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Original Article

Phytochemical study and anti-radical activity of the hydroethanolic extract of the barks of Erythrophleum suaveolens (Guill. & Perr.) Brenan

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ABSTRACT

The article presents the phytochemical study and the evaluation of the anti- radical capacities of the extract of *Erythrophleum suaveolens*. After chemical screening by colorimetric methods, a dosage of total polyphenols (PPT) and total flavonoids (FVT) by UV-visible spectrometry, the anti-radical activity is evaluated by action with DPPH. The screening of the extract shows the presence of six major groups of chemical compounds: alkaloids, flavonoids, saponosides, tannins, reducing compounds, steroids, and terpenes. The quantitative analysis of flavonoids (FVT) compounds varies between 144.42 and 193.92 mgEQt/100g DM, the amount of total polyphenols varies between 1313.13 and 3075.24 mgEGA/100g DM. This high production of polyphenolic compounds is confirmed by the highly marked anti-radical activity observed on the DPPH radical for the fractions F2 (8.1 µg/ml), F3 (7.2 µg/ml) and F4 (7.2 µg/mL). This activity remains low compared to the standard compounds used, Quercetin (2.7 µg/ml), Gallic Acid (1.6 µg/ml), but justifies the use of this bark in traditional medicine.

Keywords: *Erythrophleum suaveolens*, total polyphenols, total flavonoids, Chemical screening, antioxidant activity

INTRODUCTION

The world is continuously encountering an increasing number of emergences and re-emergence of diseases; developing countries appear as the most vulnerable [1] to cope with this issue. This is due to the high cost of medicines, the remoteness and / or insufficiency of health centers, especially in rural areas, which limits genuine treatment of public health problems [2,3]. Scientific advances in the development of natural substances from plants (leaves, bark, roots) permits to predict, in the long term, that green

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chemistry products, substitutable and competitive, replace those produced by synthetic chemistry. Nowadays, the growing interest in the use of natural antioxidants in food or pharmaceutical applications to protect humans against free radicals and delay the progression of chronic diseases reinforces this observation. Indeed, several antioxidant compounds extracted from plants have been identified as chelators of free radicals or active oxygen [4]. Controlling plagues such as bacterial and fungal infections, malaria, cancer and many others is getting complex due to the emergence of the resistance of these diseases to many conventional drugs. This resistance is taking an alarming rate, so as to overpass the development of new means of combating these diseases. Faced with these public health problems, traditional medicine could provide a therapeutic response adapted to financial means and the socio-cultural environment of the populations. Natural remedies are an alternative in primary care systems and therefore a promising avenue for the development of traditionally improved drugs. Thanks to the aforementioned arguments, we embark on Erythrophleum suaveolens, a medicinal plant widely used in traditional Congolese medicine.

This work aims to carry out a chemical analysis, the fractionation, the dosage of the polyphenols and flavonoids contents in the total extracts and in the fraction but also to evaluate the anti-radical activity in order to highlight the use of this plant in traditional Congolese medicine.

MATERIALS AND METHODS

Plant Material: *Erythrophleum suaveolens* belongs to the Leguminosae family (Caesalpiniaceae). It is found in the Guinea-Congolese rain forest from Sierra Leone, Ivory Coast, Ghana, Nigeria, Congo, Cameroonto Gabon [5]. In Congo, the bark of Erythrophleum Suaveolens is used in the treatment of skin conditions, and certain dermatoses as well as various diseases of the mouth and teeth. It is also used to treat patients with edema or rheumatism [6].In Benin, the bark of Erythrophleum suaveolens is used in the traditional treatment of Buruli ulcer [7].

The bark extract is used as a laxative in Sierra Leone [8].In Kenya, a decoction of diluted roots is used as a dewormer, mainly against tapeworms. In Malawi, a decoction of the roots and bark is used to relieve pain affecting the entire body [9].The bark extract is used to treat parsnip and taken as an anti-inflammatory in Cameroon [10]. This study was carried out with the bark of *Erythrophleum suaveolens*, harvested in February 2019, in the Etoumbi sub-prefecture (Figure 1) in the West Cuvette department of Congo. The identification was made at the National Research Institute for Exact and Natural Sciences (IRSEN).

