Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(4): 140-146
Received: 16-07-2023
Accepted: 15-08-2023

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Isolation, structural characterization and evaluation of the *in vitro* antioxidant potential of four compounds isolated from the selective leaf extracts of *Bauhinia monandra* Kurz (Fabaceae)

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DOI: https://doi.org/10.22271/phyto.2023.v12.i4b.14702

Abstract
Bauhinia monandra belongs to Fabaceae family, is used in traditional Ivorian medicine to treat diabetes and male infertility. A preliminary study conducted on the leaves, stems and roots showed fluctuating contents of total phytophenols and flavonoids in the ethyl acetate extract of the leaves. This study made it possible to isolate and elucidate the molecular structures of four phytoconstituents from selective leaf extracts: Vomifoliol (1), guibourtinidol (2), epiafzelechin (3) and D-pinitol (4). The molecular structures of the phytoconstituents were elucidated by NMR ^{1}H, ^{13}C, COSY, HMBC et HMQC. Also, There *in vitro* antioxidant activity was measured using the DPPH and FRAP spectrophotometric methods.

Keywords: *Bauhinia monandra*, structure moléculaire, activité antioxydante, RMN

1. Introduction
Orchids of the genus *Bauhinia*, called Judas tree, are dicotyledonous plants native to Madagascar and widespread in the tropics. The genus includes about 300 species growing in tropical regions around the world [1-2]. They are small trees or shrubs with showy flowers grown as avenue trees. *Bauhinia* species are often used in traditional medicine for the treatment of diabetes [2]. Ethnobotanical information obtained from local traditional herbalists indicates that *Bauhinia monandra* is used to treat diarrhoea, dysentery, fevers, leprosy, smallpox (pox), diabetes, eye diseases. Previous work on *Bauhinia* species has made it possible to isolate different classes of metabolites such as phenolic acids, esters, terpenoids, alkaloids, steroids, tannins, quinines and more commonly flavonoids [2-7]. A recent study carried out on the organs of the Ivorian species made it possible to determine the contents of total phenols and flavonoids, as well as the antioxidant potential by trapping of DPPH and by reduction of Fe^{3+} ions from organic extracts [8]. This work aims to explore the antioxidant potential of four compounds isolated from selective extracts of *Bauhinia monandra* leaves, in relation to their determined molecular structures.

2. Material and Methods
2.1. Plant material
The plant material used in this study consists of the leaves of B. monandra (BMf), harvested in Agboville (city in the south-east of Côte d'Ivoire, capital of the department of Agboville, Agnéby-Tiassa region, 5°55'41" north and 4°13'01" west). After identification by Prof. MALAN Djah François (systematic botanist in NANGUI ABROGOUA University), the organs were cleaned under running water, dried under constant air conditioning (20°C, 20 days), then reduced to powders, then preserved in hermetically sealed glass jars.

2.2. Methods
2.1. Preparation of the crude hydrométhanolic extract
352 g of leaf powder are macerated during 48 hours in 1L of methanol (MeOH, 80%) at room temperature with constant stirring. The operation is repeated three times. After vacuum filtration, the collected filtrates are concentrated using a rotary evaporator (BÜCHI) (40°C, 335 mbar) and drained under liquid nitrogen during 6 h to give 62 g of total hydromethanolic extract (BMF).

2.2. Preparation of selected extracts

To obtain selective extracts, 56 g of BMf are dissolved in 500 mL of distilled water and then successively exhausted with hexane, dichloromethane (DCM), ethyl acetate (AcOEt) and n-butanol (n-BuOH). The organic fractions obtained were dried over anhydrous magnesium sulphate (Na_2SO_4). After filtration on filter paper, hexane (BMf1), DCM (BMf2), AcOET (BMf3) and n-BuOH (BMf4) fractions are reduced on a rotary evaporator, and dried with a pump under a liquid nitrogen atmosphere for 4 h to provide selective extracts. DCM and AcOEt extracts were used for compound isolation and purification.

