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Chemical constituents of the methanolic extracts of *Ceiba Pentandra* (L.) Gaertn and the antioxidant activities of its aqueous, ethanolic and hydro-ethanolic extracts

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Abstract
Objective: This work aimed to determine the phytochemical composition of *Ceiba pentandra*, and to evaluate its antioxidant properties *in-vitro*.
Method: The study of the chemical composition was done by chromatography (LC-MS) and the evaluation of the antioxidant activity was done by DDPH, ABTS and FRAP methods.
Result: Phytochemical analysis of extracts led to the identification of 18 compounds. The study of the antioxidant activity showed that aqueous fruits extract, hydroethanolic leaves' extract and the decoction of the mixture of leaves and roots has the best antioxidant activity respectively on FRAP (IC_{50} = 13.91 µg / mL), ABTS (IC_{50} = 14.81 µg/mL) and DPPH (IC_{50} = 11.13 µg / mL) method.
Conclusion: Studies carried out on *Ceiba pentandra* extracts show the diversity of secondary metabolites in this species and significant antioxidant activity.

Keywords: *Ceiba pentandra*, chromatographic profiles, quantitative assays, antioxidant activity

1. Introduction
Human beings are often exposed to various diseases, the cause of which could be an excessive presence of free radicals in their bodies, which could lead to quick and premature aging of cells thereby facilitating the emergence of various diseases such as atherosclerosis, inflammatory problems, the aging process, cardiovascular and pulmonary diseases, cancers, diabetes [1]. Thus, the best protection against this phenomenon seems to be therapeutic plants with antioxidant properties. The best way of loading the body with antioxidants is through consumption of natural plants. Some plants have proven to possess exceptional antioxidant properties which can fight harmful elements that tend to weaken the body. However, numerous intervention studies carried out with antioxidants poorly consumed did not yield the spectacular results expected in the prevention of complications associated with this pathology. This is why researchers at the University of Douala have made research on plants with antioxidant properties a priority. Hence, our main objective is to study the antioxidant activities of the extracts of the roots (R), leaves (Fe), barks (E) and fruits (Fr) of *Ceiba pentandra* (L.) Gaertn, a plant of the African pharmacopoeia known for its antioxidant properties [2] and compare this activity to that of the decoction of a mixture of roots and leaves (R + Fe) of the same plant. As specific objectives, we studied the chemical composition of the methanolic extracts of the different parts of the plant, we also determine their total polyphenol, total flavonoid and condensed tannin contents before to evaluate antioxidant activity of their aqueous, ethanolic and hydro-ethanolic extracts.

2. Materials and Method
2.1. Plant Material
The plant material consisted of the leaves (Fe), trunk bark (E), roots (R) and fruits (Fr) of *Ceiba pentandra* (L.) Gaertn. The plant was collected in Ebolowa, in the South region of Cameroon in 2021.

It was authenticated by the botanist, Mr Nana Victor at the National Herbarium of Cameroon by comparison with an existing specimen referenced under the number 43623HNC.

2.2. Preparation of extracts

The various parts of *Ceiba pentandra* (L.) Gaertn were cut, dried in the shade and ground using an artisanal mill. 01 kg of each powder was macerated for 72 hours in 03L of each of the following solvents and solvent system: Methanol, Water, Ethanol and Ethanol-water mixture in the proportions (70:30). In addition, a decoction of a mixture of 500g of the powdered leaves and 500g of powdered roots was also prepared by boiling them in 03 L of water. All the extracts were filtered under vacuum and then concentrated using a rotary evaporator. The chromatographic profiles, total polyphenol, flavonoid and condensed tannin contents as well as the antioxidant activity of the different extracts were determined.

2.3. LC-MS analysis

LC-MS was used for the identification of bioactive compounds at the Higher Teachers Training College, of the University of Yaounde I, using a Compact Bruker (UPLC-ESI-TOF-MS) on a waters synapt G2-S HDMS spectrometer coupled to a UPLC d ' acquity central (waters, Manchester, UK), consisting of binary solvent handler, sample handler and 2 × 150 mm column. The compounds were characterized by liquid chromatography coupled to mass spectrometry according to the method described by Abay and collaborators and by comparison with literature data [3].

2.4. Quantitative analysis

2.4.1. Determination of the total polyphenolic content

The total polyphenolic content was determined by spectrophotometry, using the Folin-Ciocalteu reagent. The process is based on the quantification of the total concentration of hydroxyl groups present in the extract. The protocol used is that described by Singleton *et al***.** [4]. Briefly, in glass hemolysis tubes, a volume of 500 μL of each extract was added, with a mixture of 1mL of Folin-Ciocalteu 's reagent diluted 10 times, and 800 μL of a 7.5% sodium carbonate solution (added 04 min later). The tubes were shaken and kept for 30 min. Absorbance was read at 765 nm. A calibration curve was produced in parallel under the same operating conditions using gallic acid at different concentrations (0 to 1000 μg /mL).

2.4.2. Determination of the total flavonoid content

The quantification of flavonoids was carried out by a method based on the formation of a very stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids [5]. The protocol used is based on that described by ZHISHEN and collaborators and by KIM and collaborators [6, 7], with some modifications. In a glass hemolysis tube, 500 μL of extract, or standard, or distilled water for the control, were added to 200 μL of 5% $NaNO_2$. After 5 minutes, 200 μL of 10% $AlCl_3$ were added, and the medium mixed vigorously. After 6 minutes, a volume of 1000 µl of 1 M NaOH was added to the medium. The absorbance was read immediately at 510 nm against the control. A methanolic solution of Quercetin was prepared. Daughter solutions prepared from the stock solution at different concentrations between 0 and 1000 μg /mL were used in plotting of the calibration curve.

2.4. 3. Determination of condensed tannin content: This study was carried out by the vanillin method combined with hydrochloric acid. This method depends on the reaction of vanillin with the terminal flavonoid group of condensed tannins and the formation of red complexes [8, 9]. This is explained by the property of tannins to be transformed into red-colored anthocyanidins by reaction with vanillin [10]. The content of condensed tannins was determined by the vanillin method described by JULKUNEN-TITTO [11]. A volume of 500 μL of each extract was added to 1500 μL of the 4% vanillin/methanol solution, then mixed vigorously. This was followed by addition of 750 μL of concentrated hydrochloric acid (HCl). The mixture obtained was left to react at ambient temperature for 20 min. Absorbance was measured at 550 nm against a white background. Different concentrations between 0 and 1000 μg /mL prepared from a stock solution of catechin were used in plotting the calibration curve.

2.5. Evaluation of the antioxidant activity

2.5. 1. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

To 01 mL of DPPH solution of concentration 0.3 mM, 50 µL of each extract and of trolox previously prepared at different concentrations (250, 125, 62.5, 31.25, 15.62, 7.81 µg/mL) are added separately. For each concentration, the test is repeated 3 times. The reaction mixtures are incubated at 37°C for 30 min in the dark and at room temperature and the absorbance of each solution is measured at 517 nm using a spectrophotometer. The positive control is represented by a solution of a standard antioxidant, trolox, prepared at the same concentrations as the extract [12].

2.5.2. ABTS (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical) assay.

In practice, the ABTS cationic radical is generated by mixing 10 mL of ABTS (7 mM) in H_2O with 5 mL of potassium persulfate (2.45 mM) and the whole kept away from light at room temperature for 12 to 16 hours. The solution obtained is diluted with ethanol until an absorbance of 0.70 at 734 nm is obtained. The next step is the addition of 1.5 mL of this freshly prepared solution to 50 μL of each sample and to the trolox, at different concentrations (250 - 125 - 62.5 - 31.25 - 15.62 - *7.81* µg /mL). The absorbances are measured at 734 nm after incubation for 10 minutes in the dark at room temperature. Three tests are carried out for each concentration of product tested [13].

2.5.3. FRAP (ferric reducing ability of plasma) assay.

Practically, 0.4 mL of the sample and ascorbic acid taken as reference at different concentrations (250, 125, 62.5, 31.25, 15.62, 7.81 µg/mL) is mixed with 1 mL of phosphate buffer (0.2 M; pH=6.6) and 1 ml of potassium hexacyanoferrate $[K_3Fe(CN)_6]$ at 1%. After incubating the mixture at 50°C for 30 minutes, 1 mL of 10% trichloroacetic acid was added and the tubes centrifuged at 3000 rpm for 10 minutes. Finally, 1 mL of the supernatant from each tube was mixed with 0.2 mL of a 0.1% $FeCl_3$ solution and left to stand in the dark for 30 minutes before measuring the absorbances at 700 nm [14].

2.6. Statistical analyzes

The data were represented as mean±standard error of the mean (SEM) on Excel 2019 workbook. Ordered analysis of variance of data was done using GraphPad software Prism, version 8.01 followed by Tukey's post-test for one-way variables, or Bonferroni 's post-test for two-way variables. Antioxidant activity assays were analyzed after data normalization. A value of $p < 0.05$ was considered statistically significant.

3. Result

3.1. Chromatographic profiles of methanolic extracts of *Ceiba pentandra* L. Gaertn

The chromatographic profiles of the methanolic extracts of *Ceiba pentandra* L. Gaertn were studied in order to know the chemical composition of each part of the plant. The chromatograms obtained (FIGS. 1, 2, 3 and 4) revealed the presence of several compound peaks (m/z) as a function of the retention time (Rt) expressed in minutes.

The order of appearance of these compounds is linked to the polarity of the molecules and type of column used. The more polar compounds were eluted first from the column and detected later while others could not be identified (Nd).

The chromatographic profiles of the studied extracts show that the chemical compositions of the methanolic extracts of the barks and the leaves are slightly different while the roots and the fruits have exactly the same chemical composition.

