food and Nutrition (LARAN), Department of Biology, Faculty of Science, University of Kinshasa, Kinshasa XI, Democratic Republic of the Congo.* [3]*Institut Supérieur des Techniques Médicales/ISTM-Kinshasa, P.O. Box 35300, Kinshasa XI, Democratic Republic of the Congo.* [4]*Centre d'Etudes des Substances Naturelle d'Origine Végétale (CESNOV), Faculty of Pharmaceutical Sciences, University of Kinshasa, Kinshasa XI, Democratic Republic of Congo.* [5]*Department of Chemistry, Faculty of Sciences, University of Kinshasa, P.O. Box 190, Kinshasa XI, Democratic Republic of the Congo.*

***Corresponding author:** *Gédéon Ngiala Bongo, Department of Biology, Faculty of Sciences & Technologies, University of Kinshasa, Kinshasa, Democratic Republic of the Congo. Tel. (+243) 840355534*
E-mail address: Gedeon.bongo@unikin.ac.cd

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ABSTRACT

Objective: The aim of this study was to identify major phytoconstituents and evaluate the antioxidant activity of extracts from different organs of *Hua gabonii*. **Methods:** Phytochemical screening was carried out in order to highlight the major groups of secondary metabolites contained in the various extracts through TLC. To assess the antioxidant activity, three methods were used namely DPPH, ABTS and FRAP. The IC_{50} values for the various samples were determined using GraphPad Prism 9.0 software, while ANOVA and LSD multiple comparison test, were carried out using Statistix 10 software. **Results:** The findings showed that the ethanolic extracts of stem bark exhibited a higher activity with DPPH (IC_{50}: 0.84±0.03 µg/mL) compared to the standards (0.81±0.15 µg/mL, 0.42±0.10 µg/mL and 0.33±0.05 µg/mL for gallic acid, catchin and quercetin respectively). In addition, the analysis by the ABTS test showed that leaf extracts also showed interesting activity (IC_{50}: 13.86±0.29 µg/mL and 12.76 ±0.42 µg/mL for aqueous and ethanolic extracts respectively). The FRAP test revealed interesting activity for the aqueous extracts of leaves, stem bark and branch bark with IC_{50s} of 0.006±0.00 µg/mL; 0.02±0.00 µg/mL and 0.02±0.01 µg/mL respectively, compared with 2,41±0.00 µg/mL for ascorbic acid. In addition, the TLC showed the presence of total polyphenols, phenolic acids, coumarins, flavonoids, anthocyanins, leuco-anthocyanins, tannins, quinones, alkaloids and terpenoids. While assay analyses revealed that the total polyphenol content was high in the ethanolic extracts and in particular in the stem extracts. **Conclusion:** *H. gabonii* can be considered as good sources of natural antioxidants.

***Keywords**: H. gabonii, Extracts, Phytochemistry, Evaluation, Antioxidant*

INTRODUCTION

Hua gabonii is one of the plants used as food and like other plants in the Huacaceae family, this species is found in West and Central Africa [1]. Its leaves, fruits or seeds and the bark of its branches are used in food to season dishes [1-3]. The plant is sometimes used as a substitute for garlic and *Scorodophloeus zenkeri* [4]. The young leaves are eaten as vegetables, while the dried ones and then crushed bark are consumed as tea [3, 5]. Moreover, it is also used in traditional medicine for the treatment of different ailments due to the huge arsenal of secondary metabolites with a large spectrum of several biological activities like antioxidant, anti-inflammatory, anti-carcinogenic, etc. [1-3, 5-6].

As to natural antioxidants, several studies reported their benefits in preventing oxidative stress and chronic non-communicable diseases like cancer, diabetes, cardiovascular disease, etc. [1, 7-8]. Recently, the use of natural antioxidants has been of great interest due to studies indicating the possible adverse effects associated with the consumption of synthetic antioxidants. Yet, it is known that several plant resources are considered as natural sources of antioxidants, including aromatic plants [1, 7, 9]. Furthermore, the use of natural antioxidants has an economic impact because they can be extracted from food species that are less known scientifically, such as *H. gabonii*.

However, there is very little scientific literature on this species. Henceforth, the interest of this study is to comparatively evaluate the antioxidant properties of different extracts (leaves, bark of branches and stem) and to carry out phytochemical screening of these different extracts. Studies in this area are rare or have not been carried out.

MATERIAL AND METHODS

Plant material

The samples (leaves, stem and branch bark) of *H. gabonii* used were collected in the province of Nord Ubangi, in the Democratic Republic of Congo (DRC). This specimen was identified by a specialized botanist of the Institut National pour les Etudes et la Recherche Agronomiques (INERA), of the Faculty of Sciences & Technologies, University of Kinshasa. The specimen was also compared to the herbarium number 1262 Campère. The plant was dried in the room temperature for two weeks and then was grounded in order to obtain the powder.

Phytochemical screening

Qualitative Phytochemical screening

It is a qualitative test used to determine the major groups of secondary metabolites using specific reagents to each group. The results are read by observing changes in coloration or the formation of precipitates resulting from the reaction between the chemical groups and the specific reagents used[10-13].

Preparation of total aqueous or ethanolic extract

So, 10 grams of the powder from different samples were macerated in 100 mL of distilled water or 80% ethanol for 24 hours at laboratory temperature and then filtered using Whatman N°1. The filtrates obtained were used for phytochemical screening. The aqueous extracts were used to screen for total polyphenols, flavonoids, tannins, anthocyanins, leuco-anthocyanins, bound quinones, alkaloids and saponins, while the ethanolic extracts were used to screen for steroids, terpenoids and free quinones.

Search for total polyphenols

In fact, 3 mL of aqueous extract was introduced in a test tube and 1 mL of Burton's reagent ($FeCl_3$; $K_3Fe(CN)_6$ 1%, 1:1/V/V) was added. In the presence of polyphenols, the extract turned an intense blue colour, sometimes accompanied by a precipitate. If the test is positive, various polyphenolic compounds are systematically tested for, like flavonoids, anthocyanins, tannins, leucoanthocyanins, etc.

Search for Flavonoid

We took 3 mL of aqueous extract in a test tube and then successively added a few drops of Shinoda's reagent, a few Mangesium chips and a few drops of iso-amyl alcohol. The formation of a colored film in the supernatant layer of the iso-amyl alcohol showed the presence of: flavones (if the color is red to orange), flavonols (if the color is cherry red) or flavonones (if the color is purplish red).

Search for Anthocyanin

We took 3 mL of aqueous extract and then added 1 mL of 20% chloridric acid to the test tubes. The reaction mixture was heated in a water bath for 30 minutes. The presence of anthocyanins is evidenced by a purplish coloration of anthocyanin chloride, which may crystallise.

