Vol. 14(3), pp. 37-43, October-December 2022
DOI: 10.5897/JPP2022.0622
Article Number: 878B41170141
ISSN 2141-2502

http://www.academicjournals.org/JPP

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Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Evaluation of the phenolic content and *in vitro* antioxidant potential of Bauhinia monandra Kurz (Fabaceae)

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Received 19 October, 2022; Accepted 16 November, 2022

This work was carried out to evaluate the total phenolic content and antioxidant potential (2,2-di-phenyl-1-picrylhydrazyl [DPPH] and ferric reducing antioxidant power [FRAP] methods) of Bauhinia monandra through its organs (leaves, stems and roots). The results showed fluctuating contents with a dominance in the leaves of total phytophenols in the crude hydromethanolic and selective AcOEt extracts (57.344 ± 12.35 and 48.165 ± 4.77 mg EAG/g DM, respectively) and of total flavonoids in the AcOEt extract (100.588 ± 12.06 mg EQ/g DM). The evaluation of antioxidant activity shows that the AcOEt extract of the stems is the most active with a CR50 = 23.442 µg/ml (DPPH method) and 44.34 ± 0.1 µg EVc/100 g DM and 182394.94 ± 406.09 µg EFeSO4/g DM (FRAP method).

Key words: Bauhinia monandra, phytophenols, antioxidant activity.

INTRODUCTION

Health from a holistic point of view is man's main concern. To do so, he uses beneficial plant species both to feed himself and to maintain his well-being. Indeed, plants are the breeding ground for various families of active ingredients, including phytophenols, whose various therapeutic and antioxidant properties are recognized (Fouché et al., 2000). The close relationship between man and his environment has spearheaded the production of various medicinal molecules in order to intensify the fight against numerous pathologies and their prevention. Local and endogenous knowledge and know-how, acquired from generation to generation, are indispensable for the process of prevention and control of diseases (Pousset, 1989). It is generally accepted that flora is the natural pharmacy par excellence from which most of the world's populations (about 80%) draw their therapeutic resources because of its richness in antioxidant actives, the most representative of which are phytophenols (anthocyanins, flavonoids, tannins, etc.) (Sevindik, 2018; Maleki et al., 2019; Agati et al., 2020). Antioxidants control oxidative stress (OS). This type of natural aggression of the cell constituents at the origin of many diseases, has given rise to a craze for the research of phytocompounds that can inhibit cytotoxic free radicals

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(oxygen and nitrogen species) that induce OS (Lu and Yeap, 2000; Pousset, 2006; Varban et al., 2009; Akalin and Selamoglu., 2019). In the present study, Bauhinia monandra of the botanical Fabaceae family was the focus of our investigations through an ethnobotanical survey that we conducted in Côte d'Ivoire. The said survey concluded medicines often use the organs of this plant to treat male infertility. And OS would play a crucial role in the etiology of this disease (Aitken et al., 1994; Bui et al., 2018). Moreover, seminal reactive oxygen species are produced mainly by leukocytes or immature abnormal spermatozoa, which are a natural by-product of oxidative pathways (Wagner et al., 2017; Aitken et al., 2019). *B. monandra* is an ornamental plant native to Madagascar, now widespread in other parts of the world (Africa, Asia, Australia, North, Central, and South America, West Indies, and Pacific Ocean islands) (Website 1, ILDIS, 2014; PROTA, 2014; PIER, 2014). In addition, the literature (Website 2) indicates that the plant, in addition to its anti-inflammatory virtue, is used to treat diarrhea, dysentery, fevers, leprosy, smallpox (pox), diabetes, and eye conditions. To provide a valid answer among other traditional uses of *B. monandra* growing in Côte d'Ivoire, its phenolic content and antioxidant potential were determined by spectrophotometry.

