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Research Article

Phytochemical composition and antioxidant activity: Comparison of *Pentaclethra eetveldeana* (De Wild & T. Durand) leaf ethanolic extracts (Congo-Brazzaville)

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Abstract: It is important to know the intraspecific variability of the biological properties and chemical composition of plants in order to promote their better use. Thus, referring to the use of *Pentaclethra eetveldeana* leaves in a dementia traditional treatment, this study aims to highlight the antioxidant capacity and the chemical composition of the ethanolic extracts of *P. eetveldeana* leaves from four localities of Congo-Brazzaville. The β-carotene bleaching, diphenyl-picryl-hydrazyl (DPPH) radical-scavenging and molybdenum reduction methods were used to determine the antioxidant potency. Subsequently, the yields of the extractions, the phytochemical screening and the quantification of the phenolic compounds were carried out. The results revealed that the extracts of the four localities presented an antiradical and an antilipid peroxidation superior to those of ascorbic acid in DPPH and β-carotene bleaching methods. Moreover, among the extracts, those of the leaves from Boundji and Brazzaville presented the best antilipid peroxidation, antiradical and reducing activities as well as the greatest extraction yields, the greatest quantities of total polyphenols and proanthocyanidins against low levels of flavonoids. Furthermore, saponins, polyphenols, alkaloids, reducing sugars and cardiotonic glycosides were identified in all ethanolic extracts except sterols and triterpenes which were only identified in the extracts of leaves collected in Brazzaville. In addition, flavones were identified in the leaves from Owando and Makoua; flavonols in the leaves from Boundji and Brazzaville. This study showed that *P. eetveldeana* leaf ethanolic extracts exhibit antilipid peroxidation, antiradical properties and phytochemical that varied according to the region.

1. INTRODUCTION

Endemic to the Guineo-Congolese region, *Pentaclethra eetveldeana* (De Wild & T. Durand) is a plant of the Fabaceae-Mimosoideae family (Gillet, 2013). Traditionally, the bark of this plant is known to have emetic, purgative, analgesic, anthelmintic and antiparasitic properties (Bouquet, 1969). Furthermore, the leaves of this plant are used to treat dementia (Bouquet, 1969), a neurodegenerative disease in which free radicals, including the peroxyl type, are

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involved in the damage of neuronal cells, particularly by targeting lipid membranes and proteins (Błaszczyk, 2022).

Therefore, based on the traditional use of *Pentaclethra eetveldeana* (*P. eetveldeana* leaves to treat dementia (Bouquet, 1969), a previous study demonstrated the antiradical, antilipid peroxidation and phytochemical potential of aqueous extracts of these leaves collected in four different localities of the Republic of Congo (N'goka *et al*., 2023). Underlining the importance of knowing the intraspecific variability within a species for a better use of the plant, the extracts showed a higher antioxidant effect than ascorbic acid and an abundant presence of phenolic compounds, alkaloids and saponins (N'goka *et al*., 2023) witch are groups of secondary metabolites including sub-groups with antioxidant properties (Ashraf *et al*., 2013; Francenia Santos-Sánchez *et al*., 2019; Plazas *et al*., 2022) by releasing an electron or hydrogen atoms and inhibiting lipid peroxidation. Furthermore, in order to offer a better treatment in terms of efficacy and to research the highest yield of secondary metabolites, it is necessary to experiment different extraction process. This would provide more scientific data on the plant, particularly on variations in antioxidant potential and phytochemical composition. In this respect, antioxidant compounds such as polyphenols, which prevent the risk of neurodegenerative diseases (Francenia Santos-Sánchez *et al*., 2019), as well as saponins and alkaloids are well extractable by ethanol (Ato Koomson *et al*., 2018; Rajbhar *et al*., 2015) (LD50 = 6200 mg/kg), a non-toxic or very low toxic solvent with good biodegradability and low bioaccumulation potential (VWR International, 2007).

In accordance to the previous statements, this work aims to highlight the antioxidant activity and the phytochemical composition of the ethanolic extracts of *P. eetveldeana* leaves. Furthermore, to highlight any difference in the therapeutic potential and the phytochemical composition of these leaves, four localities of Congo-Brazzaville have been selected for the harvest of leaves.