Search for Tannins

We took 3 mL of aqueous extracts and placed them in test tubes, then Stiasny's reagent (concentrated formalin 30% and chloridric acid; 20:1) was added and heated the rational mixture in a water bath at 30°C for 30 minutes. The presence of catechic tannins is indicated by the formation of a brown precipitate. If the test was positive, the solution was filtered and then saturated with sodium acetate crystals and added 1 mL of 2% $FeCl_3$. A blackish color indicates the presence of water-soluble tannins or galic tannins.

Search for Leuco-anthocyanin

3 mL of aqueous extract were placed into test tubes, and few drops of Shinoda's reagent and iso-amyl alcohol were added. The reaction mixture obtained was heated in a water bath. The presence of leuco-anthocyanins is indicated by a purplish-red coloration in the supernatant layer.

Search for bound quinones

We removed 3 mL of aqueous extract from the test tubes and then added 1 mL of Bornstrager's reagent. The resulting reaction mixture was shaken vigorously. The appearance of an orange to bright red colour indicates the presence of bound quinones.

Search for Alkaloids

We took 3 mL of each plant extract, placed it in test tubes, acidified it with 1 mL hydrochloric acid and then added a few drops of Dragerdorff's reagent. The presence of alkaloids is indicated by a red-orange coloration.

Search for Steroids and triterpenoids

We took 3 mL of each plant extract, placed it in the test tubes and then added a few drops of Liebermann Burchadat reagent (concentrated sulphuric acid and concentrated anhydride, 2:1). A violet coloration indicates the presence of triterpenoids and steroids in mixture. Separately, the triterpenoids have a purple coloration while the steroids develop a green coloration.

Search for free quinones

We took 3 mL of each plant extract and placed it in the test tubes, then added a few drops of Borntrager's reagent (10% NaOH or 10% NH_4OH). The reaction mixture was shaken vigorously. The appearance of a color ranging from orange to bright red attests to a positive test.

Phytochemical screening by Thin Layer Chromatography

Thin layer chromatography (TLC) was performed according to the standard protocol as described in the literature [14-15].

Preparation of samples

So, 1 g of the pulverised drug from each sample was macerated in 10 mL of methanol for 24 hours at laboratory temperature. The macerates obtained were filtered using Whatmann N°1 and the filtrates were used to search for flavonoids and phenolic acids, iridoids, anthocyanins and anthraquinones (10 µL deposit).

To test for terpenoids, 1g of the pulverised drug was macerated in 10 mL of dichloromethane and placed in an ultrasound bath for 45 minutes. The macerate was filtered through Whatmann N°1 and the filtrate used for TLC (10 µL deposit).

To test for alkaloids, 1 g of the pulverised drug from each extract was macerated in 1 mL of 10% ammonia and 5 mL of ethyl acetate. The reaction mixture was left under thermal agitation for 30 minutes. The filtrate (10 µL) was then used for TLC screening.

Search for flavonoids and phenolic acids

The stationary phase was Silicagel F_{254} while the mobile phases were composed of: ethyl acetate-formic, acid-glacial acetic, acid-water (100:11:11:26) for the first phase, dichloromethane-formic acid-acetone (80:10:20) for the second phase and ethyl acetate-methanol-water (100:13.5:10) for the third phase. Rutin, hyperoside, isoquercitrin and chlorogenic acid were used as controls, and were prepared at a concentration of 1 mg/mL with methanol (deposit: 10 µL). The chromatoplates developed were observed under UV at 254 and 366 nm; and then sprayed with DPBAE/PEG reagent and observed under UV at 366 nm. The presence of flavonoids is indicated by the presence of fluorescent spots of various colors (yellow-orange-green) that vary according to the structure of the compounds detected. Blue fluorescence is often due to phenolic acids.

Research into anthraquinones

The mobile phase used was ethyl acetate-methanol-water (100:13,5:10) and the stationary phase was Silicagel F_{254}. The developed chromatoplates were observed under UV light at 254 and 366 nm. After spraying with 10% ethanolic KOH, the anthraquinones stained red and fluoresced red at 366 nm, while the anthrones (aloin) gave a yellow colour.

Search for Iridoids

The mobile phase used was ethyl acetate-methanol-water (100:13.5:10) and the stationary phase was Silicagel F_{254}. Development was carried out using 5% sulphuric acid in ethanol. The chromatoplate was heated at 100°C for 10 minutes. True iridoids gave various colours, while the other terpenes were coloured black.

Search for Anthocyanins

The mobile phase used was ethyl acetate-formic acid-water (100 :10 :40) and the stationary phase was Silicagel F_{254}. Phosphoric acid was used as a developer. The chromatoplates were heated to 100°C for 10 minutes. Anthocyanins gave pink colourations.

Search for Terpenoids

The mobile phase used was toluene-ethyl acetate (93:7) and the stationary phase was Silicagel F_{254}. thymol, menthol and oleanic acid were used as controls. They were prepared at a concentration of 1 mg/mL with

methanol (deposit: 10 µL) and sulphuric vanillin was used as a developer. Terpenes give different colours with this reagent.

Search for Coumarin research

The solution prepared for the terpene test is used: 10 µl deposit. In addition, the mobile phase used was composed of: Toluene-Ethyl acetate-Acetic acid (31 :2,3 :3) while the stationary phase was Silicagel F_{254}. The chromatoplates were observed under UV light at 366 nm. The presence of coumarins is indicated by spots coloured blue, violet or brown.

Search for Alkaloids

The mobile phase used was toluene-ethyl acetate-diethylamine (35 :10 :5) and the stationary phase was Silicagel F_{254}. Quinine was used as a control (deposit: 10 µL) while sodium nitrite (NaNO2) 5% was used as a visible light developer. In this condition, yellow and brown spots indicate the presence of alkaloids.

Determination of polyphenolic compounds

The determination of total polyphenol and anthocyanin content was performed as per the protocol described by Mbadiko et al. [7], flavonoid content as per Adepado et al. [16], tannin content as per Nabil [17] et Nabil et al. [18].

Determination of total polyphenols

Total polyphenol content was determined by the Folin-Ciocalteu method as described by Mbadiko et al. [7]. A reaction mixture comprising 0.2 mL of extract to be analyzed, 2 mL of distilled water and 0.2 mL of folin reagent was prepared. After 3 minutes of incubation, 0.4 mL sodium carbonate (20%) was added. The resulting mixture was shaken well and then incubated for one hour at laboratory temperature in the dark. We carried out the same operation for the blank, except that instead of the extract, we added 0.2 mL 80% ethanol or distilled water. After incubation, the absorbances were read on a UV-visible spectrometer (Jenway 7615) at 725 nm. Each assay was repeated three times.

