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## Phytochemical study and antioxidant activities of *Terminalia catappa* L. and *Mitragyna ciliata* Aubrev and Pellegr medicinal plants of Gabon

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### Abstract

The aim of this study was to perform phytochemical screening, to quantify the phenolic compound and to evaluate the antioxidant activity of the ethanolic, ethanolic-water and aqueous extracts of *Mitragyna ciliata* and *Terminalia catappa*, medicinal plants from Gabon. Phytochemical tests reveal that both plants contain alkaloids, flavonoids, terpenoids, saponosids, tannins and reducing compound. The quantitative analysis shows that the polar extracts of *Terminalia catappa* have high contents in phenolic compounds compared to the polar extracts of *Mitragyna ciliata* which have low contents. The polar extracts of *Terminalia catappa*, with respective inhibition concentrations of  $0.27 \pm 0.114$ ;  $0.102 \pm 0.061$  and  $0.97 \pm 0.068$  mg.mL<sup>-1</sup>, are on average given a more significant antioxidant power compared to that of the control (Vitamin C). The polar extracts of *Mitragyna ciliata* have weak antioxidant activities with respective inhibition concentrations of  $1.026 \pm 0.061$ ;  $0.804 \pm 0.071$  and  $1.16 \pm 0.055$  mg.mL<sup>-1</sup>.

**Keywords:** *Mitragyna ciliata*, Combretaceae, *Terminalia catappa*, Rubiaceae, phenolic compounds, antioxidant activity

### 1. Introduction

The use of antioxidant molecules of synthesis is questioned because of potential toxicological risks. Now, new plant sources of natural antioxidants are sought [1, 2]. Indeed, polyphenols are natural compounds widely used in plants, which are increasingly important thanks to their beneficial effects on health [3]. The role of natural antioxidants attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [4]; they are also used as additives in food, pharmaceutical and cosmetics [1]. Scientific research has been developed for the extraction, identification and quantification of these compounds from different sources, such as agricultural and horticultural crops or medicinal plants [5-7]. Recent research realized on phenolic compounds shows that these chemical compounds possess physiological properties such as antioxidant and hepato-protective activities [8, 9]. Despite the place of antihypertensive synthetic products on the market, high blood pressure constitutes until this day a public health problem worldwide, especially in developing countries like Gabon. The Gabonese flora contains several species of plants little or not studied but endowed of veritable pharmacological properties; this is the case of *Terminalia catappa* and *Mitragyna ciliata*, two medicinal plants used in the treatment of high blood pressure by people.

*Terminalia catappa* is a plant of the family Combretaceae. It is native to Malaysia and the west of Pacific, introduced in Africa; it is present in irrigated areas ranging from West Africa (Senegal) to Central Africa. In Gabon, it is present in the West, North to South, the entire coastal area [10]. It is used by the Gabonese people to treat high blood pressure, hemorrhoids and diabetes. Several studies have been made on the plant *Terminalia catappa* whose authors suggest the presence of tannins, triterpenoids, glycosides cardiac and leucoanthocyanins and show that this plant has antioxidant activity, an aphrodisiac effect, immunogenicity, anti-cancer activity, dietary activity, hepato-protective, antimicrobial activity [10, 11].

*Mitragyna ciliata*, belonging to the Rubiaceae family, is a large tree that can reach 20 to 35 meters can reach 20 to 35 meters tall with a diameter rarely reaching 0.8 to 1 meter, was 12 to 15 meters, cylindrical, and without thickening at the base. Gabonese population uses this plant

In the treatment of female infertility, for douching as a local antiseptic and bark decoction. In association with other plant species is administered as an enema in painful menstruation. The Bavili (a Gabonese ethnic group) particularly pounding the bark with raffia fruits to asphyxiate fish [12-14]. Several authors have shown that this plant has local anesthetic properties, analgesic, hypotensive, antiplasmodial, antibacterial, antioxidant, vasodilating, immunostimulant and immunogenic [15-19]. Our study consists to realize the phytochemical screening, to determine the content of chemical compounds and evaluate the antioxidant activity of the polar extracts of those two medicinal plants of Gabonese flora.