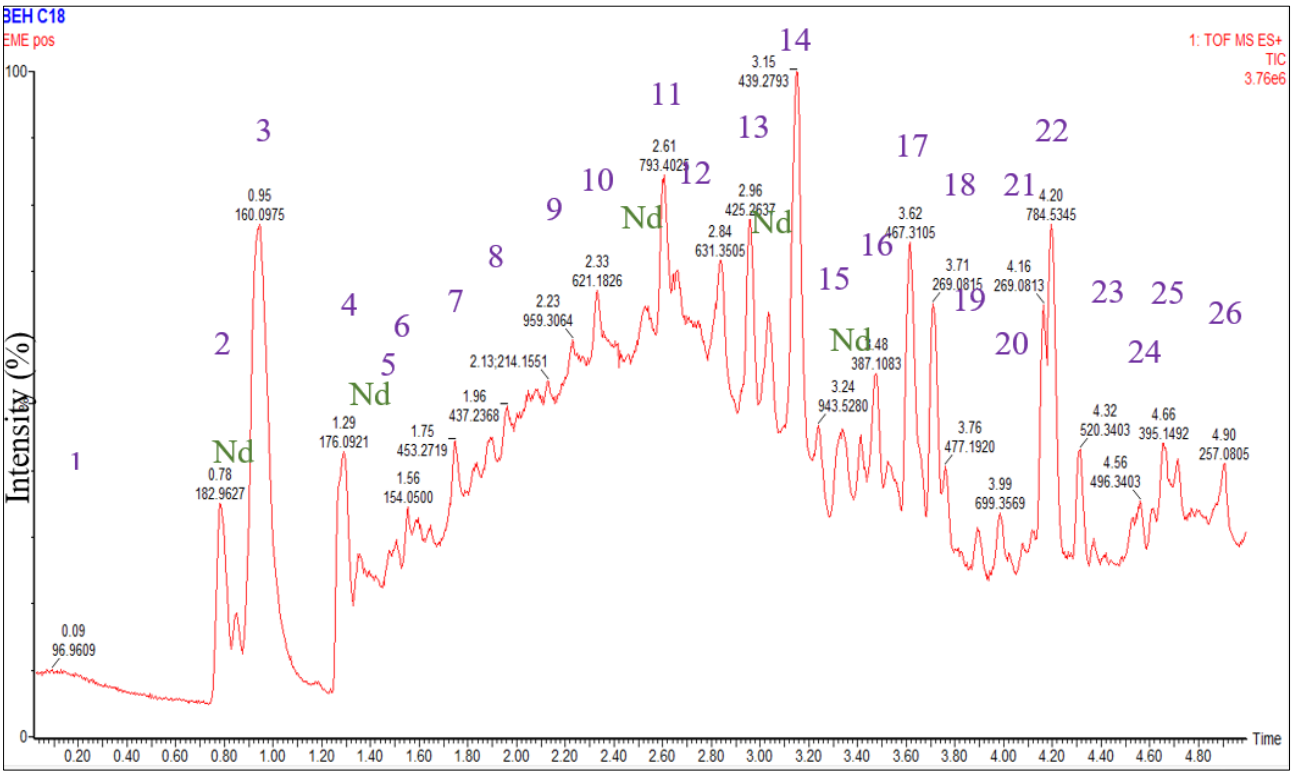

Fig 1: Chromatographic profile of the methanolic extract of *Ceiba pentandra* L. Gaertn bark

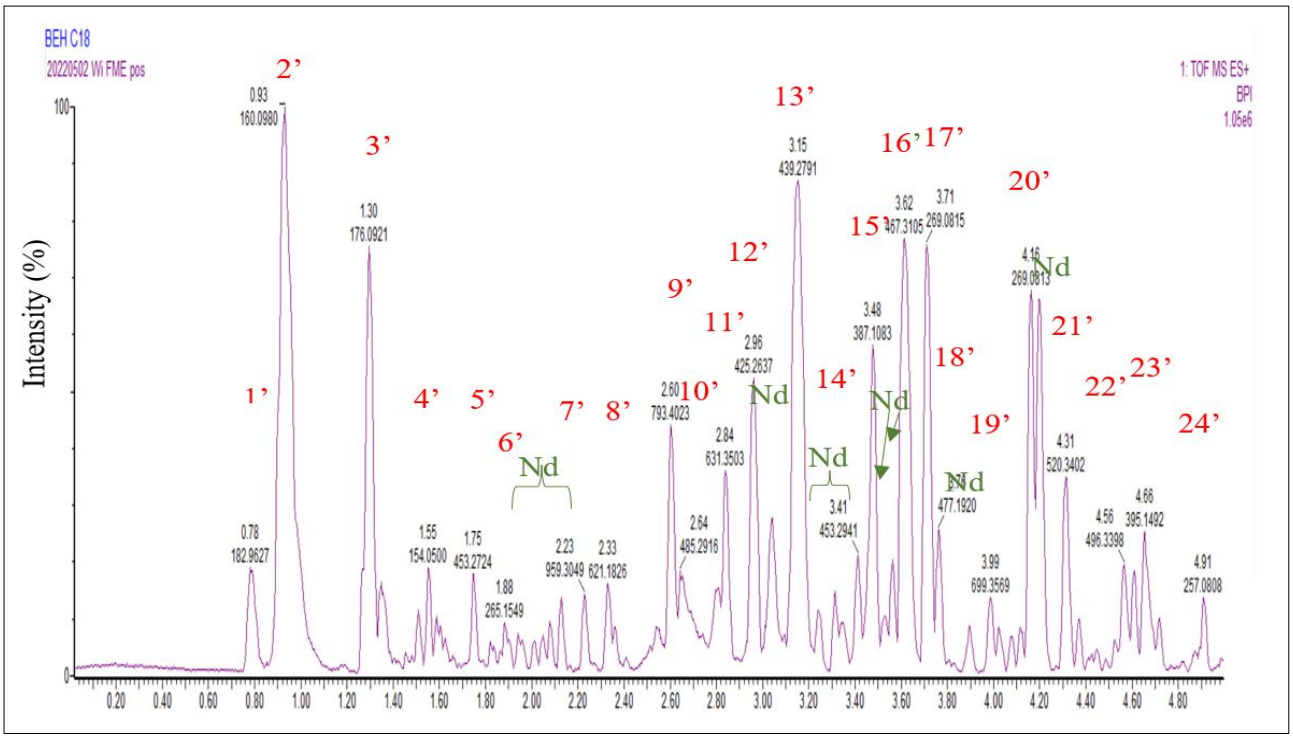

Fig 2: Chromatographic profile of the methanolic extract of *Ceiba pentandra* L. Gaertn leaves

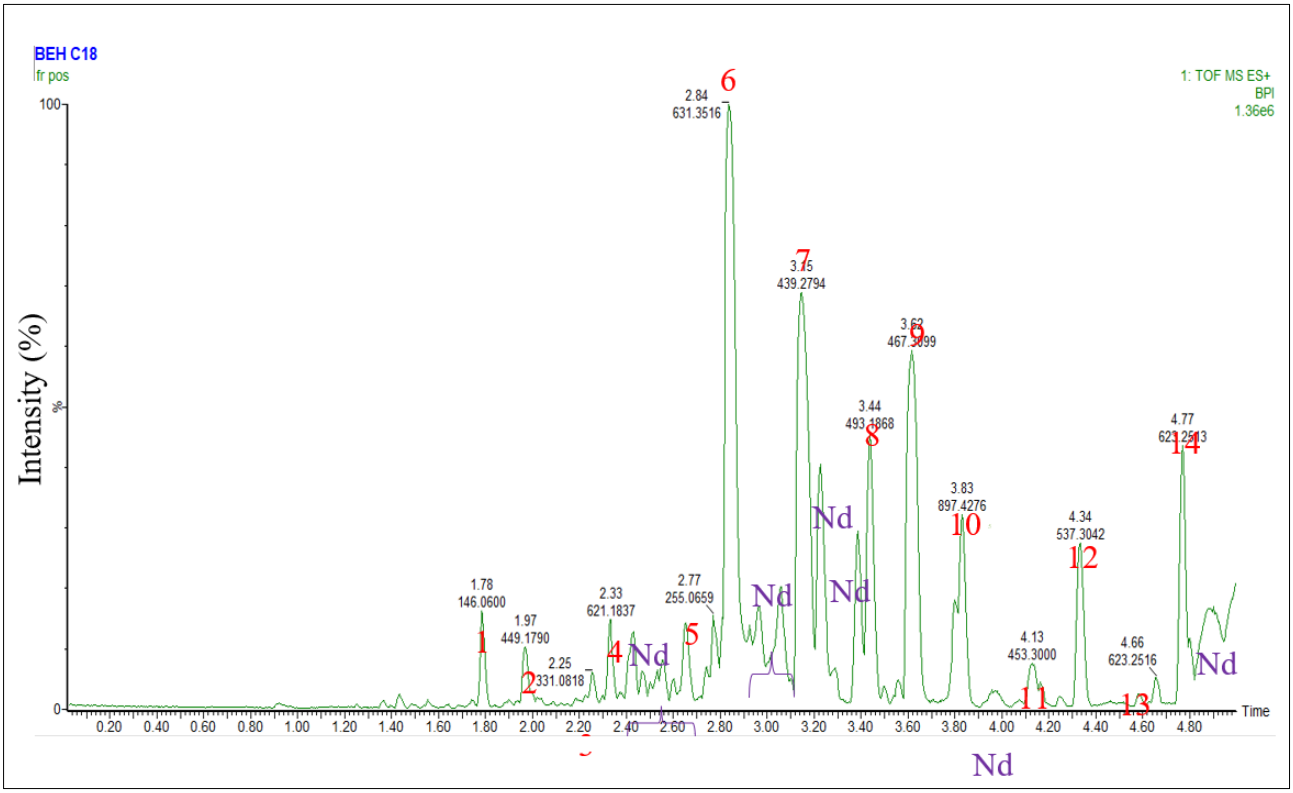

Fig 3: Chromatographic profile of the methanolic extract of *Ceiba pentandra* L. Gaertn fruits