A standard range with gallic acid (0.5 mg/mL to 0.03125 mg/mL) was prepared to calculate the concentration of total polyphenols in each extract. The results of the assay are expressed as mg gallic acid equivalent per gram of dry matter (mg AGE/g). Using the following equation from the calibration line:

$$y = 7{,}2852x + 0{,}0581 \quad [1]$$

R^2=0,99, where y is the absorbance of the extract and x is the gallic acid equivalent (mg/g).

Determination of flavonoid content

Flavonoid content was determined as described by Adepado et al. [16]. Aluminium trichloride forms a yellow complex with flavonoids that absorbs at 415 nm. We prepared a reaction mixture consisting of 1 mL of the extract analyzed and 1 mL of 2% $AlCl_3$ (dissolved in methanol). The mixture obtained was shaken well and incubated at laboratory temperature in the dark for one hour. The same operation was carried out for the blank, except that 1 mL of 80% ethanol or distilled water was added instead of the extract. Absorbance was measured using a spectrophotometer at 415 nm. Mixtures were prepared in triplicate for each analysis.

A standard range was established separately with quercetin (0.5 mg/mL to 0.03125 mg/mL) to calculate the concentration of flavonoids in each extract. The results of the assay are expressed as mg quercetin equivalent per gram of dry matter (mg EQ/g DM) using the following equation for the calibration line:

$$y = 22.382x - 0.15 \quad [2]$$

y = absorbance of the extract,
x=quercetin equivalent (mg/g).

$R^2 = 0{,}9826$

Determination of condensed tannin content

Tannins are secondary metabolites made up either of oses (usually glucose), or of catchins or triterpenoids to which galloyl units (or their derivatives) are attached, or of oligomers or polymers of flavanols. Condensed tannins, which have a more complex structure, contain no oses in their molecule and are not hydrolysed by either acids or tannases, but in the presence of strong acids or oxidising agents, they are transformed into red substances: phlobaphenes. These are polymers of flavan-3 ols, also known as catchins, and flavan-3,4-diols called leuco-anthocyanidins, or a mixture of the two [17]. Condensed tannins were assayed using the vanillin method as reported by Nabil et al. [18]. Vanillin reacts with free flavan-3-ols and the terminal units of proanthocyanidins giving a red color of which the intensity is proportional to the levels of flavanols present in the medium and which presents an absorption maximum at 500 nm.

We prepared a reaction mixture consisting of: 0.5 mL of 80% aqueous or hydro-ethanolic extracts, 1.5 mL of vanillin and 1.5 mL of chloridric acid. The mixtures obtained were shaken well and incubated for 30 minutes at laboratory temperature in the dark. After incubation, we read the absorbances with a spectrophotometer (Jenway 7615) at 500 nm. We then separately established a standard range with catchin (0.01 to 0.1 mg/mL) to calculate the concentration of tannins in the different samples analyzed.

The tannin content is expressed in mg catchin equivalent per g of the corresponding dry matter (mg EC/g DM) using the equation from the calibration line:

$$y = 34.358x - 0.0132 \quad [3]$$

R2 = 0.9994, where x is the absorbance and y the catchin equivalent (mg/g).

Analysis of antioxidant activity

Screening with the DPPH radical

Screening for antioxidant activity using the DPPH• radical assay was performed according to the protocol reported by Mbadiko *et al.* [7] and Bongo *et al.* [11]. This method is based on the reduction of the DPPH• radical. The addition of an extract containing antioxidant constituents to a solution of the DPPH• radical (violet in colour) reduces this radical by causing its decolorisation, which is measured by the spectrophotometer at 517 nm. The decolorisation of DPPH• is proportional to the antioxidant concentration.

Preparation of different concentrations of the extracts

So, 40 mg of dry aqueous or hydro-ethanolic extracts were weighed and diluted in 4 mL of ethanol 80% or distilled water to obtain a stock solution with a concentration of 10 mg/mL for each extract. From this stock solution, successive dilutions were performed to obtain a concentration range between 10 and 0.015 mg/mL.

Preparation of the DPPH radical

In fact, 3.2 mg of DPPH was dissolved in 100 mL methanol 80%. The resulting solution was kept protected from light for at least one hour. After incubation, the absorbance of the DPPH• radical solution was adjusted to 0.7 ±0.05 using methanol 80%.

Contact the sample with the DPPH radical

So, 20 µL of each extract concentration level was placed in the test tubes and 1980 µL of the DPPH radical analysis solution was added. The same operation was carried out for the control solution (DPPH solution), except that 20 µL of methanol was added in place of the extract. The reaction mixtures were incubated in the dark for 30 minutes. Each concentration was prepared in triplicate. Absorbances were read after incubation at 517 nm using a spectrophotometer (Jenway 7615). Ascorbic acid and gallic acid, quercetin and catchin were used as reference antioxidants (positive control). They were prepared under the same conditions as the plant extracts.

Determination of the inhibition power of the DPPH radical

The percentage inhibition of the DPPH radical by the sample was determined using the following formula:

$$\% \text{ inhibition} = (1 - Ax/Ac) \times 100 \quad [5]$$

Where: Ax: absorbance of the DPPH radical in the presence of the extract. Ac: absorbance of the DPPH radical (control or blank solution)

Screening with the ABTS radical

ABTS (2,2'-azino-bis-3ethylBenz-Thiazoline-6-Sulfonic Acid) forms the blue to green cationic radical ABTS•+ by reacting with potassium or sodium persulfate ($K_2S_2O_8$). However, the addition of an antioxidant reduces this radical and causes the mixture to discolor, which is measured using the spectrophotometer at 734 nm. The decolourisation of the ABTS•+ radical is proportional to the antioxidant concentration [7].

2.1.1.1. Preparation of different concentrations of extracts

So, 40 mg of total dry aqueous or hydroethanol extract was weighed and diluted in 4 mL of ethanol 80% or distilled water. From this stock solution (10 mg/mL), successive dilutions were made to obtain concentrations ranging from 10 to 0.015 mg/mL.

Preparation of the ABTS•+ solution

So, 32.49 mg of ABTS was diluted in 1500 µL of distilled water to form solution A and 8 mg of potassium persulphate ($K_2S_2O_8$) was then weighed and dissolved in 1142 µL of distilled water (solution B). The two solutions were mixed in equal volumes (1:1) and the resulting mixture was kept in the dark for 12 to 16 hours to obtain the stock solution of the ABTS•+ radical, which was then diluted x times with methanol to obtain an analysis solution with an absorbance ranging from 0.800 to 1.000.

Contact the sample with the ABTS radical

We placed 20 µL of the extract to be analyzed for each concentration level in a test tube and then added 1980 µL of the ABTS•+ radical solution. The same procedure was followed for the control solution (ABTS•+ radical solution), except that 20 µL of 80% ethanol or distilled water was added instead of the extract. The reaction mixtures were prepared in triplicate. Ascorbic and gallic acids, quercetin and catchin were used as reference antioxidants and were prepared under the same conditions as the extracts analyzed. After 30 minutes of incubation of the reaction mixtures in the dark, we took successive absorbance readings on a spectrophotometer (Jenway 7615) at 734 nm for the blank (methanol), the control solution (three replicates) and the sample solutions (three replicates per concentration level).

