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Phytochemical Properties and *in vitro* Antioxydant Activities of the *Lannea Microcarpa* Extracts

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Abstract

This study investigates the phytochemical composition and antioxidant activity of extracts from the trunk bark of *Lannea microcarpa*, a tree traditionally used in Mali for various medicinal purposes. The sample was collected in Kayes. The polyphenols, flavonoids, tannins and anthocyanins of the extracts were quantified using spectrophotometry and identified via HPLC. The antioxidant activity was assessed using 2, 2-azinobis-3-ethylbenzothiaz oline-6-sulfonic acid (ABTS) and 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radicals scavenging capacities. The extracts were mainly rich in flavonoids; Polyphenols, tannins and anthocyanins were very low. The values were 44.50 mg ECt; 1.89 mg EAG; 1.01 mg ECt; 0.57 mg ECG per 1 g of dry matter (DM) respectively for flavonoids, polyphenols, tannins and anthocyanins. The HPLC analysis of the sample revealed the presence of a Caffeic acid 37.51 µg per 100 mL. Our extracts showed good ABTS and DPPH radical scavenging capacities. The antioxidant activities of sample correlated with their contents in total phenolic and total flavonoids.

Keywords

Lannea microcarpa, Extracts, Phytochemistry, Antioxidants-Potential

1. Introduction

Since ancient times, natural products, particularly those of plant origin, have always been an important source of therapeutic agents. Currently, approximately

25% - 30% of all drugs available for the treatment of diseases are derived from natural products (from plants, animals, bacteria and fungi).

Lannea microcarpa or true grape tree is a tree which can reach 12 to 13 m high whose bark is smooth, grayish white in color, it becomes rough and comes off in patches in old trees. The fruits are ellipsoid drupes, glabrous, dark purple in color when ripe. They are 1.4 cm long and have 2 to 4 small teeth at the top [1]. Literature studies revealed that *L. microcarpa* is used as herbal medicine in 58.3% of the countries where the species is indigenous. The plant would be cited in the treatment of wounds in the mouth, fever, amenorrhea, inflammation, leprosy, dysentery, and cough [2].

Free radicals are responsible for the alteration of DNA and cellular aging which is the basis of certain diseases such as atherosclerosis, cancer, Alzheimer's disease or Parkinson's disease [3].

An antioxidant is any substance which, when present in low concentration compared to that of the oxidizable substrate, significantly delays or prevents the oxidation of this substrate. Antioxidants of natural origin are present in all parts of higher plants. These are phenolic compounds (flavonoids, xanthones, coumarins, carotenoids, phenolic acid derivatives, tannins, anthocyanins, etc.). These components can act by directly capturing free radicals or by inhibiting the enzymes responsible for the regeneration of ROS (reactive oxygen species), or by capturing metal ions [4] [5].

The antioxidant activity of flavonoids can take several forms in the regulation of oxidative stress with respect to the deleterious effects of free radicals. The phytochemical study of the acetone extract of the plant revealed the presence of polyphenols, flavonoids as well as significant antioxidant power [6].

In order to provide protection against serious diseases and to prevent foods from undergoing deterioration, many chemicals with strong antioxidant activity are used as additives, such as butylated hydroxyanisole, butylated hydroxytoluene, and *n*-propyl gallate. Moreover, their use in foodstuffs is restricted or prohibited in some countries due to their undesirable consequences on human health [7]. Therefore, natural antioxidants have attracted more and more interest because of their safety and wide distribution. [8] [9] and [10].

Given the richness of our plant in phenolic compounds and the link of these with antioxidant activity. The objective of this study was to identify the polyphenolic compounds present in bark of the trunk of *Lannea microcarpa* and to evaluate their antioxidant effects which would justify the uses of the plant in traditional medicine.

2. Material and Methods

2.1. Material

The samples (trunk bark) of *Lannea microcarpa* were collected in Kayes (Mali). They have been transported and identified to the Department of Traditional Medicine (DMT) under the number (0376). Folin-Ciocalteu, Gallic, Protocatechic,

Chlorogenic, Caffeic acids, Lawsone, Rutin, Apigenin, Quercetin, Kaempferol. ABTS and DPPH were provided by the companies SIGMA-Aldrich (France) and across organics (Belgium). All other chemicals and solvents used were obtained from a commercial source and were of analytical grade.

2.2. Methods

2.2.1. Samples Preparation

After the initial cleaning process, the samples were dried in the shade and at room temperature in the Natural.

