GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Phytochemical, antioxidant and anticholinesterase profiles of *Musanga cecropioïdes* R. Br. (Urticaceae) from Côte d'Ivoire

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GSC Biological and Pharmaceutical Sciences, 2021, 16(02), 094–102

Publication history: Received on 02 July 2021; revised on 11 August 2021; accepted on 13 August 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.16.2.0224

Abstract

This study is devoted to *Musanga cecropioides* (Urticaceae), a tropical plant whose leaves and root bark are used in traditional medicine in the treatment of various pathologies. The objective of this work was to study qualitative and quantitative composition of phenolic compounds in extracts of the leaves (McF) and root bark (McR) *Musanga cecropioides* and to evaluate their antioxidant and anticholinesterase properties. Phytochemical screening using TLC showed that the extracts contain, along with phenolic compounds such as coumarins, flavonoids and tannins, other bioactive phytocompounds namely sterols, terpenes and alkaloids. Quantitative analysis of phenolic phytoconstituents by spectrophotometry showed that contents of total flavonoids and polyphenols in leaves (7.753% and 119.389 mg EAG / g, respectively) are higher than those in the root bark (1.41% and 105.944 mg EAG / g, respectively). The antioxidant activity of total and selective extracts evaluated by FRAP and DPPH methods was found to be significant compared to vitamin C. All the extracts of *Musanga cecropioides* showed good anticholinesterase activity with percentages of inhibition of the acetylcholinesterase (AChE) ranging from 51.952 to 63.589%.

Keywords: *Musanga cecropioides*; Chemical composition; Antioxidant; Anticholinesterase activities

1. Introduction

The Urticaceae family, with more than 2000 species of morphological diversity, is distributed in tropical and temperate regions, with the majority of general and species in tropical Asia. They are herbaceous plants, usually deciduous and sometimes creepers shrubs. The *Musanga cecropioïdes* (Urticaceae) species, widespread in the tropical forests of Africa from Guinea to the Congo Basin [1], is a plant used in traditional medicine in West and Central Africa for the treatment of pathologies such as constipation, rheumatism, cough, schizophrenia, lung infections, leprosy, high blood pressure and malaria [2, 3]. Numerous scientific studies have shown that extracts from the various organs of *M. cecropioides* exhibit hypotensive, hypoglycemic, antidiabetic, antidiarrhoeal and antibacterial properties [4-7]. In Côte d'Ivoire, according to information gathered from ethnobotanical surveys, the root bark of *M. cecropioides* are used to treat mental illnesses. In view of this virtue of *M. cecropioides*, we took an interest in it, and this, by considering studying chemical composition of its organs and their inhibitory activity of acetylcholinesterase (AChE). Phytocompounds such as alkaloids, terpenoids, cardiac glycosides, saponins and coumarins have a beneficial effect on the central nervous system by increasing the activation of cholinergic neurons and stimulating cognitive functions such as acetylcholinesterase inhibitors (AChE) [7, 8]. This makes it possible to consider them promising in the prevention of neurodegenerative diseases of the Alzheimer type. Indeed, cholinergic deficiency is a hallmark of Alzheimer's disease (AD), which is associated with the selective death of cholinergic neurons in the initial stages of the disease [9]. We know that the brain is more sensitive to damage

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caused by free radicals than other tissues. The effectiveness of antioxidant system in the brain gradually decreases with aging, and in the brains of patients with AD, this decrease occurs even more strongly. All of this determines the advisability of using natural antioxidants in the prevention and treatment of AD [10].

2. Material and methods

2.1. Plant material

The plant material consists of the leaves and root bark of *M. cecropioides* (Cecropiaceae) collected in Zokolilié (Lakota, south-western Côte d'Ivoire), and identified at the National Floristic Center (CNF) of Abidjan under the No. 17543. After cleaning with water and drying in a ventilated room for 15 days, the organs were pulverized using an electric grinder and kept in tightly closed glass jars.

2.2. Preparation of extracts

20 g of powder macerated in 150 ml of MeOH (80% v / v) for 24 hours. The operation is repeated three times. After reduction by simple distillation, 2/3 of the filtrate is kept in an oven at 50°C for 24 hours to obtain the total hydromethanolic extracts of the leaves (McF) and of the roots (McR). A selective liquid / liquid extraction of 1/3 of the filtrate was carried out with solvents of increasing polarity to obtain the extracts with hexan (McF^{I} McR^{I}), chloroform (McF^{II} McR^{II}), ethyl acetate (McF^{III} McR^{III}) and n-butanol (McF^{IV} McR^{IV})).

