Original Research Article

Phytochemical screening and comparative evaluation of antioxidant activity of different parts of *Anonidium mannii* (Oliv) Engl. et Diels.

ABSTRACT

Aims: this study aimed to identify the phytochemical constituents, to evaluate and compare the *in vitro* antioxidant potential of the methanolic extracts of the leaves, trunk (wood and bark) and roots (wood and bark) of *Anonidium mannii* (Oliv) Engl. And Diels which is used in Cameroon for the treatment of rheumatism.

Study design: This is an experimental study.

Place and Duration of Study: The work was conducted at the Pharmacochemistry and Natural Substances Laboratories of the Faculty of Medicine and Pharmaceutical Sciences, University of Douala. All of this weas carried out from the 15th October 2019 to the 31th July 2020.

Methodology: Phytochemical screening was based on conventional techniques focusing on color reactions and precipitation. The evaluation of the antioxidant power was carried out by the method of scavenging the free radical DPPH of the extracts and by reading the absorbance for each concentration at 517 nm.

Results The phytochemical screening of the extracts revealed the presence of alkaloïds, flavonoïds, anthraquinones, coumarin, terpenes, steroïds, saponins, phenols and anthocyanins. All extracts tested showed antioxidant activity, particularly root wood with an IC_{50} = 1.85 µg/mL identical to the standard (BHT: Butylhydroxytoluène).

Conclusion: these results justify the use of the leaves or the stem bark of *A. mannii* in the traditional pharmacopeia for the treatment of rheumatism. However, the wood of the roots having presented the best activity (IC_{50} = 1.85 µg/mL). It would be beneficial to suggest this plant part to the local populations for the management of that pathology. This study is the first comparative biological study conducted on the different parts of *A. mannii*.

Keywords: Phytochemical screening; antioxidant activity; Anonidium mannii; Medicinal plant.

1. INTRODUCTION

The use of some synthetic antioxidant molecules is currently a concern because of potential toxicological risks. An antioxidant is a substance which prevents or slows down oxidation by neutralizing free radicals which, in excess, are responsible for cellular damage, in particular on DNA and can promote diseases.

Now, new plant sources of natural antioxidants are being sought [1,2]. In fat, polyphenols are natural compounds widely distributed in the plant kingdom which have a growing importance, in particular thanks to their beneficial effects on health [3]. Their role as natural antioxidants is attracting more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [4]. They are also used as additives in the food, pharmaceutical and cosmetic industries [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].
New and bioactive molecules have been isolated from medicinal plants from Cameroon and across the world. In order to contribute to the enhancement of floristic biodiversity in general and in particular to the study of *Anonidium mannii* (Oliv) Engl. and Diels, a medicinal plant from Cameroon, we directed our research to the evaluation of the antioxidant activity of the different parts of this plant, motivated on the one hand by its use in traditional medicine in the treatment of rheumatism [8,9]. On the other hand, a literature review revealed few studies on *A. mannii* including the phytochemical study, the evaluation of the antiprotozoal and cytotoxic activity of the stem bark, the antimycobacterial activities of the leaves and twigs, the antiplasmodial activities of the leaves and stems and the antioxidant, cytotoxic and antibacterial activities of the leaves [10-13]; the other parts have not been studied.
Indeed, this study aimed to identify the classes of secondary metabolites and to evaluate and compare the antioxidant activity of the leaves, trunk wood, stem bark, root wood, root bark of *Anonidium mannii* in order to determine the parts displaying the best activity.

2. MATERIALS AND METHODS

2.1 Plant material

The plant material used consisted of leaves, trunk and roots of *Anonidium mannii*. They were harvested in October 2019 in a forest of the village Nkolotou'outou, District of Sangmélima, Department of Dja et Lobo, South region of Cameroon. The botanical identification of the plant was made in the National Herbarium of Cameroon by comparison with the botanical collection of D.W.Thomas N° 2188 registered in the National Herbarium of Cameroon under N° 50327/HNC.