2.3. Chromatographic separation of BMf2 and BMf3 fractions

2.3.1. Isolation of compound (1) from the BMf2

245 mg of the DCM fraction are introduced into an open column containing 50 g of silica gel 60 GF254 (Merck) (length 45 cm, diameter 4 cm, height of the silica 17 cm), eluted with a gradient of solvents (DCM/ AcOEt) increasing the polarity to 100% AcOEt (90/10-70/30-50/50-40/60-20/80 - 00/100). The elution of the compounds was controlled by TLC (60 F254, aluminum support, Merck). Subsequently, 170 fractions were collected and grouped according to their similarity into 11 sub-fractions (BMf2-1 to BMf2-11). The BMf 2-9 subfraction (23 mg) showed a single molecular spot by TLC visible under UV/256 nm.

2.3.2 Isolation of compounds (1-4) from BMf3

3 g of AcOEt extract dissolved in MeOH are introduced into an open column. The elution was carried out with two solvent gradients: the first elution was carried out with a DCM/AcOEt mixture in the proportions of 20/80 (v/v) with an increasing increase in polarity up to 100% AcOEt. The second elution was performed with AcOEt/MeOH (9/1) up to 50% MeOH. Two elutions gave 81 fractions grouped into 9 sub-fractions, the purification of which made it possible to collect 6 other sub-fractions (BMf3-$_1$ to BMf3-$_6$). The BMf3-$_1$ sub-fraction (53 mg) was subjected to preparative chromatography, eluted with a solvent mixture DCM/AcOEt, 60/40 (v/v) to give 3 bars (BMf3-$_1$A fractions (12 mg), BMf3-$_1$B (10 mg), BMf3-$_1$C (14 mg) of compounds, after migration, observation under UV light 254-366 nm and visualization with phosphomolybdic acid. The BMf3-$_1$A fraction (12 mg) gave the sole compound (1) (12 mg). Compound (2) (14 mg) is isolated from BMf3-$_1$C. The BMf3-$_2$ subfraction (86 mg), purified and analyzed by preparative plate chromatography, provided compound (3) (41 mg) from BMf3-$_2$D. Purification of the BMf3-$_5$ subfraction (131 mg) made it possible to isolate compound (4) (45 mg).

2.4. Structural characterization of isolated compounds

The characterization of the isolated compounds was done by nuclear magnetic resonance techniques (NMR ^1H, ^{13}C, COSY, HMBC, HMQC) on a spectrometer (Shimadzu, 400 MHz).

2.5. Determination of antioxidant potential (PO) of isolated compounds

DPPH Test

To 1 mL of the ethanolic extract (obtained from each isolated compound) are added 2.5 mL of an ethanolic solution (0.03 mg/mL) of the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). The stirred mixture is incubated for 30 min in the dark. The decrease in the absorbance of the violet color of the DPPH solution is measured at 517 nm on a UV-visible spectrophotometer (Spectro AL 800) against a white [9-10]. Plant extracts and quercetin are prepared in the concentration range from 1500 to 2.24 µg/mL. The reduction percentage (PR) is determined by the following formula:

$$PR\ (\%) = 1 - \frac{extract\ absorbance}{DPPH\ absorbance} \times 100$$

The median DPPH reduction concentration (CR_{50}) reflecting the antioxidant efficacy parameter of the extract, is determined graphically from the PR [11-14]. All tests are performed in triplicate.