2.3. Qualitative analysis by TLC

The phytochemical screening was carried out on chromatoplates (aluminum support, silica gel 60 F254, thickness 0.2 mm; Merck) according to the identification methods taken from the literature [11-13]. The different developing agents used are: n- C_6H_{14} / AcOEt, 6: 1.5 (v / v) for the hexane extracts; $CHCl_3$ / AcOEt / n-C_6H_{14}, 5: 5: 2.5 (v / v / v) and $CHCl_3$ / AcOEt / n-C6H14, 5: 5: 7.5 (v / v / v) for the chloroform extracts; ($CHCl_3$ / AcOEt / CH_3COOH, 4: 5: 2 (v / v / v) ethyl acetate extracts and MeOH / CH_3COOH, 4: 0.5 (v / v) n-butanol extracts.

Depending on type of secondary metabolites to be revealed, specific reagents used are Liebermann-Bürchard reagent for sterols and terpenes; sulfuric vanillin; Godin's reagent for sterols, terpenes and flavonoids, 5% (v / v) methanolic KOH for coumarins; AlCl3 for flavonoids; Dragendor'ff's reagent for alkaloids; 2% (v / v) FeCl3 for tannins and phenolic compounds.

2.4. Quantitative analysis

2.4.1. Determination of total phenols (PT)

PT was quantified in the crude extracts (McF and McR) by the spectrophotometric method [14]. 1 ml of extract diluted 1 / 50 added 1.5 ml of a solution of Na_2CO_3 (17%, m / v) and 0.5 ml of Folin-Ciocalteu reagent (0.5 N). The whole is incubated at 37°C for 30 min and the absorbance read at 720 nm against a blank without extract taken as a reference. A calibration with different concentrations of gallic acid (from 1.95 µg / ml to 62.5) is carried out under the same conditions. The PT contents are calculated according to formula (1) and expressed in µg equivalent of gallic acid per g of dry matter (µg EAG / g DM).

$$PT = (V \times C \times d) / m \quad (1)$$

V**:** volume of the crude extract (ml); **C:** mean concentration (µg / ml); d**:** dilution factor; **m:** mass of the pulverized dry matter (g).

2.4.2. Determination of total flavonoids (FT)

The quantification of FT is performed by spectrophotometry [15, 16]. To 2 ml of each crude extract diluted to 1 / 20th were added 100 µl of NEU reagent, the absorbance is measured at 404 nm and compared to that of quercetin (0.05 mg / ml) taken as standard. The percentage of FT is calculated according to expression (2) and converted into µg equivalent of gallic acid per g of dry matter (µg EAG / g DM)

$$\% FT = [(0{,}05 \times A_{ext} \times d) / A_q \times C_{ext}] \times 100 \quad (2)$$

A_{ext}: Absorption of the extract; Aq: Absorption of quercetol; C_{ext}: Concentration of the extract (2.5 mg / ml); d: dilution

2.4.3. Dosage of tannins

Hydrolyzable tannins (TH)

This method is based on the formation of the violet-red coloring complex between tannins and iron trichloride ($FeCl_3$), the absorbance of which is read with a UV-visible spectrophotometer (Analytic AL800) [17]. 0.4 g of powder are macerated in 20 ml of MeOH (80%) for 18 h. To 1 ml of the macerate are added 3.5 ml of a solution of FeCl3 (0.01 M in 0.001 M HCl) and the absorbance is read at 660 nm. The TH content is calculated to formula (3):

$$TH\ (\%) = (Abs \times M \times V) / E\ mole \times P \quad (3)$$

Abs: absorbance, E mole: 2169 of gallic acid (constant expressed in mole), M: mass = 300, V: volume of the extract used, P: mass of the sample.

Condensed tannins (TC)

To 400 µl of sample (0.5 mg / ml) are added 1.5 ml of vanillin solution (4% in MeOH) and 0.8 ml of concentrated HCl. After 15 min of incubation, the absorbance is read at 500 nm [18, 19]. The TC contents are deduced from the calibration curve produced with the catechin (0-150 µg / ml) and expressed in µg of catechin equivalent per mg of dry extract (µg ETC / mg).