2. MATERIAL and METHODS

2.1. Plant Material

Plant material was constituted by four collections of *P. eetveldeana* leaves namely from the districts of Makoua, Owando, Boundji and the department of Brazzaville in the Republic of Congo. These leaves collected (September, 2022) between 6 to 7 a.m. were dried and crushed for the study. The herbarium number: collection B. DESCOINGS n°6999, 05/06/1961.

2.2. Preparation of Extracts: Maceration

65 g of dried leaves were incubated for 72 hours in 400 mL of ethanol 90°. After filtration, the filtrate was evaporated at 60°C in an oven (Ang & Manuales, 2022; Mbengui *et al*., 2013). Thus, the ethanolic dry extract was obtained. Finally, the dry extract was weighed and the formula below was used to calculate yields. $M_{extract}$ represent the mass of dry ethanolic extract while M_{leaves} represent the mass of dry crushed leaves.

$$\text{Yield (\%)} = (M_{extract}/ M_{leaves}) \times 100$$

2.3. Phytochemical Screening

2.3.1. *Polyphenols*

The mixture of 2 mL of the extract with few drops of $FeCl_3$ give a bluish black color (N'goka *et al*., 2023).

2.3.2. *Gallic tannins*

The extract (2 mL) mixed with few drops of $FeCl_3$ (5%) give a green color for gallic tannins or a brown color for pseudo tannins (N'goka *et al*., 2023).

2.3.3. *Alkaloids*

An orange-colored precipitate appears in the test tube containing the extract (2 mL) and few drops of Dragendroff's reagent (N'goka *et al*., 2023).

2.3.4. *Flavonoids*

In the test tube containing extract (2 mL), the subsequent addition of hydrochloric alcohol (HCl/ethanol, 50:50, v/v) then magnesium shavings leading to a red color means the presence of flavonols and an orange color is for the presence flavones (N'goka *et al*., 2023).

2.3.5. *Saponins*

The saponins presence is demonstrated by a persistent foam for two or three minutes later after a vigorous shaking of the test tube containing 2 mL of the extract (N'goka *et al*., 2023).

2.3.6. *Cardiotonic Glycosides*

This test consists to observe a reddish-brown color in the tube when 2 mL of chloroform and then 2 mL of sulfuric acid are added to 2 mL of extract (N'goka *et al*., 2023).

2.3.7. *Reducing sugars*

A brick red precipice in the test tube containing extract (2 mL) and 1 mL of Fehling's liquor demonstrates the reducing sugar presence (N'goka *et al*., 2023).

2.3.8. *Sterols and triterpenes*

First 2 mL of chloroform are added in the test tube containing extract and then 2mL of sulfuric acid from the sides of the test tube: when a red ring appears, the sterols presence is justified and a reddish-brown color shows the triterpenoids presence (N'goka *et al*., 2023).

2.4. Quantification of Secondary Metabolites

2.4.1. *Total polyphenols (TP)*

Based on the Folin–Ciocalteu method (Aryal *et al*., 2019), 0,25 mL of the ethanolic extract prepared at 1 mg/mL was added to 1.25 mL of the reagent Folin–Ciocalteu 10% (w/v). Then, the addition of 1 mL of Na_2CO_3 (20%). Thus, the mixture was incubated for 10 min at room temperature in the dark. After that, by using a Thermo Scientific Genesys 10S UV-VIS spectrophotometer (Waltham, Massachusetts, USA), the absorbance was measured at 765 nm. Finally, the result was expressed as gallic acid equivalents in microgram per gram of extract (µg GAE/g).

2.4.2. *Tannins (TN)*

Based on the Obame Engonga (Obame-Engonga, 2009) described method, with few modifications, in the test tube a mix was made with 1.25 mL of distilled water, 0.25 mL of ferric ammonium citrate (28%), 0.25 mL of ethanolic extract and finally 1 mL of aqueous ammonia (10%). A period of 10 min of incubation followed. After that, at 525 nm, the absorbance was obtained. Then, in microgram of tannic acid equivalent/g (µg TAE/g), results were determined.

