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Phytochemical Screening and Antioxidant Potential of Aqueous Extracts of *Millettia laurenti*, *Lophira alata* and *Milicia excelsa*, Commonly Used in the Cameroonian Pharmacopoeia

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Authors' contributions

This work was carried out in collaboration among all authors. All members contributed in designing the study. Authors FLEE, BRTT and REKD wrote the protocol and managed the analyses of the study. Author HTM performed the statistical analysis together with authors FLEE and BRTT who wrote the first draft of the manuscript. Authors ADKT, AACN and GNM supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: As the world nowadays is turning towards the research of biologically active natural compounds, this work aimed at assessing the antioxidant potential of compounds contained in the aqueous extracts of three common Cameroonian pharmacopoeia plants, namely *Millettia laurenti* (*Wengé*) seeds, *Lophira alata* (*Azobé*) leaves and *Milicia excelsa* (*Iroko*) barks and the associated bioactive compounds.

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Methodology: After being dried and ground, they were macerated in water and the polyphenols, tannins, flavonoids, and alkaloids quantified. The antioxidant potential of the extracts was evaluated through DPPH free radical scavenging, NO scavenging, phosphomolybdate method (TAC), and iron-reducing power (FRAP).
Results: The mean concentrations obtained ranged between 527 and 1213 µg GAE/g DM for polyphenols, 0.39 and 0.65 µg GAE/g DM for tannins, 19.79 and 27.06 µg QE/g DM for flavonoids, 15.72 and 16.02 µg QuE/g DM for alkaloids. Aqueous extracts of *Wengé* (AE-WG) and *Azobé* (AE-AZ) exhibited the highest and significantly similar contents. AE-AZ presented the highest iron reducing power (0.015 µg AAE/g DM at 10 mg/mL) and NO scavenging (IC_{50}=3.63 mg/mL) while AE-WG showed the highest DPPH scavenging activity (IC_{50} = 4.20 mg/mL) and total antioxidant capacity (0.39 µg AAE/g DM at 10 mg/mL). No significant correlation was observed between studied bioactive compounds and the different antioxidant responses except flavonoids and tannins with TAC ($p<0.05$).
Conclusion: AE-*AZ* and AE-*WG* exhibited different antioxidant mechanisms and are therefore of high interest for potential use in the food industry and medicine with reserves to toxicological studies.

Keywords: Aqueous extracts; Wengé seeds; Azobé leaves; Iroko barks; antioxidant potential.

ABBREVIATIONS

GAE : Gallic Acid Equivalent
QE : Quercetin Equivalent
QuE : Quinine Equivalent
AE : Aqueous extract
AE-WG : Wengé aqueous extract
AE-AZ : Azobé aqueous Extract
AE-IR : Iroko aqueous extract

1. INTRODUCTION

Antioxidants can be defined as compounds that can prevent or counteract the deleterious effects of oxidation. They are used for medical purpose to fight against oxidative stress which results from a deficit in antioxidants in favor of free radicals in the body which may lead to cancer, cardiovascular diseases, neural disorders, aging, mental illnesses, and atherosclerosis [1,2,3,4]. Antioxidants are also used as food preservatives to oppose lipid oxidation or inhibit reactions promoted by oxygen or peroxides. Indeed, lipid oxidation leads to food spoilage through the rancidity of fat, off-odor, and off-color [5]. In contrast to natural antioxidants, synthetic ones like butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) were reported having side effects on human health (skin allergies, gastrointestinal problems, increasing of risk of cancer, etc) and their use in the food industry have been restricted [1,6,7]. Substantial evidence showed that the consumption of foods with high antioxidant potential is quite important for disease prevention [8]. However, to avoid a competition for the consumption of such foods that may negatively impact food security especially in developing countries, research for natural antioxidant sources is nowadays turned toward plants that are not used as food. The search for new plant-derived compounds with effective antioxidant activities known as bioactive compounds appears as a challenge worldwide [5,6]. Above their biological properties, the interest for these natural components is also related to their economic value. Most of them may be extracted from food by-products and under-exploited plant species [9]. Various plant extracts used as antioxidants like grape seeds, pine bark, green tea, and pomegranates, have exhibited similar or better properties compared to synthetic antioxidants [10,11]. Bioactive compounds present in plants such as polyphenols, tannins, flavonoids, alkaloids, saponins, phytates, etc., are generally associated with the health benefits of these plants [12,13]. That is why plants with medicinal attributes known as “medicinal plants” are considered as a good source of bioactive compounds [6,8]. *Millettia laurentii* De Wild commonly known as *Wengé* (trade name) or *Awoung* (*Nfon* language, Cameroon) is a characteristic species of the dense tropical forests of the Congo Basin belonging to the Fabaceae family. The seeds are used by some indigenous people as a dietary supplement or seasoning. Some studies reported that different parts of the plant of the species *Millettia* (roots, barks, and leaves) exhibited anti-tumor, antiviral, antiparasitic, or even anti-inflammatory activities [14]. *Lophira alata* Banks ex Gaertn. F. better known under the name of *Azobé* (trade name) or *Abang* (*Nfon* language, Cameroon) is a plant of the family Ochnaceae. Its leaves are elongated,