2.5. Evaluation of the antioxidant potential by spectrophotometry

DPPH test

The antioxidant activity is evaluated by the reduction of the DPPH radical according to the methodologies described in the literature [20, 21]. The reference antioxidant used is ascorbic acid (vitamin C) prepared under the same conditions as the samples.

FRAP test

The method is based on the reduction by the antioxidant of the ferrous tripyridyltriazine complex TPTZ-Fe^{3+} to TPTZ-Fe^{2+} of blue color, the absorbance of which is read at 593 nm with a UV-visible spectrophotometer (Analytic AL800). The standard curve was constructed from the Trolox calibration curve. The results are expressed in mM Trolox equivalent [22, 23].

2.6. Evaluation of anticholinesterase activity

The inhibition of acetylcholinesterase (AChE) evaluated to methods described literature [24, 25]. 10 µl of AChE (1 U / ml) and 150 µl of methanolic extract (2 mg / ml) and 1.80 ml of Tris-HCl buffer. The whole was incubated at 37 ° C for 15 min. Subsequently, 20 µl DTNB (0.01M) and 10 µl ATCI (75 mM) was added, and incubated at room temperature for 5 min. The control mixture (150 µl of galanthamine (Reminyl®) and a blank (150 µl of MeOH) was prepared under same conditions as the extracts. The absorbances was read with a spectrophotometer at 412 nm every 30 s for 6 min. AChE inhibition (I) calculated as a percentage (%) to formula:

$$I\ (\%) = 100 - [(Absorbance\ extract / Absorbance\ control) \times 100] \quad (4)$$

3. Results and discussion

3.1. Phytochemical profile of selective extracts

The phytochemical screening by TLC of the selective extracts of. M cecropioides has shown the presence of several phytocompounds using appropriate specific developers (**Table 1**). Liebermann-Bürchard and Godin reagents and sulfuric vanillin demonstrated sterols, terpenes, oleanan-type triterpenes and ursane in hexane and $CHCl_3$ extracts [11].