METHODOLOGY

Plant materials

Of all the species listed during the ethnobotanical survey, *B. monandra* was chosen for its frequency of use in the treatment of pathologies. The plant material used specifically for this study consists of leaves, stems and roots of the said species, harvested in July 2020 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 Doctor Malan Djah François (botanist at the University NANGUI ABROGOUA), the samples were cleaned with running water, dried under constant conditioned air (20°C, 20 days), then reduced to powders, then preserved in hermetically sealed glass jars.

Preparation of hydromethanolic crude extracts

Powder (170 g) each BM specie, are macerated in 500 ml of methanol (MeOH, 80%) for 48 h at room temperature under constant stirring. The operation is repeated three times. After vacuum filtration, the collected filtrates are concentrated in a rotary evaporator (BÜCHI) (40°C, 335 mbar) and pump dried under liquid nitrogen for 4 h to give the crude hydromethanol extracts: BMf (Leaves), BMt (Stems), and BMr (Roots).

Preparation of selective extracts

Crude extracts (15 g) were dissolved in 200 ml of distilled water, then successively exhausted with hexane, dichloromethane (DCM) and ethyl acetate (AcOEt). Anhydrous magnesium sulfate was used to dry the various organic fractions obtained. After filtration on filter paper, the different fractions with hexane, DCM and AcOEt, are concentrated in rotary evaporator, and dried with pump under liquid nitrogen for 3h time to provide the selective extracts BMf1 (Leaves), BMt1 (Stems), BMr1 (Roots), BMf2 (Leaves), BMt2 (Stems), BMr2 (Roots), BMf3 (Leaves), BMt3 (Stems), and BMr3 (Roots), respectively.

Determination of the total phytophenol (TP) content of BM organs

To 0.3 ml of 1:10 diluted aqueous extract (obtained from crude and selective extract of each organ), 2.5 ml of Folin-Ciocalteu reagent (diluted 1:10) and 2 ml of sodium carbonate (Na_2CO_3) solution (75 g/l, m/v) were added. The reaction mixture is heated in a water bath for 15 min at 50°C and then incubated for 2 min in the dark after cooling. The absorbance of blue coloration, proportional to the amount of oxidized phytophenols, is measured at 760 nm on a UV-visible spectrophotometer (Spectro AL 800) against a blank without extract. A linear calibration ($y = 1.0673 x + 0.0015$; $R^2 = 0.9937$) with gallic acid (from 0.056 to 0.7127 mg/ml) is performed under the same conditions. The result is expressed as milligram of gallic acid equivalent per gram of dry matter (mg GAE/g DM) (Wood et al., 2002). All the tests are done in triplicate.

Determination of the total flavonoid (TF) content of BM organs

To 2.5 ml of aqueous extract (obtained from crude and selective extract of each organ), 0.75 ml of aluminium trichloride solution ($AlCl_3$, 10%, w/v) and 0.75 ml of sodium nitrite ($NaNO_2$, 5%, w/v) were added. After 5 min of incubation, 5 ml of sodium hydroxide solution (NaOH, 1M) was added to the mixture. The reaction mass, adjusted to 25 ml with water is vigorously stirred with a magnetic stirrer. The absorbance is measured at 510 nm on a UV-visible spectrophotometer (Spectro AL 800) against a blank without extract. A linear calibration with quercetin (from 0.045 to 5.810 mg/ml) is performed under the same conditions. The result is expressed as milligram equivalent of quercetin per gram of dry matter (mg EQ/g DM) (Kouamé et al., 2021). All the tests are done in triplicate.

In vitro determination of the antioxidant potential (OP) of BM organs using the DPPH method

To 1 ml of ethanolic extract (obtained from crude and selective extract of each organ) are added 2.5 ml of an ethanolic solution (0.03 mg/ml) of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The shaken mixture is incubated for 30 min in the dark. The decrease in absorbance of the violet color of the DPPH solution in the presence of phytoantioxidants is measured at 517 nm on a UV-visible spectrophotometer (Spectro AL 800) against a blank (Espin et al., 2000; Slandjana et al., 2012). Plant extracts and quercetin are prepared at concentrations (1500 to 2.24 µg/ml). The percent reduction (PR) is determined by the following equation:

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

The median DPPH reduction concentration (CR_{50} or EC_{50}) reflecting the antioxidant efficiency of the extract is determined graphically (Etekpo et al., 2018; Tanoh et al., 2019a, b; Etekpo et al., 2022). All trials were done in triplicate.