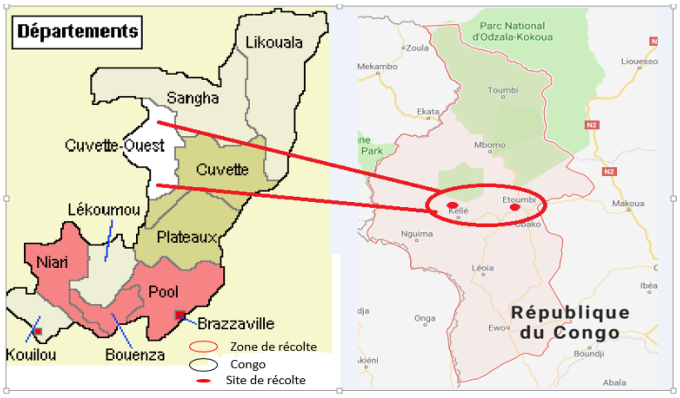

Figure 1: Map of the study area

Preparation of extracts: The bark was dried at a room temperature, for about two months. The dry vegetable matter is ground with an IKA-WERKE Gmbh-CO-KG, D-79219, Staufen-type device, with a sieve of granulometry 0.25mm. For measurements, hydro-ethanolic (EtOH-H_2O, 50:50 v/v) was obtained on mixing 100 g of vegetable matter with 2 × 500 mL. The mixture is shaked up during 72 hours, then filtered.

The filtrate dried concentrated with a rotary evaporator is kept in a cool place (+4 °C) a waiting to be analyzed. The extraction rate is calculated by the following formula:

$$\text{Extraction rate (\%)} = \frac{m}{m_0} \times 100$$

With m_0: the mass of the powder before extraction and m the mass of the resultant dry extract.

Chemical Screening and Qualitative Analysis: EtOH-H_2O (50:50 v/v) is screened for their classes of bioactive compounds using standard procedures [11-16]. The extracts were tested qualitatively for the presence of chemical constituents such as tannins, saponins, flavonoids, polyphenols, anthocyanins, alkaloids and reducing sugar. We used the methods developed by Békro et al. [11] to test these chemical families.

Fractionation of the extract: 1g of the hydroethanolic extract was chromatographed on an open column of Polyamide 6 (Fluka) 1.5 mm in diameter and 50 cm long. The elution is carried out with a water-ethanol mixture with decreasing polarity, in the proportions: 100% H_2O, EtOH-H_2O (30:70 v/v), EtOH-H_2O (70: 30 v / v), and 100% EtOH.

The different fractions collected are monitored by TLC analysis with silica gel on an aluminum support. The plates are firstly visualized in the UV (λ = 254 and 366 nm) then revealed by the reagent from NEU [12] followed by another visualization in the UV-366 nm.

Measurement of Total Polyphenols [17]: We used the reagent of Folin-Ciocalteu to evaluate the total phenols of hydroethanolic extract. Folin-Ciocalteu is a mixture of phosphotungstene acid ($H_3PW_{12}O_{40}$) and phosphomolybdène ($H_3PMo_{12}O_{40}$) of yellow color. The method is based on the oxidation of the phenolic compounds by this reagent. This oxidation draws the formation of new complex molybdenumtungsten of blue color that absorbs to 725 nm.

The evaluation of Total Phenolic compounds is done by comparing the optic density (D.O) observed to the one resulting from a stallion of known acid Gallic concentration. The total phenol compounds are measured as follows: 0.1ml of the extract hydroethanolic is introduced in an Eppendorff tube of 2 ml, the extract is diluted with 0.9 ml of distilled water. 0.9ml of the reagent of Folin-Ciocalteu (1N) is immediately put after the addition of 0.2 ml of Na_2CO_3 (20%) solution.

The resultant mixture is hatched to the ambient temperature during 40 minutes safe from light. The absorbance is measured with the spectrophotometer at 725 nm against a solution of ethanol used like white (control). The right of standardization achieved previously with the Gallic acid under the same conditions that the samples to analyze, permitted to calculate the total phenols contents. The results are expressed in terms of mg equivalent to Gallic acid by gram of dry matter (mg EGA/gDM).