2.4.3. *Proanthocyanidins (PR)*

In accordance with Dicko *et al*. 2005 described method, proanthocyanidins quantification were assessed by mixing 2.33 mL of a butanol hydrochloric acid solution (30%) with 0.17 mL of ethanolic extract. This mixture was then, for 2 hours at 100°C, heated followed by a cooling step. At 550 nm, the absorbances were read and finally, in microgram apple proanthocyanidins equivalent/g (µg APE/g), results were determined.

2.4.4. *Flavonoids (FL)*

With some changes, using the $AlCl_3$ assay reported by Quettier-Deleu (Quettier-Deleu *et al*., 2000), flavonoids quantity was determined in microgram of quercetin equivalent/g (µg QE/g).

This method consists in the mix of 0.5 mL of $AlCl_3$ (2 % in methanol) with 0.5 mL of extract (1 mg/mL in methanol). Then, after waiting 10 min, at 430 nm the absorbances were determined.

2.5. Antioxidant Assay

2.5.1. *DPPH scavenging assay*

Following the method reported by Abdullahi *et al*. (2018), with some changes, 10 g of 1.1-diphenyl-2-picrylhydrazyl (DPPH) powder were prepared at 50 µg/mL in methanol (100%). Then, a mix of 1 mL of ethanolic extract or reference antioxidant (12.5, 6.25, 3.125, 1.5625 and 0.78125 µg/mL in methanol) with 1 mL of DPPH solution was made followed by an incubation in the dark, for 30 min. After that, all absorbances were read at 517 nm. The negative control consisted of a mixture of 1 mL of distilled water with 1 mL of DDPH solution. The inhibition percentages of DPPH radical were calculated using the formula below.

$$\text{Inhibition (\%)} = (\text{AbsC} - \text{AbsS}/\ \text{AbsC}) \times 100$$

AbsC means the absorbance of the negative control and AbsS is the absorbance of the extract or reference antioxidant (ascorbic acid).

2.5.2. *Molybdenum assay*

In line with Abubakar *et al*. (2013), the molybdenum method for the assessment of total antioxidant capacity consisted of the following mixture: 0.3 mL of extract with 3 mL of molybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Test tubes containing the mixtures were then heated for 1 hour 30 min at 70°C and cooled at room temperature. Finally, after the measurement of the absorbances at 695 nm, the total antioxidant capacity was determined as microgram of ascorbic acid equivalent/g (µg AAE/g).

2.5.3. *β-carotene bleaching assay*

The β-carotene linoleate assay like described in a previous study was used with few changes (Ghedadba *et al*., 2014). The reagent was prepared using 500 µg of β-carotene mixed with 1 mL of chloroform, 0.05 mL of linoleic acid and 0.5 µL of tween (20%). Then, the addition of 100 mL of distilled water followed the step of evaporating chloroform at 50°C. The mixture was vigorously shaking and an emulsion was obtained. The reaction mixture was constituted by 2.5 ml of the emulsion and 0.5 mL of extract (1mg/mL) or reference antioxidant (ascorbic acid, 1 mg/mL) or distilled water (negative control). Then, the mixtures were incubated for 2 hours at 50°C for the generation of linoleic acid free radical and 48 hours later the absorbances were measured at 470 nm. Finally, using the following formula, the relative antioxidant activity (RAA) expressed in percentage was calculated.

$$\text{RAA (\%)} = (\text{AbsS}_{(48h)}/\ \text{AbsP}_{(48h)}) \times 100$$

$\text{AbsS}_{(48h)}$ means the absorbance of the ethanolic extract or the absorbance of the negative control while $\text{AbsP}_{(48h)}$ represents the absorbance of the positive control (ascorbic acid).

2.6. Statistical Analysis

All results were analyzed using Microsoft Office Excel 2019. They were expressed as mean ± standard deviation (*SD*) and significance difference was evaluated at p =0.05. Then, using a regression analysis, the concentration of ethanolic extract that inhibit 50 % of the DPPH radicals (IC_{50}) were calculated for the data obtained from DPPH assay. Finally, to assess the link between the antioxidant activity and phytochemicals, a correlation analysis was done.