narrow, coriaceous, simple, alternate, and tufted at the branched ends. In the traditional pharmacopoeia, many properties are attributed to different parts of the plant (bark, roots, seeds, and leaves). The barks had analgesic and anti-inflammatory activities, they also treated fever and gastrointestinal disorders. The roots are used against yellow fever and young leaves are used for the treatment of respiratory problems [15]. *Milicia excelsa* known as *Iroko* (trade name) or *Bang* (*Nfon* language, Cameroon) is a plant of the family Moraceae; with outer barks gray to dark brown or black, lenticellar, becoming scaly. The inner barks are thick, fibrous, cream-colored spotted with orange-brown spots, exuding a white or yellowish latex; spreading of the crown; obliquely ascending branches. This plant has been reported to have aphrodisiac properties and to be helpful in treating a variety of health problems like throat obstructions, asthma, edema, diarrhea, dysentery, rheumatism, hypertension, tumors, and even skin problems [16]. In Cameroon, the use of these three plants is quite recommended by the traditional medicine for the above-mentioned properties. One can, therefore, hypothesize an important content of bioactive compounds in these plants. This work was carried out to assess the antioxidant potential of their aqueous extracts and the associated bioactive compounds.

2. MATERIALS AND METHODS

2.1 Biological Material

Leaves of *L. alata* Banks ex Gaertn (*Azobé*), seeds of *M. laurentii* De wild (*Wengé*) and barks of *M. excelsa* (*Iroko*) were harvested in the mini-forest of the National School of Water and Forests of Mbalmayo (3°31'00'' N and 11°30'00 E), Centre region of Cameroon. Botanical identification was facilitated through the database of the different species available at that Institute. These biological materials were transported to the lab for analysis.

2.2 Chemical Reagents

All analytical grade reagents used in this study (Folin-Ciocalteu, Sodium Carbonate, Chloride acid, 1,10-phenanthroline, 2,2-diphenyl-1-picrylhydrazyl, Naphtylenediamine chloride, Gallic Acid, Aluminum Chloride, Potassium Acetate, Quercetin, Ethanol 99%, Vanillin, Hydrochloric Acid, Iron Chloride, Ascorbic Acid, Sulfuric Acid, Sodium dihydrogen phosphate, Dibasic hydrogen phosphate, Ammonium molybdate, Potassium ferricyanide, Trichloroacetate, Sodium Nitroprusside, Sulfanilic acid, Acetic acid, N-(1-naphthyl)-Ethylenediamine dihydrochloride, and Methanol) were purchased from *Merck* (Germany).

2.3 Plant Materials Processing

The collected samples were washed, rinsed with distilled water, and dried for one week at 60°C (*Heraeus*, Germany) until constant weights. The dried samples were ground (*Moulinex*, France) to obtain powders of approximately 1 mm granulometry. The powders were introduced into clean glass bottles, sealed and stored in the darkness at room temperature for analysis.

2.4 Preparation of Aqueous Extracts

Aqueous extracts of *Azobé* leaves (AE-*AZ*), *Wengé* seeds (AE-*WG*), and *Iroko* barks (AE-*IR*) were prepared as follows: 190 g of sample was dissolved in distilled water at ratio 1:10 (w/v) in a 2000 mL Erlenmeyer flasks. The mixture was homogenized and kept for a 24 h maceration at room temperature (25±1°C). The obtained mixture was centrifuged at 3500 g for 20 min using a *Rotofix 32 A, Hettich Zentrifugen*; filtered (Whatman filter paper No. 2) and the supernatant was evaporated at 70°C for 72 h until a semisolid residue was obtained. The extracts were stored at 4°C (*Labcold, Basingstoke Hants*) and used later for analysis.

2.5 Phytochemical Analysis of the Aqueous Extracts

2.5.1 Determination of the total polyphenols

The total phenolic content of the extracts was evaluated following the method described by Singleton and Rossi [17]. Briefly, an aliquot of 0.1 mL of extract (4 mg/mL) was mixed with 0.75 mL of Folin-ciocalteu reagent (10-fold diluted) and kept at room temperature for 5 min. Then, 0.75 mL of sodium carbonate (6%, w/v) was added. The mixture was homogenized and incubated at 25±1°C in darkness for 90 min. The absorbance of the solution was read at 725 nm (*UVmini-1240, UV-Vis Spectrophotometer*, *Shimadzu*-Japan) against the blank reagent. Gallic acid (0-1000 µg/mL) was used as standard. The total

polyphenol contents of the different extracts were calculated from the calibration curve (r^2 = 0.97) and expressed as microgram of gallic acid equivalent per gram of dry matter (µg GAE/g DM).

