

Qualitative, Quantitative Phytochemical Screening and Antioxidant Study of Root Bark Extracts of *Terminalia avicinniodes* from Central African Republic

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Abstract The species we have chosen is *Terminalia avicinniodes* from the *combretaceae* family is a medium-sized tree that can reach about 30 m in height and is widespread in tropical Africa, known locally in the Central African pharmacopoeia. It is a species widely used in traditional medicine for its therapeutic properties. **Objectives:** As part of the development of medicinal plants in the Central African Republic, the interest of this study is focused on the traditional uses, the phytochemical study and the antioxidant activity of *Terminalia avicinniodes*. **Results:** The results of the various extracts obtained showed that the methanol extract has the best yield with a percentage of 9.02%. Qualitative analyzes revealed the presence of flavonoides, tannins, les saponins, sterols and triterpenes. The results showed that the root extracts of *Terminalia avicinniodes* contain more polyphenols than the other families (tannins, anthocyanins and flavonoids). The quantified total polyphenols of the different extracts varied between 9.35 ± 8.77 and 41.00 ± 31.66 mg of gallic acid equivalent (mg EqAG/g). The methanol extract has the best rate of polyphenols 41.00 ± 31.66 mg of gallic acid equivalent (mg EqAG/g), than the other extracts. It is followed by the ethyl acetate extract with a level of 28.60 ± 2.10 mg of gallic acid equivalent (mg EqAG/g). The quantified tannins have a rate that varies between 2.21 ± 11.54 and 9.17 ± 2.97 mg Catechin Equivalent/g. The best correlation coefficient obtained is $R^2=0.899$ between antioxidant activity and flavonoids. **Conclusion:** The phytochemical study revealed the presence of certain molecules such as polyphenols and alkaloids, which can give *Terminalia avicinniodes* its therapeutic properties.

Keywords: *Terminalia avicinniodes*, phytochemical, 2,2-diphényl 1-pyrcilhydrazyle and antioxidant activity

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1. Introduction

The medicinal plants used for the treatment of various pathologies possess chemical substances assimilated to secondary metabolites (alkaloids, phenolic compounds and terpenoids) whose biological properties have been demonstrated at length in the literature. Indeed, more than 25% of existing drugs belong to the secondary metabolisms of plants [1].

Polyphenolic compounds are among the molecules sought for their physiological and pharmacological activities [2]. These are natural compounds widely distributed in the plant kingdom. Their role as natural antioxidants is attracting more and more interest in the prevention and treatment of cancer, inflammatory and

cardiovascular diseases. Polyoxygenated sterols and triterpenes have selective cytotoxicity and antitumor properties [3].

In view of the beneficial effects assigned to polyphenols and their derivatives, to alkaloids and other natural compounds, our research is oriented towards the class of chemical molecules endowed with biological activities. *Terminalia avicinniodes* of the *Combretaceae* family, with the vernacular name Dafo (in Banda), Bebera (in Mandjas) and Begbakoa (in Gbanou), was selected from among the medicinal plants used in the Central African pharmacopoeia.

This plant is recognized in the Central African Republic for its use in the treatment of certain diseases. Generally, it is used for its virtues against diarrhea, viral hepatitis, cancer, headaches, tooth decay, yellow fever, asthma and cough. Pharmacological studies have shown that the barks

of *Terminalia avicinniodes* are used in traditional medicine in Nigeria in the treatment of painful menstruation (dysmenorrhea), menorrhagia, trypanosomiasis, diarrhea, tuberculosis, cough, wounds especially for a better healing of skin infections, gastrointestinal disorders, ulcers, dental caries [4,5].

The aim of this work is to carry out the phytochemical study of *Terminalia avicinniodes* and to evaluate its antioxidant activity.

2. Materials and Methods

2.1. Study and Sample Collection Area

Origin of the plant: The study was carried out on the roots of *Terminalia avicinniodes* collected in July 2021, in the Yassara region, which is a subdivision of the Begoua region 15 kilometers from Bangui towards the village of Voudambala in the Central African Republic. The plant (Figure 1) was identified at the Center for Studies and Research in Pharmacopoeia and Traditional African Medicine (CERPHAMETA) by Doctor Denis BEINA. The harvested parts were dried in the open air at the LAARSN Laboratory for two weeks and reduced to powder.

2.2. Phytochemical Study

2.2.1. Preparation of the Extracts

The maceration method was used. One hundred grams (100g) of *Terminalia avicinniodes* bark powder was successively extracted with four solvents of increasing polarity (petroleum ether, dichloromethane, acetone and methanol). A quantity of 100g of powder was put in

400mL of petroleum ether with stirring for 4 hours at room temperature. After filtration, the extract is evaporated using a rotavapor at 35°C and the residues are taken up with the following solvent.