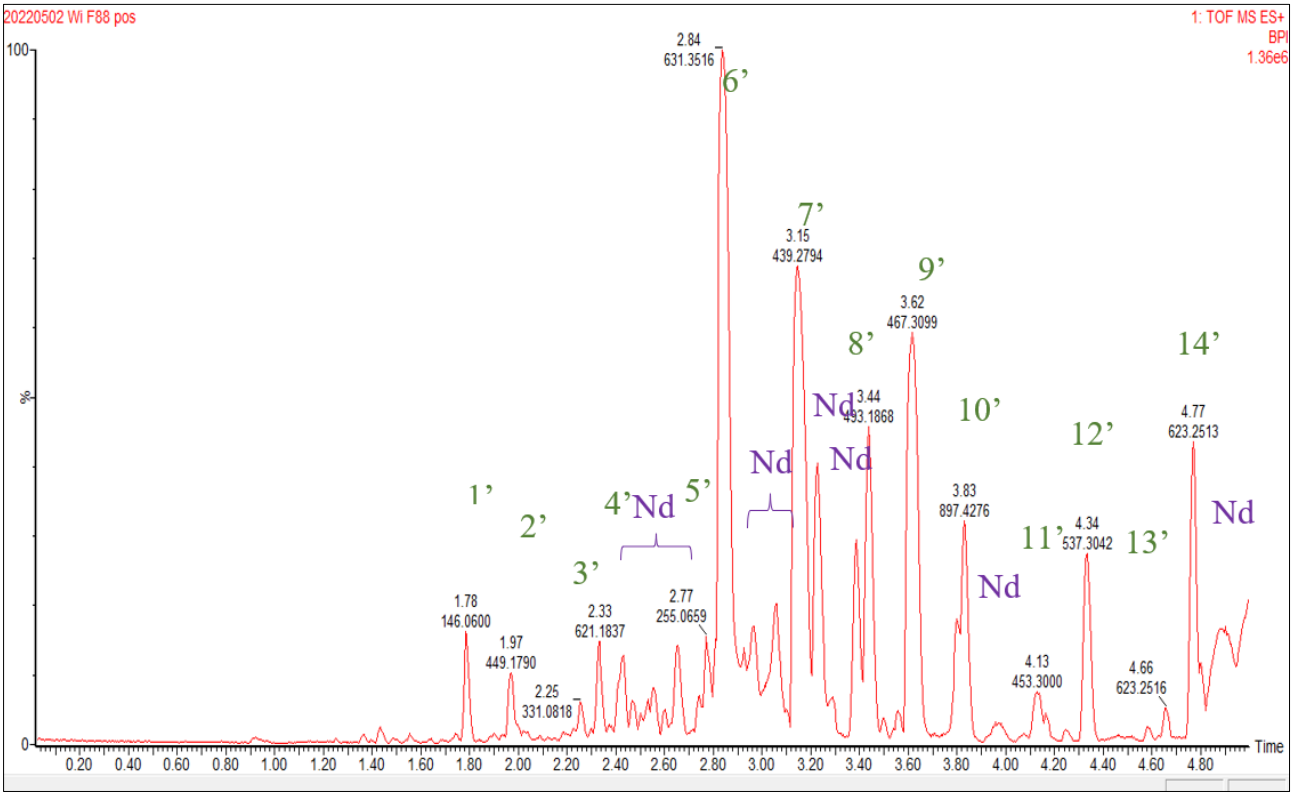

Fig 4: Chromatographic profile of the methanolic extract of *Ceiba pentandra* L. Gaertn roots

Among the compounds detected, 10 were identified according to the ratio (Rt ; m/z) for the leaves, 10 for the barks, 09 for the fruits and 09 for the roots. The compounds identified in the fruit and root extracts are identical while *β*- sitosterol was identified in the bark extract and but absent in the leaf extract. There are therefore a total of 18 different compounds identified for the four extracts. The structures of the compounds identified are recorded in the appendix, while the names of the compounds identified are recorded in Tables 1 and 2.

Table 1: Identification of the chemical compounds of the methanolic extract of bark and leaves of *Ceiba pentandra* L. Gaertn

Order		(Rt ; m/z)	Nomenclature	Raw formulas	Family of compounds	References
Barking	Sheets					
2	1'	(0.78; 182. 9627)	(R)-6-[(Z)-1-heptenyl]-5,6-dihydro-2H-pyran-2-one	C12H18O2 _ _ _ _	Lactone	[15]
6	5'	(1.75; 453.2719)	α, δ-tocopherol	$C_{29}H_{50}O_2$	Tocopherol	[16]
7	-	(1.96; 437.2368)	β-sitosterol	$C_{29}H_{50}W$	Steroid	[17]
-	ten'	(2.64; 485, 2916)	Cyanidin 3-O-glucoside	$C_{9:21}PM$ _	Anthocyanin	[18]
13	12'	(2.96; 425.2637)	lupenone	C30H48O _ _	Triterpenes	[19]
14	13'	(3.15; 439.2793)	β, γ-tocopherol	$C_{28}H_{48}O$	Tocopherol	[16]
16	15'	(3.48; 387,1083)	Cleomyscosin A	$D_{20}h_{18}S$	Coumarin	[20]
18	17'	(3.71; 269.0815)	Heptadecenoic acid	$C_{5:32}PMO_2$	Fatty acid	[21]
26	24'	(4.90; 257.0805)	Hexadecanoic acid	$C_{16:32}O_2$ _	Fatty acid	[22]

Table 2: Identification of chemical compounds of the methanolic extracts of the fruits and roots of *Ceiba pentandra* L. Gaertn

Order		(Rt ; m/z)	Nomenclature	Raw formulas	Family of compounds	References
Fruits	Roots					
2	2'	(1.97; 449.1790)	Luteolin-7- *O* - β -D-glucoside	$C_{9:20\ p.m.}M_{11}$ _	Flavone	[23]
3	3'	(2.25; 331.0818)	Tricine	$C_{5:14}PM$ _ _ _	Flavone	[23]
5	5'	(2.77 ; 255.0659)	Palmitoleic acid	$C_{16}H_{30}O$ _	Fatty acid	[24]
7	7'	(3.15 ; 439.2794)	β, γ-tocopherol	$C_{28}H_{48}O$ _	Tocopherol	[16]
8	8'	(3.44; 493.1868)	vavain-3'- *O* - *β-D* -glucoside	$C_{23}H_{24}O_{12}$	isoflavonoid	[25]
11	11'	(4.13; 453.3)	Cinchonaine la, lb	$C_{24}H_{20}O_9$	Flavonolignane	[26]
13	13'	(4.77)	Verbascoside		phenylpropanoid	[27]

3.3. Quantitative characterization of plant extracts

The total polyphenols, total flavonoids and condensed tannin contents are given in the table below.

Table 3: Content of total polyphenols, total flavonoids and condensed tannins in the extracts of *Ceiba pentandra* L. Gaertn

Extracts	Total polyphenols (*μg* EAG/mL)	Total flavonoids (*μg* EQ/mL)	Condensed Tanins (μg *ECAT* /mL)
FeEtOH	25,540±0,04[b]	5,940±0,4	0,494±0,07[a]
FeEtOH/H_2O	47,846±0,19[c]	21,573±0,5[c]	0,308±1,1[a]
FeH_2O	20,713±0,12[a]	1,623±0,57[a]	0,194±[0,23 a.m]
REtOH	17,326±[0,18 a.m]	6,670±0,33 [a.m]	0,731±0,18 [b]
REtOH / H2O	24,186±[0,29 a.m]	±0,27 [a.m]	0,636±0,19 [b]
EEtOH	23,880±0,32[a]	5,530±0,03[a]	0,702±1,14[b]
EEtOH/H_2O	35,353±0,11[b]	13,410±0,03[b]	0,703±0,19[b]
EH_2O	34,200±0,04[b]	6,550±0,08[a]	0,589±0,15[a]
FrEtOH	27,846±0,21[b]	4,586±0,19[a]	0,517±0,08[a]
FrEtOH/H_2O	18,736±0,038[a]	3,380±0,31[a]	0,625±0,04[b]
FrH_2O	21,640±0,34[a]	4,253±0,09[a]	1,311±0,21[c]
R+Fe	52,226±0,28[c]	14,050±0,06[b]	0.527±[0.04A]

3.3.1. Total polyphenol content

The content of total polyphenols estimated by the Folin-Ciocalteu method for each extract was established from a standard range with different concentrations of gallic acid. The standard equation for the calibration curve is: y = 0.0001x + 1.7192 with R^2 = 0.9428 (Appendix, Figure 17). The results obtained are expressed in *μg* equivalent of gallic acid per milliliter of extract solution. From these results, it can be seen that all the plant extracts studied are rich in polyphenols but by different amounts. The results (Table 3) show that the aqueous extract from the decoction of the mixture of leaves and roots has the highest content of polyphenols (52.226±0.28 μ g EAG/mL) followed by the hydroalcoholic extract of the leaves FeEtOH/ H_2O (47.846±0.19 *μg EAG* /mL).

The polyphenol contents of the aqueous fraction of the leaves (FeH_2O), hydroethanolic fraction of the fruits (FrEtOH /H_2O), and ethanolic fractions of the roots are the lowest with respective values of 20.713±0.12 *μg EAG* /mL; 18.736±0.03 *μg* EAG/mL and 17.336±0.18 *μg* EAG/mL.

3.3.2. Total flavonoid content

The total flavonoid content was estimated from a standard range of standards established with different concentrations of quercetin. The equation for the standard calibration curve is: y = 0.0001x + 0.0767; R^2 = 0.7937 (ANNEX, figure 18). The results obtained, expressed in *μg* equivalent of Quercetin per mL of extract (μg EQ/mL of extract), are presented in Table 3. The flavonoid content fluctuates between 21.573±0.5 μg EQ/mL and 3.880±0.31 μg EQ/mL respectively for the *hydroethanolic* fractions of the leaves (FeEtOH/H_2O) and the hydroethanolic fractions of the fruits (FrEtOH /H_2O). It should be noted however that the hydroethanolic fractions of the leaves and the decoction of the mixture of leaves and roots have the highest values in flavonoids, compared to the other extracts. The respective values are 21.573±0.5 μg EQ/mL and 14,050±0.06 *μg* EQ/mL.

3.3. 3. Condensed tannin content

The condensed tannin content was determined by the vanillin method. For each extract from an established standard range with different concentrations of catechin. The equation for the standard calibration curve is: y = 0.0046x + 0.585; R^2 =

0.933. (ANNEX, Figure 19). The results obtained (Table 3), show that the condensed tannin contents vary between 1.311±0.21 μ g ECAT/mL and 0.194±0.23 μ g ECAT/mL respectively for the aqueous fractions of the fruits (FrH_2O) and leaves (FeH_2O). The highest content of tannins is that of the aqueous fraction of the fruits (1.311±0.21 µg *ECAT* /mL of extract.), followed by the ethanolic fraction of the roots (0.731±0.18 and µg *ECAT* /mL of extract). The lowest tannin contents are attributed to leaf extracts with values of 0.494±0.07 µg *ECAT* /mL for the ethanolic extract (FeEtOH); 0.308±1.1 µg ECAT/mL for the hydroalcoholic extract (FeEtOH/H $_2$ O) and *0.194*±0.23 µg *ECAT* /mL for the aqueous extract (FeH_2O).

2.4. Antioxidant activity

2.4.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging

The DPPH radical abundance inhibition test on the root, leaf, bark and fruit extracts of *Ceiba pentandra* L. Gaertn is shown in figures 5, 6, 7 and 8 respectively.