2.5.2 Determination of the total flavonoids

The colorimetric method described by Aiyegoro and Okoh [12] was used to evaluate the total flavonoid content of the different plant extracts. Briefly, an aliquot of 0.5 mL of extract (4 mg/ mL) was introduced into a tube containing 1.5 mL of methanol, followed by addition of 0.1 mL of aluminum chloride (10%, w/v), 1 mL of potassium acetate 1M and 2.8 mL of distilled water. The mixture was homogenized, incubated at 25±1°C for 30 min and the absorbance was read at 415 nm against blank reagent. Quercetin at concentrations ranging from 0 to 1000 µg/mL was used as standard. The content of total flavonoids was calculated from the calibration curve (r^2 = 0.99) and expressed as microgram of quercetin equivalent per gram of dry matter (µg QE/g DM).

2.5.3 Determination of the total tannins

The method described by Bainbridge et al. [18] was used to estimate the tannins content of the different extracts. One mL of the extract (4 mg/mL) was mixed with 5 mL of working solution (50 g of vanillin + 4 mL of HCl 1N in 100 mL of distilled water) and the mixture was incubated at 30°C for 20 min. The absorbance was read at 500 nm against the blank. Gallic acid (0-1000 µg/mL) was used as standard and the calibration curve obtained (r^2 = 0.98) was used to calculate the tannins content of the extracts. The results were expressed as microgram of gallic acid equivalent per gram of dry matter (µg GAE/g DM).

2.5.4 Determination of the total alkaloids

Quantification of the total alkaloids in the different plant extracts was performed according to the method described by Singh et al. [19] with slight modifications. Briefly, 100 mg of extracts were dissolved in 10 mL of ethanol solution (80%, v/v). The mixture was homogenized and centrifuged at 5000 rpm for 10 min. A 1 mL volume of the supernatant was introduced into a test tube, followed by the addition of 1 mL of acidified $FeCl_3$ solution (0.025 M; 0.5 M HCl) and 1 mL of an ethanolic solution of 1,10-phenanthroline (0.05M). The mixture was homogenized and incubated for 30 min at 100°C in a water bath (*Poly Science*, USA). The absorbance of the reddish complex formed was read at 510 nm against the blank. Quinine at a concentration of 10 µg/mL was used as standard and the alkaloid content was expressed as microgram of quinine equivalent per gram of dry matter (µg QuE/g DM).

2.6 Antioxidant Potential of the Aqueous Extracts

2.6.1 Determination of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging

The ability of the different aqueous extracts (AE) to scavenge the DPPH radical was evaluated by the method described by Sanchez-Moreno et al. [20] with some modifications. Briefly, 0.05 mL of the methanolic solution of AE at different concentrations (0.5-10 mg/mL) was added to 1.95 mL of a methanolic solution of DPPH (0.025 g/L). The mixture was homogenized and incubated in darkness at room temperature (25±1°C) for 30 min. Methanol was used as a negative control while ascorbic acid was used as a positive control. The controls were prepared as for the test sample except that AE was replaced with either methanol or ascorbic acid. Absorbance was read at 515 nm against blank reagent. The inhibition percentage of the DPPH radical (%I_{DPPH}) was calculated according to the following formula (Eq 1):

$$I_{DPPH}(\%) = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100 \qquad (1)$$

The IC_{50} value corresponding to the concentration of antioxidants required for a 50% reduction of the DPPH radical, was estimated graphically based on the curve plotted. As the IC_{50} value of an extract is low, the extract is considered as active.

2.6.2 Determination of the scavenging of NO radical

The scavenging capacity of NO radical was evaluated using the method described by Marocci et al. [21]. To a 2 mL solution of sodium nitroprusside (10 mM) prepared in phosphate buffer (50 mM, pH 7.4) was added 0.5 mL of AE (1-10 mg/mL), and the whole mixture was incubated at room temperature (25±1°C). A 0.5 mL volume of the mixture was then removed and 0.5 mL of Griess reagent (1 mL of sulfanilic acid

0.33% in acetate 20% incubated for 5 min at room temperature + 1 mL of naphtylenediamine chloride 0.1% w/v) supplemented. The obtained solution was incubated again at 25±1°C for 30 min and the absorbance was read at 540 nm against the blank. Ascorbic acid (0.25 µg/mL) was used as a standard.

2.6.3 Determination of the reducing ferric capacity (FRAP: Ferric Reducing Antioxidant Power)

The reducing power of iron (Fe^{3+}) in aqueous extracts was determined according to the method of Oyaizu [22]. In the protocol, 1 mL of AE at different concentrations (0.5-10 mg/mL, distilled water) was mixed with 2.5 mL of phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ potassium ferricyanide solution. The mixture was incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloro-acetic acid (TCA) solution was added to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant was subsequently combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% aqueous solution of iron ($FeCl_3$) chloride. The absorbance of the mixture was read at 700 nm against the blank. Distilled water and ascorbic acid (0.25 µg/mL) treated under the same conditions as the samples were used as negative and positive controls, respectively. The reducing capacity of iron (Fe^{3+}) was expressed in µg equivalent of ascorbic acid per gram of dry matter (µg EAA/g DM).