Table 1 Phytocompounds identified by TLC in the selective extracts

Extracts		Rf extracts, color, family of compound possible
Hexan	*McR*^{I}	0,02 Vi^{e**}:Ter ; 0,10 Br^{c*} J^{C**} :St ; 0,18 Br^{c*} J^{C**} :St ; 0,25 Vi^{d*e**}:Ter ; 0,34 Vi^{e**} :Ter; 0,37 :Vi^{d*e**}:Ter ; 0,41 B^{c*}:St ; 0,49 O^{c**} Vi^{d*e*} :TrL ; 0,54 Vi^{C*} R^{c**}:TrOU ; 0,58 B^{c*} R^{c**}:TrOU; 0,60 V^{c*} B^{d*} :St; 0,63 B^{e} R^{c**} : TrOU ; 0,67 Br^{C*} J^{c**} :St ; 0,70 Vi^{c*} : Ter; 0,75 Vi^{d*,e**} :Ter; 0,8 B^{d*e*}: St ; 0,83 J^{c**} :St; 0,92 Br^{c*} B^{d*e*} : St
	McF^{I}	0,02 Vi^{e**} :Ter ; 0,18 Br^{c*} B^{d*} :St ; 0,25 R^{c**} :TrOU ; 0,28 Vi-O^{d*}: Ter ; 0,33 Vi^{c*e**} :Ter ; 0,35 O^{d*} :Ter ; 0,41 Vi^{e**} :Ter; 0,46 Vi^{d*} : Ter; 0,47 V-J^{c**} :St; 0,50 R^{c**} :TrOU; 0,53 O^{d*} :Ter; 0,58 V^{c*} :St; 0,60 R^{c**} : TrOU; 0,63 B^{d*} :St; 0,70 Vi^{c*} :Ter; 0,78 Vi^{d*} :Ter; 0,83 Vi^{e**} :Ter; 0,85 Vi^{e**} :Ter ; 0,92 Vi^{e**} :Ter
$CHCl_3$	*McR*^{II}	0,02 Vi^{e**} :Ter ; 0,1 Br^{c*} :St ; 0,18 Br^{c*} :St ; 0,25 Vi^{d*e**}:Ter ; 0,28 B^{b**}:Cou ; 0,33 Vi^{d*} :Ter; 0,37 Vi^{d*e**} : Ter ; 0,41 Br^{c*} :St; 0,45 Vi^{b**} :Anth ; 0,49 O^{c**} Vi^{d*,e*} :TrL; 0,60 V^{c*} B^{d*} :St; 0,67 J^{c**} :St; 0,74 Vi^{d*e**} :Ter ; 0,80 B^{d*e*} :St; 0,83 J^{c**} :St; 0,85 V^{b**}:Cou; 0,92 Vi^{d*e**} :Ter
	McF^{II}	0,18 Vi^{d*e**} :Ter ; 0,25 Vi^{d*e**} :Ter; 0,28 Vi-O^{d*} :Ter; 0,33 Vi^{e**} :Ter; 0,35 O^{d*} :Ter; 0,37 R^{c**} :TrOU; 0,41 Vi^{e**} :Ter; 0,47 J-V^{c**}: St; 0,53 O^{d*} :Ter; 0,57 V^{c*} :St; 0,58 Gr-O^{d*}:Ter ; 0,60 B^{e*} :St; 0,74 B^{b**} :Cou ; 0,79 Vi^{b**} :Cou; 0,80 Vi^{c*} :Ter ; 0,83 Vi^{e**} :Ter; 0,88 Vi^{e**} :Ter ; 0,92 Vi^{d*e**} :Ter; 0,95 Vi^{b**} :Cou
AcOEt	*McR*^{III}	0,02 O^{h*} :Al; 0,05 Gr^{i*}:Tan ; 0,24 J^{g**} : Fl
	McF^{III}	0,02 J^{g*} :Fl ; 0,05 Gr^{i*} :Tan ; 0,24 J^{g*} : Fl ; 0,16 Gr^{i*} :Tan ; 0,22 J^{g*} : Fl; 0,33 B^{g**} : Fl ; 0,37 B^{g**} :Fl; 0,47 B^{g**} :Fl
n-BuOH	*McR*^{IV}	0,02 O^{h*}: Al ; 0,05 Gr^{i*} :Tan^{i} ; 0,24 J^{g*} :Fl
	McF^{IV}	0,02 J^{g*} :Fl ; 0,05 Gr^{i*} :Tan ; 0,24 J^{g*} :Fl; 0,16 Gr^{i*} :Tan ; 0,22 J^{g*} :Fl ; 0,33 B^{g**} :Fl ; 0,37 B^{g**} :Fl ; 0,47 B^{g**} :Fl

J / yellow; Vi / violet; O / orange; B / blue; V / green; R / red; Jv / Yellow-green; Br / brown; Gr / Gray; Anth / Anthracene; St / sterol; Ter / Terpene; TrL / Triterpene of lupane type, TrOU / Triterpene of oleanane and ursane type; Fl / Flavonoid; Neck / Coumarin; Al / Alkaloid; Tan / Tannins; b / KOH; c / Libermann-Buchard; d / Sulfuric vanillin; e / Godin; g / $AlCl_3$; h / Dragenddor'ff; i / FeCl3; * / visible to the naked eye; ** / at UV 365 nm

The yellow molecular fingerprints perceptible to the naked eye (Table 1) revealed by 5% (w / v) of methanolic KOH solution indicate the presence of coumarins. This coloration may intensify or turn blue or green under UV light / 365 nm [11, 13]. KOH solution (5%) allow to identify 1, 2-dioxyanthracene (Rf = 0.45) under UV at 365 nm as a purple spot in the $CHCl_3$ extract of the root bark. [11]. The alkaloids, flavonoids and condensed tannins were revealed in the AcOEt and n-BuOH extracts. Indeed, Dragenddor'ff's reagent revealed alkaloids in orange spots, while $AlCl_3$ revealed flavonoids in yellow to the naked eye and blue under UV / 366nm. Condensed tannins were detected by $FeCl_3$ as gray spots [13]. Comparing our results with those of studies of extracts from the bark of the trunk of M. cecropioides from Nigeria, we observed some similarity in the presence of tannins, flavonoids and alkaloids [26]. In addition, Togolese researchers have also shown the presence of phenols, coumarins, tannins, flavonoids and alkaloids in the leaves and in the stem bark of the species from Gabon [27].