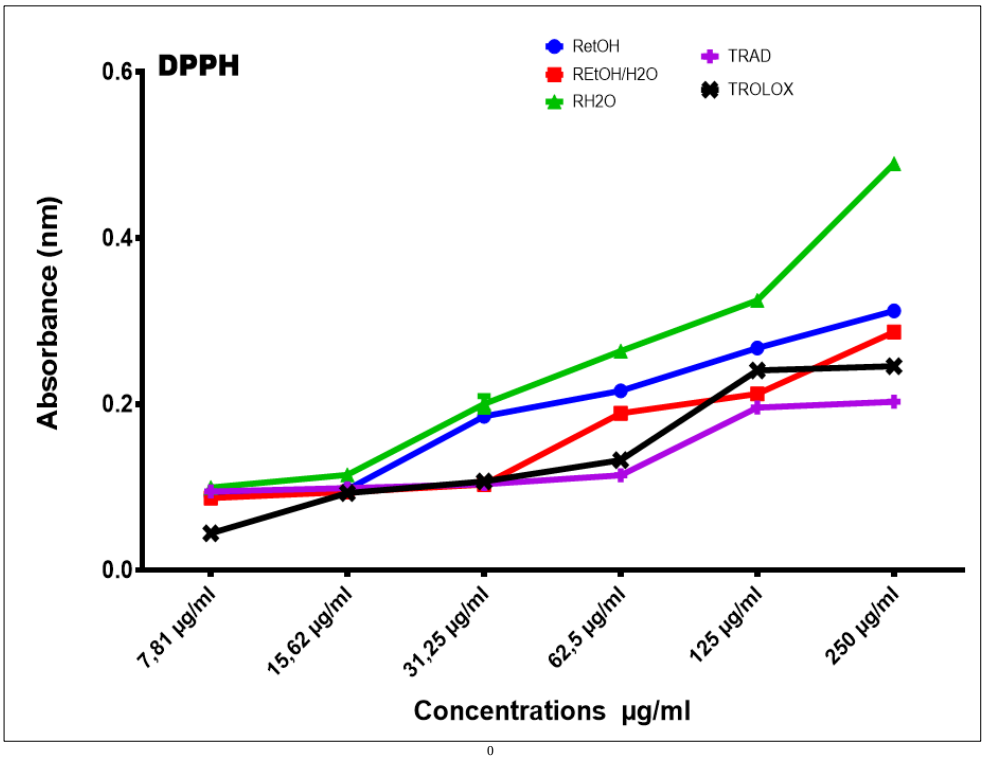

Fig 5: Absorbance of the DPPH radical by trolox and the ethanolic, aqueous and hydroethanolic extracts of the roots of *Ceiba pentandra*

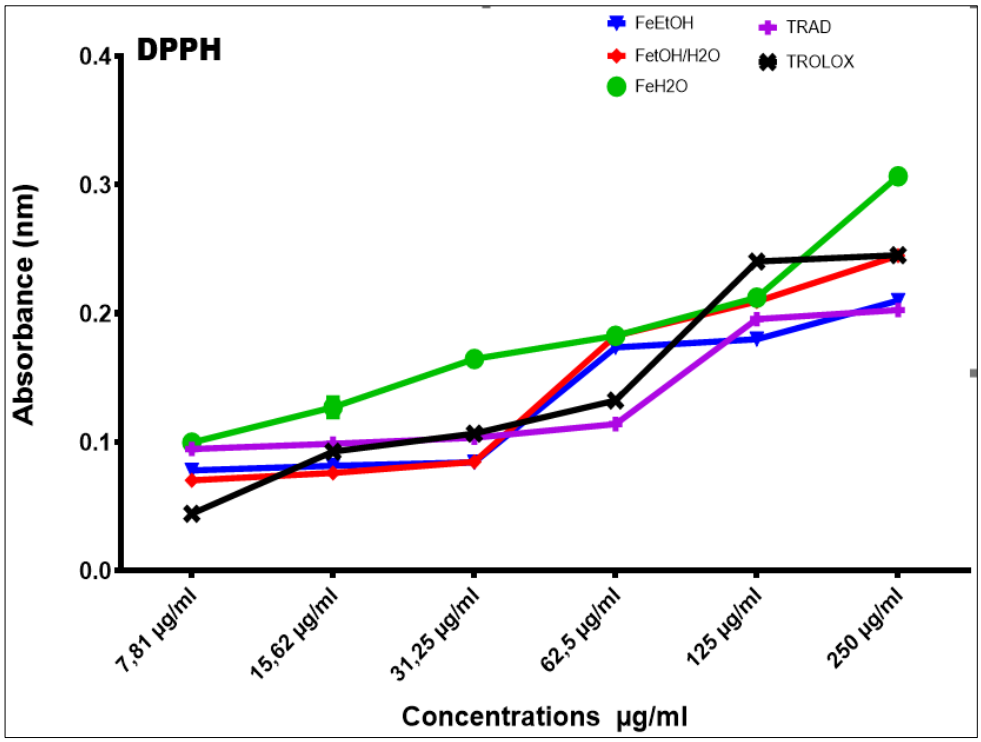

Fig 6: Absorbance of the DPPH radical by trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* leaves

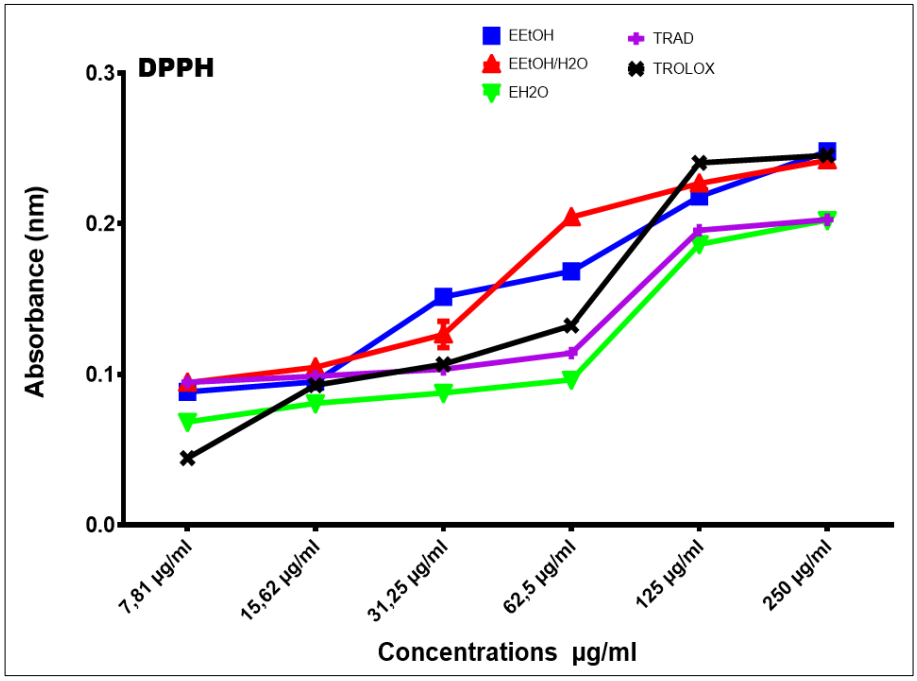

Fig 7: Absorbance of the DPPH radical by trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* bark

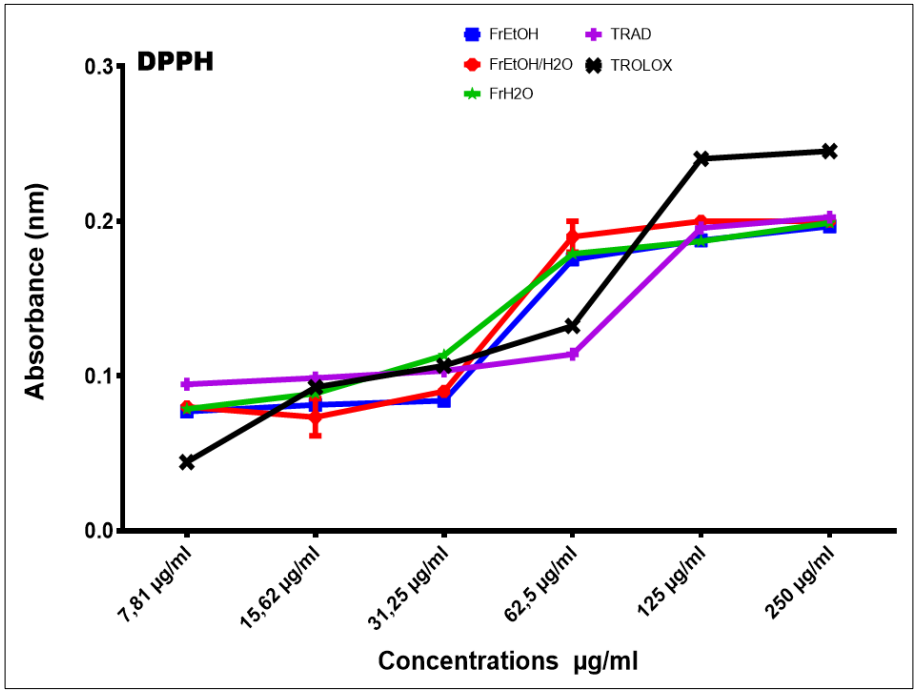

Fig 8: Absorbance of the DPPH radical by trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* fruits

Their analysis reveal that almost all the extracts exhibit antioxidant activity at certain concentrations.

The hydroalcoholic extract of the roots (REtOH /H_2O) and the aqueous extract of the barks (RH_2O) show activities similar to those of trolox at all concentrations except at 7.81 µg/mL.

For the leaves, we note a trapping activity greater than that of trolox for the ethanolic extracts (FeEtOH) at the concentrations of 15.62 µg/mL, 31.25 *µg* /mL, 125 µg / mL, and 250 *µg* /mL; for the hydroethanolic extract ($FeEtOH/H_2O$) at the concentrations of 15.62 µg/mL and 31.25 *µg* /mL with significant values of P (0.0001) for the two extracts at these concentrations and finally for the aqueous extract of the leaves (FeH_2O) at a concentration of 125 *µg* /mL. On the other hand, at a concentration of 250 µg/mL, the hydroethanolic extract (FeEtOH /H_2O) behaves similar to trolox (P > 0.9999)

For the barks, the aqueous extract (EH_2O) showed higher activity than that of trolox at all concentrations (P<0.0001). The same is true for the ethanolic EEtOH and hydroethanolic (EEtOH /H_2O) extract at a concentration of 125 µg/mL. However, at 250 *µg* /mL, their activities are similar to those of trolox with respective P values of 0.8146 and 0.9080.

With regards to the fruits, the ethanolic (FrEtOH) and hydroethanolic ($FrEtOH/H_2O$) extracts showed higher activities than that of trolox at concentration of 31.25 *µg* /mL; while at 125 *µg* /mL and 250 *µg* /mL, the activities of all the fruit extracts exceed those of trolox with a highly significant P value (P<0.0001).