Determination of the inhibition power of the ABTS radical

The percentage inhibition of the $ABTS^{\cdot+}$ radical by the sample is determined using the following formula:

$$\% \text{ inhibition} = (1 - A_x/A_c) \times 100 \quad [6]$$

A_x: absorbance of the ABTS radical in the presence of the extract

A_c: absorbance of the ABTS radical (control or blank solution)

FRAP screening (Ferric Reducing antioxydant Power)

The protocol described by Bougandoura and Bendimerad [19] and Ghizlane [20] was used to screen antioxidant activity using the FRAP method. This method consists of evaluating the reducing power of ferric iron (Fe^{3+}) to iron salt (Fe^{2+}) by a plant's antioxidants. This is the ability of the extract to donate an electron when iron is converted from the Fe^{3+} form to the Fe^{2+} form; this reaction is manifested by the appearance of a blue colour that can be measured at 700 nm. A high absorbance therefore indicates that the extract has a high reducing power.

Then 10 mg of dry aqueous or hydro-ethanolic extracts from each sample were diluted in 10 mL of distilled water or 80% ethanol. We then successively took 0.5 mL, 1 mL, 1.5 mL and 2 mL of each extract, which were placed in test tubes. We added 2.5 mL, 2 mL, 1.5 mL and 1 mL of distilled water respectively to bring the volume of the extracts to 3 mL and obtain different concentrations of the extracts analysed. These extracts were mixed with 1 mL of a 10% phosphate buffer solution (0.2 M and pH 6.6) and 1 mL of a 0.016 M potassium ferricyanide $K_3Fe(CN)_6$ solution. The tubes were incubated for 20 minutes in a water bath at 50°C.

After incubation, 1 mL of 10% trichloroacetic acid (TCA) was added to stop the reaction. The tubes were centrifuged at 3000 rpm for 10 minutes. An aliquot (1.5 mL) of the supernatant was combined with 1.5 mL of distilled water and 0.5 mL of 0.1% (0.02 M) aqueous Fe trichloride ($FeCl_3$). The absorbance of the reaction solutions was read at 700 nm. The control solution was prepared in the same way, replacing the extract with distilled water or ethanol 80%. Ascorbic acid, an antioxidant standard, was used as a positive control and its absorbance was measured under the same conditions as the plant extracts.

The reducing power of iron was calculated using the following equation:

Reducing power of iron (%) = $[\{A_1 - A_0 / A_1\}] \times 100$ [7]

Where:

A_o: is the absorbance of $FeCl_3$ in the absence of the extract (Control)

A_1: is the absorbance of $FeCl_3$ solution in the presence of the extract.

Data analysis

The IC_{50} values for the various samples were determined using GraphPad Prism 9.0 software, while ANOVA and LSD multiple comparison test, were carried out using Statistix 10 software. The CI was of 95% (p-value $\leq$ 0.05). Graphs were produced using Origin 6.1 software.

RESULTS AND DISCUSSION

Determination of secondary metabolites

Phytochemical screening

The qualitative phytochemical screening is presented in **Table 1**.

Table 1. Composition of secondary metabolites in different organs of *H. gabonii*

Secondary metabolites		Used parts		
		Leaves	Stem bark	Branch bark
Total polyphenols		+	+	+
Flavonoids	Flavones	-	-	-
	Flavonones	+	-	-
	Flavonols	-	+	+
Anthocyanins		+	+	+
Leucoanthocyanins		+	+	+
Tannins	Catechic	+	+	+
	Gallic	+	+	+
Linked quinones		+	+	+
Free quinones		+	+	+
Alkaloids		+	+	+
Steroids		-	-	-
Triterpenoids		+	-	-
Saponins		-	-	-

It was observed that different extracts from *H. gabonii* organs contain polyphenolic compounds like flavonoids, anthocyanins, leucoantocyanins, tannins, quinones and alkaloids. With regard to flavonoids, the table shows that the leaves of *H. gabonii* contain flavonones, while the stem bark and branches contain flavonols. It should be noticed the presence of triterpenoids in leaves only.

Furthermore, those extracts did not contain steroids and saponins. Several studies report these major groups identified are responsible for the medicinal properties of plants [1, 18, 20-23]. Mbadiko et al. [1] reported on the presence of tannins and flavonoids in the bark and fruit of H. *gabonii*.

Chemical screening on TLC

Different plates of TLC from different extracts are presented in these figures below.

The presence of flavonoids is revealed by fluorescent spots of various colors (yellow-orange-green) that vary according to the structure of the compounds detected, while blue fluorescence indicates the presence of phenolic acids (**Figure 1.a, b, c**).

Anthraquinones and anthrones (aloin) (**Figure 3**) fluoresce red and yellow respectively. Anthocyanins are revealed by pink fluorescence (**Figure 4**) and terpenes (**Figure 5**) are revealed by spots with fluorescence of various colors. The presence of coumarins is revealed by spots colored blue, violet or