3.2. Total phenols and flavonoids content

The PT content is obtained from the linear regression equation for gallic acid (y = 0.0232 x + 0.0002; R_2 = 0.9983) and expressed in g EAG / g DM. The values obtained show that the leaf extract (McF) contains more PT (119.389 mg EAG / g) compared to the McR root bark (105.944 mg EAG / g) (**Figure 1**)**.** The FT contents are also higher in the leaves (7.753%) than in the root bark (1.41%).

A study carried out on the leaves and stem bark of the species from Gabon showed the contents of total phenols (40.69 ± 6.43 and 40.26 ± 3.12 mg / g, respectively) lower compared to to those found in our case [27]. In general, the PT and FT are higher in the leaves than in the stems and roots, which is explained by the fact that the synthesis of flavonoids occurs under UV irradiation, therefore in the part of the plant. The most exposed to solar rays [28, 29].

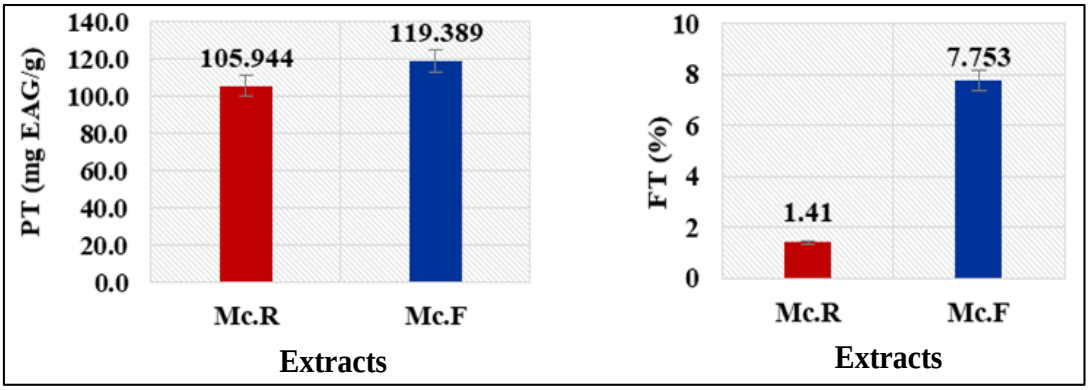

Figure 1 Content of total polyphenols and flavonoids

3.3. Hydrolyzable (TH) and condensed (TC) tannin content

In plants, TH and TC occur simultaneously, with the predominance of one class. In our case, the quantification of the tannins showed that TH and TC are present in two parts of *M. cecropioides* with the predominance of TC (**Figure 2**). Previously, depending on the result of the reaction with FeCl3 solution, TC was found to predominate in the study extracts (**Table 1**). However, the highest tannin content is found in the aerial part of *M. cecropioides*, the lowest in its underground part. Tannins in significant quantities accumulate, mainly in underground organs, in the bark and wood of trees and shrubs, less often in the leaves (green and black tea, strawberry, bramble, raspberry) [30]. Knowing that these leaves are great classics of herbalists thanks to their richness in tannins, we can also recommend using the leaves of *M. cecropioides* in the form of herbal teas.