Measurement of Total Flavonoids (FVT) [17]: We have used the colorless solutions of sodium nitrite ($NaNO_2$, 5%) and aluminum chloride ($AlCl_3$, 10 %) for the evaluation of total flavonoids in hydroethanolic extract. The method is based on the oxidation of the flavonoids by these reagents; oxidation that draws the formation of a brownish complex that absorbed at 510 nm. The comparison of the optic density (D.O) observed to the one deriving from a stallion of known concentration Quercetin permits to value the total content in flavonoids by colorimetric effect. In a ball of 10 ml are introduced 250 µl of extract and 1 ml of distilled water successively. At the initial time (0 minute) is added 75µl of a $NaNO_2$ (5%) solution. After 5 min, 75µl of $AlCl_3$ (10%) is added; 6 minutes later, 500µl of NaOH (1N) and 2.5 ml of distilled water are added successively to the mixture. A curve of standardization is elaborated with standard solutions of Quercetin prepared at different concentrations. The results are expressed in terms of mg equivalent to Quercetin by gram of dry matter (mg EQt/gDM).

Determination of the Radical Scavenging Activity [17]: The quantitative analysis of the scavenging activity has been evaluated on mixing 5 mL of the solution of 1,1-diphenyl-2-picrylhydrazyle (DPPH) at 10 mg in 250 ml of ethanol and 50µL of extract or the fractions at the concentrations of 10 mg/mL; 5 mg/mL; 1.25 mg/mL; 0.625 mg/mL and 0.3125 mg/mL. The activity has been measured at 517 nm in the shelter of the light after 30 minutes of incubation to darkness using a UV-visible spectrophotometer**.** The percentage of inhibition was calculated using the following relation: [(*A*517 white - *A*517 of the sample) / *A*517 white] × 100. *A*517: Absorbance at 517 nm. The concentration which inhibits 50% of DPPH (IC_{50}) was determined by proportion.

RESULTS AND DISCUSSION

Determination of the major chemical families: Table 1 presents the results of the phytochemical screening of the extracts of bark of *Erythrophleum suaveolens*. The analysis identifies six major groups of chemical compounds. These are alkaloids, flavonoids, saponosides, tannins, reducing compounds, steroids and terpenes. These results are very significant and in agreement with the literature [7,8]. Indeed, previous studies have shown that these large groups of chemical compounds are responsible for several pharmacological effects, in particular: anti-inflammatory, antiseptic and anticicatrizing, antimicrobial, antiviral, antioxidant, anticancer, antiparasitic, antiradical, anti-tumor, antiallergic, surfactant and cardiotonics [18,19]. This could justify the use of this plant in traditional medicine.

Table 1: Results of chemical screening of extract of *Erythrophleum suaveolens*

Chemical families	Extract
Alkaloids	++++
Flavonoids	++++
Saponosides	++++
Cardiotonicheterosides	++
Tannins	+
Terpenes	+

Legend: ++++ = very abundant; ++ = abundant; + = medium

Fractionation and Chemical Analysis by Thin Layer Chromatography (TLC): Extraction with a hydroethanolic mixture (50% v/v) permits to obtain a mass of 9.8g of crude extract, which corresponds to an extraction production of 9.8%. The fractionation of 1g of this crude extract on a polyamide column with a decreasing gradient elution, (100% H_2O, 30% H_2O-CH_3CH_2OH, 70% H_2O-CH_3CH_2OH, 100% CH_3CH_2OH), allowed to obtain four (04) Fractions, F1, F2, F3 and F4, the masses and productions which are shown in Table 2.

Table 2: Fractionation yield of the crude extract

Fractions	Mass (g)	yield (%)
F_1 (100% H_2O)	0.5 g	50
F_2 (30% H_2O-CH_3CH_2OH)	0.2 g	20
F_3 (70% H_2O-CH_3CH_2OH)	0.09 g	9
F_4 (CH_3CH_2OH)	0.05 g	5

The high amount of the F1 fraction shows that the extract contains more hetero and protein compounds [17]. The aglycones are insufficient in the extract because they are found only in the F3 and F4 fractions, which represent only 2% of the total mass of the extract deposited on the stationary phase. The thin layer chromatographic analysis of the extract and the four (04) fractions show after visualization of the plate with a UV-366nm lamp [12], a succession of spots materializing the presence of compounds with polyphenolic structures.