3. RESULTS

In the following line, the ethanolic extract of *P. eetveldeana* leaves from Boundji, Brazzaville, Makoua and Owando are respectively eetBO, eetBR, eetMA and eetOW.

3.1. Extraction Yields

It was found that the ethanolic extract of the leaves from Brazzaville presented the greatest yield like reported in Table 1. Among the others yields, that of the leaves from Boundji showed the highest value while the lowest value is shown by the ethanolic extract of the leaves from Owando.

Table 1. Yields of the ethanolic extracts of *P. eetveldeana* leaves.

Ethanolic extracts	Dry extracts mass (g)	Yields (%)
eetMA	2.45	3.76
eetBO	3.58	5.50
eetBR	5.04	7.75
eetOW	1.94	2.98

3.1. Phytochemical Composition

The Table 2 reports the presence of alkaloids, saponins, polyphenols, flavonoids, tannins, cardiotonic glycosides and reducing sugars in all ethanolic extracts while sterols and triterpenes were only found in the ethanolic extract of the leaves from Brazzaville.

Table 2. Metabolites identified in the ethanolic extracts of *P. eetveldeana* leaves.

Extracts	Alkaloids	Saponins	Polyphenols	Flavonoids	Tannins	ST	CG	RS
eetMA	+++	+	+++	+ (a)	++	-	+	++
eetBO	+++	+	+++	+ (b)	+++	-	+	+++
eetBR	+++	+	+++	+ (b)	+++	++	++	+++
eetOW	+	+	+++	+ (a)	++	-	+	+

Very abundant: +++, Abundant: ++, less abundant: +, not detected: -. ST: sterols and triterpenes, CG: cardiotonic glycosides, RS: reducing sugars. (a): flavones, (b): flavonols.

3.2. Phenolic Compounds Content

The Figure 1 shows the quantities of phenolic compounds from the ethanolic extracts of *P. eetveldeana* leaves. It was found that eetBO (TP: 1373.05 ± 10.35 µg GAE/g, PR: 1663.22 ± 42.50 µg APE/g), eetBR (TN: 744.66 ± 16.44 µg TAE/g) and eetOW (FL: 146.86 ± 4.31 µg QE/g) possess respectively the greatest quantity of total polyphenols, proanthocyanidins, tannins and flavonoids followed respectively by eetMA (TP: 1096.11 ± 8.42 µg GAE/g), eetBR (PR: 886.00 ± 11.54 µg APE/g), eetOW (TN: 512.07 ± 31.03 µg TAE/g) and eetMA (FL: 112.17 ± 2.03 µg QE/g)). Furthermore, the lowest quantities of total polyphenols (642.50 ± 26.78 µg GAE/g), proanthocyanidins (576.00 ± 28.03 µg APE/g), tannins (169.48 ± 20.55 µg TAE/g) and flavonoids (32.07 ± 2.62 µg QE/g) were found to be those of eetOW (TP and PR), eetBO (TN) and eetBR (FL).

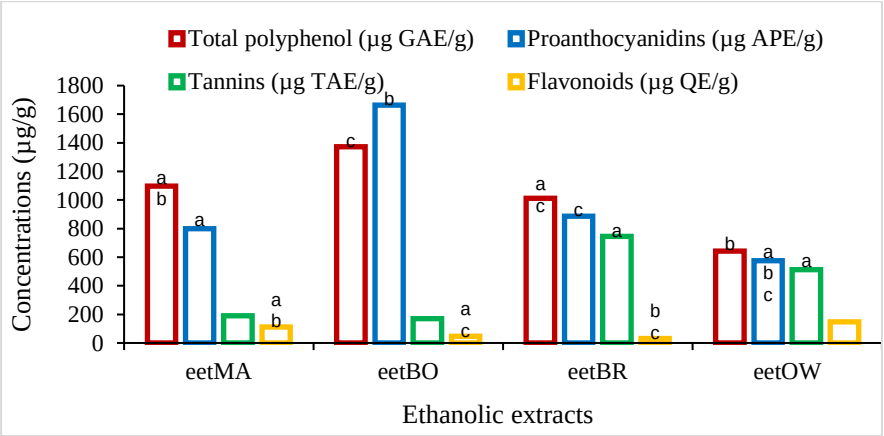

Figure 1. Phenolic contents of the ethanolic extracts of *P. eetveldeana* leaves. For each phenolic compound, the same letter means a significant difference (p=0.05).