However, the extract from the decoction of the mixture of roots and leaves (R+Fe) shows better activity than that of trolox at concentrations of 62.5 µg / mL, 125 *µg* /mL and 250 µg/mL, with a highly significant *P value* equal to 0.0001 at

each of the concentrations. In order to better appreciate the scavenging activities and to determine the extract with the best trapping activity, we determined the IC_{50} values for each extract as shown in table 4 below

Table 4: IC_{50} values and correlation coefficients of *Ceiba pentandra extracts* for the DPPH test

Extracts	REtOH	REtOH/H_2O	RH_2O	FeEtOH	FetOH/H_2O	FeH_2O	EEtOH
$IC_{50(DPPH)}$	37,73	14,36	33,81	26,70	13,65	38,8	35,21
R square	0,91	0,91	0,90	0,93	0,96	0,99	0,93
Extracts	EEtOH/H_2O	EH_2O	FrEtOH	FrEtOH/H_2O	FrH_2O	R+Fe	Trolox
$IC_{50(DPPH)}$	33,05	15,55	26,00	32,43	15,40	11,13	18,69
R square	0,92	0,97	0,91	0,91	0,95	0,99	0,98

These results show that the extract from the aqueous decoction of the leaves and roots (R+Fe) has the best activity, IC_{50} = *11.3* µg/mL, followed by the hydroethanolic extract of the leaves (FetOH/H_2O), IC_{50} = 13.65 µg/mL, hydroethanolic extract of the roots (REtOH /H_2O) IC_{50} = *14.36* µg/mL, and aqueous extract of fruits, IC_{50} = *15.40* µg/mL, compared with trolox, IC_{50} = *18.69* µg/mL, used as reference.

2.4.2. ABTS

The results of the ABTS radical abundance inhibition test by the *extracts of C. pentandra* are given by figures **9, 10, 11** and **12.**

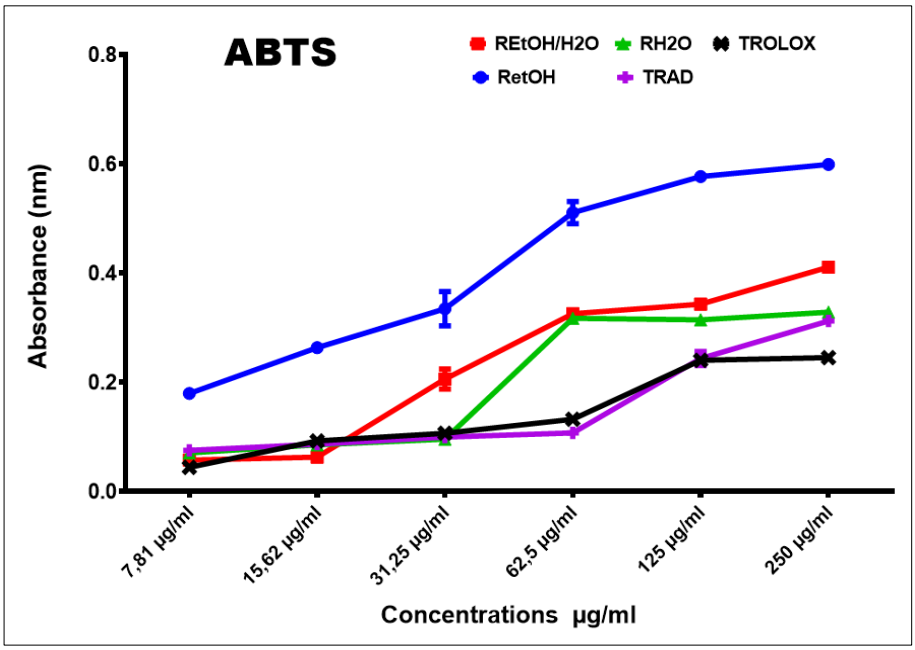

Fig 9: Absorbance of the $ABTS^+$ cation by trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* roots

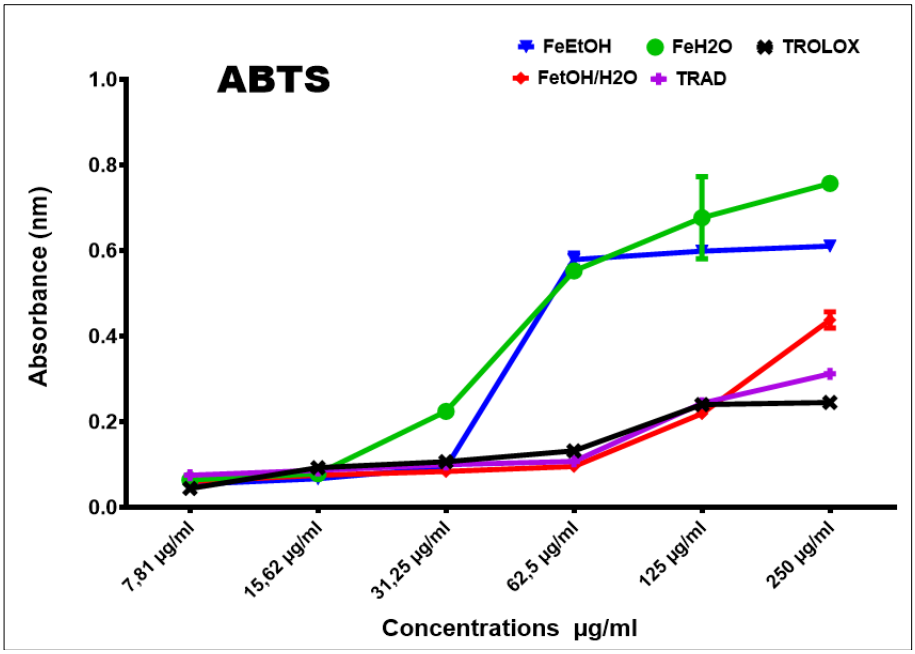

Fig 10: Absorbance of the $ABTS^+$ cation by trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* leaves

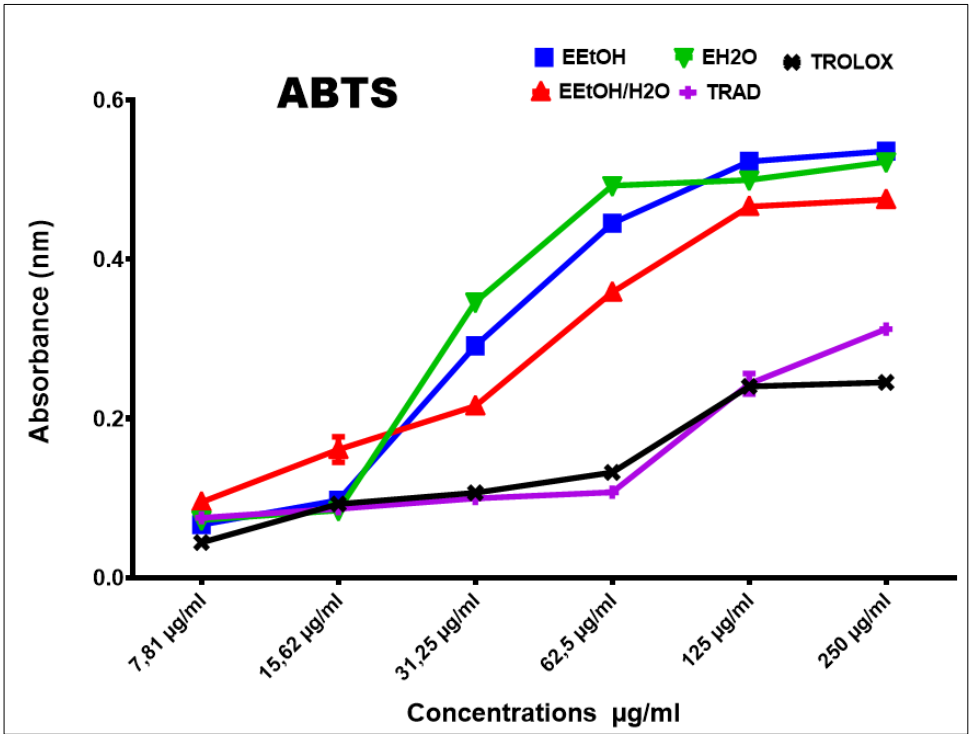

Fig 11: Absorbance of the $ABTS^{+}$ cation by trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* bark

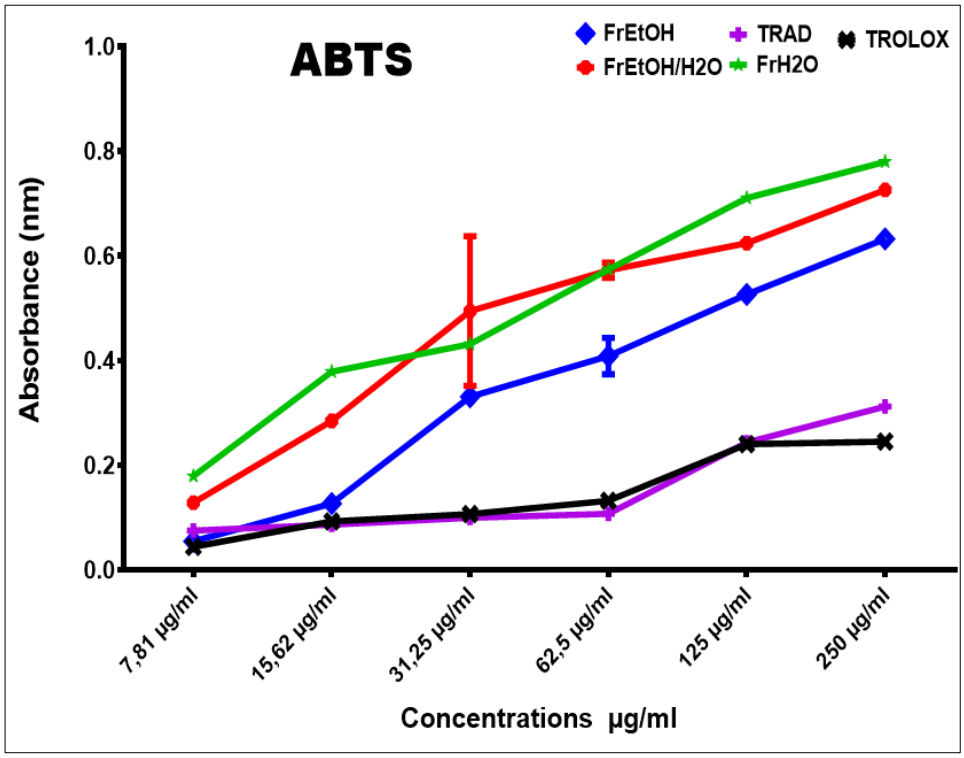

Fig 12: Absorbance of the $ABTS^{+}$ cation by trolox and ethanoli c, aqueous and hydroethanolic extracts of *Ceiba pentandra* fruits

T 0 heir analysis shows on the whole, the inactivity of the barks and the fruits. On the other hand, the extracts (R+Fe) and FeEtOH/H_2O exhibit activities similar to those of trolox at concentrations of *15.62 µg* /mL; 31.25 *mcg / mL;* 62.5 *µg / mL;* 125 µg/mL with respective P values of 0.9872, 0.9774, 0.2441, 0.9987 for the decoction and 0.9630; 0.9066; 0.6251; 0.9227 for the hydroalcoholic extract of the leaves. A better activity than that of trolox was for the aqueous extract of the roots at concentrations of 7.81 µg / mL and 31.25 *µg* /mL with a very significant P value of 0.0001 at these two concentrations.