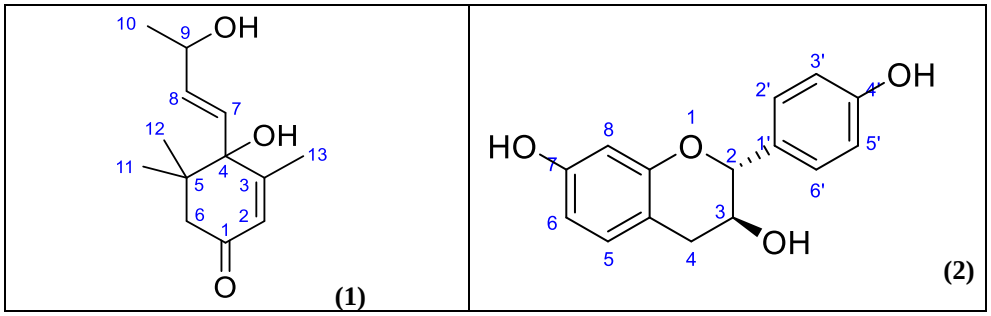
FRAP Test

The reagent used is a mixture of sodium acetate buffer (0,3 M; pH= 3,6) and 2, 4, 6-Tri (2-pyridyl)-s-triazine (TPTZ) (10 mM) prepared in hydrochloric acid solution (HCl, 40 mM) and iron tricholride ($FeCl_3$, 20 mM) in a volume ratio 10: 1: 1. 3 mL of FRAP reagent freshly prepared and preheated to 37 °C in a water bath are added to 100 µL of plant extract (0, 25 mg/mL). The absorbance of the intense blue color induced by the reduction of the colorless ferric complex (TPTZ- Fe^{3+}) into ferrous cations (Fe^{2+}) is read at 593 nm on a UV-visible spectrophotometer (Spectro AL 800) after 4 min of incubation. Linear calibrations are carried out with ferrous sulphate ($FeSO_4.H_2O$), 80% pure (standard), at concentrations (0,78125; 1,5625; 3,125; 6,25; 12,5 et 25 µg/mL). Ascorbic acid (vitamin C) was the control antioxidant. The results are expressed in micrograms $FeSO_4$ equivalent per 100 g of extract (µg ESF/100 gE) [15-16]. All tests are performed in triplicate.

3. Results and discussion

3.1. Molecular structures of isolated compounds

Figure 1 presents the molecular structures of the compounds isolated from the BMf2 and BMf3 fractions of the leaves of *B. monandra.*



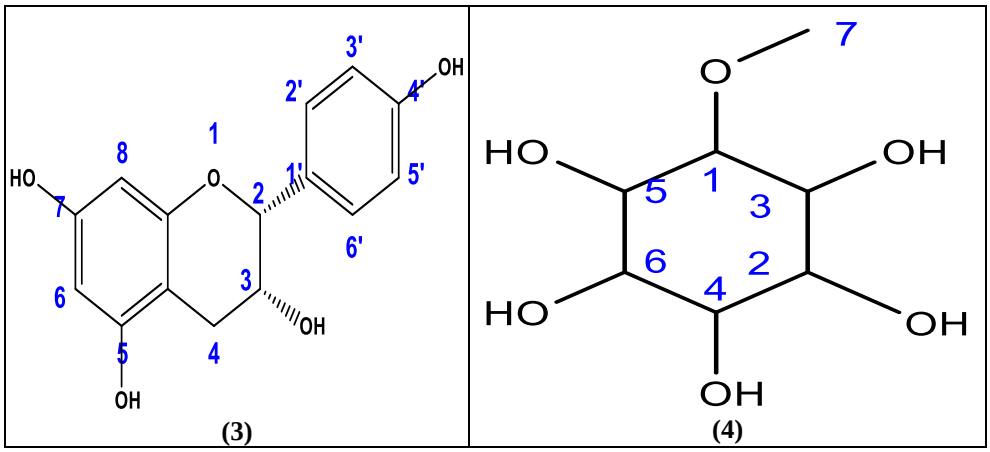


Fig 1: Molecular structures of isolated compounds

Compound (1)

Based on spectral data NMR (^{1}H, ^{13}C(APT)) (400 MHz, $CDCl_3$) (Table 1) and COSY, HMBC (Figure 2), and in comparison, to reported spectral data [17-18] the compound (1) is Vomifoliol.