## 2. Material and Methods

### 2.1. Plant material

The trunk bark of *Mitragyna ciliata* synonymous *Hallea ledermannii* and leaves of *Terminalia catappa* were collected in Franceville, in the Province of Haut-Ogooué (South-Eastern of Gabon) in March 2014.

	DD Coordinates	DMS Coordinates
Latitude	- 1.6196441	N-S 1° 37' 10.719''
Longitude	13.6015462	E-O 13° 36' 5.556''
Altitude	291 meters	

Identification of the species was carried out at the "Institut de Recherche en Ecologie Tropicale" (IRET), Libreville (Gabon). The samples were deposited at the National Herbarium of National Center of Scientific and the Technical Research (CENAREST) of Libreville (Gabon). They were dried at Laboratory of Natural Substances and Organometallic Synthesis (LASNSOM), Department of Chemistry of the Faculty of Sciences of USTM (Franceville), protected from light and at ambient temperature (27-29°C) during 5 weeks. The dry bark and leaves were crushed and preserved in bottles out of glass safe from the light and moisture for later analyses.

### 2.2 Preparation of plant extract

The total extracts were prepared from the dry powder of the plant material. 100 g of powder were set to macerate in 1 L of solvent, respectively ethanol, water ethanol (500/500, v/v) and water, with magnetic stirring for 48 hours. The macerate was then filtered and the solvent was evaporated to dryness under reduced pressure at 75°C at help BÜCHI R-210 rotary evaporator. The extracts were concentrated and kept in glass bottle for analysis.

### 2.3 Phytochemical Screening

The chemical characterization of the secondary metabolites contained in the plant extracts was carried out according to the conventional methods described by Bouquet [11]. Thus, we carried out the tests of alkaloids (reactive of Dragendorff and Mayer), anthocyanins (HCl 20%), anthraquinones (KOH 10%), flavonoids (reaction to the cyanidin), triterpenoids (reaction of Liebermann-Bouchard), saponosids (foam index), Gallic tannins and catechic tannins (ferric chloride, Stiany), reducing compounds (reagent of Fehling) and carotenoids. Thin-layer chromatography (TLC) was associated to confirm the presence or absence of certain chemical families such as the alkaloids, flavonoids and terpenoids. Different eluents and developers were used: for flavonoids we used ethyl acetate/formic acid/ water (8/1/1, V/ V/V) as eluent and Neu as a developer; Petroleum ether/ethyl acetate (7/3, V/V) as eluent and Anisaldehyde and Liebermann-bürchard as developer for terpenes and sterols; Ethyl

acetate/methanol/ammonia (9/1/1, V/V/V) as eluent and Dragendorff as a developer for alkaloids.

### 2.4 Phenolic content

The total phenolic contents of the different extracts were determined by the method of Folin-Ciocalteu [20]. A quantity of 200 µL of the extract is mixed with 1 mL reagent of Folin-Ciocalteu coldly prepared (10 times diluted) and 0.8 mL sodium carbonate solution (7.5%). The unit is incubated at ambient temperature during 30 min and the reading is carried out against a white using a spectrophotometer with 765 nm. All analyses were done in triplicate and results (average of triplicate analysis) are expressed in milligrams equivalent of Gallic acid per gram of dry vegetable matter.

### 2.5 Flavonoids content

The content in flavonoids of the extracts was given by using the colorimetric method with aluminum chloride [21]. A quantity of 100 µL of the extract was mixed with distilled water 0.4 mL and thereafter with 0.03 mL sodium nitrite solution (5%). After 5 min, 0.02 mL aluminum chloride solution (10%) was added. One adds with the mixture 0.2 mL sodium carbonate solution (1 M) and 0.25 mL distilled water after 5 min with rest. The unit is agitated using a vortex and the absorbance was measured to 510 nm was recorded after 30 min of incubation. A standard calibration plot was generated using known concentration of quercetin. The results are expressed in milligrams equivalent of quercetin per gram of dry vegetable matter.