The determination of the IC_{50} values, (Table **5)** revealed that the hydroethanolic extract of the leaves had the best antiradical activity with $ABTS^{+}$ (IC_{50} = 14.81 µg/mL), followed by the (R + Fe) extract with (IC_{50} = 16.69 µg / mL). These two extracts are more active than trolox (IC_{50} = 19.77 µg / mL) used as a reference.

Table 5: IC $_{50}$ values and correlation coefficients of *C. pentandra extracts* for the ABTS test

Extracts	REtOH	REtOH/H_2O	RH_2O	FeEtOH	FeEtOH/H_2O	FeH_2O	EEtOH
$IC_{50\ (ABTS)}$	33,66	38,99	33,95	34,85	14,81	31,09	37,48
R square	0,97	0,96	0,92	0,99	0,97	0,95	0,99
Extraits	EEtOH/H_2O	EH_2O	FrEtOH	FrEtOH/H_2O	FrH_2O	R+Fe	Trolox
$IC_{50\ (ABTS)}$	37,02	47,93	36,76	46,91	34,54	16,69	19,77
R square	0,98	0,99	0,96	0,92	0,96	0,98	0,92

Ferric Reductive Antioxidant Potential (FRAP)

The iron-reducing antioxidant power *of Ceiba pentandra extracts* is presented in the following figures 13, 14, 15 and 16.

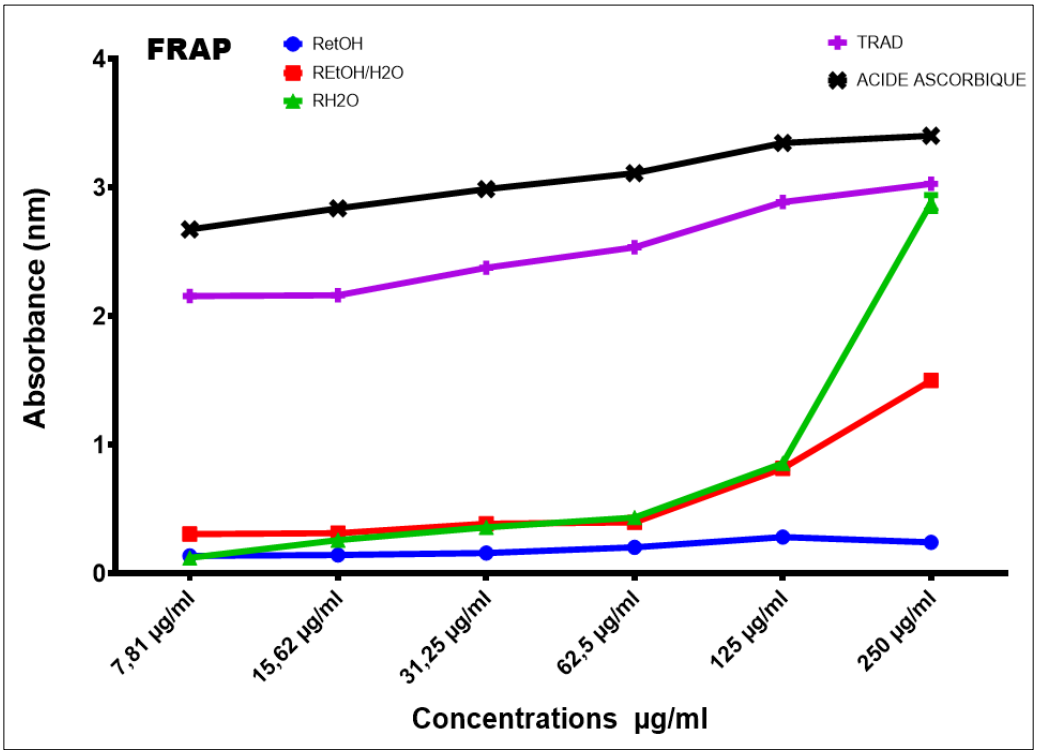

Fig 13: reducing power of the ferric ion Fe^{3+} of trolox and ethanolic, aqueous and hydroethanolic extracts of the roots of *Ceiba pentandra*

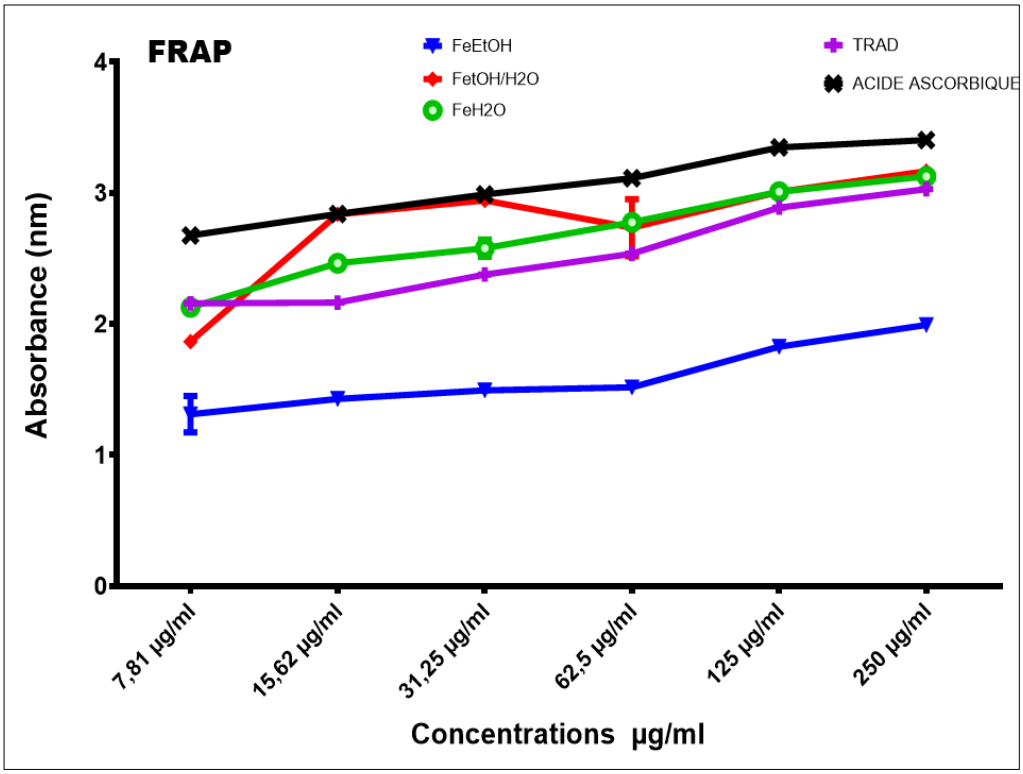

Fig 14: reducing power of the ferric ion Fe^{3+} of trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* leaves

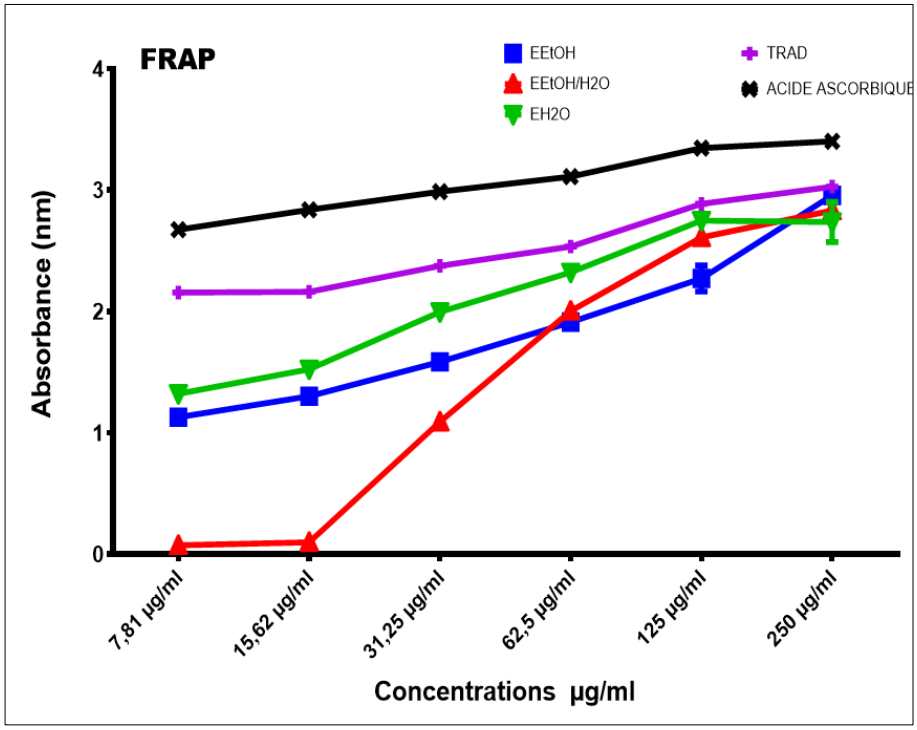

Figure 15: reducing power of the ferric ion Fe^{3+} of trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* bark

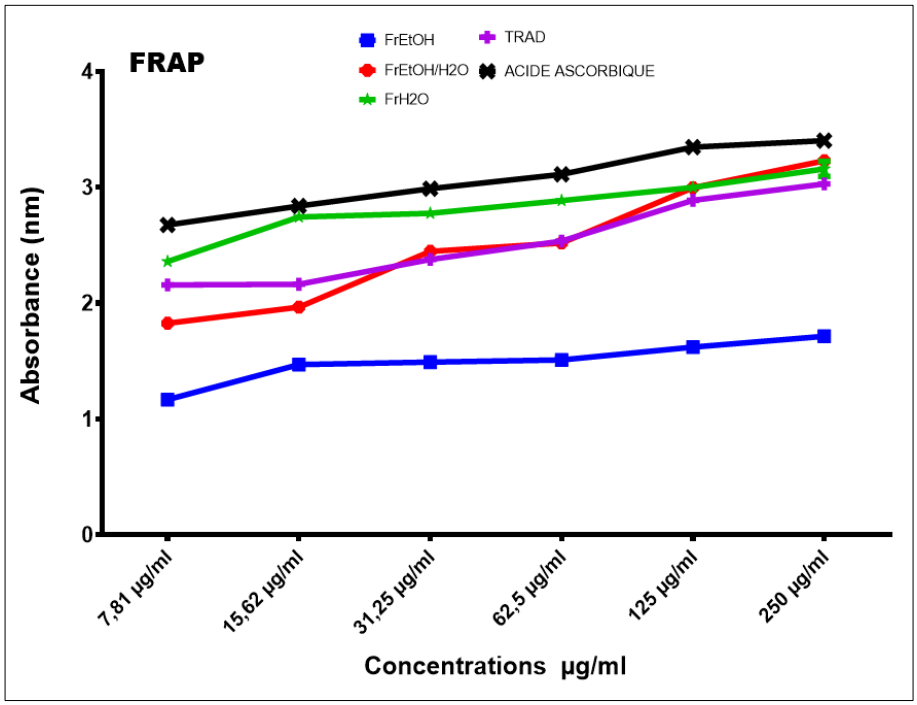

Fig 16: reducing power of the ferric ion Fe^{3+} of trolox and ethanolic, aqueous and hydroethanolic extracts of *Ceiba pentandra* fruits

Their analysis shows that the FeEtOH/H_2O extract has a reducing antioxidant power similar to that of the reference at concentrations of 15.62 μ g/mL (P >0.9999) and 31.25 μ g/mL (P = 0.9697). Bark extracts showed no scavenging activity. On the other hand, the extract resulting from the decoction of the mixture of roots and leaves shows a highly significant scavenging activity compared to that of ascorbic acid, taken as a reference (P<0.0001), at all concentrations except at 125 μ g/mL. The aqueous extract of the fruits has a moderately significant activity (P = 0.0026) compared to that of ascorbic acid.