In vitro determination of the antioxidant potential (OP) of BM organs using the ferric-reducing antioxidant power (FRAP) method

The antioxidant potential of *B. monandra* organ extracts resulting in the reduction of chelated ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) was also evaluated. The reagent used is a mixture of sodium acetate

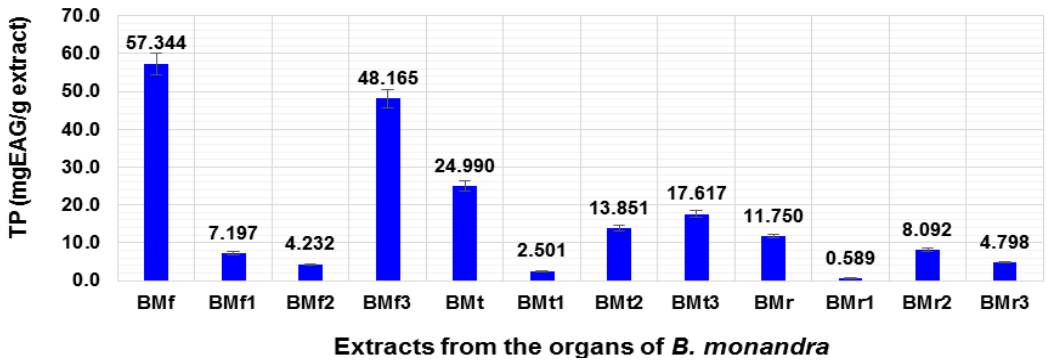

Figure 1. TP content of organs of *B. monandra.* Crude hydromethanol extracts BMf (Leaves), BMt (Stems), BMr (Roots). Hexane selective extracts: BMf1 (Leaves), BMt1 (Stems), BMr1 (Roots); DCM; BMf2 (Leaves), BMt2 (Stems), BMr2 (Roots); AcOEt: BMf3 (Leaves), BMt3 (Stems), BMr3 (Roots).
Source: Author

buffer (0.3 M; pH= 3.6) and 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) (10 mM) prepared in a solution of hydrochloric acid (HCl, 40 mM) and iron trichloride ($FeCl_3$, 20 mM) in a volume ratio of 10: 1: 1. To 3 ml of freshly prepared FRAP reagent preheated to 37°C in a water bath, 100 µl of plant extract (0.25 mg/ml) was added. The absorbance of the intense blue color induced by the reduction of the ferric complex (TPTZ-Fe^{3+}) to the ferrous form (Fe^{2+}) is read at 593 nm on a UV-visible spectrophotometer (Spectro AL 800) after 4 min incubation. Linear calibration is performed with the control reference, ferrous sulfate ($FeSO_4.H_2O$; purity 80%) at concentrations (0.78125, 1.5625, 3.125, 6.25, 12.5 and 25 µg/ml) and ascorbic acid (vitamin C) was used as reference antioxidant. The results are expressed in micrograms $FeSO_4$ equivalent per 100 g extract (µg ESF/100 gE) (Benzie and Strain, 1999; Gong et al., 2016). All the tests were done in triplicate.

Statistical analysis

Single-criteria analysis of variance (ANOVA) was used to compare values using STATISTICA software 7.1 (Statsolf, 2005). When there was a significant difference between the means ($P < 0.05$), the Fisher multiple comparison test was performed (Dagnelie, 2001). Results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

TP content of organs of *B. monandra*

TP contents (in mg EAG/g ES) were determined by linear calibration from gallic acid taken as standard (Figure 1).