### 2.6 Tannins content

The content in tannins of the extracts was determined by the reference method of European community. A volume of 1 mL of extract to be proportioned is mixed with the vortex with 5 mL distilled water, 1 mL ferric ammonium citrate (28% of iron; 3.5 g.L<sup>-1</sup>) going back to 24 h and 1 mL from ammoniac (NH<sub>3</sub> 8 g.L<sup>-1</sup>). After 10 min of incubation, the absorbance were measured to 525 nm. The results are expressed in equivalent microgram of catechin per gram of dry vegetable matter.

### 2.7 Flavonols content

The content flavonols of the extracts was determined by the method of Yermakov *et al* [22]. One mixes 2 mL each extract (1 mg.mL<sup>-1</sup>) with 2 mL aluminum chloride solution (20 g.L<sup>-1</sup>) and 6 mL sodium acetate solution (50 g.L<sup>-1</sup>). After 2 h and half of incubation to 20°C, the reading of the absorbance was made to 440 nm. The results are expressed in equivalent microgram of quercetin per gram of dry vegetable matter.

### 2.8 Evaluation of antioxidant activity on Thin-Layer Chromatography (TLC)

This test was done according to the method described by Takao *et al* [23] with some modifications. 5 µL of solution of each extract at a concentration of 10 mg.mL<sup>-1</sup> were deposited on the plate of silica 60 F<sub>254</sub> Merck aluminum. Plaque development is carried out in the solvent system: butanol/ethyl acetate/water (15/3/5, v/v/v). After migration, the chromatograms are dried using the electric dryer and then revealed to the ethanol solution of DPPH 1%.

### 2.9 Evaluation of antioxidant activity on spectrophotometer

The test on DPPH Spectrophotometer was performed according to the method described by Sanchez-Moreno *et al* [24] (1998). 50 µL of each ethanolic extracts at various

concentrations (0.0625 to 4 mg.mL<sup>-1</sup>) are added to 1.95 mL of the ethanolic solution of DPPH (0.025g.L<sup>-1</sup>). In parallel, a negative control was prepared by mixing 50µL of ethanol with 1.95 mL of the ethanolic solution of DPPH. The absorbance reading is made against a blank prepared for each concentration at 515 nm after 30 min of incubation in the dark at room temperature. The positive control is represented by a standard solution of an antioxidant; ascorbic acid, whose absorbance has been measured under the same conditions as the sample for each concentration and the test was repeated 3 times. The results were expressed as percentage of inhibition (I %). The percentage of inhibition of free radical DPPH (I %) is calculated as follows:

$$I\% = [(A \text{ white} - A \text{ sample}) / A \text{ white}] \times 100$$

With:

A white: Absorbance of control (DPPH in ethanol)

A sample: Absorbance of the solution of test

The concentration of 50% inhibition of DPPH (IC<sub>50</sub>) was determined from the regression line.

### 2.10 Statistical analysis

The experimental results are expressed as mean ± SEM. All measurements were duplicated three times. The IC<sub>50</sub> values were calculated from the linear regression graph showing the percentages of inhibition depends on the concentration of the extract.

*Terminalia catappa* and *Mitragyna ciliata* are rich in secondary metabolites. The ethanolic and ethanolic-water extracts of *Terminalia catappa* have significant contents of phenolic compounds. Both plants have antioxidant properties. The antioxidant activity of ascorbic acid nevertheless remains lower than that of *Terminalia catappa* but superior to that of *Mitragyna ciliata*. But it is a question of the rough extracts which contain compounds which, once purified, could present a more interesting antioxidant activity more. These extracts could thus constitute an alternative to certain synthetic additives. For better apprehending the synergy which exists between the polyphenolic compounds present in the extracts of plants and the antioxidant activity of these extracts, it would be interesting to identify, insulate and purify the various compounds of this plant