2.2 Preparation of extract

After the harvest, the barks were separated from the wood on the one hand and from the roots on the other. The parts obtained, namely the leaves, trunk wood, stem bark, root wood, root bark, were then cut into small pieces, dried and then machine-pulverized into fine fibers. The preparation of methanolic extracts of the plant was carried out in accordance with the method described by Bidié *et al*. (2008) with some modifications [14]. Eight hundred grams (800 g) of shredded plant leaves were mixed with 5 l of 96% methanol;one thousand grams(1000g)of shredded wood from the roots of the plant with 6 l of 96% methanol; one thousand one hundred grams (1100 g) of shredded wood from the trunk of the plant with 5 l of 96% methanol; one thousand one hundred grams (1100 g) of shredded bark from the roots of the plant with 6 l of 96% methanol; one thousand one hundred grams (1100 g) of ground bark from the trunk of the plant with 7 l of 96% methanol. The mixture obtained was shake using a spatula once day for 2 days at room temperature (25°C), to facilite the dissolution of the compounds contained in the ground material; then the mixture was filtered three times on cotton and on büchner with 3 mm wathman filter paper. The filtrate was evaporated under reduced pressure at 40°C using a rotary evaporator. The crude extracts obtained were used to carry out the various tests. The yields (Rd) expressed as a percentage (%) were calculated using the formula below:

$$Rd = \frac{initial\ mass}{final\ mass} \text{ X } 100$$

2.3 Phytochemical screening

The secondary metabolites in the extracts were dividence following the methods described by Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Békro et *al*. (2007) [15-18]. These methods are based on color and precipitation reactions. Qualitative analysis of methanolic plant extracts was carried out by testing for the presence of alkaloids, flavonoids, anthraquinones, anthocyanes, coumarins, terpenes, phenols, saponins, steroids.

2.4 Antioxidant activity

2.4.1 Determination of total polyphenols

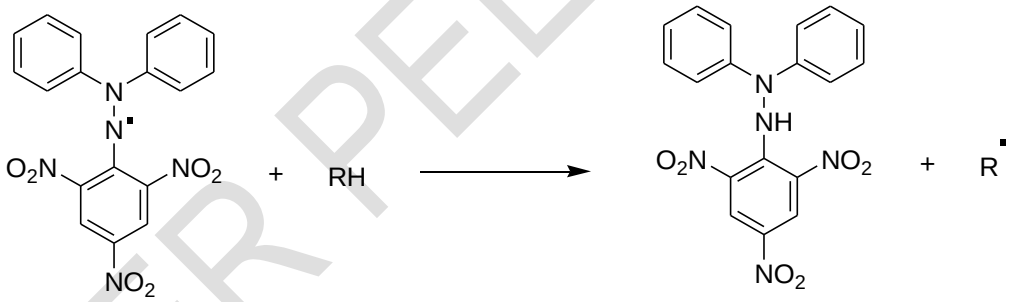
This test makes it possible to determine the content of total phenols in the methanolic extracts of the different parts of *Anonidium mannii*. The content of phenolic compounds in the various extracts was estimated by the Folin-Ciocalteau methods, which is based on the reduction of the mixture in the alkaline medium phosphotungstic ($(W_{12}O_{40})^{2-}$) phosphomolybdic ($(Mo_{12}O_{40})^{2-}$) of Folin-Ciocalteau reagent by the oxidizable groups of the phenolic compounds, leading to the formation of blue-colored reduction products. The latter exhibit an absorption maximum at 765 nm, the intensity of which is proportional to the quantity of polyphenols present in the sample. 1 ml of Folin-Ciocalteau reagent (1/10) was added to 200 µl of extract or standard with suitable dilutions; after 4 min, 800 µl of a sodium carbonate solution (75 mg/ml) were added to the reaction medium; After 2 hours of incubation at room temperature, the absorbance was measured at 765 nm; The concentrations of total polyphenols contained in the extracts were estimated from the regression equation of the calibration range established with gallic acid (0200 µg/ml) used as standard. The total polyphenol content was calculated and expressed as milligram gallic acid (GA) equivalent per milligram extract (mgEqGA/g extract) [19].

$$\mathrm{T} = C \times \frac{V \times D}{Ps}$$

T: Content of total phenols; C: Concentration of polyphenols in gallicacid equivalent deduced from the curve; V: Volume of extract; D: Dilution factor; Ps: Weight of dry matter.