2.6.4 Determination of the total antioxidant capacity

The total antioxidant capacity of the aqueous extracts was assessed following the phosphomolybdenum method described by Prieto et al. [23]. For that, 0.2 mL of AE at different concentrations (0.5-10 mg/mL) was mixed with 2 mL of working reagent solution (0.6 M H_2SO_4 98%, 28 mM NaH_2PO_4 and 4 mM ammonium molybdate). The tubes were screwed and incubated at 95°C for 90 min in the water bath. After cooling at room temperature (25±1°C), the absorbance of the solutions was measured at 765 nm against blank reagent (2 mL of the reagent solution and 0.2 mL of distilled water) treated under the same conditions as the samples. Ascorbic acid at a concentration of 0.25 µg/mL was used as a standard. The total antioxidant capacity was expressed in µg equivalent of ascorbic acid per gram of dry matter (µg EAA/g DM).

2.7 Statistical Analyses

All experiments were performed in triplicate. The results obtained were expressed as mean ± standard deviation. A One-way ANOVA (Least Squared Difference) was performed using Statistical Package for Social Science (SPSS) software version 20.0 to assess the significance of the difference between the values obtained ($p<0.05$). The principal component analysis was performed using the statistical software XLSTAT 2014 (Addins of, Inc., New York, USA) to visualize the relationship between the bioactive compounds of the AE of plants and their antioxidant activities assessed through the different tested methods. The strength of the association between these variables was determined using the Neyman-Pearson correlation.

3. RESULTS AND DISCUSSION

3.1 Bioactive Compounds of the Different Aqueous Extracts

The bioactive compounds of the different aqueous extracts were quantified and the results are presented in Table 1. Their concentrations vary from one plant extract to another, except for alkaloids were the values were significantly the same (15.72-16.02 µg QuE/g DM). Concerning polyphenols and flavonoids contents, the lowest contents (527.667 ± 5.433 µg GAE/g DM and 19.792 ± 2.892 µg QE/g DM, respectively) were recorded with the AE of *Iroko* bark. In contrast, AE of *Wengé* seeds presented the highest contents (1213.222 ± 13.426 µg GAE/g DM and 27.060 ± 2.477 µg QE/g DM, respectively), contents that were statistically similar to the ones of AE of *Azobé* leaves (1078.778 ± 19.116 µg GAE/g DM and 22.292 ± 1.531 µg QE/g DM, respectively). This polyphenols content of AE of *Wengé* and *Azobé* was almost twice the one of *Iroko* extract. For tannins, the highest and lowest contents were also observed with AE-*WG* (0.654 ± 0.017 µg GAE/g DM) and AE-*IR* (0.386 ± 0.026 µg GAE/g DM), respectively.

3.2 Antioxidant Activities of the Aqueous Extracts of Plants

3.2.1 DPPH radical scavenging capacity

The DPPH radical scavenging activity of the different plant AEs can be observed in Fig. 1. Whatever the plant extract, this scavenging activity rises with the extract concentration. The

maximum inhibition percentage of DPPH radical (64.84%) was observed with AE-*WG* at 10 mg/mL. AE-*AZ* showed the lowest activity with just 14.15% inhibition at that concentration. Based on the gotten inhibition curves, the IC_{50} values were estimated at 4.50 mg/mL for AE-*WG* and above 10 mg/mL for AE-*IR* and AE-*AZ*. This is far higher compared to the value obtained with the reference antioxidant (ascorbic acid) we used (IC_{50} = 3.916 µg/mL).

3.2.2 Nitric oxide (NO) radical scavenging capacity

Plant AEs exhibited an ability to scavenge the NO radical that was also concentration-dependent (Fig. 2). The calculation of their IC_{50} gave values of 3.630 mg/mL for AE-*AZ* and 4.518 mg/mL for AE-*IR*, which is better than the 5.906 mg/mL obtained with the reference antioxidant (ascorbic acid). The least active plant AE was the one derived from *Wengé* (AE-*WG*) which exhibited an IC_{50} of 7.016 mg/mL.

3.2.3 Iron reducing power

Fig. 3 presents the data on the iron-reducing power of the different AE of plants. The AE-*WG* and AE-*IR* presented a weak activity of 0.006 µg AAE/g DM which did not vary with the increase of the tested concentration. This was not the case for AE-*AZ* where a rise of the iron-reducing activity with the concentration of the extract was noticed. The highest iron-reducing activity of 0.015 µg AAE/g DM was observed at the AE-*AZ* concentration of 10 mg/mL.