## 3. Results and Discussion

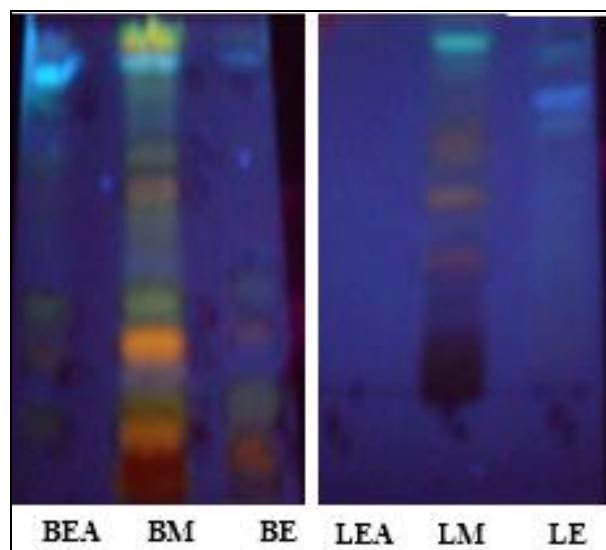
### 3.1 Phytochemical screening

Phytochemical screening was carried out to identify the main chemical groups present in plant extracts. Due to the results shown in Table 1, it appears that *Mitragyna ciliata* and *Terminalia catappa* contain alkaloids, flavonoids, terpenoids, saponosides, catechic and Gallic tannins, and reducing compounds. In addition to these compounds, *Mitragyna ciliata* also contains anthocyanins and anthraquinones. Both plant species not contain carotenoids. Chromatograms (Figure 1) confirm the presence of terpenes and sterols (appearance, after revelation, of violet, brown, blue, greenish yellow, yellow, red, green spots), Flavonoids (yellow-orange, yellow, green-yellow fluorescent spots) and alkaloids (appearance of an orange trail on a purple background after revelation) [14].

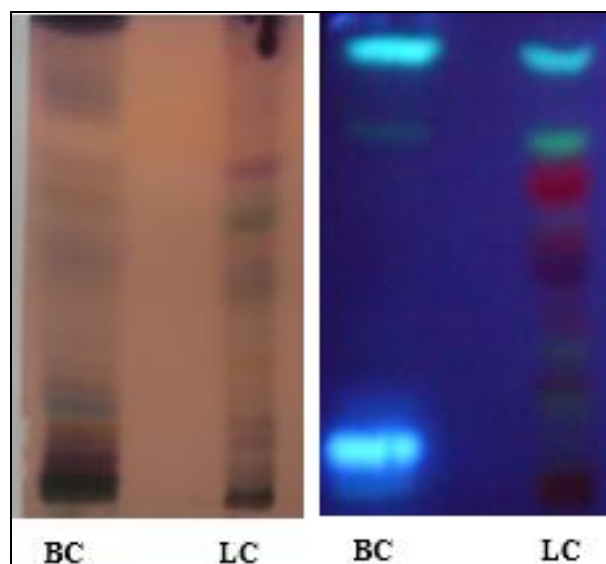
**Table 1:** Results of phytochemical screening

Chemical groups		<i>Mitragyna ciliata</i>	<i>Terminalia catappa</i>
Alkaloids	Dragendorff	++	-
	Mayer	++	-
Anthocyanins		+++	-
Anthraquinones		+++	-
Flavonoids		++	+++
Terpenoids		+++	+++
Saponins		+++	+
Catechic tannins		+	++
Gallic tannins		++	+++
Reducing compound		+++	+++
Carotenoids		-	-

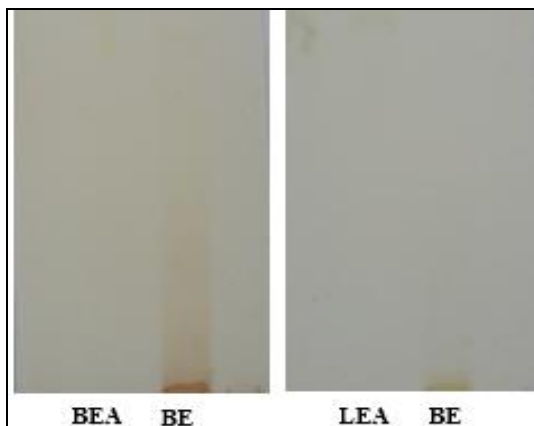
+++ : Very abundant, ++ : Abundant, + : Rare, - : Not detected.