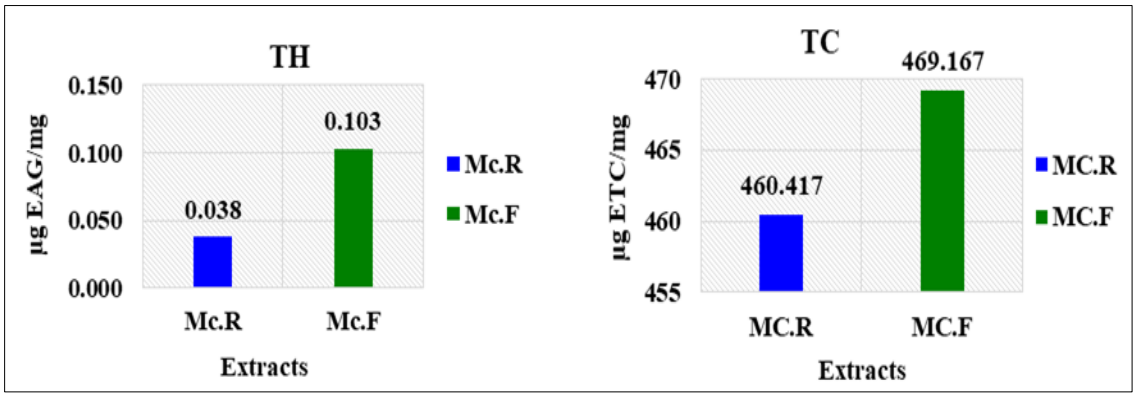

Figure 2 Hydrolyzable (TH) and condensed (TC) tannin content

3.4. Antioxidant DPPH and FRAP profiles

There are different methods for determining the antioxidant activity (AOA) of plant extracts. The use of a single method does not give a reliable result and the data obtained by different methods are not always comparable [31]. In this regard, in this work, we have used two methods for the determination of the antioxidant activity of crude and selective extracts of the leaves and root bark of M. cecropioides: the method based on the interaction of antioxidants with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and the FRAP method based on the determination of the reducing capacity of extracts when they interact with the Fe complex with tripyridyltriazine. The results of the measurements are shown in **Figures 3 and 4**.

The higher percentages of reduction of the DPPH radical were observed for the crude extracts McR (15.35-72.70%), McF (15.35-72.70%), ethyl acetate McR^{III} (66.78-81.41%), McF^{III} (60.59-85.26%) and n-butanol McR^{IV} (34.91-82.20%), McF^{IV} (48.87-69.61%). The reduction of DPPH by these extracts is dose-dependent (**Figure 3**). However, the values obtained are lower than that of vitamin C (49.49-88.49%), taken as standard. For a better appreciation of this potential, the median concentration of sample which reduces 50% of the $DPPH^{\bullet}$ (CR_{50}) was determined and the values are recorded in **Table 2**. This value is all the more important (effective), if the graphically determined CR_{50} is small.