The highest contents were found in BMf = 57.344 ± 12.353 mg EAG/g DM, BMf3 (48.165 ± 4.770 mg EAG/g DM), BMt = 24.990 ± 3.830 mg EAG/g DM, and in BMt3 (17.617 ± 1.671 mg EAG/g DM). Thus, it appears that the PT contents obtained are variable, depending on the plant organ (Falleh et al., 2008) and the nature of the extractor (solvent) (Garcia-Salas et al., 2010; Koffi et al., 2010; Ben Salah et al., 2021). Cechinel-Zanchett et al. (2019) reported that PTs are present in leaves of the Brazilian species in varying proportions depending on the type of extraction solvent. Itharat et al. (2016) showed fluctuating levels of PT in the leaves of *Bauhinia strychnifolia*. Other studies conducted on species of the same genus (Ferreira-Filho et al., 2020) and on other *Bauhinia* species (Aliyu et al., 2009; Chew et al., 2011) resulted in the same finding. In addition, experimental and clinical studies have shown that phytophenols enhance endothelial formation of vaso-protective factors such as nitric oxide (NO) (Auger and Valerie, 2014; Djamilatou et al., 2021).

TF content of organs of *B. monandra*

The contents of Total Flavonoids were determined in the different fractions and the results obtained are variable. The results obtained are illustrated by the histograms (Figure 2).

The most significant contents are seen in the ethyl acetate-selective extracts of leaves (BMf3 = 100.588 ± 12.063 mg EQ/g DM), stems (BMt3 = 63.298 ± 2.727 mg EQ/g DM) and crude hydromethanol of leaves (BMf = 47.804 ± 1.86 mg EQ/g DM), respectively. Furthermore, from the analysis of Figure 2, it is clear that the FT contents vary, on one hand considerably, and on the other hand, inequitably in the organ extracts. This suggests that the concentration of these secondary actives is dependent on the type of organ and the polarity of the extractor used. Studies conducted on several species of the genus *Bauhinia* have shown the presence of FTs (Fernandez et al., 2012; Farag et al., 2015; Ferreira-Filho et al., 2020). Farag et al. (2015), of O- and C-glycosylated flavonoids and proanthocyanidins in 9 species of the genus *Bauhinia*. Flavonoids are considered one of the most predominant phytochemical subfamilies within phytophenols in species of the genus *Bauhinia* (Da Silva and Cechinel Filho, 2002). Several glycosylated and aglycone flavonoids have been isolated from *Bauhinia variegata* (Reddy et al., 2003; Yadava and Reddy, 2003; Maheswara et al., 2006; Rao et al., 2008;

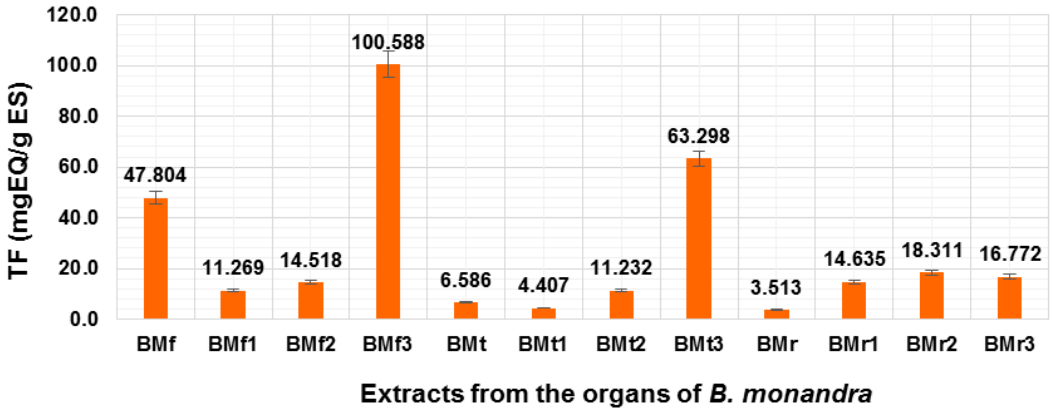

Figure 2. TF content of organs of *B. monandra* crude hydromethanol extracts BMf. Crude hydromethanol extracts BMf (Leaves), BMt (Stems), BMr (Roots). Hexane selective extracts: BMf1 (Leaves), BMt1 (Stems), BMr1 (Roots); DCM; BMf2 (Leaves), BMt2 (Stems), BMr2 (Roots); AcOEt: BMf3 (Leaves), BMt3 (Stems), BMr3 (Roots).
Source: Author