The analysis of the IC_{50} values of the various extracts (Table 4), revealed that the extract resulting from the decoction of the mixture of roots and leaves (R+Fe), had the best ferric reductive antioxidant potential with IC_{50} = 13.9 μg / mL, followed by the aqueous extract of the fruits (IC_{50} = 14.06 µg / mL) and the hydroethanolic extract of the leaves (IC_{50} = 15.4 μg / mL). These three extracts have a ferric reducing power greater than that of ascorbic acid estimated at 20.38 µg / mL.

Table 6: IC_{50} values and correlation coefficients of *C. pentandra extracts* for the FRAP test

Extracts	REtOH	REtOH /H_2O	RH $_2$O	FeEtOH	FetOH /H2O	FeH $_2$O	ETOH
$IC_{50\ (FRAP)}$	35.60	28.2	33.8	17.57	15.40	37.52	31.8
R-square	0.90	0.95	0.97	0.98	0.92	0.96	0.95
Extracts	EtOH /H $_2$O	EH $_2$O	FrEtOH	FrEtOH /H $_2$O	FrH $_2$O	R+Fe	Ascorbic acid
$IC_{50\ (FRAP)}$	35.05	33?35	26.70	24.67	14.06	13.91	20.38
R-square	0,99	0,91	0,96	0,90	0,96	0,99	0,90

Discussion

Thanks to the information found in the literature, the use of polar solvents made it possible to extract from the fruits, roots, leaves and bark of *Ceiba pentandra*, polar compounds such as polyphenols, fatty acids, terpenoids and lactones. This notion of polarity seems to be confirmed by phytochemical screening which reveals the presence of polar compounds such as polyphenols, flavonoids, terpenoids, coumarins, tannins and saponins in the various extracts studied. All the compounds detected by HPLC-MS in the different methanolic extracts of *Ceiba pentandra* could not be identified. This could be explained by the method of identification by chromatographic profiles taken from literature which made it possible to identify for all the extracts only 18 different compounds compared to the usual methods (NMR, COZY, DEPT, etc.). Note however that there is a great structural homogeneity between the compounds identified in the leaves and the bark on the one hand and between those identified in the roots and the leaves on the other hand. Only β -sitosterol identified in the barks was not identified in the leaves. As for the fruits and roots, the same compounds were detected in both extracts. The α, δ β, γ -tocopherols were identified in the 04 extracts. This structural homogeneity is explained by the fact that the 04 extracts all come from the same plant. The chromatographic profiles indicate that polyphenols and flavonoids constitute the major classes of secondary metabolites present in the different extracts studied. These results are in agreement with previous work done on the plant [27, 28]. The results of the quantitative assays carried out on the extracts also made it possible to confirm these results by revealing that the decoction resulting from the mixture of the root and leaves and the hydroalcoholic extract of the leaves present the greatest quantities of total polyphenols, total flavonoids and condensed tannins. These classes of secondary metabolites are among the main components of plants with antioxidant activity [29, 30, 31]. This justifies its strong antioxidant power observed from the three methods used: ABTS, FRAP and DPPH.

A combined analysis of the antioxidant potency results revealed that the extract (R + Fe) from the decoction of the mixture of leaves and roots has the best antioxidant potency according to the DPPH (11.3 µg / mL) and FRAP (IC_{50} =13.91 µg /mL) while the ABTS method showed a better antioxidant power for the hydroalcoholic extract of the leaves (IC_{50} = 14.81 µg / mL). These results are better than those obtained by Fofié and collaborators on the antioxidant activity of the decoction and a maceration of the stem bark of *Ceiba pentandra* by DPPH method which gave IC_{50} values of 87.84 and 54.77 µg /mL respectively [32]. This discrepancy in results may be due to the fact that since the roots and leaves have different chemical compositions, mixing them gave the extract a variety of chemical compounds with antioxidant properties. This can also be explained by the fact that the mixture generated significant synergistic effects between the different molecules which constitute it or could also be due to the presence of substances which present absorption bands at the same wavelength as the DPPH radical in the R+Fe extract [33].

Furthermore, it should be noted that according to the ABTS method, the best activity which is that of the hydroethanolic extract of the leaves (FeEtOH/H_2O) whose IC_{50} value of 14.81 μg /mL is much lower than that of trolox used as reference and with IC_{50} of 19.77 µg /mL. This could be explained by the presence of a large quantity of substances which exhibit absorption bands at the same wavelength as the ABTS radical in the FeEtOH/H_2O extract. This could also be justified by the large number of polyphenols (47.846±0.19 μg EAG/mL) and flavonoids (21.573±0.5 μg EQ/mL) contained in the extract.

Conclusion

A phytochemical study has been carried out on the aqueous, ethanolic and hydroethanolic extracts of *Ceiba pentandra* (L.) Gaertn, followed by evaluation of their antioxidant potential. The results revealed the diversity of secondary metabolites in the species *C. pentandra* (L.) Gaertn notably alkaloids, terpenoids, phenolic compounds, flavonoids, tannins, steroids, coumarins and anthocyanins. Combined analysis of antioxidant potential using the DPPH and FRAP methods revealed that the extract from the decoction of the mixture of roots and leaves of *Ceiba pentandra* (L.) Gaertn has the best antioxidant power while the FeEtOH/H_2O extract has the best antioxidant power according to the ABTS method.

Additional studies are necessary on the one hand to isolate and characterize the compounds from the active extracts and evaluate the cytotoxicity of both the pure compounds and extracts. The profound study will be necessary for the correlation of the metabolic composition and the antioxidant assays performed.

Acknowledgement

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Appendices

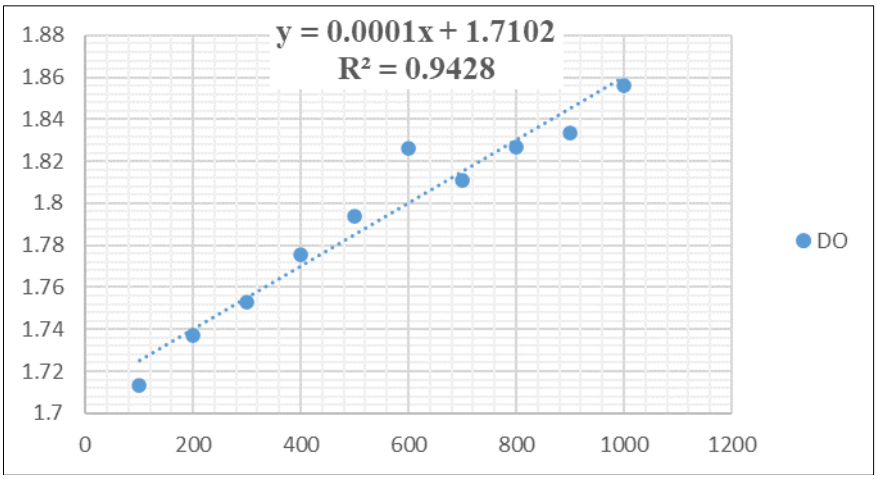


Fig 17: Gallic Acid Calibration Line ((Mean ± SD of three runs)

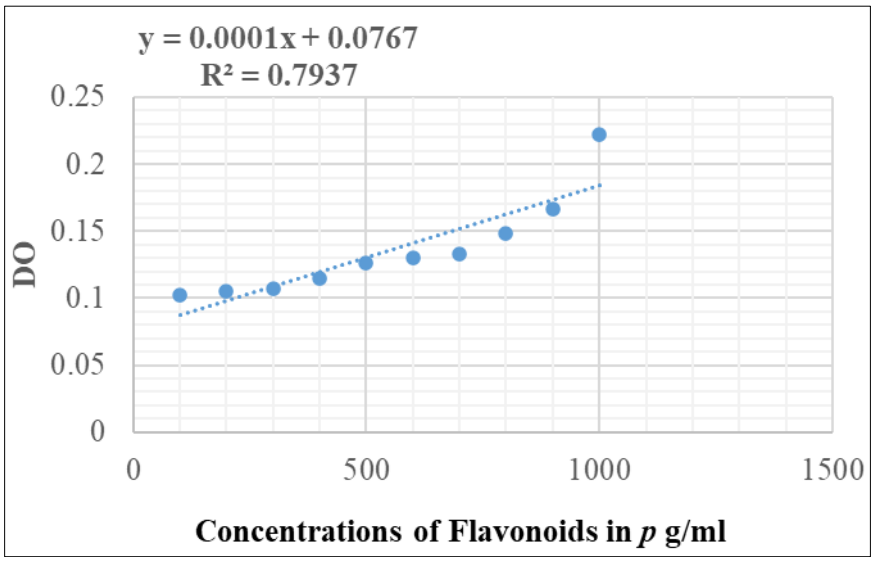


Fig 18: Quercetin Calibration Line (Mean ± SD of Three Trials)