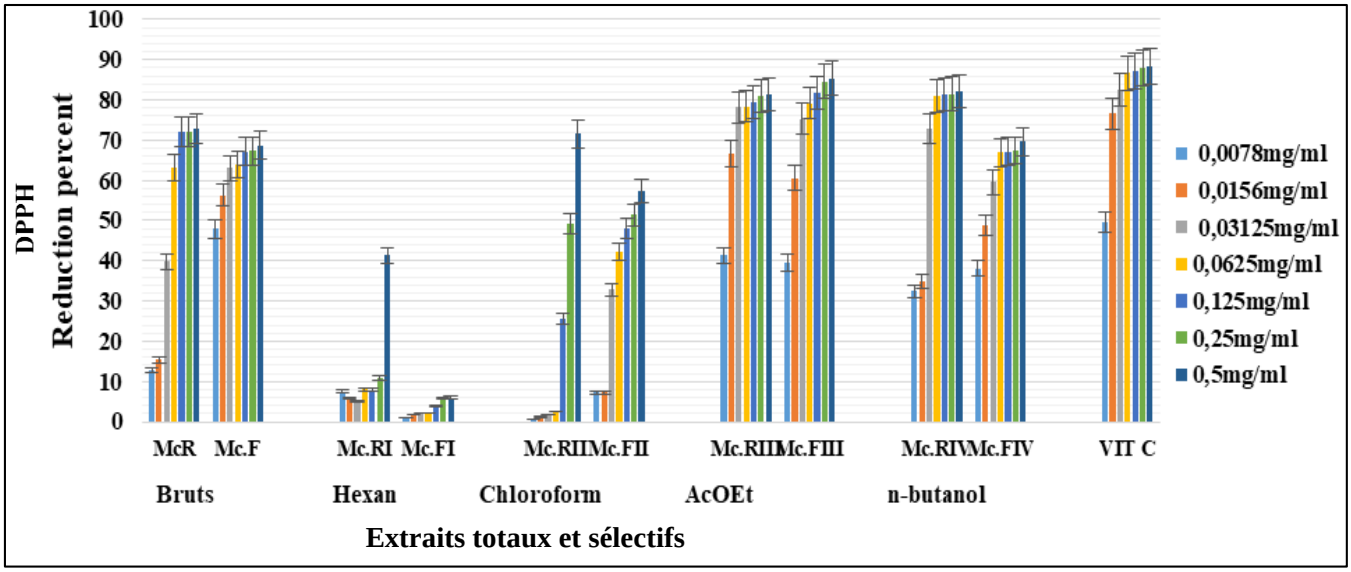

Figure 3 Percentages of reduction of DPPH● by crude and selective extracts

Table 2 CR_{50} values of crude and selective extracts

	Bruts	**Hexan**	**$CHCl_3$**	**AcOEt**	**n-BuOH**	**Vit C**
Mc.R	0,0465	> 0,5	0,3022	0,0099	0,0217	0,0027
Mc.F	0,0088		0,1996	0,0123	0,0132	

The extracts with AcOEt and n-BuOH of two studied organs of *M. cecropioides* have CR_{50} values close to the value of vitamin C. This anti-free radical potential could be explained by the presence of phenolic compounds such as flavonoids, coumarins and tannins in these extracts (**Table 1; Figures 1 and 2**). The results obtained agree with those obtained with the extract of the root bark and leaves of the species from Gabon which respectively presented CR_{50} equal to 0.29 ± 0.02 µg / ml and 6.36 ± 0.89 µg / ml [27].

The FRAP test made it possible to evaluate antioxidant activity *in vitro* by measuring their reduction potential from Fe^{3+} to Fe^{2+} [32] as a function of the calibration line of Trolox (standard antioxidant) y = 0.9864 x + 0, 0353 (R^2 = 0.9884). The antioxidant activity of extracts of *M. cecropioides*, determined by their reducing power and that of quercetin (standard) are illustrated by histograms in **Figure 4**.

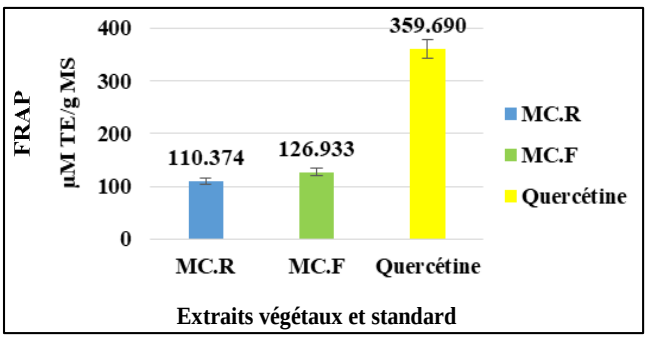

Figure 4 Reducing power of Fe^{3+} by extracts and quercetin

Leaf extracts (126.933 µM TE / g MS) have the ability to reduce Fe is slightly higher compared to that of root bark (110.374 µM TE / g MS). This difference is due, in fact, to the maximum quantity of phenolic compounds recorded in the

leaves (**Figures 1 and 2**). Indeed, FRAP antioxidant activity could be linked to the presence of these compounds because they are antioxidants recognized by their reducing power [32]. However, the antioxidant activity of the study extracts is lower than that of quercetin (359.690 µM TE / g MS). Also, the FRAP antioxidant activity of *M. cecropioides* is lower than that of the leaves of different varieties of green tea (272-1144 µM TE / g) [33].

As a result of this study, it was found that regardless of the method used (DPPH or FRAP), the extract of the leaves of *M. cecropioides* showed higher antioxidant activity than that of its root bark. The leaves of *M. cecropioides* can be considered a source of phenolic antioxidants.

3.5. Anticholinesterase activity

The inhibitory activity from 0 to 360s of the extracts of *M. cecropioides* tested on AChE gave more or less variable percentages of inhibition (PI) compared to Reminyl® (Galanthamine, commonly used reference alkaloid) (**Figure 5**).