Table 1. Contents in the tested bioactive compounds of different plants aqueous extracts

Aqueous extracts	Flavonoids (µg QE/g DM)	Polyphenols GAE/g DM)	Tannins (µg GAE/g DM)	Alkaloids (µg QuE/g DM)
AE-*WG*	27.060 ± 2.477^{a}	1213.222 ± 13.426^{a}	0.654 ± 0.017^{a}	16.016 ± 0.239^{a}
AE-*IR*	19.792 ± 2.892^{b}	527.667 ± 5.433^{b}	0.386 ± 0.026^{c}	15.718 ± 0.203^{a}
AE-*AZ*	22.292 ± 1.531^{a}	1078.778 ± 19.116^{a}	0.499 ± 0.022^{b}	15.881 ± 0.077^{a}

n=3 replications, AE-WG: Aqueous Extract of Wengé, AE-IR: Aqueous Extract of Iroko, AE-AZ: Aqueous Extract of Azobé, µg: micrograms, g: grams, DM: Dry Matter, GAE: Gallic Acid Equivalent, QE: Quercetin, QuE: Quinine Equivalent. Mean ± Standard Deviation bearing the same letter in superscript in the same column are not significantly different (p<0.05)

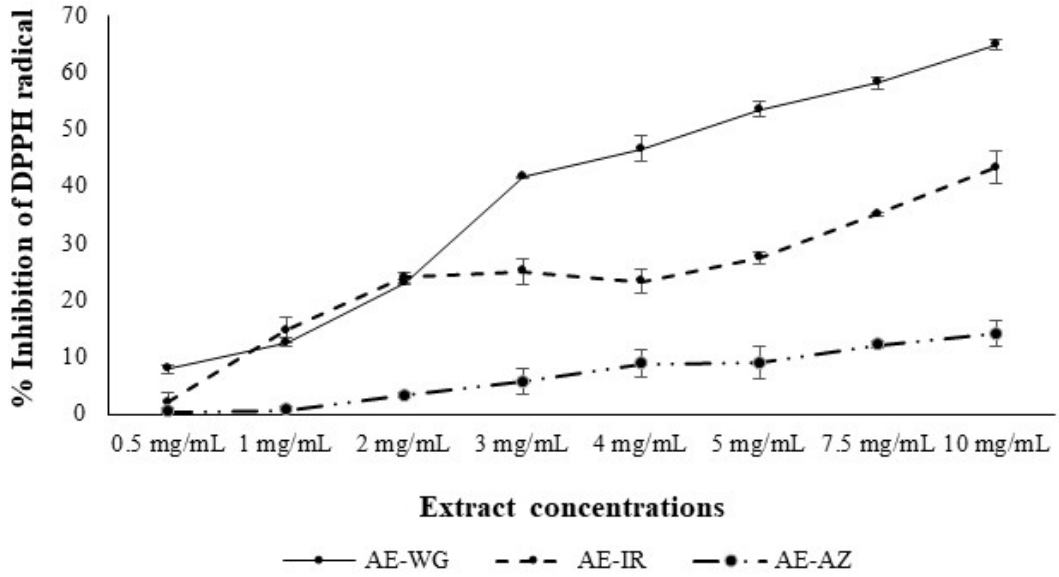

Fig. 1. Scavenging capacity of the DPPH radical by the different plant extracts (AE-*WG*: *Wengé* aqueous extract; AE-*AZ*: *Azobé* aqueous Extract; AE-*IR*: *Iroko* aqueous extract)

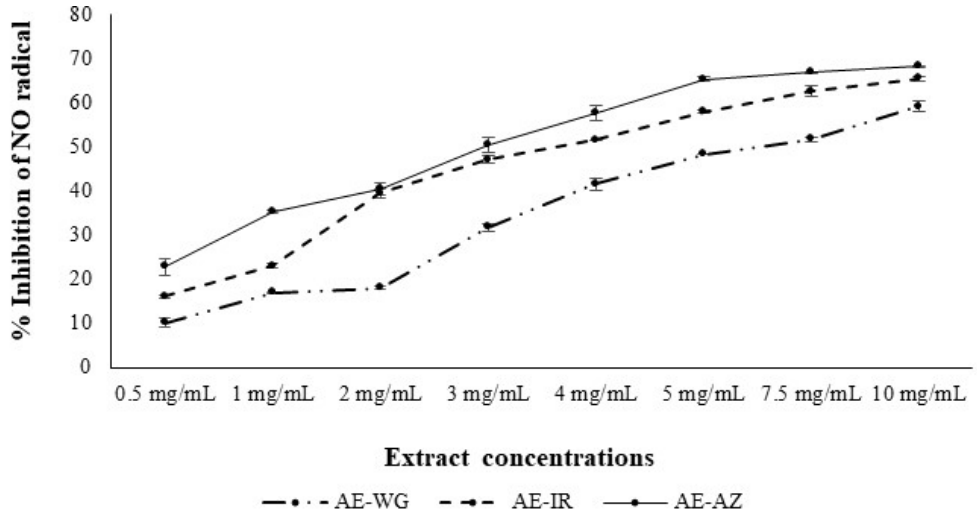

Fig. 2. NO radical scavenging capacity of different aqueous extracts of plants (AE-*WG*: *Wengé* aqueous extract; AE-*AZ*: *Azobé* aqueous Extract; AE-*IR*: *Iroko* aqueous extract)