Table 1: NMR ^{1}H and ^{13}C spectral data

Position	Compound (1)	
	δ_C (ppm)	δ_H, mult. (J in Hz)
1	198.3	
2	127.0	5.89 : p; (1.3); 1H
3	163.2	
4	79.2	
5	41.3	
6	49.8	2.22: dd;(17.1; 1.2); 1H$_a$
		2.43: dd;(17.1; 0.8); 1H$_b$
7	129.2	5.78: dd;(15.6; 0.2); 1H
8	135.8	5.83: dd; (15.6; 5.5); 1H
9	68.2	4.39: qdd; (6.4; 5.5; 0.2); 1H
10	23.8	1.28 : d; (6.4); 3H
11	24.2	1.00 : s; 3H
12	23.0	1.06 : bs; 3H
13	19.1	1.88 : d; (1.4); 3H

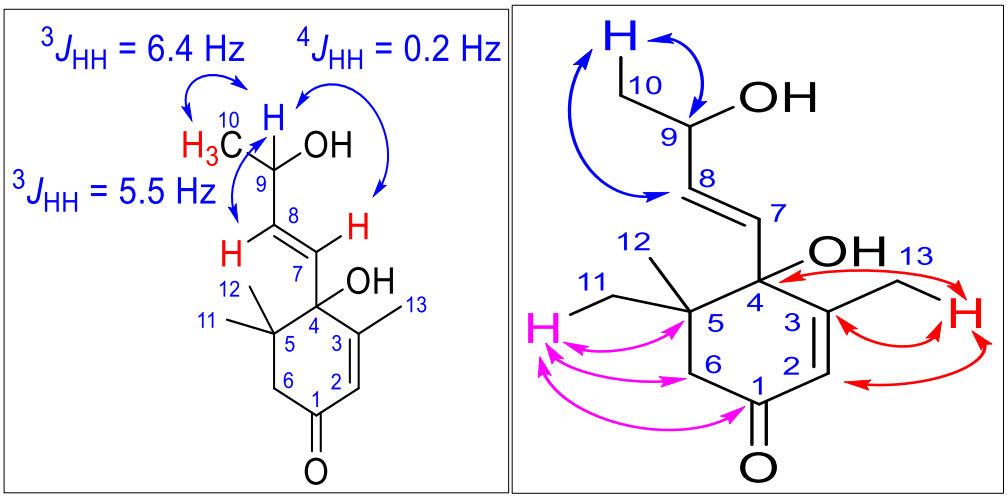


Fig 2: Main COSY and HMBC corrélations of Vomifoliol

Vomifoliol is a derivative of megastigma [19-20]. It exhibits moderate anticholinesterase and antileishmanial activity [21]. Natural derivatives of megastigma also exhibit ant proliferative effects [22], anticancer and cytotoxic [23].

Compounds (2) and (3) : The compounds (2) and (3) are yellow-orange amorphous solids whose spectral data NMR (^{1}H, ^{13}C(APT)) (400 MHz, (CD_3OD) (Table 2) are consistent with data reported in the literature. The compoud (2) (Figure 3) is guibourtinidol ((2R,3S)-4',7-dihydroxyflavan-3-ol) [24-25]. The compoud (3) is epiafzelechin (2R,3R)-4',5,7-trihydroxyflavan-3-ol) [24, 26-28].