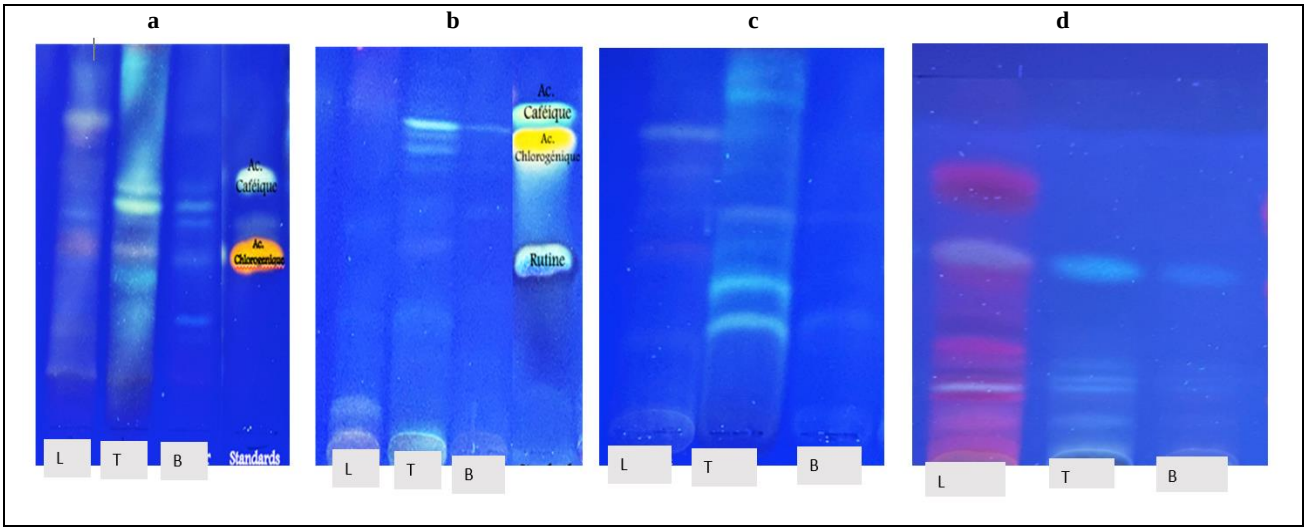

Figure 1. TLC chromatoplates for flavonoids and coumarins. a. Flavonoid chromatoplate, Chromatographic condition: Stationary phase: Silica gel 60 F254, Mobile phase 1: ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26). **b**. Flavonoid chromatoplate: Chromatographic condition: Stationary phase: Silica gel 60 F254, Mobile phase 2: dichloromethane-formic acid-acetone (80:10:20). **c**. Flavonoid chromatoplate: Chromatographic condition: Stationary phase: Silica gel 60 F254, Mobile phase 3: ethyl acetate/methanol/water (100:13.5:10). **d**. Coumarin chromatoplate: Chromatographic conditions: Stationary phase: Silica gel 60 F254, Mobile phase: Toluene-Ethyl acetate-Acetic acid (31 :2,3 :3). ***Legend**: L: Leaves ; T: Stem Bark; B: Branch Bark.*

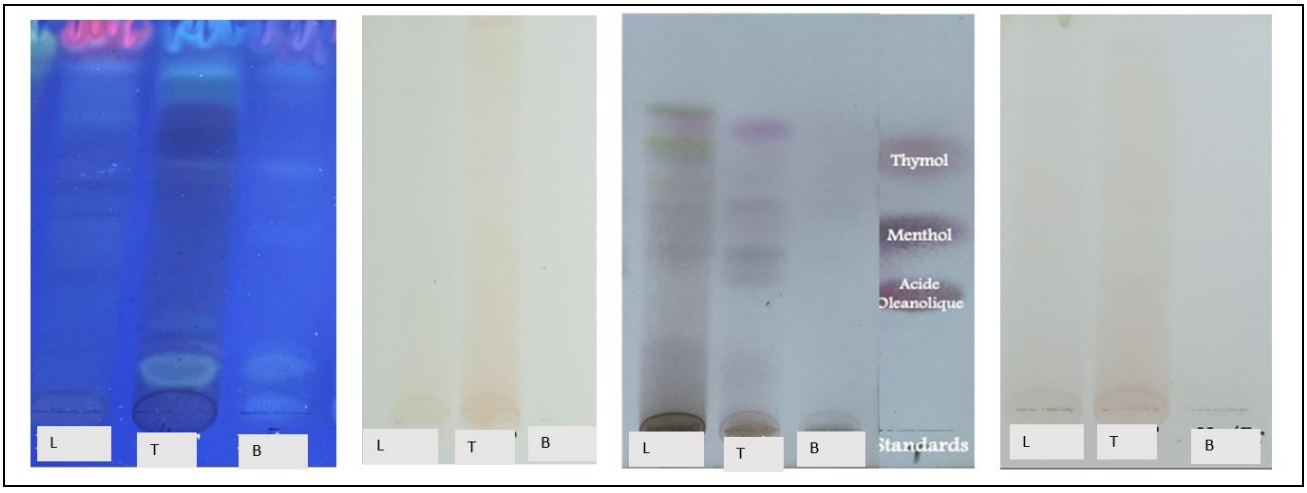

Figure 3. Chromatoplate of anthraquinones. Chromatographic condition: Stationary phase: Silica gel 60 F_{254}. Mobile phase: Ethyl acetate-Methanol-Water (100 :13.5 :10).

Figure 4. Chromatoplate of anthocyanins. Chromatographic condition: Stationary phase: Silica gel 60 F_{254}. Phase mobile: Ethyl acetate -formic acid formique -eau (100 :10 :40).

Figure 5. Chromatoplate of terpenes. Chromatographic condition: Stationary phase: Silica gel 60 F_{254}. Mobile phase: toluene-ethyl acetate (93:7).

Figure 6. Chromatoplate of iridoids. Chromatographic condition Stationary phase: Silica gel 60 F_{254}. Mobile phase: Ethyl acetate-Methanol-water (50:6.7:5).

***Legend**: L: Leaves ; T: Stem Bark; B: Branch Bark.*

brown (**Figure 2**) while that of alkaloids is shown by yellow and brown spots (**Figure 7**). The presence of iridoids in the chromatographic condition used in this study is shown by spots of various colors (Figure 6). Leaf extracts (L), stem bark (T) and branch bark (B) were analyzed.

Different spots presented below (**Figures 1-7**) also indicate that the extracts contained various phytocompounds, mainly flavonoids, phenolic acids, coumarins, anthocyanins, terpenoids and alkaloids. Although the phytochemical profile varied according to the part used.

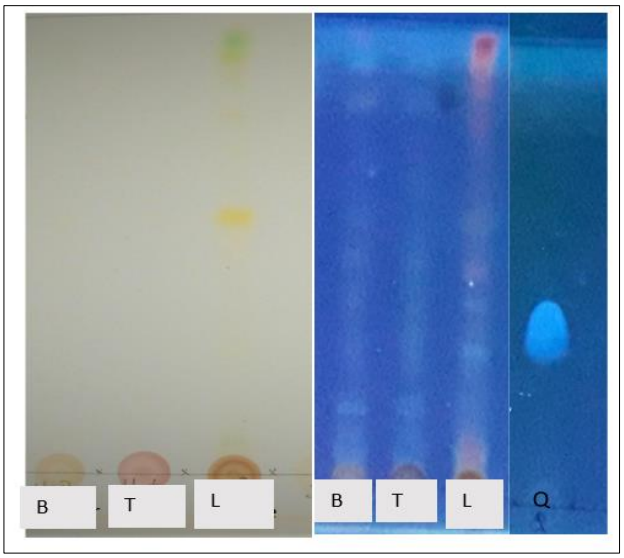

Figure 7. Alkaloid chromatoplate. Chromatographic condition: Stationary phase: Silica gel 60 F_{254}. Mobile phase: toluene-ethyl acetate-diethylamine (35:10:5). ***Legend**: L: Leaves ; T: Stem Bark; B: Branch Bark.*

Determination of polyphenolic compounds

Table 2 presents the determination of polyphenolic compounds contained in different extracts of *H. gabonii*

Comparing total polyphenol concentration in aqueous extracts from different organs of *H. gabonii*, the ANOVA showed a highly significant difference (p-value: 0.0000), while the LSD multiple comparison test showed that stem bark extracts had a high total polyphenol content (524 ± 0.37 mg/GAE/g), followed by leaf extracts (295.75 ± 0.60 mg/GAE/g) and branch bark extracts (84.36 ± 0.00 mg/GAE/g) (Table 2). As for the ethanolic extracts, the LSD multiple comparison test showed that stem bark extracts had a high total polyphenol content (783.22 ±0.17 mg/GAE/g), followed by leaf extracts (463.57 ±0.16 mg/GAE/g) and branch bark extracts (272.68 ±0.46 mg/GAE/g). In addition, the analysis of variance showed that there was a highly significant difference in the concentration of total polyphenols in the hydro-ethanol extracts of the samples analyzed (p-value: 0.000) (**Table 2**).