2.2.2. Qualitative Analyzes

To search for the major families of secondary metabolites, we used standard methods based on color reactions in tubes. Anthocyanins by the ammonia reaction, alkaloids by Mayer's tests, tannins by the FeCl_3 test, foam tests were used for saponosides. As for triterpenes and steroids, the Liebermann-Burchard test was used [6].

2.2.3. Dosage of Polyphenols

A modification made to the method used by Worowounga et al [7] made it possible to measure the polyphenols. A volume of 20 μL of extract was added to 100 μL of Folin's reagent solution (0.2 N). The mixture was stirred for 30 seconds followed by incubation for 5 minutes in the dark. After incubation, a volume of 80 μL of Na_2CO_3 was added. The mixture is again stirred for 30 seconds with 15 minutes of incubation at room temperature. The absorbance was read at 620 nm. The reference used was gallic acid.

2.2.4. Dosage of Condensed Tannins

The vanillin solution was prepared at 1% in H_2SO_4 (7 M). A volume of 150 μL of this solution was added to 50 μL of extract. After incubation for 15 minutes at 25°C, the absorbance was measured at 500 nm [7]. The results are expressed in mg catechin equivalent/g dry matter (DM) and the catechin was the reference used. Different concentrations between 0 and 1000 $\mu\text{g/mL}$ prepared from a stock solution of catechin, made it possible to plot the calibration curve.



Figure 1. Leaves and trees of *Terminalia avicinniodes*

2.2.5. Anthocyanin Assays

For rapid and accurate measurement of total anthocyanins even in the presence of degraded polymerized pigments and other interfering compounds, the differential pH spectrophotometric method is recommended [8]. It is based on the determination of the absorbance of extractive solutions diluted with buffer solutions of pH = 1 and pH = 4.5. Anthocyanins are reversibly transformed under the influence of pH. The structural change associated with the modification of the chromophores determines the different color of the solutions of the anthocyanins according to the pH. The colored form (oxonium) predominates at pH=1 and the colorless form (hemiacetal) at pH=4.5. Two solutions of different pH were prepared. Solution at pH=1 contains KCl (0.2 M) and HCl (0.2 M). That at pH=4.5 is a mixture of CH₃COOH (0.2 M), CH₃COONa (0.2 M) and H₂O. A volume of 100 µL of extract was added to 100 µL of the pH=1 solution in four wells, the next four wells contain 100 µL of extract and 100 µL of the pH=4.5 solution. The absorbance of the extract was measured at two wavelengths, 450 and 620 nm after incubation for 15 minutes at 25°C [8]. The results are expressed in mg cyanidin-3-glucoside equivalent/g plant material.

2.2.6. Dosage of Flavonoids

The quantification of flavonoids was carried out by a method based on the formation of a very stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids [9]. The protocol used is based on that described by Worowunga et al [7]. The solution of AlCl₃ (0.02 g/mL) in methanol was prepared. A volume of 100 µL of this solution is added to 100 µL of extract (3 mg/mL). For the control blank, 100 µL of methanol is added to 100 µL of solvent used to dissolve the extract. The measurement is made after 15 minutes of incubation at room temperature; the

absorbance of the extract is measured at 415 nm. An aqueous solution of quercetin was prepared. Daughter solutions prepared from the stock solution at different concentrations between 0 and 1000 µg/mL will allow the calibration curve to be plotted.

2.3. Antioxidant Activity

A solution of DPPH (0.35 M) prepared in methanol (MeOH) was diluted 10 times. From the *Terminalia aviccinoides* extract solution (3mg/mL), a range of 16/20 dilutions; 1/2 and 1/5 was prepared. A volume of 180 µL of DPPH is added to 20 µL of the diluted solution of *Terminalia aviccinoides* extract. The mixture is stirred for 30 seconds, followed by incubation for 25 min. Absorbance is measured at 450 nm [7]. Ascorbic acid is used as a reference (0.25 mg/mL). The percentage inhibition of DPPH by the plant extracts was obtained, using the following formula:

$$\%Inhibition = [(Ab - Ae) / Ab] \times 100$$

Ab: Absorbance white

Ae: extract absorbance

3. Results and Discussion

3.1. Extraction Yield

The extraction yield of the *Terminalia aviccinoides* root powders obtained varies between 0.80 and 9.20% (Figure 2). The methanol extract has the best yield of 9.20%. The work of Musa et al on the methanol extract of *Terminalia aviccinoides* bark gave a yield of 17.8% [10]. This yield is double what we found for the methanol extract from the same plant.