Table 2: NMR ^{1}H and ^{13}C spectral data of compounds (2) and (3)

Position	Compound 2		Compound 3	
	δ_C (ppm)	δ_H, mult. (J in Hz)	δ_C (ppm)	δ_H, mult. (J in Hz)
2	83.1	4.67 : d;(7.6); 1H	79.9	4.86 : bs; 1H
3	68.8	4.01 : ddd; (8.5; 7.7; 5.2); 1H	67.4	4.18 : ddd; (4.6; 3.0; 1.4); 1H
4	33.6	2.71 : dd; (15.8; 8.5); 1H	29.3	2.74 : dd; (16.8; 3.0); 1H
		2.89 : dd; (15.7; 5.2); 1H		2.88 : dd; (16.8; 4.6); 1H
5	131.2	6.87 : d; (8.3); 1H	157.3	
6	109.5	6.35 : dd; (8.3; 2.4); 1H	96.5	5.92 : d; (2.3); 1H
7	157.9		157.6	
8	103.6	6.28 : d; (2.4); 1H	95.9	5.96 : d; (2.3); 1H
9	156.2		157.9	
10	112.6		100.1	
1’	131.5		131.6	
2’/6’	129.5	7.21 : m; 2H	129.1	7.41-7.26 : m; 2H
3’/5’	116.1	6.79 : m; 2H	115.8	6.83-6.72 : m; 2H
4’	158.5		158.0	

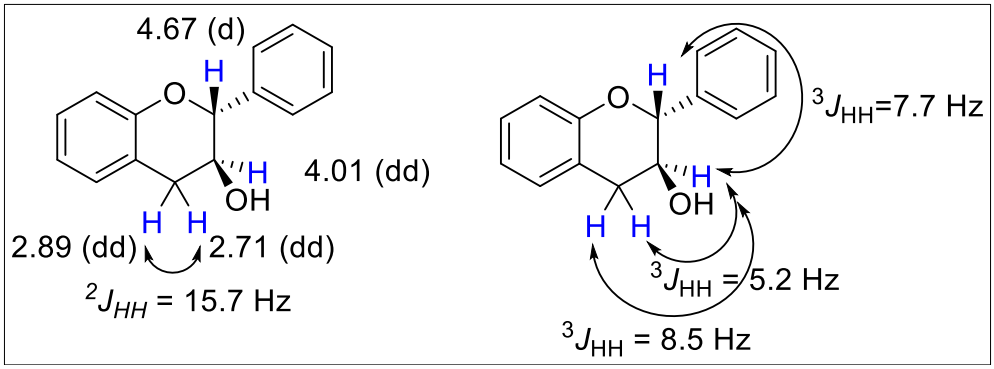


Fig 3: Main COSY corrélations of guibourtinidol

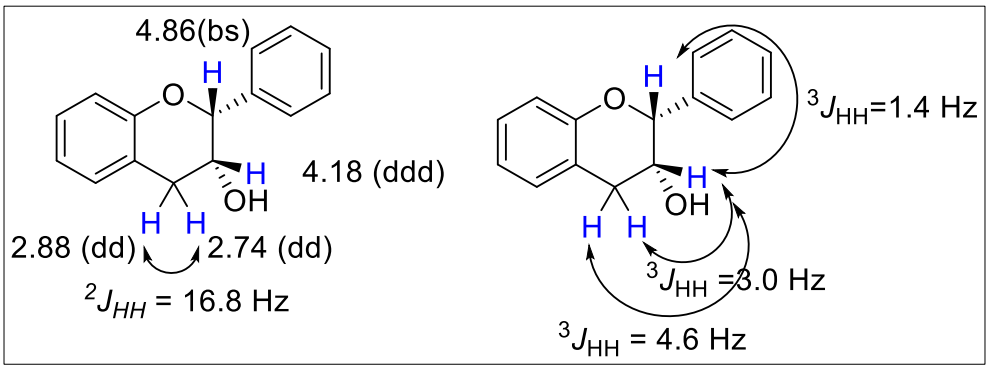


Fig 4: Main COSY corrélations of epiafzelechin

Guibourtinidol and epiafzelechin are the flavanols which have been described and isolated, respectively in *Cassia sieberiana* [25, 29], *Cassia sieberiana* [30-31] and *Celastrus orbiculatus* [27]. These compounds are known for their pharmacological interest [32-35].