These fluorescence and spots can be attributed to the following structures (Table 3):

- ✓ The dark blue fluorescence with frontal retention (0.9), clearly highlighted in the extract and the fractions F1 and F2, could be attributed to the derivatives of gallic acid [9].
- ✓ The light green fluorescence observed specific in the extract and the fractions F3 and F4 with frontal retention (0.7 and 0.8), could be attributed to derivatives of chlorogenic acid.
- ✓ Finally, the orange-yellow fluorescence with frontal retention (0.95), clearly highlighted in the F4 fraction, could be attributed to derivatives of a flavonol ortho-di-hydroxylated in position 3 'and 4' [9].

Table 3: Qualitative analysis of the extract and fractions by TLC

Sample	Nomber of Spots	Revealing		Probable structures NEU [12]
		DPPH [20]	NEU [12] UV-366 nm	
EHE	05	Strong trail on purple bed	FJO (0.95)	Flavonoids (Flavonol)
			FB (0.9)	Acid phenols (derived from gallic acid)
F1	01	-	FB (0.2)	Acid phenols (derived from gallic acid)
F2	03	CJC (0.4; 0.5 et 0.9)	FB (0.9)	Acid phenols (derived from gallic acid)
F3	04	Strong trail on purple bed	FVC (0.7 et 0.8)	Acid phenols (derived from chlorogenic acid)
F4	04	Strong trail on purple bed	FJO (0.95)	Flavonoids (Flavonol)
			FVC (0.7 et 0.8)	Acid phenols (derived from chlorogenic acid)

Dosage of Total polyphenols (PPT) and Total flavonoids (FVT): The calibration curve of gallic acid (y = 3.9089x + 0.1257) is used for the determination of total polyphenols (PPT) and that of quercetin (y = 1.6954x + 0.2816) for the dosage of total Flavonoids (FVT). The results are expressed in mg gallic acid equivalent per gram of dry matter (mgEGA/gDM) for polyphenols and in mg Quercetin equivalent per gram of dry matter (mgEQt/gDM) for flavonoids. The two calibration curves are established with correlation coefficients (R^2) of 0.988 and 0.993 respectively. During the determination of total polyphenols in the bark, a blue color appeared after 40 min from the addition of the Folin-ciocalteu reagent and sodium carbonate, which confirms the presence of total polyphenols in the crude extract and fractions [4]. A yellowish color formed in the extract and bark fractions after the addition of Aluminum Chloride ($AlCl_3$) solution. This coloration reveals the presence of flavonoids in the extracts analyzed. The UV-visible spectrophotometer analysis of the crude extract of the bark of *Erythrophleum suaveolens* (Fig. 2).

The contents of total flavonoids in extract and the fractions are 144.42 (extract), 5.04 (F1); 165.53 (F2),188.83 (F3) and 193.92 (F4) mgEQt/100gDM and those in polyphenols are 2489.55; 1313.13; 2973.18; 2290.89; 3075.24 mgEGA/100gDM respectively for the extract and the fractions F1, F2, F3 and F4 (Fig. 2). Indeed, these results show that F4, F3 and F2 fractions are richer in total polyphenols than F1 fraction.

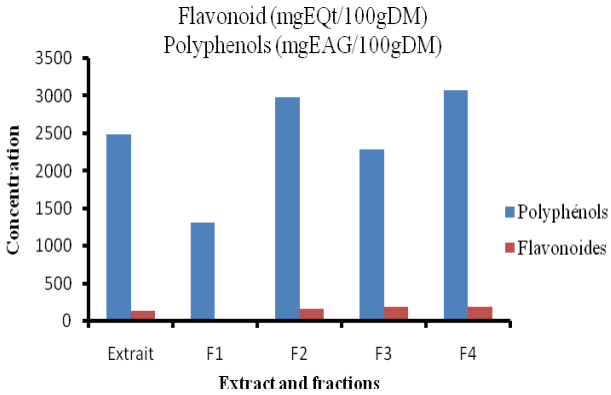

Figure 2: Content of total polyphenols and flavonoids in the bark of *E. suaveolens*