With regard to flavonoids, the LSD multiple comparison test revealed that, for the ethanolic extracts, stem bark extracts had a high flavonoid content (38.72±0.87 mg QE/g), followed by leaf extracts (36.93±0.33 mg QE/g) and branch bark extracts (34.22±0.50 mg QE/g). Furthermore, the ANOVA revealed that there was a highly significant difference in flavonoid content in the ethanolic extracts (p-value: 0.0003). As for the aqueous extracts, the LSD multiple comparison test revealed that flavonoid content was significant in stem bark (34.90±1.92 mg QE/g), followed by branch bark (24.45±0.79 mg QE/g) and leaf extracts (24.00±0.27 mg QE/g); and that there was no significant difference in flavonoid concentration in the aqueous extracts of branch bark and leaf. Furthermore, when comparing the flavonoid content in the ethanolic and aqueous extracts, the LSD multiple comparison test showed that ethanolic extracts of stem bark include a significant concentration of flavonoids followed by ethanolic extracts of leaves, followed by aqueous extracts of stem bark and ethanolic extracts of branches, followed by aqueous extracts of branch bark and leaves. The LSD multiple comparison test also showed that there was no significant difference in flavonoid concentration in the aqueous extracts of stem bark and ethanolic extracts of branch bark and in the aqueous extracts of branch bark and leaves. In addition, the ANOVA showed a highly significant difference in flavonoid concentration in the aqueous and ethanolic extracts (p-value: 0.0000).

With regard to tannins, the LSD multiple comparison test revealed that the aqueous extracts of stem bark had a high tannins content (26.52±4.35 mg CE/g), followed by leaves (21.02±0.27 mg CE/g), and last branch bark (14.43±0.22 mg CE/g). Besides, the ANOVAshowed that there was a significant difference in the tannins content of the aqueous extracts of the different organs analyzed (p-value: 0.0032). As for the ethanolic extracts, the LSD multiple comparison analysis also showed that stem bark had a high tannins content (26.11±1.42 mg CE/g) followed by leaves (26.11±1.42 mg CE/g) followed by branch bark (9.34±1.35 mg CE/g). Moreover, the ANOVA showed a highly significant difference in the concentration of tannins in the ethanolic extracts of the *H. gabonii* organs (p-value: 0.0000).

Furthermore, when comparing the total polyphenol content of the aqueous and ethanolic extracts of different *H. gabonii* organs analyzed, the ANOVA showed a highly significant difference (p-value: 0.0000), while the LSD multiple comparison test revealed that the ethanolic extracts of each organ had a higher total polyphenol content than their aqueous extracts (**Figure 8**).

Biological activity

Antioxidant activity using DPPH and ABTS tests

The antioxidant activity of extracts of leaves stem bark and branch bark of *H. gabonii* is presented in the table below.

For DPPH test, the aqueous extracts of different organs of *H. gabonii* had very low antioxidant activity compared with the standards. Although the LSD multiple comparison test showed that the aqueous extracts of leaves had a relatively interesting activity (IC_{50}: 56.35±0.54 µg/mL) compared to the aqueous extracts of other organs (stem bark: IC_{50}: 735.3±3.25 µg/mL;

Table 2. Total polyphenolic content in leaves, stem bark and branches of *H. gabonii*

Parts used	Polyphenolic compounds					
	Total polyphenols (mg/ GAE/g)		Flavonoids (mg QE/g)		Tannins (mg CE/g)	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Leaves	295.75 ± 0.60 [b]	463.57 ±0.16 [b]	24.00±0.27 [b]	36.93±0.33 [b]	21.02±0.27 [b]	20.27±1.02 [b]
Stem bark	524± 0.37 [a]	783.22 ±0.17 [a]	34.90±1.92 [a]	38.72±0.87 [a]	26.52±4.35 [a]	26.11±1.42 [a]
Branch bark	84.36 ± 0.00 [c]	272.68 ±0.46 [c]	24.45±0.79 [b]	34.22±0.50 [c]	14.43±0.22 [c]	9.34±1.35 [c]
p-value	0.0000	0.0000	0.0001	0.0003	0.0032	0.0000
F	955126.30	2250655.05	74.51	41.29	17.35	133.67
LSD	0.8180	0.5950	2.4324	1.2203	50274	2.5479

***Legend**: **GAE/g**: Gallic Acid Equivalent per gram; **QE/g**: Quercetin Equivalent per gram; **CE/g**: Catchin Equivalent per gram. The superscript letters on the averages reveal the concentration levels of the polyphenolic compounds. These concentration levels are presented in the following order: a>b>c. Within the same column, the averages with the same letters have a similar content of the compound analyzed.*

branch bark: IC_{50}: 3098±4.75 µg/mL). Furthermore, the ANOVA showed a highly significant difference (p-value: 0.0000) in the antioxidant activity for aqueous extracts from different organs. In addition, the LSD multiple comparison analysis showed that theethanolic extracts (stem bark) exhibited an antioxidant activity similar to the standards, with IC_{50} values of: 0.84±0.03 µg/mL; 0.81±0.15 µg/mL; 0.33±0.05 µg/mL; 0.42±0.10 µg/mL for the ethanolic stem bark extract, gallic acid, quercetin and catchin respectively. This activity is higher than that of the one of branch bark (IC_{50}:15.83±1.86 µg/mL) and leaves (IC_{50}: 15.54±1.55 µg/mL) and ascorbic acid (IC_{50}: 2.6±0.17 µg/mL). Although the multi-step comparison test did not reveal any significant difference in the antioxidant action of leaf extracts and branch bark.

For the ABTS test, it was observed that leaves from aqueous extracts have a high antioxidant activity (IC_{50}: 13.86±0.29 µg/mL) compared to the stem bark (IC_{50}: 255±0.47 µg/mL) and branch bark (959.8±1.1 µg/mL) or catchin (IC_{50}: 22.32±0. 34 µg/mL) and quercetin (IC_{50}: 16.41±0.14 µg/mL) used as reference antioxidants. While for the ethanolic extracts, comparison of the IC_{50} values obtained after screening with ABTS (**Table 3**) shows a significant activity with leaves, followed by stem bark and branch bark. This activity was more significant than the one of the standards namely: catchin and quercetin.