2.4.2 DPPH RADICAL SCAVENGING ACTIVITY ASSAY

To study the antiradical activity of each extract, the diphenyl picryl-hydrazyl (DPPH) method was used following a modifie protocol described by Mansouri *et al*. in 2005 [20]. This test was made to determine, at constant concentration, the antioxidant activity of methanolic extracts of the leaves, trunk wood, stem bark, root wood, root bark of *Anonidium mannii.* The principle is based on the reduction by diphenyl picryl-hydrazyl antioxidants having a violet color to a yellow compound, the color intensity of which is inversely proportional to the ability of the antioxidants present in the medium to donate protons [21].



DPPH **DPPHH**

Procedure:
Solution 1 (S1) was obtained by introducing 100000 µg of extract in a test tube and by adding 1000 µg of methanol. The solution obtained had a concentration of 100 µg/µl.
Solution of DPPH was obtained by introducing 20 ml of methanol in 39.4 mg of DPPH: a solution A (SA) with a concentration of 5 Mm is obtained. By diluting to the fifth SA (1ml of SA + 4 ml of methanol), we have acquired solution B (SB) with a concentration of 1 Mm. by diluting the SB to one tenth (1ml of SB+ 9 ml of methanol) we have acquired solution C (SC) with a concentration of 0.1 Mm. Each DPPH solution obtained is stored away from light.

Spectrometric measurements:

For each concentration range, a solution of DPPH is tested as a control (blank), in order to estimate its decomposition in the absence of the extract studied. For each extract to be analyzed, tests of the ability to reduce the concentration of DPPH with different dilutions of the stock solution S1 were carried out. Using these results, the concentrations were adjusted to obtain a range of extract concentrations for which the curve of the percentage of DPPH consumed in relation to the concentration of extract is within the range of linearity. The stock solutions of DPPH (1 ml) and extract to be assayed are added to test tubes in order to obtain solutions (2 ml) with a concentration varying from 0.1 µM to 0.5 µM of DPPH and antioxidant between 1-125 µg/ml. Each series is respectively passed through the spectrophotometer for the

absorbance reading for each concentration at 517 nm. The tubes containing DPPH were previously incubated for 30 min in the dark and at room temperature. The positive control is represented by a solution of a standard antioxidant: BHT (Butylhydroxytoluène), the absorbance of which was measured under the same conditions as the samples and for each concentration. Each series was carried out in triplicate for each extract as well for the BHT.
The scavenging activity was estimated according to the equations below:

Abs sample = (Abs samples + DPPH) – (Abs sample + MeOH)

% of scavenging activity = [(Abs Control-Abs sample) / Abs control] * 100

The values of the IC_{50} (Median inhibitory concentration) were determined graphically by linear regression.

3. RESULTS

3.1 Yield of extraction

After drying, grinding, maceration, filtration and evaporation of the methanol using a rotary evaporator, we obtained 15.02 g (1.80%) of crude extract of leaves, 13.09 g (1,2%) of crude extract of stem bark, 40,57 g (13.7%) of crude extract of Trunk wood, 7.09 g (0.6%) of crude extract of Root bark, and 10.06 g (1.0%) of crude extract of root wood (Table1).

Table 1. Extraction yield of the methanolic extracts of leaves, trunk and roots of *Anonidium mannii*

Vegetal material	Steps	Initial mass (g)	Final mass (g)	Yield (%)
Leaves	Drying	15000	5000	33,30
	Grinding	5000	2200	44,00
	Maceration +Evaporation	800	15,02	1,80
Stem bark	Drying	7500	3000	40,0
	Grinding	3000	900	30,0
	Maceration +Evaporation	1100	13,09	1,2
Trunk wood	Drying	10000	4000	40,0
	Grinding	4000	1400	35,0
	Maceration +Evaporation	1100	7,09	0,6
Root bark	Drying	7500	3000	40,0
	Grinding	3000	900	30,0
	Maceration +Evaporation	1100	40,57	3,7
Root wood	Drying	17500	7000	40,0
	Grinding	7000	2700	38,6
	Maceration +Evaporation	1000	10.06	1,0

3.2 Phytochemical screening

Phytochemical analysis show that the methanolic extract of the leaves of *A. mannii* contain alkaloïds, phenols, anthocyanes, saponins,terpenes and sterols. Stem bark contain alkaloïds, flavonoïds, phenols, anthocyanes and saponins. Root bark contains alkaloïds, flavonoïds, phenols, coumarins, saponins and terpenes. Trunk wood contains alkaloïds, phenols, anthraquinones, and terpenes. Root wood contains alkaloids, flavonoids, phenols, anthocyanes, anthraquinones, saponins, and terpenes (Table 2).