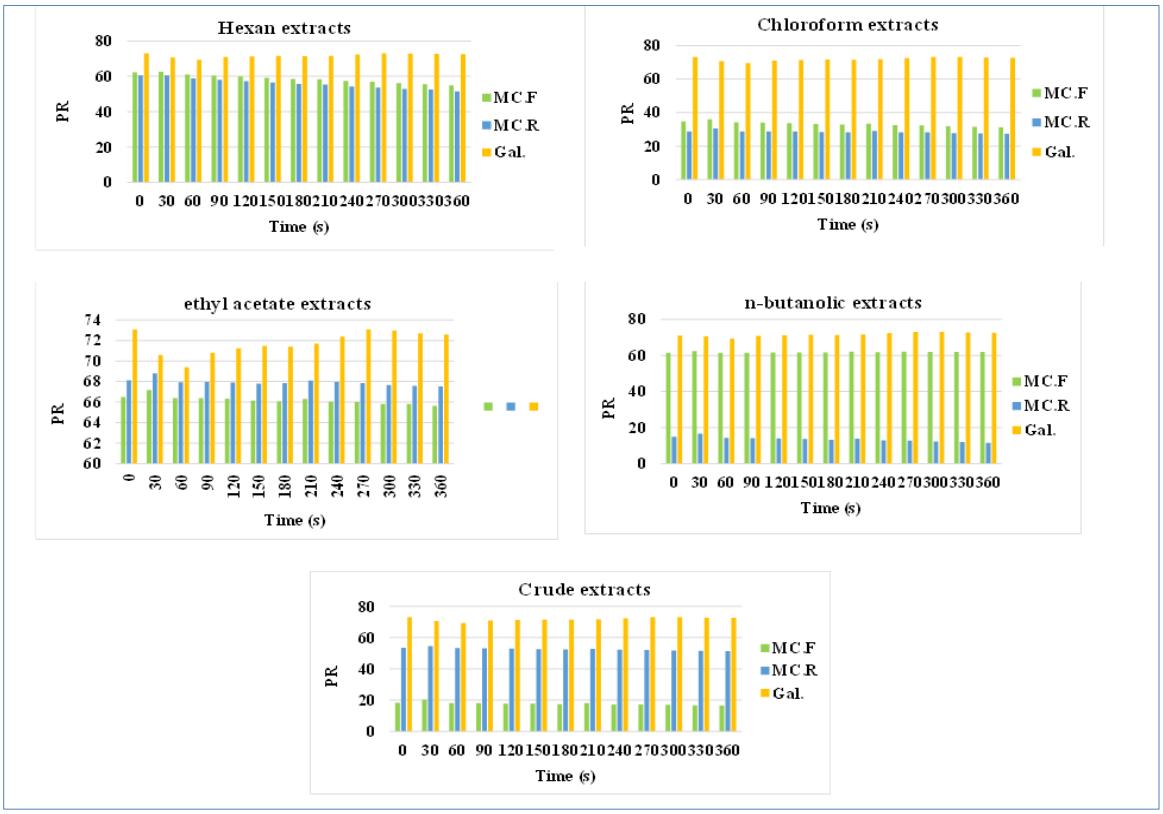

Figure 5 Anticholinesterase activity of crude and selective extracts of *M. cecropioides*

The hexane (McF^{I}) and n-butanol (McF^{IV}) extracts from the leaves showed variable PI values with a maximum inhibition of over 62%. The PIs recorded for chloroform extracts (McF^{II}) are the lowest and vary between 27 and 33%. As for the AcOEt extracts, they showed maximum inhibition of the AChE enzyme (McR^{III}: 67.510 - 68.780% and McF^{III}: 65.633 - 67.160%). Also, the n-BuOH leaf extract (McF^{IV}) recorded high PIs (61.459 - 62.442%). By comparing the inhibitory activity of the AChE enzyme by extracts tested with that shown by galanthamine (69.380 - 73.098%), we can say that the extracts with AcOEt and n-BuOH signed a good anticholinesterase activity, which could be due to the synergistic action of the phytocompounds present in the extracts tested (**Table 1; Figures 1 and 2**). Indeed, the extracts contain secondary metabolites such as alkaloids, terpenoids, coumarins, flavonoids and tannins which are the main families of compounds recognized as AChE inhibitors [8, 33].

4. Conclusion

The results of our study showed that extracts from the leaves and root bark of *M. cecropioides* contain several bioactive phytocompounds which are responsible for their DPPH and FRAP antioxidant activities with overt AChE enzyme inhibitory activity. These results could explain the therapeutic virtues widely attributed to this species in unconventional medicine.

Compliance with ethical standards

Acknowledgments

We would like to thank LCBOSN for allowing us to carry out this work.

Disclosure of conflict of interest

The authors declare no financial or any other conflicts of interest in this work.

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