Table 3: NMR ^{1}H and ^{13}C spectral data of compound (4)

Position	Compound 4	
	δ_C (ppm)	δ_H, mult. (J in Hz)
1	84.9	3.26 : t; (9.6; 9.1; 1H
2	72.6	3.76 : dd; (9.7; 2.6); 1H
3	73.7	3.91 : d; (3.4); 1H
4	73.4	3.60 : t; (9.4); 1H
5	74.3	3.90 : s; (3.4); 1H
6	72.0	3.70 : dd; (9.7; 2.6); 1H
O-CH_3	60.6	3.61 : s; 3H

Compound (4)

The compound (4) is D-pinnitol (yellow amorphous solid). NMR (^{1}H, ^{13}C(APT)) (400 MHz, (CD_3OD) Spectral data in Table 3 are consistent with those reported in the literature [36-37].

3.2. Antioxidant potential of isolated compounds

The antioxidant potential of the compounds was evaluated using the DPPH and FRAP tests.

3.2.1. DPPH antioxidant profile

In general, the isolated compounds reduce DPPH. Figure 5 presents the significant and variable reduction percentages (PR) of the different compounds isolated: 87.332±0.179% for vomifofiol ; 89.053±0.182% for epiafzelechin; 66.719±0.175% for guibourtinidol and 51.126±0.061% for D-pinnitol.

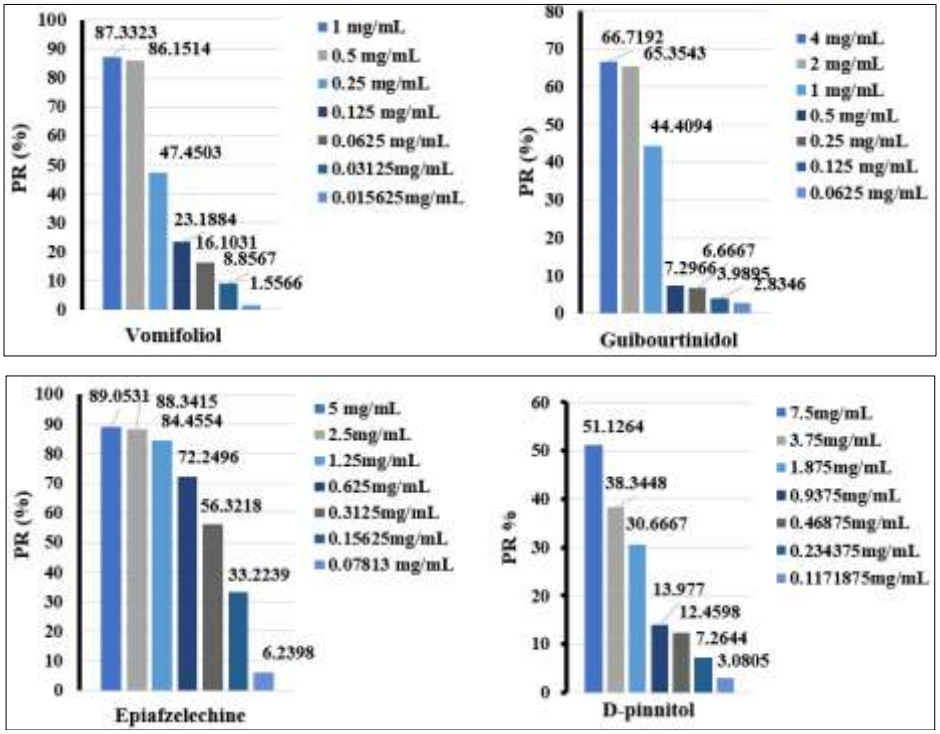


Fig 5: DPPH reduction percentages of molecules isolated from *B. monandra* leaves

Table 4 highlights the antioxidant efficacy of the compounds isolated, which is reflected by the determination of the CR_{50}. This parameter reflects the efficiency of the sample. The lower its value, the more significant the antioxidant activity [38, 14].