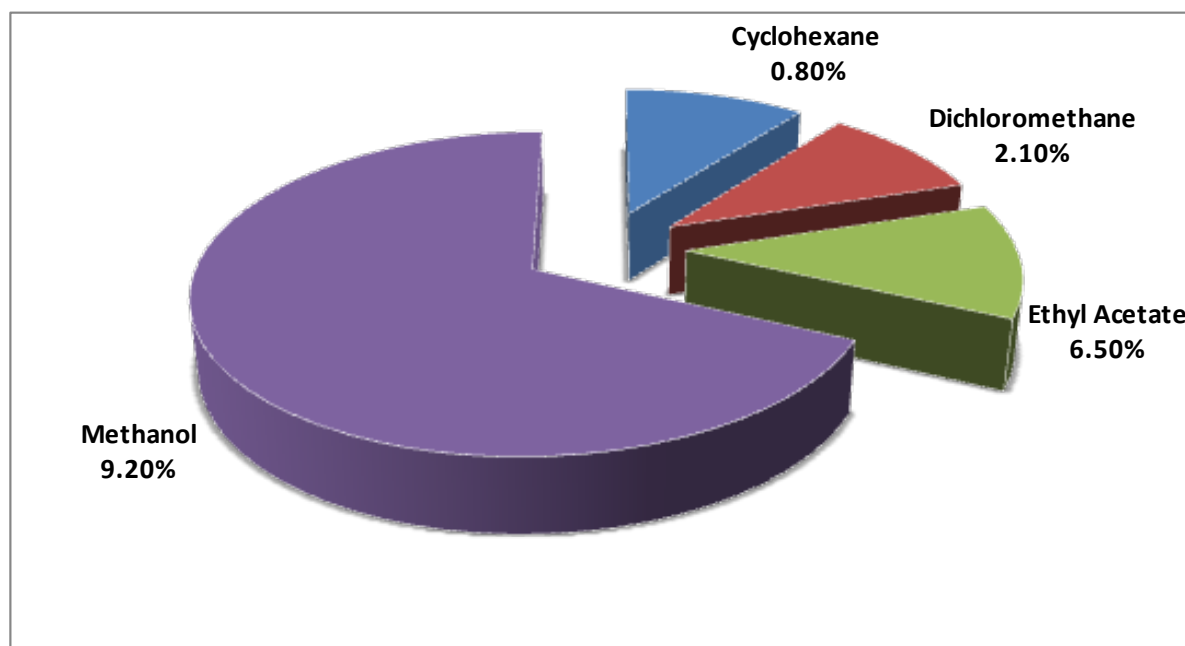


Figure 2. Extraction yield of *Terminalia aviccinoides*



Figure 3. Foam index of saponifies

Table 1. Qualitative analysis of *Terminalia avicinnoides*

Echantillons	Alkaloids	Flavonoids	Tannins	Anthocyanins	Sterols et triterpenes	Saponins	
						Presence	Foam index
<i>Terminalia avicinnoides</i>	-	++	++	-	+++	+++	627,08

- : absence, ++ : abundant presence, +++ : very abundant presence.

3.2. Qualitative Analysis

The tube, which has a color which is accentuated by acidification and which turns blue-violet in a basic medium, for the presence of anthocyanins, was obtained. The height of the foams (Figure 3) indicating the presence of saponins was observed. The saponification index of this extract was 627.08. The results of qualitative analysis of the roots of *Terminalia avicinnoides* indicated the presence of flavonoids, tannins, saponosides, sterols and triterpenes. We noted however, the absence of alkaloids, anthocyanins (Table 1). The work of the qualitative analyzes of the extract of *Terminalia avicinnoides* carried out by Jules Yoda et al [11], by Louis-Claire Ndel Famen et al [12], revealed the presence of alkaloids, anthocyanins, tannins, saponosides, sterols and triterpenes.

3.3. Chemical Composition in Polyphenols, Tannins, Flavonoids and Anthocyanins

The results in Table 2 showed that the root extracts of *Terminalia avicinnoides* contain more polyphenols than the other families (tannins, anthocyanins and flavonoids). By family of compounds, the quantified total polyphenols of the different extracts varied between 9.35 ± 8.77 and 41.00 ± 31.66 mg of gallic acid equivalent (mg EqAG/g). The methanol extract has the best rate of polyphenols 41.00 ± 31.66 mg of gallic acid equivalent (mg EqAG/g), than the other extracts. It is followed by the ethyl acetate

extract with a level of 28.60 ± 2.10 mg of gallic acid equivalent (mg EqAG/g). Yoda et al [11] found a polyphenol level of 223.16 ± 3.93 mg GAE/g in the alcoholic extract of the same plant. This rate is much higher than that obtained from the methanol extract (Table 2).