**Fig 1a:** TLC of the flavonoids of the polar extracts of trunk barks of *Mitragyna ciliata* and the leaves of *Terminalia catappa* Revelation with Neu



**Fig 1b:** TLC of the terpenoids of the chloroform extracts of trunk barks of *Mitragyna ciliata* and the leaves of *Terminalia catappa* Revelation to Anisaldéhyde Revelation with the reagent of Liebermann- Burchard



**Fig 1c:** TLC of the alkaloid of the polar extracts of trunk barks of *Mitragyna ciliata* and leaves of *Terminalia catappa* Revelation with Dragendorff

**Fig 1:** Results of TLC tests of flavonoids, alkaloids, terpenes and sterols. B = Bark; L= Leaf; EA = Ethyl acetate extract; M = Methanolic extract; E = Ethanolic extract; C = Chloroform extract.

These results are in agreement with those obtained by Mibindzou [15] and Souleymane [17] on the plant species harvested respectively in the Abidjan region of Côte d'Ivoire and in Libreville in southern Gabon. On the other hand, they are different from the results found by Babayi *et al* [18] and Bidié *et al* [19], who claim respectively that the bark of *Mitragyna ciliata* harvested in the central western forest area in the Issia department (Côte d'Ivoire) does not contain Gallic tannins and *Terminalia catappa*, harvested in Nigeria, do not contain flavonoids [18-19]. These differences in chemical compositions could be explained from several parameters,

the environment, genotype, geographical origin, harvest time, climate and extraction method [25]. The results show that both plants are rich in secondary metabolites. Thus, the proven efficacy of these species in the traditional treatment of cardiovascular diseases, in this case hypertension, could be explained by the representativeness of the polyphenols in their breasts. Indeed, several studies show, on the one hand, that polyphenols have a vasculo-protective action, and on the other hand that they have a vasodilating activity and many others affirm that the flavonoids have a vasculo-relaxing effect [26-28].

### 3.2 Polyphenolic content

The determination of the phenol, flavonoid, tannin and total flavonol contents of plant extracts by spectrophotometric assays was carried out using the different colorimetric methods: Folin-Ciocalteu for phenols, aluminum trichloride (AlCl<sub>3</sub>) for flavonoids, The reference method of the European Community with some modifications for tannins and the Yermakov *et al* method [22] for flavonols. The results are shown in Table 2. The phenol contents of the extracts are expressed in milligrams equivalent of Gallic acid per gram of dry plant material (Standard Equation of the Calibration Curve:  $Y = 0.0004X + 0.0053$ ;  $R^2 = 0.9929$ ); the total flavonoid and flavonol contents (standard equations of the respective calibration curve:  $Y = 0.0005X + 0.0044$ ;  $R^2 = 0.9963$  and  $Y = 0.0575X - 0.2123$ ;  $R^2 = 0.9982$ ) are expressed in milligrams equivalent of dry plant material and the quantities of tannins are expressed in milligrams equivalent of tannic acid per gram of dry vegetable matter (mg EAT/g MS), the standard equation of the calibration curve of which is  $Y = 0.0009X + 0.2088$ , with  $R^2 = 1$ .