3.3. Antioxidant Activity

The Table 3 reveals that the ethanolic extracts of *P. eetveldeana* leaves possess antioxidant activity in all methods tested. As shown below, in the case of DPPH (lowest IC_{50} represents greatest scavenging activity) and molybdenum assays, eetBR followed by eetBO exhibited the greatest scavenging power and total antioxidant capacity while concerning β-carotene bleaching assay, it was found that eetBR followed by eetMA exhibited the greatest relative antioxidant activity. Moreover, eetOW showed the lowest scavenging power, the lowest total antioxidant capacity and also the lowest relative antioxidant activity. Finally, both in DPPH (except eetOW) and β-carotene assays, the ethanolic extracts of *P. eetveldeana* leaves showed the greater activities than ascorbic acid.

Table 3. Antioxidant activity of *P. eetveldeana* leaf ethanolic extracts.

Extracts and reference	DPPH IC_{50} (µg/mL)	Total antioxidant capacity (µg EAA/g)*	Relative antioxidant activity (%)
eetMA	3.47 ± 0.20 $^{a, b}$	206.62 ± 19.67 ^{a}	179.67 ± 2.85 $^{a, e}$
eetBO	0.35 ± 0.07 ^{a}	408.91 ± 81.02 ^{b}	165.93 ± 11.03 ^{b}
eetBR	0.25 ± 0.04 ^{b}	300.79 ± 19.36 ^{c}	223.26 ± 10.21 ^{c}
eetOW	5.72 ± 0.65	126.62 ± 1.08 $^{a, b, c}$	147.25 ± 0.95 d, ^{e}
Ascorbic acid	10.27 ± 0.27	-	100.00 ± 0.00 $^{a, b, c, d}$

In each column, the same letter means a significant difference (p=0.05).
*µg AAE/g: microgram of ascorbic acid per gram of dry extract.

3.4. Correlation Analysis

Table 4 shows the results of the correlation analysis. With correlation coefficients in blue, a strong positive correlation was observed between total polyphenols and proanthocyanidins; total antioxidant capacity, total polyphenols and proanthocyanidins; DPPH scavenging activity and flavonoids.

Table 4. Correlation coefficients.

	TP	PR	Tannins	Flavonoids
PR	0.890079	1		
Tannins	-0.57122	-0.50412	1	
Flavonoids	-0.69985	-0.6656	-0.18231	1
DPPH	-0.78658	-0.72814	-0.05555	0.991331
TAC	0.894808	0.934102	-0.23841	-0.88735
RAA	0.241945	-0.00836	0.544111	-0.71607

PR: proanthocyanidins, TP: total polyphenol, Blue: positive correlation, Red: negative correlation, Clear blue or red: weak correlation.

Furthermore, with correlation coefficients in red, a negative correlation was observed between DPPH scavenging activity and total polyphenols; DPPH scavenging activity and proanthocyanidins; total antioxidant capacity and flavonoids; relative antioxidant activity and flavonoids

4. DISCUSSION

The results showed a variability in yields and phenolic compound levels of ethanolic extracts of *P. eetveldeana* leaves from one collection region to another, as did the results for aqueous extracts of the same leaves (N'goka *et al*., 2023). However, the rates recorded with aqueous extracts are higher (N'goka *et al*., 2023) than those obtained in the present study. The variation in the quantities of secondary metabolites accumulated could be justified by the difference in soils depending on where the leaves were collected, as well as by the age of the leaves (Li *et al*., 2016; Vázquez-León *et al*., 2017). Alternatively, the availability of carbohydrates or nutrients could be responsible for the variability in phenolic compound quantities and yields (Dar *et al*., 2016; Jaafar *et al*., 2012). The solvent used also has an influence, as compounds such as polyphenols and alkaloids are more soluble in ethanol and study carried out by Mbengui and al showed the greater yield of ethanolic extract compared to aqueous extract (Mbengui *et al*., 2013). Moreover, in agreement with our study, Tine *et al.* (2019) showed a variation in phenolic compound content in *Combretum micranthum* leaves from three localities.