Figure 9 compares the antioxidant potential of aqueous and ethanolic extracts of different *H. gabonii* organs analyzed by the DPPH radical test. It was observed that the ethanolic extracts have a greater antioxidant activity than the aqueous extracts. However, the LSD of IC_{50} revealed that the ethanolic extracts of stem bark have a high activity, followed by leaf and branch bark extracts. We believe that the interesting antioxidant activity of the ethanolic extracts is associated with their high content of polyphenolic compounds. Besides, the phytochemical screening showed that the ethanolic extracts contained higher levels of total polyphenols than the aqueous extracts. Mbadiko et al. [7] reported that the DPPH radical test evaluates the potential of mainly polyphenolic compounds. The high content of polyphenolic compounds in ethanolic extracts of stem bark justifies this interesting antioxidant activity.

The antioxidant potential of ethanolic and aqueous extracts using the ABTS radical test is displayed in the figure below.

Figure 10 shows that the ethanolic extract of leaves exhibited high activity in the ABTS test, followed by the aqueous extract of the leaves. Furthermore, the LSD multiple comparison test showed that the activity of leaf extracts was higher than the two other of ethanolic extracts and the standards namely quercetin and catchin. According to Mbadiko et al. [7], the ABTS radical test evaluates the activity of the cocktail formed by polyphenolic compounds and non-polyphenolic compounds (essential oils and other hydrophobic compounds). We believe that the interesting antioxidant power of leaf extracts is linked to the synergistic action of polyphenolic and non-polyphenolic compounds such as essential oils. Plant antioxidants are increasingly recommended these days, given the effects of synthetic antioxidants [1,7]. Moreover, according to some sources in the literature, eating plants with antioxidant properties as nutraceuticals may help in the prevention of chronic non-communicable diseases [1].

Antioxidant activity by FRAP assay

The FRAP antioxidant activity of aqueous and ethanolic extracts is presented in the table below.

It was observed that all the extracts from the aqueous extracts have similar activities but their potential is higher than ascorbic acid. For ethanolic extracts, the same observation was performed as for the aqueous extracts but their activity is as similar as the ascorbic acid.

Table 3. Antioxidant properties of extracts from different organs of *H. gabonii* (IC_{50} in µg/mL)

Samples	IC_{50} (µg/mL)			
	DPPH		ABTS	
	Aqueous extracts	Ethanolic extracts	Aqueous extracts	Ethanolic extracts
Stem bark	735.3±3,25^{b}	0.84±0.03^{c}	255±0.47^{b}	15.35±0.92^{c}
Branch bark	3098±4.75^{a}	15.83±1.86^{a}	959.8±1.1^{a}	15.39±0.14^{c}
Leaves	56.35±0.54^{c}	15.54±1.55^{a}	13.86±0.29^{e}	12.76±0.42^{d}
Gallic acid	0.81±0.15^{d}	0.81±0.15^{c}	0.78±0.06^{g}	0.78±0.06^{f}
Ascorbic acid	2.6±0.17 ^{d}	2.6±0.17^{b}	3.2±0.38^{f}	3.2±0.38^{e}
Quercetin	0.33±0.05^{d}	0.33±0.05^{c}	16.41±0.14^{d}	16.41±0.14^{b}
Catchin	0.42±0.10^{d}	0.42±0.10^{c}	22.32±0.34^{c}	22.32±0.34^{a}
p-value	0.0000	0.0000	0.0000	0.0000
F	833741	183.94	1472055	937.14
LSD	3.82	1.61	0.88	0.75

The superscript letters on the IC_{50} averages indicate the degree of antioxidant power of the extracts analyzed, in the following order: g>f>e>d>c>b>a. Within the same column, averages with the same letters show a similar antioxidant potential. Low IC_{50} values indicate high antioxidant activity.

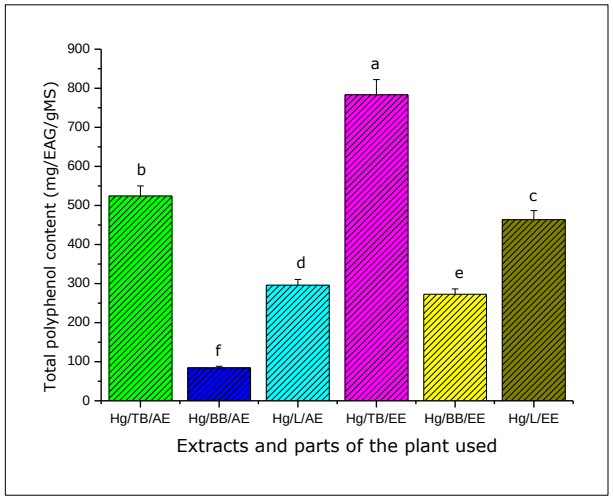

Figure 8. Comparison of total polyphenol content in aqueous and ethanolic extracts of different organs of H. *gabonii*.

Legend: *Hg/TB/AE: Hua gabonii/ Aqueous extracts of stem bark; Hg/BB/AE: Hua gabonii /Aqueous extracts of branch bark; Hg/L/AE: Hua gabonii/ Aqueous extracts leaf extracts; Hg/TB/EE: Hua gabonii /Ethanolic extracts of stem bark ; Hg/BB/EE: Hua gabonii /Ethanolic extracts of branch bark; Hg/L/EE: Hua gabonii /Ethanol extracts from leaves. Superscript letters indicate levels of total polyphenol content. These concentration levels are presented in the following order: a>b>c>d>e>f. P-value: 0.0000; F: 1371076.27; Critical Value for Comparison (LSD): 0.6369.*

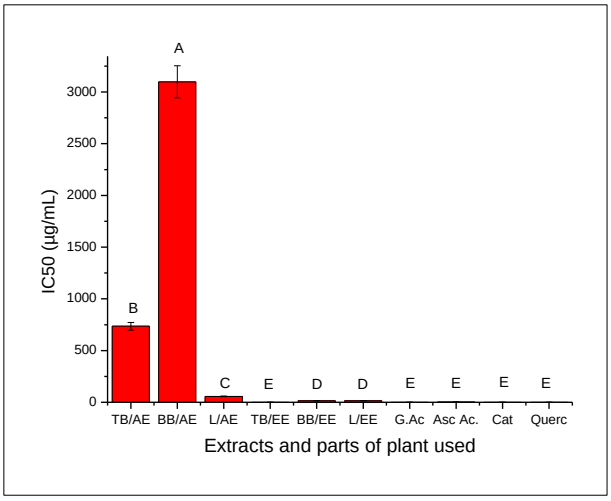

Figure 9. Comparison of the antioxidant potential of aqueous and ethanolic extracts of different organs of *H. gabonii*, analyzed by the DPPH radical test.