**Table 2:** Total phenol content (TPC), total flavonoid content (TFC), total tannin content (TTC) and total flavonol content (TfC) of polar extracts of *Mitragyna ciliata* and *Terminalia catappa*

Extractions	Yield of extracts (%)	TPC (mg GAE/ g dm)	TFC (mg QE/ g dm)	TTC (mg TAE/ g dm)	TfC (mg QE/ g dm)
EE of Tc	6.246	21.56175 ± 0.331	1.0812 ± 0.115	0.37022 ± 0.167	0.02239 ± 0.213
EWE of Tc	15.009	13.43675 ± 0.378	0.8832 ± 0.167	0.45244 ± 0.091	0.01655 ± 0.05
AE of Tc	8.756	10.22175 ± 0.272	1.3112 ± 0.206	0.228 ± 0.049	0.01355 ± 0.084
EE of Mc	6.545	2.78675 ± 0.110	1.2272 ± 0.197	0.078 ± 0.056	0.01635 ± 0.075
EWE of Mc	13.245	3.55675 ± 0.083	0.7232 ± 0.187	0.13278 ± 0.091	0.01388 ± 0.116
AE of Mc	7.172	3.27425 ± 0.101	0.8072 ± 0.129	0.1143 ± 0.052	0.01374 ± 0.068

Tc = *Terminalia catappa*; Mc = *Mitragyna ciliata*; EE = Ethanolic extract; EWE = Ethanolic-water extract; AE = Aqueous extract; MS = Dry matter

The results obtained show that *Terminalia catappa* has higher polyphenol contents compared to *Mitragyna ciliata* (Table 2). Indeed, ethanolic, ethanolic-water and aqueous extracts of *Terminalia catappa* have respectively the contents highest of phenol (21.56175 ± 0.331 mg EAG/g ms), tannins (0.45244 ± 0.091 mg EAT/g ms) and flavonoids (1.3112 ± 0.206 mg EQ/g ms) compared with those of *Mitragyna ciliata*. On the other hand, flavonoids, tannins and flavonols are more representative of phenols in *Mitragyna ciliata* extracts than in *Terminalia catappa*. Flavonoids, tannins and flavonols represent respectively 44.037%; 2.799% and 0.587% of phenols in the ethanol extract while they represent respectively 5.014%; 1.717% and 0.104% of phenols in the ethanolic extract of *Terminalia catappa*. These results are different from those found by Adeleke *et al* [16] and Kuo-Ching *et al* [29] respectively on *Mitragyna ciliata* leaves harvested in Nigeria (total phenols 88 mg EAG/g ms) and on *Terminalia catappa* leaves, harvested in Taichung County (total phenols 102.0 ± 0.2 µg GAE/mg). In Malaysia and Brazil, some authors found respectively very high contents of

phenols (432.90 ± 1043 mg EAG/g ms and 338.09 ± 4.26 mg/g ms) on leaves of *Terminalia catappa* and on fruit of *Terminalia catappa* of very low phenol contents (142.84 ± 2.09 mg EAG/100 g ms and 244.33 ± 18.86 mg EAG/100 g ms) [7, 30].

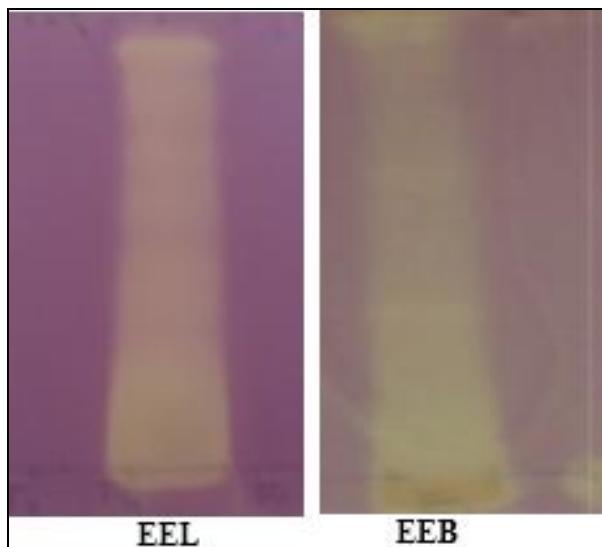
### 3.3 Antioxidant activity

The antioxidant activity of ethanolic, ethanolic-water and aqueous extracts of *Mitragyna ciliata* and *Terminalia catappa*, with respect to the free radical DPPH, was evaluated on the one hand on TLC (thin layer chromatography) and on the other with the aid of a spectrophotometer following the reduction of the free radical which is accompanied by its passage from the violet color (DPPH) to the yellow color (DPPH-H) measurable at 515 nm.