Table 2. Phytochemical screening of different parts of *Anonidium mannii*

Types of tests	Family of compounds	Observations	Results				
			AMMsb	AMMrb	AMMtw	AMMrw	AMMle
Dragendorff	Alkaloïdes	Purple coloring	+	+	+	+	+
Ferric Chlorid ($FeCl_3$)	Phenols	Blue or purple coloring	+	+	+	+	+
Schinoda	Flavonoïds	Orange or purplish pink coloring	+	+	-	+	-
Bornsträger	Anthraquinones : Bound quinones	Yellow or orange coloring	-	+	+	+	-
	Free quinones	Purplish red coloring	-	-	-	-	-
Coumarins test	Coumarins	Coloration varying from blue to purple yellow	-	+	-	-	-
Anthocyanes test	Anthocyanes	Greenish purplish blue coloration	+	-	-	+	+
Foam index	Saponins	Presence of foam	+	+	-	+	+
Liebermann-Buchard	Terpenes	Brick red coloring turning purple	-	+	+	+	+
Liebermann-Buchard	Steroïds	Violet blue coloring	-	-	-	-	+

AMMle (Methanolic leaves extract of Anonidium mannii), AMMsb (Methanolic extract from the stem bark of Anonidium mannii), AMMtw (Methanolic extract of the trunk wood of Anonidium mannii); AMMrb (Methanolic extract of the root bark of Anonidium mannii) AMMrw (Methanolic extract from the root wood of Anonidium mannii); (+) = presence ; (-) = absence

3.3 Evaluation of antioxidant activity

3.3.1 Total phenol content

Polyphenol concentrations were determined from the calibration curve (y = 1.4937X + 0.4025, R^2=0.9935) plotted using gallic acid as standard. This curve made it possible to calculate the concentrations of the total phenols contained in the extracts (figure 1).

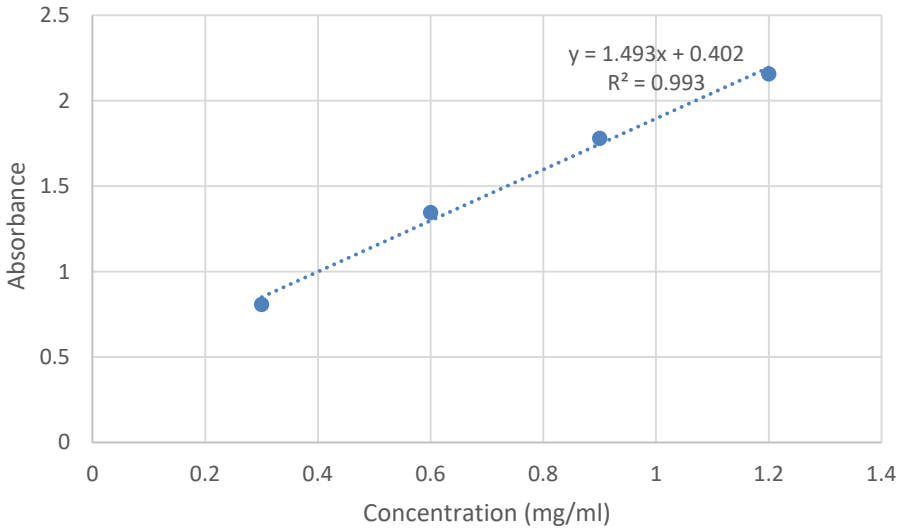


Fig. 1. Gallic acid calibration curve

The concentrations obtained enabled us to calculate the total phenol content of the various extracts. The values obtained were illustrated in figure 2. In the methanolic extract of stem bark the polyphenol content is estimated at 0.11 mgEqAG/g (milligrams equivalents of gallic acid per gram of extract), followed by the root wood 0.08 mgEqGA/g, the trunk wood, and leaves at 0.07 mgEqGA/g and finally root bark at 0.03 mgEqGA/g Thus a higher total phenol contained is in stem bark compared to the four other methanolic extracts of *A. mannii*.