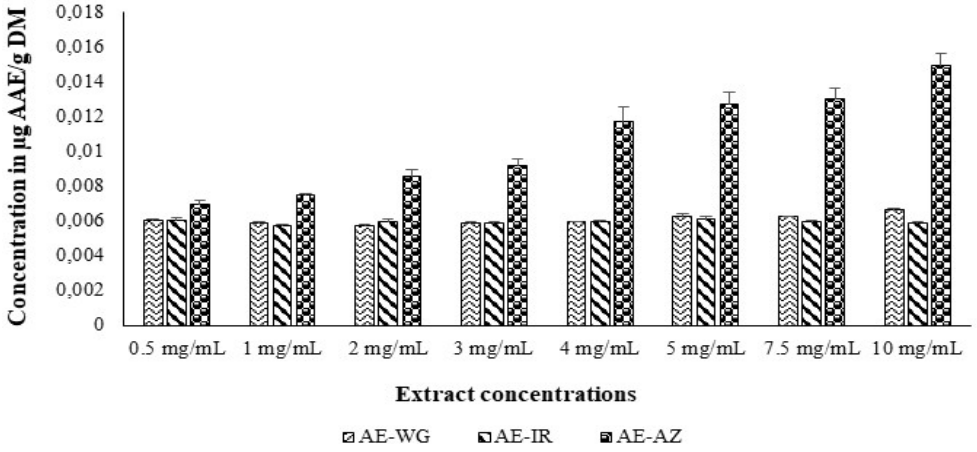

Fig. 3. Iron reducing power (FRAP) of different aqueous extracts of plants (AE-*WG*: *Wengé* aqueous extract; AE-*AZ*: *Azobé* aqueous extract; AE-*IR*: *Iroko* aqueous extract)

3.2.4 Total antioxidant capacity (TAC)

As shown in Fig. 4, the different plant AEs tended to reduce Mo(VI) to Mo(V) in a concentration-dependent manner. At 10 mg/mL, AE-*WG* presented the high total antioxidant capacity (0.39 µg AAE/g DM), followed by AE-*AZ* (0.22 µg AAE/g DM) and AE-*IR* (0.14 µg AAE/g DM).

3.2.5 Correlation between the tested bioactive compounds content and antioxidant activities of the extracts

From the principal component analysis (PCA) of the data, two main groups could be distinguished (Fig. 5). A first group where AE-*WG* appears and which reveals that the tested bioactive compounds are positively correlated with the antioxidant activities DPPH, FRAP, and especially TAC by flavonoids and tannins. Indeed, as it could be seen with the associated Newman Pearson correlation analysis (Table 2), these two bioactive compounds groups had a significant effect on the TAC observed (r^2 =1.00, P = .01 and r^2 = 0.99, P = 0.03). At the opposite, the second group is formed by the AE-*AZ* and antioxidant activity NO. Negative and non-significant correlations were noted in this case with the different tested compounds groups.

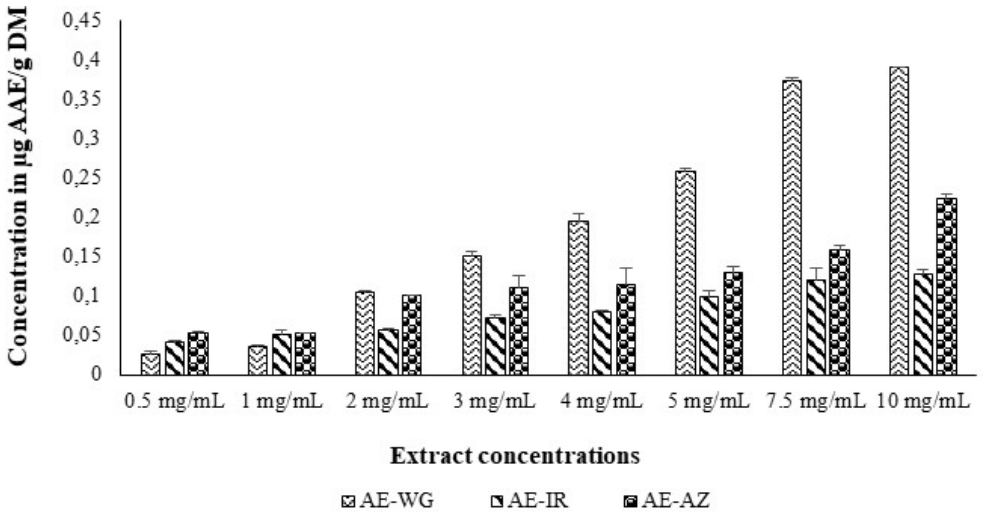

Fig. 4. Total antioxidant capacity (TAC) of the various aqueous extracts of plants (AE-*WG*: *Wengé* aqueous extract; AE-*AZ*: *Azobé* aqueous Extract; AE-*IR*: *Iroko* aqueous extract)