Table 4: CR_{50} of isolated compounds and quercetin

Compounds	CR_{50} (µg/mL)
Vomifoliol	278.035
Guibourtinidol	1266.89
Epiafzelechin	269.73
D-pinnitol	7136.44
Quercetin	3.519

According to the analysis of Table 4, epiafzelechin and vomifofiol show the best antioxidant efficacy with regard to DPPH. However, in comparison to quercetin, an antioxidant potential, epiafzelechin and Vomifoliol exhibit low antioxidant efficacy. One study reported the antioxidant behavior of epiafzelechin extracted from the root bark of *Cassia siberiana* harvested in Togo [30].

3.3.2. FRAP antioxidant profile

The antioxidant potential of the isolated compounds was explored by the reduction of ferric ions (Fe^{3+}) of the (Fe^{3+} TPTZ) complex to ferrous ions (Fe^{2+}) of the Fe^{2+} TPTZ complex. For this purpose, a linear calibration (y = 0.0197x - 0.0187; R^2 = 0.9991) was carried out. Figure 6 presents the results obtained.

In light of Figure 6, Vomifoliol (268.42±0.689 µMolEFeSO$_4$/g ES) and epiafzelechin (702.578±4.196 µMolEFeSO$_4$/g ES) show weak reducing capacities compared to vitamin C. However, the reducing activity of epiafzelechin is 2.61 times greater than that of vomifofiol.

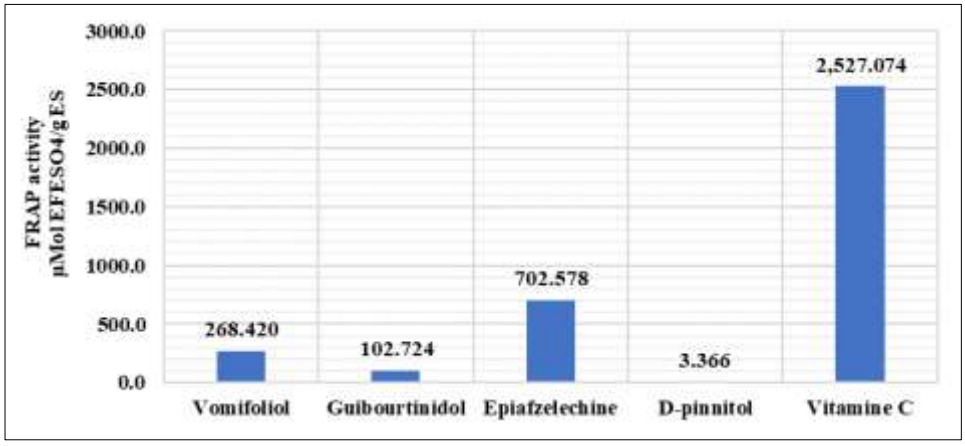


Fig 6: FRAP antioxidant profile of samples and reference

4. Conclusion

Bauhinia monandra is a plant species used in traditional Ivorian medicine in Côte d'Ivoire for the treatment of male infertility and diabetes. This study carried out on the selective extracts of the leaves made it possible to isolate, purify and elucidate the molecular structures of four compounds. These compounds were isolated from the leaves of the species distributed in Côte d'Ivoire for the first time. Two

complementary spectrophotometric methods for measuring free radical scavenging (DPPH test) and ferric reduction (FRAP test) have made it possible to assess their antioxidant potential. The antioxidant properties clearly expressed through these methods by epiafzelechin and vomifofiol, isolated from the leaves of *Bauhinia monandra* would explain, among other things, the use of the plant and its benefits in traditional medicinal practices in response to pathological conditions.

5. Acknowledgment

The authors would like to thank the EIFFEL-2020 Program and the National School of Chemistry of Montpellier. Also, they express their gratitude and thanks to Prof. MALAN Djah François for the identification of the plant.

6. Conflict of interest

The authors have not declared any conflict of interests.

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