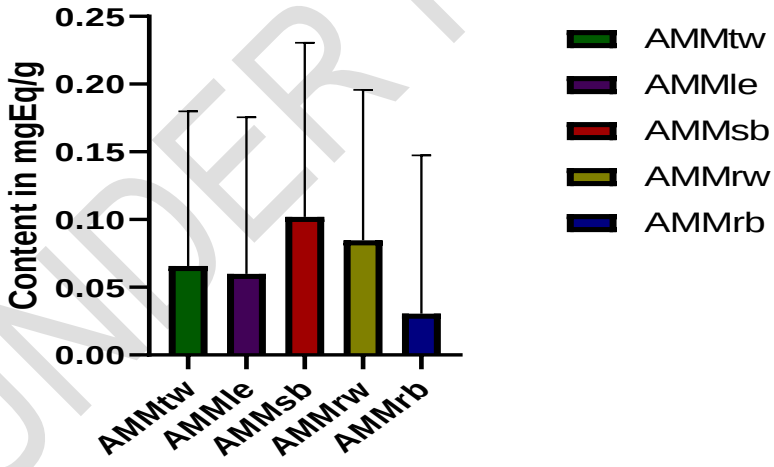


Fig. 2. Total polyphenol content of *Anonidium mannii* expressed as milligrams equivalents of gallic acid per gram of extracts (mg EGA/g)

AMMle (Methanolic leaves extract of Anonidium mannii), AMMsb (Methanolic extract from the stem bark of Anonidium mannii), AMMtw (Methanolic extract of the trunk wood of Anonidium mannii); AMMrb (Methanolic extract of the root bark of Anonidium mannii) AMMrw (Methanolic extract from the root wood of Anonidium mannii);

3.3.2 Antiradical activity

After reading the absorbance on the spectrophotometer, the percentage inhibition of DPPH was calculated and the IC_{50} values were determined respectively for each extract of the different parts of *A. mannii*. After the analysis of this curve, all extracts showed an ability to reduce the absorbance at 517 nm and therefore to reduce the concentration of the DPPH radical in solution with IC_{50} values respectively equal to 1.85 µg/ml for the root wood, 2.6 µg/ml for the trunk wood, 3.2 µg/ml for the leaves, 3.2 µg/ml for the root bark and 18.5 µg/ml for the stem bark (figure 3).