### 3.4 Method by thin layer chromatography (TLC)

The chromatograms of the ethanolic extracts of the two plants, obtained after revelation to the ethanolic solution of 1% DPPH, show light yellow color spot on violet background; characteristics of the antioxidant activity of chemical compounds (Figure 2) [31].





EEL= Ethanolic extract of the leaves of *Terminalia catappa*  
 EEB= Ethanolic extract of the bark of *Mitragyna ciliata*

**Fig 2:** Chromatographic profiles of the antioxidant activity of polar extracts of *M. ciliata* and *T. catappa*

### 3.5 Antioxidant activity on spectrophotometer

The antioxidant activity of the plant extracts was evaluated by the DPPH free radical scavenging method. The results

obtained are reported in Table 3. The antioxidant power is determined by a reduction in the absorbance induced by antiradical substances [32].

**Table 3:** Antioxidant activity of *Mitragyna ciliata* and *Terminalia catappa* extracts by DPPH free radical scavenging method

Extract/standard	Equation of the regression curve	R <sup>2</sup>	IC <sub>50</sub> (mg.mL <sup>-1</sup> )
EE of Mc	Y = 39.04X + 9.9289	0.9939	1,026 ± 0.061
EWE of Mc	Y = 47.977X + 11.419	0.9973	0,804 ± 0.071
AE of Mc	Y = 36.428X + 7.6659	0.9572	1,160 ± 0.055
EE of Tc	Y = 158.71X + 7.597	0.9834	0,270 ± 0.144
EWE of Tc	Y = 293.55X + 19.899	0.9916	0,102 ± 0.061
AE of Tc	Y = 39.752X + 11.356	0.9932	0,970 ± 0.068
Vit C	Y = 77.368 - 2.788	0.9897	0,680 ± 0.047

Tc = *Terminalia catappa*; Mc = *Mitragyna ciliata*; EE = Ethanolic extract; EWE = Ethanolic-water extract; AE = Aqueous extract; Vit C = Vitamin C IC<sub>50</sub> values are the mean of three repetitions ± standard error on mean (ESM)

The ethanolic extract of *Terminalia catappa* has the highest antioxidant activity compared to the ethanolic extract of *Mitragyna ciliata*. Indeed, the yellow trail on violet background is optimal on the chromatogram of the ethanolic extract of *Terminalia catappa*. The results found on spectrophotometer confirmed those obtained on TLC. Ethanolic-water and ethanolic extracts of *Terminalia catappa* owns significant antioxidant activity compared to the standard antioxidant (ascorbic acid). Indeed, the antioxidant power depends on the ability to reduce the free radical DPPH (IC<sub>50</sub>). Because, the lower the concentration of inhibition (IC<sub>50</sub>), the stronger the antioxidant activity. The polyphenols contained in the polar extracts of *Mitragyna ciliata* and *Terminalia catappa* are probably responsible to their antioxidant activities. Because, it is demonstrated that molecules such as flavonoids and tannins reduce and discolor DPPH because of their ability to yield hydrogen [33]. This antioxidant activity is dependent on the mobility of the hydrogen atom of the hydroxyl group of the phenolic compounds of the plant extracts. In the presence of a free radical DPPH, the hydrogen atom is transferred into the latter then transformed into a stable molecule DPPH, this causes a decrease in the concentration of the free radical and also the absorbance during the reaction time until the depletion of antioxidant capacity hydrogen donor [34]. These results are different from those obtained by Marques *et al* [7] on *Terminalia catappa* (alcohol extract: 85.99 and aqueous extract: 0.70 µg.mL<sup>-1</sup>)

and by Bidie *et al* [19] on *Mitragyna ciliata* (IC<sub>50</sub> = 10.5 ± 0.288 µg.mL<sup>-1</sup>)

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