Legend: *TB/AE: Aqueous extracts of stem bark; BB/AE: Aqueous extracts of branch bark; L/AE: Aqueous extracts of leaves; TB/EE: Ethanolic extracts of stem bark; BB/EE: Ethanolic extracts of branch bark; L/EE: Ethanolic extracts of leaves. G Asc.: Gallic acid; Asc Ac.: Ascorbic acid; Cat.: Catchin Querc.: Quercetin. The letters on the histograms indicate the antioxidant power levels of the extracts analyzed. These levels are presented in the following order: E>D>C>B>A. Histograms with the same letters show similar antioxidant potential. P-value: 0.0000; F: 728672; Critical value for comparison (LSD): 3.37.*

Table 4. FRAP antioxidant activity of different extracts.

Samples	IC_{50} (µg/mL)	
	Aqueous extracts	Ethanolic extracts
Stem bark	0.02±0.00[b]	2.91±0.98[a]
Branch bark	0.02±0.01[b]	3.10±0.06[fa]
Leaves	0.006±0.00[b]	3.25±0.64[a]
Ascorbic acid	2.41±0.00[a]	2.41±0.00[a]
p-value	0.0000	0.3478
F	74430.13	1.27
LSD	0.0144	1.04

The superscript letters of the IC_{50} averages indicate the degree of Fe^{3+} to Fe^{2+} reducing power of the extracts; in the following order: b>a. Within the same column, averages with the same letters predict a similar reducing potential. In addition, low IC_{50} values indicate a high reducing potential.

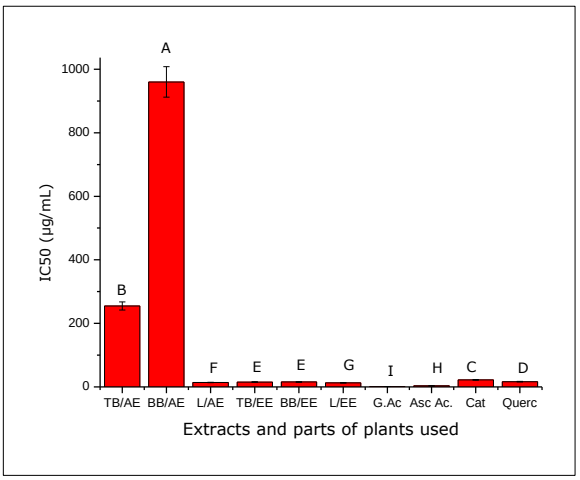

Figure 10. Comparison of the antioxidant potential of aqueous and hydroethanolic extracts of different organs of *H. gabonii*, analysed by the ABTS radical test.

Legend: TB/AE: Aqueous extracts of stem bark; BB/AE: Aqueous extracts of branch bark; L/AE: Aqueous extracts of leaves; TB/EE: Ethanolic extracts of trunk bark; BB/EE: Ethanolic extracts of branch bark; L/EE: Ethanolic extracts of leaves. G Asc.: Gallic acid; Asc Ac.: Ascorbic acid; Cat.: Catchin Querc.: Quercetin. The letters on the histograms indicate the antioxidant power levels of the extracts analysed. These levels are presented in the following order: I> H>G>F >E>D>C>B>A. Histograms with the same letters show similar antioxidant potential. P-value: 0.0000; F: 959917.43; Critical value for comparaison (LSD): 0.9060.

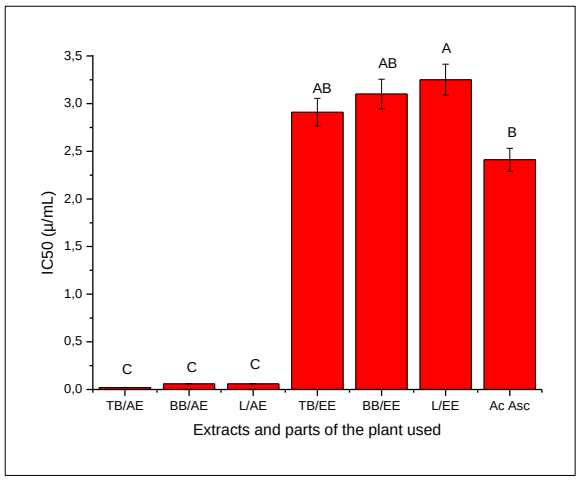

Figure 11. Comparison of the antioxidant potential of aqueous and ethanolic extracts of different organs of *H. gabonii*, analyzed by the FRAP test.

Legend: TB/AE: Aqueous extracts of trunk bark; BB/AE: Aqueous extracts of branch bark; L/AE: Aqueous extracts of leaves; TB/EE: Ethanolic extracts of stem bark; BB/EE: Ethanolic extracts of branch bark; L/EE: Ethanolic extracts of leaves; Asc Ac.: Ascorbic acid.: Quercetin. The letters on the histograms indicate the antioxidant power levels of the extracts analysed. These levels are presented in the following order: C>B>A. Histograms with the same letters show similar antioxidant potential. P-value: 0.0000; F: 42.00; Critical value for comparaison (LSD): 0.73.

The reducing potential of aqueous and ethanolic extracts of different organs of *H. gabonii* is presented in the figure below.

This high activity of aqueous extracts is thought to be linked to the synergy between polyphenolic compounds, bound quinones and alkaloids revealed in aqueous extracts through phytochemical screening.

It can be observed that the aqueous extracts showed greater activity than the ethanolic extracts. Although the aqueous extracts of leaves showed high activity compared to all the extracts screened as well as the ascorbic acid. The FRAP method evaluates the capacity of an extract to donate an electron in order to convert iron from the Fe^{3+} to the Fe^{2+}. Fe^{3+} contributes to

the formation of free radicals, including the peroxy radical (ROO^-) during lipid peroxidation [23]. In addition, the oxidation of Fe^{2+} to Fe^{3+} transforms haemoglobin into methaemoglobin ($HbFe^{3+}$) through the oxidation of Fe^{2+} to Fe^{3+} [24-26].

CONCLUSION

This study showed interesting antioxidant activity of different extracts of *H. gabonii*. Although screening using DPPH radical showed that ethanolic extracts have high antioxidant power. This high activity is thought to be associated with their high total polyphenol content. While the leaf extracts of aqueous extracts showed significant activity using the ABTS radical test. The FRAP test revealed significant activity in aqueous extracts particularly leaves. This high level of activity in aqueous extracts may be associated not only with the presence of phenolic compounds, but also with the presence of bound quinones or alkaloids, which have been shown to be present in aqueous extracts. This plant is used in food as a condiment or as a vegetable (young leaves), and its antioxidant properties are thought to help prevent chronic non-communicable diseases.

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Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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