Mohamed et al., 2009). Kaempferol and quercetin derivatives were also isolated from leaf and flower extracts of *Bauhinia forficata* (Pizzolatti et al., 2003). A chemical study of leaf flavonoids from 9 species of *Bauhinia* belonging to the subgenera *Bauhinia* and *Phanera* also identified mainly flavonol, kaempferol, quercetin and isorhamnetin derivatives (Farag et al., 2015). AL-Ishaq et al. (2019) also demonstrated the hypoglycemic virtues of flavonoids. This shows their interest in herbal medicine.

In vitro antioxidant activities of *B. monandra*

Antioxidant profile by the DPPH method

Quercetin was the reference antioxidant used. Figure 3 highlights the concentration-dependent DPPH (PR) reduction percentages of the different organ extracts of *B. monandra*. In general, the results show that the extracts show a PR towards DPPH at different concentrations, but lower than that of quercetin. Also, it was noted that the AcOEt extract of BMt3 stems shows PRs (92.404 - 4.074%) approaching that of quercetin (96.779 - 34.608%). The lowest PRs are found in BMf (80.977 - 2.663%), BMf3 (86.436 - 0.507%), BMt (89.241 - 4.410%) and BMt2 (91.916 - 9.599%).

On the other hand, the antioxidant efficiency of the extracts compared to quercetin was translated by determining the median reduction concentration (CR_{50}) of DPPH inducing a concentration-effect response at the midpoint of the oxidation process (Table 1). The lower this parameter, the greater the antioxidant efficiency of the extract (Falleh et al., 2006; Etekpo et al., 2022). All extracts from *B. monandra* organs have CR_{50} higher than that of quercetin (3.519 µg/ml). Therefore, in terms of efficacy, their antioxidant response is low compared to the reference antioxidant. However, it was noted that among the extracts tested, BMt3 and BMf3 still have a better antioxidant efficacy, corroborated by their CR_{50} of 23.442 and 70.181 µg/ml, respectively. This virtue seems to be governed by their content in phenolic and flavonoid antiradical actives (Figures 1 and 2). On the other hand, works have reported the antioxidant activity of *Bauhinia* spp., namely *B. forficata, B. vareigata, B. vareigata var candida, Bauhinia galpini, Bauhinia rufescens, B. strychnifolia, Bauhinia kockiana* and *Bauhinia purpurea* (Aliyu et al., 2009; Chew et al., 2011; Farag et al., 2015; Itharat et al., 2016; Alshammari et al., 2021).

Antioxidant profile by the FRAP method

Figure 4 presents the results obtained in the evaluation of the antioxidant potential of crude and selective extracts of leaves, stems and roots of *B. monandra*. The results show variable reduction capacities.

They reveal that the extracts with AcOEt, BMf3 (182394.94 ± 406.09 µg $EFeSO_4$/g ES), and BMt3 (174263.96 ± 507.61 µg $EFeSO_4$/g ES) present the most significant reducing capacities. The DCM extracts of BMt2 stems (126016.92 ± 117.23 µg $EFeSO_4$/g ES), BMf2 leaves (65103.21 ± 117.23 µg $EFeSO_4$/g ES), and BMr2 roots (60365.48 ± 0.00 µg $EFeSO_4$/g ES) are average, but the stems are dominant. Nevertheless, its values are lower than that of ascorbic acid (429407.783 ± 586.14 µg $EFeSO_4$/g ES) taken as reference antioxidant. Overall, the AcOEt extracts show a better reduction profile. And their richness in electron donating phytophenols and flavonoids are responsible for this (Argolo et al., 2004; Aderogba et al., 2006; Kumaran and Karunakaran, 2007; Alade et al., 2011; Ferrari et al., 2019).