For the phytochemical screening, phenolic compounds including tannins and flavonoids, alkaloids, saponins, reducing sugars and cardiotonic glycosides identified in all ethanolic extracts and sterols and triterpenes only present in the extract of leaves collected in Brazzaville show that these results are similar to those obtained with aqueous extracts (N'goka *et al*., 2023) of *P. eetveldeana* leaves collected in the same localities. The presence of these secondary metabolites is justified by the fact that they are soluble in ethanol. In agreement with our results, Dhayalan *et al*. (2018) also identified phenolic compounds, alkaloids, saponins and cardiotonic glycosides in the ethanolic extracts of *Spathiphyllum cannifolium* (Dryand ex Syns) leaves. In addition, the presence of sterols and triterpenes only in the leaves collected in Brazzaville could be explained by a polymorphism within the species or a herbivore-induced change that activates plant's defense system (Moore *et al*., 2014) as well as by the diversity of synthetic pathways depending on cell type or growth locality (Patra *et al*., 2013). In relation with our study, it was shown that the same species of lettuce had two dfferent metabolic strategies in terms of the type of metabolites produced (Corrado *et al*., 2021).

Finally, with regard to antioxidant potential, the free radical scavenging and the antilipid peroxidation effects of ethanolic extracts are superior to those of ascorbic acid and aqueous extracts of the same leaves as reported in the literature (N'goka *et al*., 2023), while the total antioxidant capacities of aqueous extracts reported in the literature are superior to those of ethanolic extracts (N'goka *et al*., 2023). On the one hand, the presence of polyphenols, which are known to be better antioxidants than ascorbic acid (Sharma *et al*., 2012), could explain the fact that ethanolic extracts have better antiradical and antilipid peroxidation effects than ascorbic acid. On the other hand, the high phenolic compound content of ethanolic extracts could explain their superior effects to aqueous extracts of the same plant. Indeed, the antioxidant effect of polyphenols is proportional to the number of hydroxyl groups they may contain (Lv *et al*., 2021). Furthermore, the high phenolic and alkaloid content of ethanolic extracts, as well as the presence of saponins, could explain their antioxidant power. Flavonoids and certain types of alkaloids are well documented for their antioxidant effects, which are linked to their ability to donate an electron or hydrogen atom to stabilize reactive oxygen species, and their capacity to inhibit lipid peroxidation (Ashraf *et al*., 2013; Banjarnahor & Artanti, 2015; Francenia Santos-Sánchez *et al*., 2019; Plazas *et al*., 2022). Furthermore, the difference in antioxidant effect observed between the different ethanolic extracts could be attributed to the proportion of hydroxyl or O-CH3 groups in the phenolic compounds, alkaloids and/or saponins of each extract (Lv *et al*., 2021). The antioxidant effect would also increase with the number of hydroxyl

groups on the B ring of flavonoids (Lv *et al*., 2021). This could explain the fact that the lowest flavonoid content corresponds to the highest antiradical activity.

5. CONCLUSION

This study focused on the variability of antioxidant properties and phytochemical composition of ethanolic extracts from *P. eetveldeana* leaves, depending on the region where the leaves were harvested. All ethanolic extracts of *P. eetveldeana* have antioxidant effects through inhibition of lipid peroxidation and scavenging of free radicals. These effects vary according to the qualitative and quantitative variation in secondary metabolites in the extracts.

The primary and secondary metabolites identified in the extracts, notably phenolic compounds including tannins and flavonoids, alkaloids, saponins, cardiotonic heterosides and reducing sugars, are therefore extractable by ethanol. A homogeneous production of these metabolites was observed according to the harvesting regions considered in the present study, with the exception of sterols and triterpenes, which were only identified in leaves harvested in the Brazzaville department.

These results complement those obtained from aqueous extracts, and together provide the information needed to make better use of this plant, while opening up prospects for further research into the traditional use of *P. eetveldeana* leaves to treat dementia.

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Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

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