The quantified tannins have a rate that varies between 2.21 ± 11.54 and 9.17 ± 2.97 mg catechin equivalent/g. the cyclohexane and ethyl acetate extracts have a similar rate. However, the flavonoid and anthocyanin contents are very low compared to the polyphenols of the different extracts (Table 2).

3.4. Antioxidant Activity

The results of the antioxidant activity of the extracts are represented in Figure 4. We note from these results that the percentages of inhibition of DPPH by the extracts of the *Terminalia avicinnoides* roots varied from 116.60 to 394, 50mg/L. The methanol extract inhibited DPPH with the $IC_{50} = 116.60 \pm 8.9$ mg/L, this methanol extract inhibited DPPH better compared to the other three extracts of the same plant. However, all the plant extracts moderately inhibited DPPH compared to the quercetin used as a reference which gave the $IC_{50} = 6.50 \pm 0.40$ mg/L. The antioxidant activity of the hydro-alcoholic decoction extract of the stem bark of *Terminalia avicinnoides* [11] gave the $IC_{50} = 11.09 \pm 0.22$ μ g/mL). Therefore, they obtained better inhibition than our result.

Table 2. Content of polyphenols, flavonoids, tannins and total anthocyanins

Extracts	Polyphenols	Flavonoids	Tanins	Aanthocyanins
Cyclohexane	nd	0.07 ± 0.06	6.76 ± 3.32	6.26 ± 0.43
Dichloromethane	9.35 ± 8.77	0.07 ± 0.05	9.17 ± 2.97	nd
Ethyl Acetate	28.60 ± 2.10	0.05 ± 0.02	6.36 ± 4.63	nd
Methanol	41.00 ± 31.66	0.05 ± 0.00	2.21 ± 11.54	nd

nd: not determined.

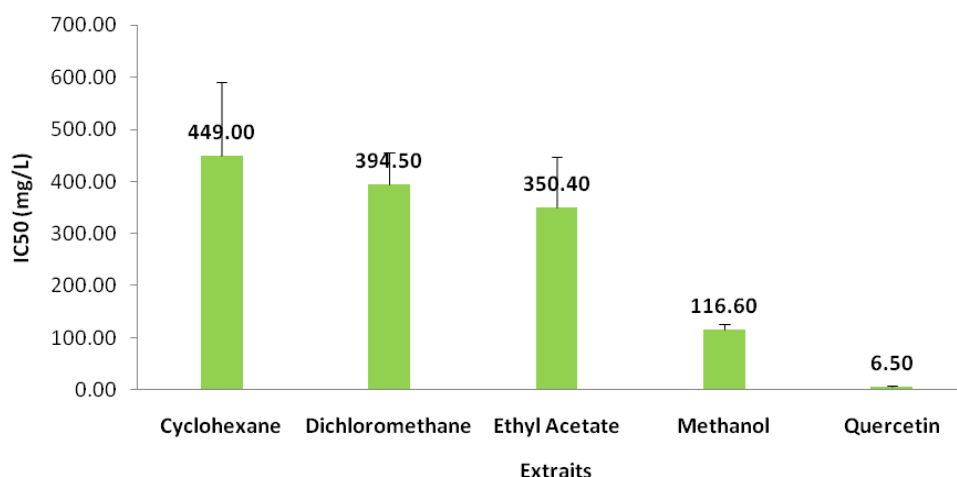


Figure 4. Antioxidant activity of *Terminalia avicinnoides* extracts

3.5. Correlation

The best correlation coefficient obtained is $R^2=0.899$ between antioxidant activity and flavonoids. The correlation coefficients between the inhibition of DPPH and polyphenols, then between antioxidant activity and tannins are respectively $R^2=0.770$ and $R^2=0.774$.

4. Conclusion

The results of qualitative analyzes revealed the presence of flavonoids, tannins, saponosides, sterols and triterpenes. Quantitative analyzes of the different extracts from the roots of *Terminalia avicinnoides* gave a high rate of polyphenols such as tannins, flavonoids and anthocyanins. The results of the antioxidant activity of the extracts of the roots of this plant nevertheless remain significantly lower than that of the quercetin that we used as a reference. Therefore, the root extracts of this plant moderately inhibited DPPH. We obtained a good correlation between antioxidant activity and the three families of quantified compounds (polyphenols, tannins and flavonoids). In perspective we plan to realize the antibacterial activities of the extracts of *Terminalia avicinnoides*.

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