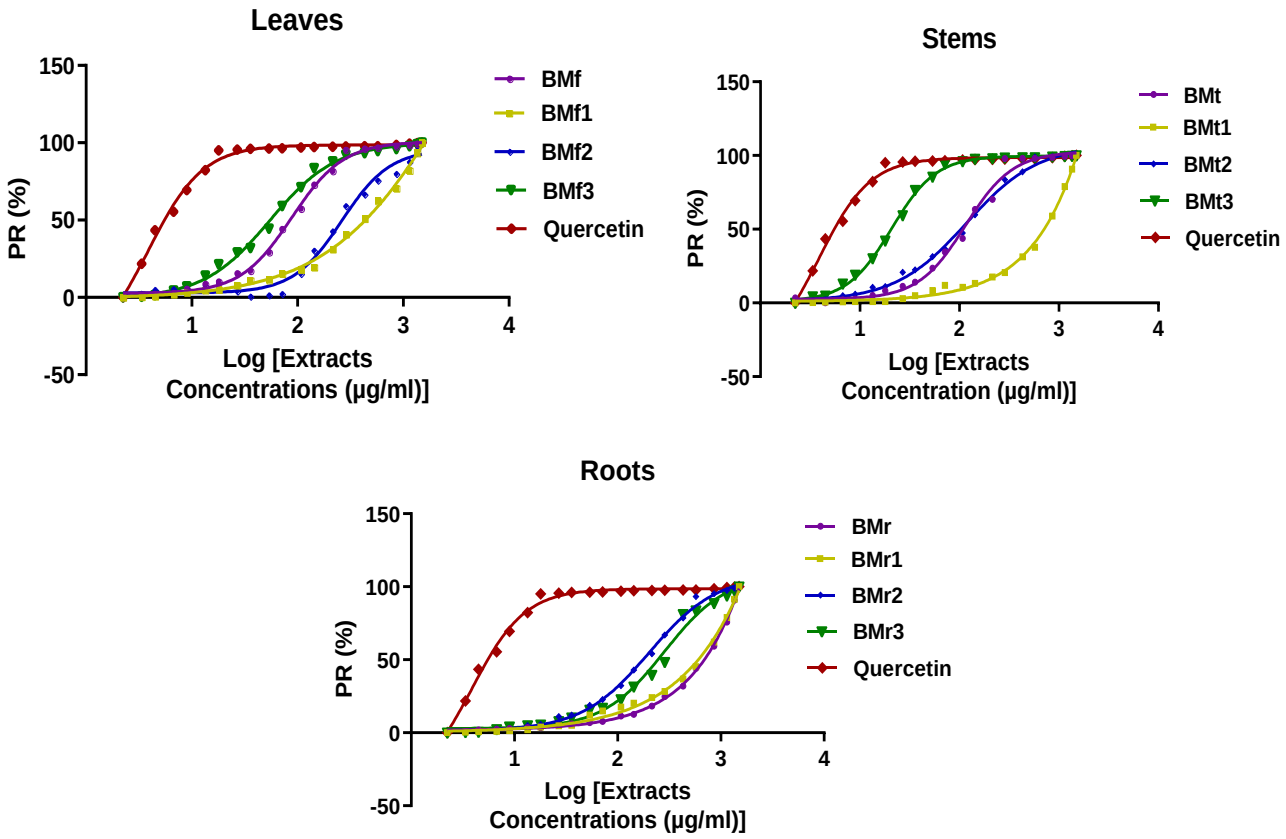

Figure 3. Antioxidant profile of crude and selective extracts of leaves, stems and roots of *B. monandra* and quercetin against DPPH. Crude hydromethanol extracts BMf (Leaves), BMt (Stems), BMr (Roots). Hexane selective extracts: BMf1 (Leaves), BMt1 (Stems), BMr1 (Roots); DCM; BMf2 (Leaves), BMt2 (Stems), BMr2 (Roots); AcOEt: BMf3 (Leaves), BMt3 (Stems), BMr3 (Roots).
Source: Author