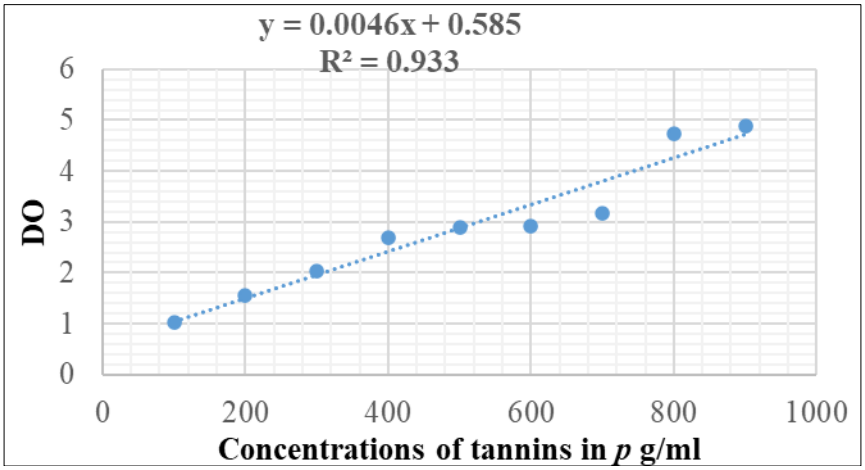
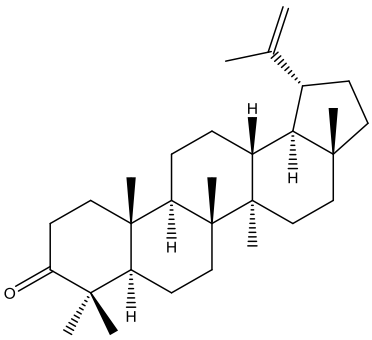
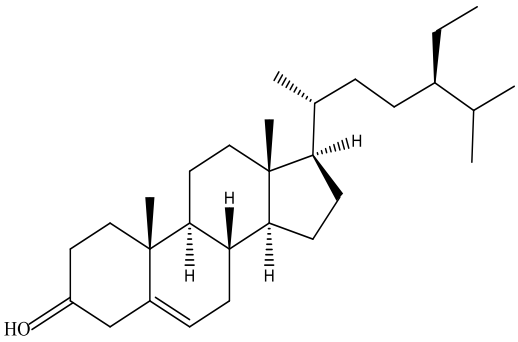


Fig 19: Catechin Calibration Line (Mean ± SD of Three Trials)

Compounds identified by LC-MS



Lupénone



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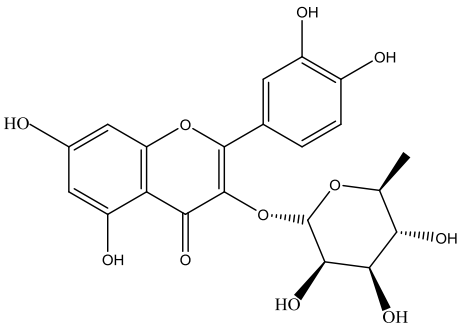

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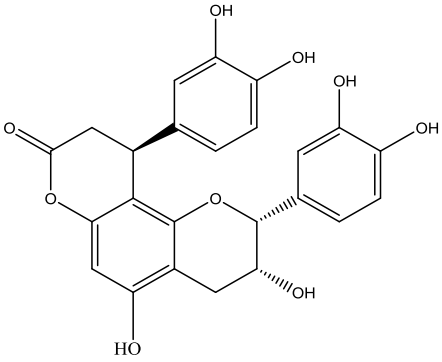

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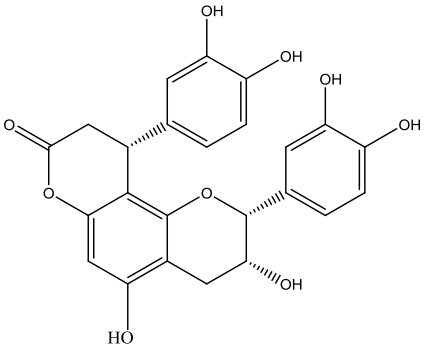
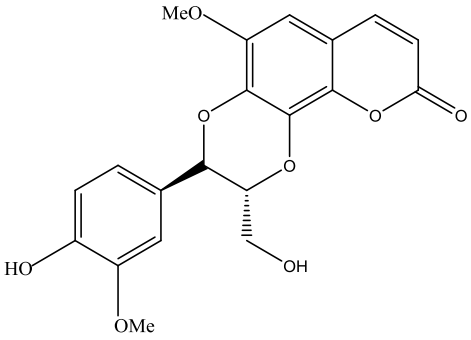


Cinchonaine la



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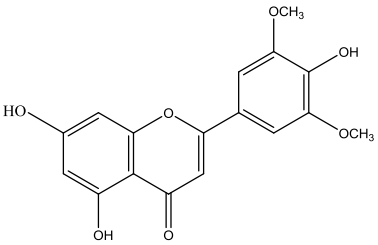
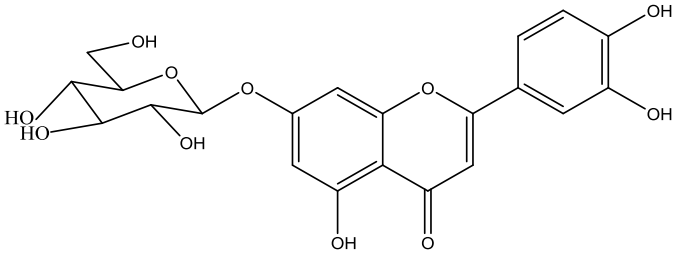


Tricine



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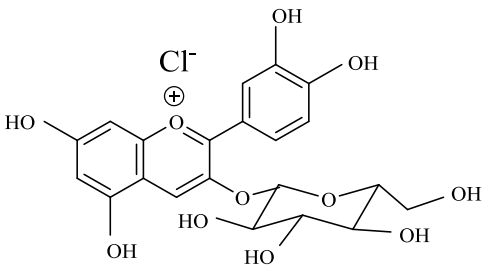

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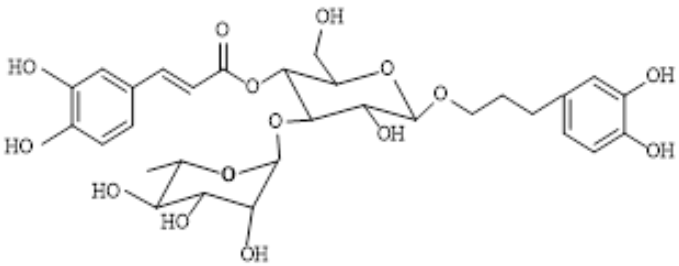
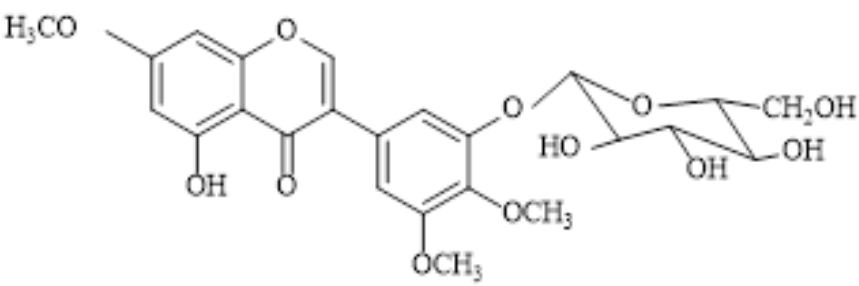
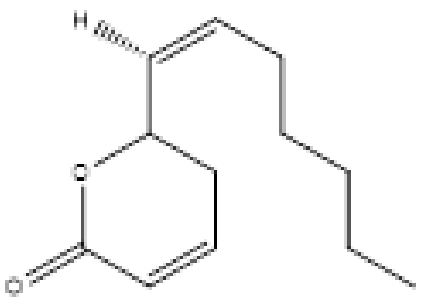
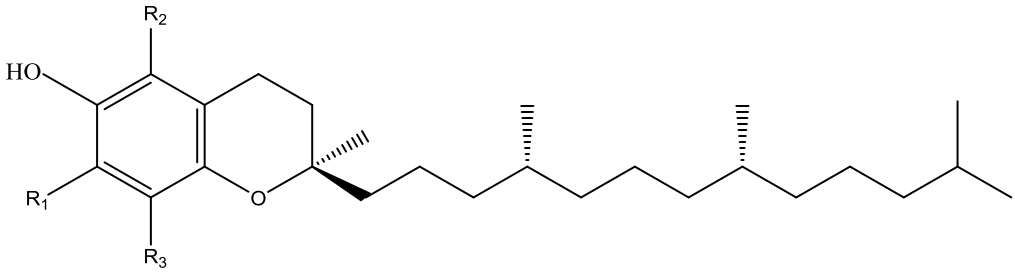


Verbascoside

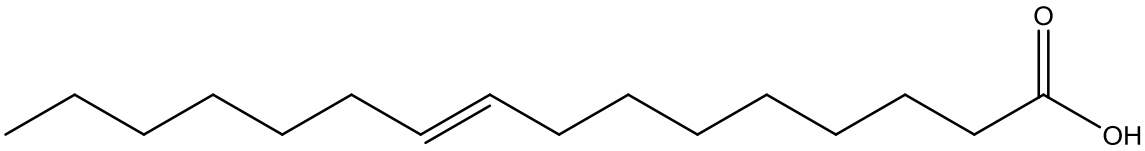




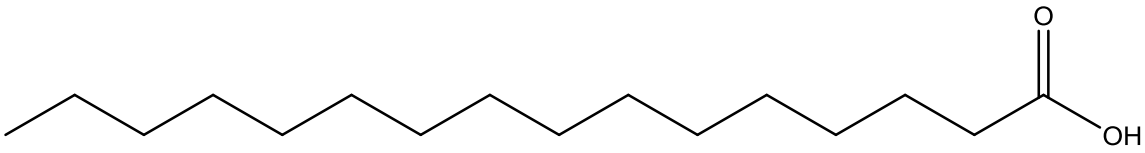
vavain-3'- *O* - *β-D* -glucoside (R)-6-[(Z)-1-heptenyl]-5,6- dihydro-2H-pyran-2-one



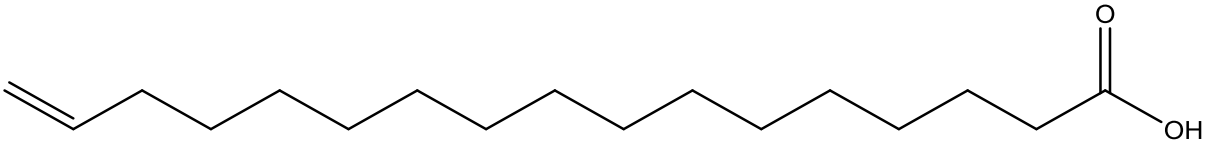
R1	R2	R3	
Me	Me	Me	α-Tocopherol
Me	H	Me	β-Tocopherol
H	Me	Me	γ-Tocopherol
H	H	Me	δ-Tocopherol



Palmitoleic acid (cis-9-hexadecenoic acid)



Palmitic acid (Hexadecanoic acid)



Heptadecenoic acid (Margaroleic acid)