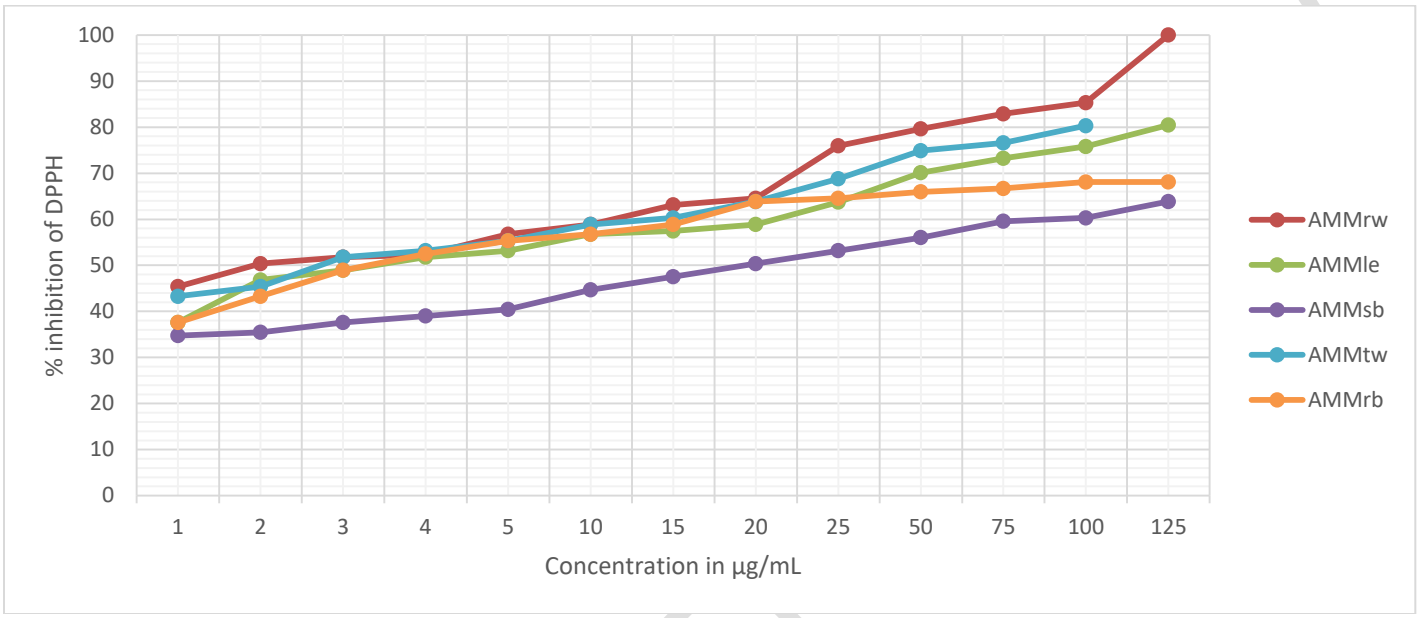


Fig. 3. Plot of percentage inhibition of DPPH as a function of different concentrations of extracts

The root wood (AMMrw) exhibited the best activity and was compared to BHT. It can be seen from figure 3 that AMMrw presented an antioxidant activity close to that of BHT with an IC_{50} value of 1.85 µg/ml (figure 4).

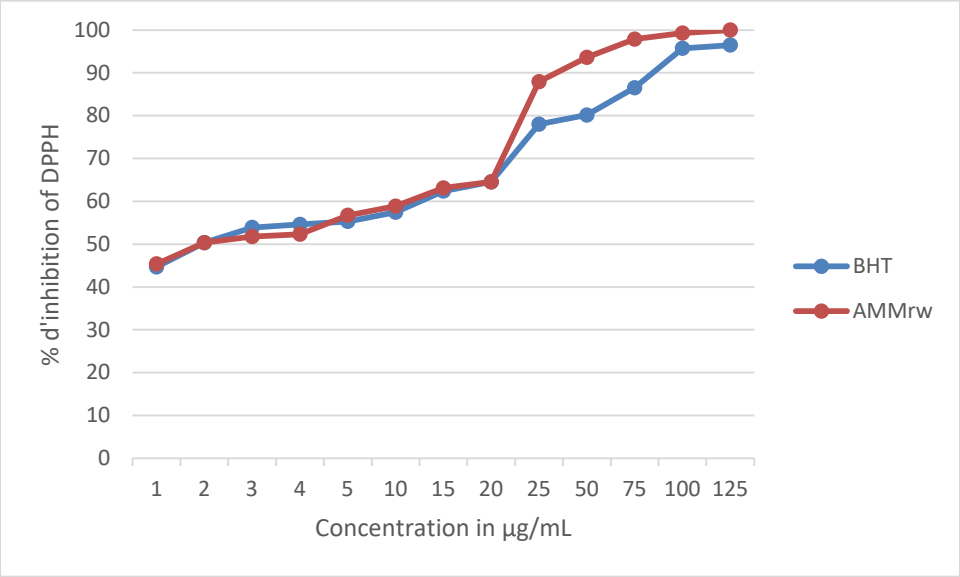


Fig. 4. Comparative curve of the percentage of inhibition of DPPH according to different concentrations of BHT and AMMrw

4. DISCUSSION

4.1 Extraction

The solvent used for our study was methanol, it was chosen in this study for its low boiling temperature, which is around 65°C, thus minimizing the risks to damage the secondary metabolites during the evaporation of the macerate. Methanol also possesses the ability to dissolve large proportions of polar and non-polar compounds [22].
The drying yield is 40.0% for all parts of *A. mannii* with the exception of the leaves, where the yield is 33.3% and therefore lower than the other parts. This could be due to the low water content of the leaves compared to other parts of *A. mannii* (Table 1).
The grinding yield is 44.0%, 30.0%, 35.0%, 38.6% respectively for leaves, stem bark, trunk wood, root bark, and root wood. These yields could be due on the one hand to losses during handling. On the other hand, they could be due to the texture of the different ground materials obtained (Table1). The maceration yield is 1.8%, 1.2%, 0.6%, 3.7%, 1.01% respectively for the leaves, stem bark, trunk wood, root bark, and root wood (Table 1).