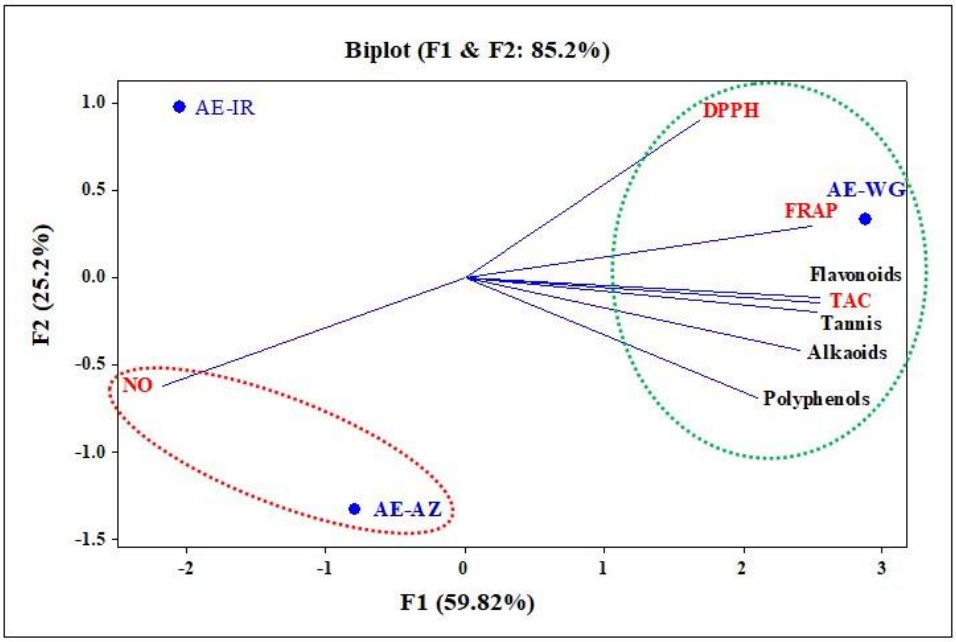

Fig. 5. Distribution of the bioactive compounds of the different plant extracts and the antioxidant activity assessed through different methods on the F1xF2 axis

Table 2. Correlation coefficients and *p*-values between the bioactive compounds of the different plant extracts and the different methods used to assess the antioxidant activity

	DPPH	FRAP	TAC	NO
Polyphenols	r^2=0.09 (*P*=.93)	r^2=0.65 (*P*=.54)	r^2=0.87 (*P*=.31)	r^2=-0.39 (*P*=.74)
Flavonoids	r^2=0.57 (*P*=.60)	r^2=0.94 (*P*=.22)	r^2=1.00 (*P*=.01)*	r^2=-0.79 (*P*=.41)
Tannins	r^2=0.51 (*P*=.65)	r^2=0.91 (*P*=.26)	r^2=0.99 (*P*=.03)*	r^2=-0.75 (*P*=.45)
Alkaloids	r^2=0.35 (*P*=.77)	r^2=0.82 (*P*=.38)	r^2=0.97 (*P*=.15)	r^2=-0.61 (*P*=.57)

**indicates that the correlation is significative at P= .05*

4. DISCUSSION

The use of plants bioactive compounds to address food safety and stability problems or for therapeutic purposes is gaining interest since the last decades, especially since these compounds are "Generally Recognized As Safe" [1,24].

The results obtained from the analyses of the three studied plant extracts showed a similar alkaloids content but a significant difference in their polyphenols, flavonoids, and tannins contents; AE-*WG* and AE-*AZ* showing the highest values. The production yields of bioactive compounds are known to vary from one plant to another [25], as well as the part of the plant where these secondary metabolites are mainly present [26]. This also varies from one region to another (environmental growth condition). Ngoua-Meye-Misso et al. [27] reported a total phenolic and total flavonoid contents of 4205 µgGAE/g of extract and 730 µgQE/g of extract, respectively with *Lophira procera* barks aqueous extract from Gabon, which is higher compared to the 1078 µgGAE/g DM and 22 µgQE/g DM obtained in this study with *Lophira alata* leaves extract. The polyphenols and alkaloids noticed in WG seeds were reported by Teugwa et al. [28] to be absent from the leaves of this plant that they also collected in Cameroon.

Considering the variety of oxidation processes and reactions that have been reported [29,30], a single method cannot be enough to provide a clear idea of the antioxidant potential of a compound. In the present study, four different methods were used to evaluate the antioxidant activity of the plant AEs: Two based on the antiradical scavenging activity (DPPH* and NO*) and two others based on the reducing potential (TAC and FRAP). It appeared that the ranking order of activity of the three extracts was depending on the specific test performed. This suggests a difference of content or in the nature of compounds associated with the specific activity evaluated. The correlation test between the different bioactive compounds content and the antioxidant activity of the extracts at different concentrations was taken as indicative.