However, there is a moderately high level of flavonoids in the F2, F3 and F4 fractions. F1 corresponds to the fraction which is the least rich in flavonoids and in polyphenolic compounds. The high concentrations of polyphenolic compounds in the fractions can be justified by the elution gradient used, because the phenolic compounds are more soluble in alcohol than in water, hence, their strong presence in the fractions F2, F3 and F4. The high specific contents of polyphenolic compounds in F2 and F4 fractions, respectively 2973.18 mgEAG/100gDM and 3075.24 mgEAG/100gDM would be linked to the chromatographic profiles which clearly show a derivative of gallic acid and that of a flavonol ortho di-hydroxylated in position 3'and 4', respectively in the fractions F2 and F4.

Based on the results in polyphenolic resultant compounds, we can classify this plant among plants rich in polyphenolic compounds. F2 and F4 correspond to the richest fractions. The high levels of polyphenolic compounds explain the high use of plant extracts in the African pharmacopoeia, thanks to the antidiabetic, anthelmintic, anti-malarial, antibacterial, antifungal and anti-inflammatory properties recognized by these compounds [21].

Anti-radical Activity: The anti-radical effect of the crude extract and the *E. suaveolens* fractions with respect to the DPPH radical evaluated using a spectrophotometer is accompanied by the change from its purple color (DPPH.) to yellow (DPPH-H) measurable at 517 nm. This reduction capacity is determined by a decrease in absorbance induced by antioxidant substances [22]. The results of the activity of the hydro-ethanolic extract and the fractions F1, F2, F3, and F4 after separation on a polyamide column are presented in Figure 3.

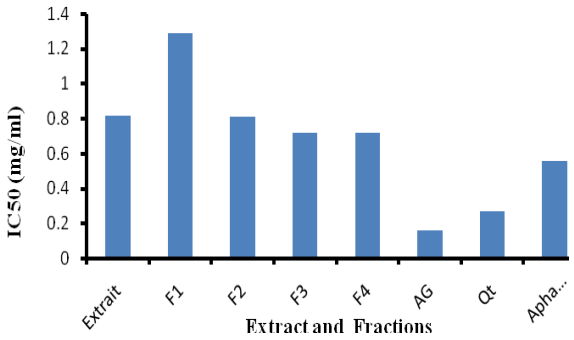

Figure 3: IC_{50} of the extract and Fractions

We note that the inhibitory concentrations 50% (IC_{50}) of the F2 fractions (8.1 µg/mL), F3 (7.2 µg/mL) and F4 (7.2 µg/mL) are very close to quercetin (2.7 µg/mL), Gallic Acid (1.6 µg/mL) and alpha-tocopherol (5.6 µg/mL), three reference compounds used in our study. The crude extract has good significant activity and very close to the F3, F4 and F2 fractions while the F1 fraction moves away from the latter. This strong inhibition of the free radicals of the fractions could be justified by their high concentrations of polyphenolic compounds and by the chromatographic profiles which reveal the compounds of flavonic types and phenolic acids of types C_6-C_3 [8].

Indeed, polyphenolic compounds are well-known as powerful compounds having a power to reduce free radicals [8].The high content of phenols and flavonoids in the bark of E. suaveolens justifies the strong antioxidant activity of extracts from this African pharmacopoeia. The bark of *E. suaveolens* can be used as a natural antioxidant to replace synthetic antioxidants in the prevention of diseases caused by oxidative stress such as cardiovascular diseases.

CONCLUSION

Medicinal plants always remain the reliable source of the active ingredients since they are known for their therapeutic properties. The phytochemical study of the barks of Erythrophleum suaveolens has highlighted the phenolic and flavonoid content of this plant, justifying its use in the Congolese pharmacopoeia. It's chemical composition and the reaction of these extracts to DPPH show its effectiveness in the reduction of free radicals responsible for several pathologies resulting from oxidative stress. This plant could therefore be used as a natural antioxidant to substitute synthetic antioxidants for the prevention of degenerative stress diseases.

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