4.2 Phytochemical screening

The results obtained in table 2 depicts the different families of compounds present in the plant materials. These results corroborate the work of Kuete *et al*. in 2013 and Djeussi *et al.* in 2013 reporting the presence of the same secondary metabolite classes in the leaves of *A. mannii* [13;23]. They also agree with the results obtained by Achenbach et al in 1985 who isolated molecules of the class of prenylated bisindole alkaloids from the barks of the stems of *A. mannii* [24].The methanolic extract of the root bark of *A. mannii* was found here to be the extract containing the largest number of compounds' families namely: alkaloïds, flavonoïds, anthraquinones, coumarins, saponins and terpenes.

4.3 Evaluation of the antioxidant activity

The dosage of total polyphenols revealed that the methanolic extract of the stem bark had the richest content in polyphenols, followed by the methanolic extract of the root wood, the leaves, then the trunk wood and finally, the root barks proved to be the poorest in polyphenols (figure 2). The phytochemical screening demonstrated the presence of phenols and other classes of polyphenols in each of the extracts**.** The difference observed could be due to the types and amount of phenols present in the different parts of the plant. The study of the antioxidant activity of the extracts suggested that all the methanolic extracts of *A. mannii* tested possess antioxidant activity (figure 3). However, the methanolic extract of the root wood showed the best activity with an IC_{50} of 1.85 µg/ml, close to that of BHT, used as the standard antioxidant in this study. Moderate antioxidant activity was observed for the methanolic extracts of the trunk wood with an IC_{50} of 2.6 µg/ml lower than that of BHT, then leaves, and root bark with an IC_{50} of 3.2 µg/ml and finally the stem bark showed the lowest antioxidant activity with an IC_{50} of 18.5 µg/ml (figure 4).

According to the phytochemical screening of the extracts tested, four classes of polyphenols tests (flavonoïds, anthraquinones, anthocyanes and saponins) were found in the root wood that also exhibit the best antiradical activity with IC_{50} of 1.85 µg/ml, while only two or three classes in the other plant parts. This variation in IC_{50} values could thus be due to the rate of polyphenol present in each extracts Except for the stem bark, the phenol levels for the other plant parts are in agreement with the antioxidant activities observed. Our work is in agreement with that of Dzoyem et *al.* in 2014 which showed that the acetone extract of the leaves of *A. mannii* had an antioxidant activity by inhibition of following standard free radicals: DPPH, ABTS, FRAP, with respectively IC_{50} values of 165.3; 216.28; 0.19 and a total phenol content of 69.0 mgEqGA/mg. On the other hand, the anti-inflammatory activity of of the acetone extract of the leaves of *A. mannii* exhibited > 40% inhibition of 15 lipoxygenase, the anti-inflammatory potency being attributed to the antioxidant potency of *A. mannii* leaves [9].

4. CONCLUSION

The phytochemical screening of the extracts from *A. mannii* revealed the presence of alkaloïds, flavonoïds, anthraquinones, anthocyanins, coumarins, terpenes, steroïds, saponins, phenols. The evaluation of the antioxidant activity revealed that the methanolic extracts of the leaves, trunk wood, stem bark, root wood, root bark of *A. mannii* are all revealed antioxidant activity. The root wood presented the best antioxidant activity close to that of BHT with an IC_{50} value of1.85 µg/ml**.** These results could justify the use of the leaves and stem bark of *A. mannii* in the treatment of rheumatism, as recommended by the traditional pharmacopoeia.
This work was the first study on the wood of the trunk, the bark and the wood of the roots of *A. mannii*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors declare that 'ethical clearance was obtained from the Institutional Ethics Committee of the University of Douala for the conduct of this study and for the publication of this article'. All experiments were reviewed and approved. A copy of the ethical clearance is available for review by the editorial office/editor/editorial board members of this journal.

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