For the scavenging activity investigation, the synthetic DPPH radical is widely used. All the plant AEs were able to convert DPPH radicals to a more stable species (DPPH-H or DPPH-R). AE-*WG* displayed the highest DPPH radical scavenging activity with an IC_{50} of 4.50 mg/mL, an activity which appeared to be positively but non-significantly linked to flavonoids (r^2 = 0.57, p = .60), tannins (r^2 = 0.51, *P* = .65) and alkaloids (r^2 = 0.35, *P* = .77). A very weak correlation with polyphenols content (r^2 = 0.09, *P* = .93) was observed, which is in contrast to many works generally linking both parameters [31,32], and which therefore questions the nature of polyphenols that are present in the extract. Indeed, the class of polyphenols has been proven to determine the free radical scavenging activity, the flavanols being the most effective [31,33]. These flavonols were not detected in *Lophira procera* barks aqueous extract [27]. The low and non-significant correlation coefficients observed suggest the involvement of other bioactive compounds groups in the scavenging activity of AE-*WG*. On the other side, the study of this scavenging activity rather with the biological radical NO*, which is known as a product of biological metabolism that can lead to diseases like cancer, atherosclerosis, cataracts or as Parkinson's and Alzheimer's diseases [31,34], also shown similar tendency. Despite the increase in the scavenging percentage with the concentration of the extract for all the 3 plants, a non-significant correlation with the 4 studied bioactive compounds groups was noticed. The NO radical scavenging activity was negatively associated with polyphenols (r^2 = -0.39, *P* = .74), flavonoids (r^2 = -0.79, *P* = .41), tannins (r^2 = -0.75, *P* = .45) and alkaloids (r^2 = -0.61, *P* = .57). These negative correlations were because it was IC_{50} values that were used to perform the correlation analysis (As IC_{50} value was low, the NO scavenging activity was high). A weak correlation between the NO radicals scavenging activity of Walnut kernels (*Juglans regia* L.) and the phenolic content (r^2 = 0.08) as well as the flavonoid content (r^2 = 0.07) was also cited by many studies (2012) [35,36]. However, there is for instance–positive correlation between the phenolic contents of *Amygdalus communis* and the NO radical scavenging activity [37]. This therefore also question the nature of the compounds present in the extracts. The more as AE-*WG* was not the most active NO* Scavenging extract like with DPPH, but rather AE-*AZ*.

The ability of the different AE of plants to inhibit Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^-$) through their iron-reducing activity was also assessed in this study. During the Fenton reaction, free radicals involved in the oxidation of ferrous ions to ferric ions by H_2O_2 are generated. The resulting ferric ions, hydroxyl radical and a hydroxyl anion can also accelerate lipid peroxidation by decomposing

lipid hydroperoxides into peroxyl and alkoxyl radicals that can perpetuate the chain [30,31,38]. At the maximal tested concentration (10 mg/mL), only the AE of *Azobé* leaves was active, with an iron-reducing activity of 0.015 µg AAE/g DM. A positive but non-significant correlation was noted with the flavonoids (r^2 = 0.94, *P* = .22), tannins (r^2 = 0.91, *P* = .26) alkaloids (r^2 = 0.82, *P* = .38) and polyphenols (r^2 = 0.65, *P* = .54) contents which were rather closed to AE-*WG* as shown by the PCA analysis. Kaurinovic and Vastag [13], as well as Erol et al. [35], highlighted that flavonoids are strong iron reducers and that the property originated from their catechol moiety. This PCA analysis helped to understand that those two extracts were at the opposite. AE-*WG* which showed the highest content of all the tested bioactive compounds but no iron-reducing activity probably obey to the rule which states that this activity depends more on structure and conformation of the different bioactive compounds than their concentrations [10,13] while AE-*AZ* may contain other compounds significantly linked to this antioxidant activity.

Regarding the total antioxidant capacity, the AEs of the three plants were active in reducing Mo(VI) to Mo(V) in a dose-dependent manner. Similar observations have been reported in the literature [39] and the phenomena was explained as a result of the exponential development of reducing power as the concentration of the extract increased. AE-*WG* showed the highest total antioxidant activity which was significantly associated to its contents in flavonoids (r^2 = 1.00, *P* = .01) and tannins (r^2 = 0.99, *P* = .03), and non-significantly to polyphenols (r^2 = 0.87, *P* = .31) and alkaloids (r^2 = 0.97, *P* = .15). Besides, the results from total antioxidant capacity were positively correlated to those obtained from iron-reducing capacity (r^2 = 0.93, *P* = .023). Thus, indicating that almost the same mechanisms or the same compounds are involved in both activities. That compound might be flavonoids as it showed the highest correlation with the total antioxidant capacity (r^2 = 1.00, *P* = .01) and the iron-reducing capacity (r^2 = 0.94, *P* = .22). It can, therefore, be suggested a quantitative dependence of TAC with flavonoids and tannins but rather a qualitative one (nature of the compounds) with polyphenols and alkaloids [33,40,41].

4. CONCLUSION

This study demonstrated that the aqueous extracts of *Azobé* leaves, *Wengé* seeds, and *Iroko* barks contained bioactive compounds such as polyphenols, flavonoids, tannins, and alkaloids at concentrations varying from one extract to another. Aqueous extracts of *Azobé* leaves, *Wengé* seeds appeared as the ones with the highest antioxidant potential, a potential exhibited in DPPH* scavenging activity for *Wengé*, and NO* and Iron reducing power for *Azobé*. These differences in the antioxidant mechanism can be exploited in food industries (bio-preservatives) or in the medical field for the management of metabolic diseases (fight against oxidative stress). Further studies on the toxicity and the structural characterization of bioactive compounds of these aqueous extracts need to be performed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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