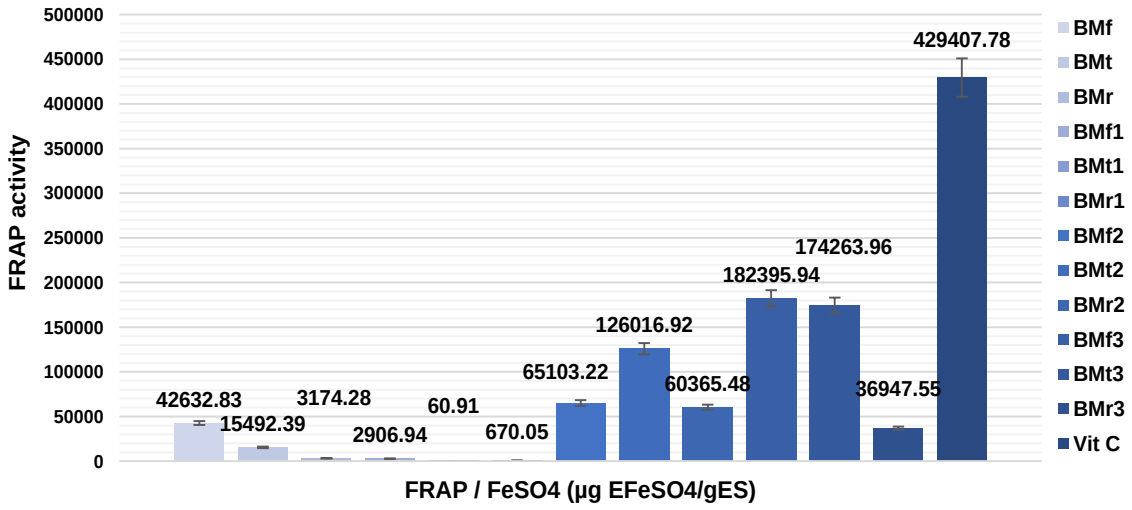

Figure 4. Antioxidant profile of crude and selective extracts of leaves, stems and roots of *B. monandra* by FRAP assay. Crude hydromethanol extracts BMf (Leaves), BMt (Stems), BMr (Roots). Hexane selective extracts: BMf1 (Leaves), BMt1 (Stems), BMr1 (Roots); DCM; BMf2 (Leaves), BMt2 (Stems), BMr2 (Roots); AcOEt: BMf3 (Leaves), BMt3 (Stems), BMr3 (Roots).
Source: Author

Table 1. CR_{50} of *B. monandra* organ extracts and quercetin

CR_{50} (µg/ml)	MeOH	Hexane	DCM	AcOEt
Feuilles	113,222	<1500	<1500	70,181
Tiges	127,561	1011,183	103,500	23,442
Racines	1263,708	< 1500	211,163	157,536
Quercétine		3,519		

Source: Author

The reducing activity by NO inhibition and FRAP assay of *B. strychnifolia* leaf and stem extracts has been shown (Itharat et al., 2016; Praparatana et al., 2022).

Conclusion

The species *B. monandra* growing in Côte d'Ivoire is used in traditional medicine to treat male infertility. However, its health benefits have not been studied. This study was designed to determine the phenolic and flavonoid content of its organs, which seems to govern their antioxidant potential determined, on one hand, by DPPH scavenging and by Fe^{3+} ions reduction on the other hand. Thus, it is established that *B. monandra* is a source of active antioxidants which would corroborate its use in traditional care. Moreover, the study opens the way to the development of phyto-preparations based on this plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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