Substances laboratory of the FST. After drying, the samples were pounded using a laboratory scale hammer miller and the resulting powder sieved until a fine powder was obtained.

2.2.2. Preparation of Extracts

The preparation of the sample extracts was carried out by the method described by Abu Bakar *et al.* [11] with slightly modified. To 5 g of plant powder were added 2 times 20 mL of a 50/50 (V/V) hydromethanolic solution for UV-Visible Spectroscopy and HPLC. The mixtures were stirred for 6 hours, and then filtered through a 0.45 µmmillipore membrane. The filtrates collected were centrifuged at 1500 × g for 20 min. The extracts obtained have been cooled and conserved at (+4˚C) in bottles before analysis.

2.2.3. Dosage of Polyphenolic Compounds

1) Total phenolic content (TPC)

The total polyphenol contents were determined by the Folin-Ciocalteu method described by Konaré *et al.* [12] with some modifications. One hundred microliters (100 µL) of each extract were introduced into a test tube. Then 100 µL of Folin Ciocalteu reagent were added to the mixture and stirred. After 5 min, 1 mL of a 7% sodium bicarbonate (Na_2CO_3) solution was added with stirring and the final volume was immediately increased to 2.5 mL with distilled water and vigorously stirred. After a 90 minute incubation at room temperature (30˚C - 35˚C), the absorbances were readed at 765 nm against a blank prepared with distilled water with a spectrophotometer. The results were compared to a calibration curve previously established before analysis with Gallic acid at different concentrations according to correlation coefficient $R^2 = 0.9757$. The polyphenol levels, expressed as mg Gallic acid equivalent per 1 g (DM). All samples were analyzed at least three times.

2) Total flavonoid content (TFC)

The content of total flavonoids was evaluated by calorimetric according to Koné *et al.* [13]. To 250 µL of each extract, 1 ml of distilled water and 75 µL of $NaNO_2$ at (5%) were added. After 5 minutes, then 75 µL of $AlCl_3$ at (10%) was added. After 6 minutes, 500 µL of NaOH (1N) and 600 µL of distilled water were added to the stirred mixture. The Absorbance of the mixture was determined at 510 nm relative to a blank prepared with water. The calibration curve was developed with standard

solutions of catechins prepared at different concentrations. Total flavonoids are expressed in mg equivalents catechins per 100 g of dry matter (mg ECt/1 g (MD). The calibration curve has been established with a correlation coefficient R^2 = 0.9901. All samples were analyzed at least three times.

3) Total tannin content (TTC)

The total tannin content was determined according to the method used by Villareal-Lozoya *et al.* [14] with a slight modification. In a test tube containing 1.5 mL of concentrated sulfuric acid, 50 µL of extract and 3 ml of a 4% methanol-vanillin solution were added. The mixture was left to stand for 15 minutes. Absorbance has been measured at 500 nm against a blank prepared with methanol. The calibration curve was developed with standard solutions of catechins prepared at different concentrations. The calibration curve has been established with a correlation coefficient R^2 = 0.9899. The results are expressed in mg equivalent catechins per 1 g of dry matter (mg ECt/1 g DM). All samples were analyzed at least three times.

4) Total anthocyanin content (TAC)

Total anthocyanin compounds (TAC) were evaluated by the differential pH method according to Lako *et al.* [15]. The method used is based on a variation of absorbances using two buffers: one containing potassium chloride (KCl) (pH = 1) at 0.025 M and the other sodium acetate (CH_3COONa) (pH = 4.5) at 0.4 M. 200 µL of extract samples were mixed with 1.8 mL of each of the buffer solutions. The absorbance of the solution has been determined at 510 nm and at 700 nm against a blank made with methanol. The change in absorbances was calculated by the following formula.

$$\Delta A = \left[\left(A510 - A700\right) \times pH1\right] - \left[\left(A510 - A700\right) \times pH4\right] \quad (1)$$

The concentration of anthocyanin pigment in the extract was expressed in mg equivalent cyanidin-3-glycoside per liter of solution.

The calibration curve was established with cyanidin-3-glycoside.

$$C\left(mg/L\right) = \Delta A \times Mm \times Df \times \left(100/Ma\right) \quad (2)$$

ΔA is the change in absorbances, Mm the molecular mass of cyanidin (449.2 g/mol), Df is the dilution factor, Ma the molecular absorptivity (26.900).

2.2.4. HPLC Analysis

The HPLC analysis was carried out according to the method described by Muanda *et al.* [16] with a slight modification, using an elution gradient consisting of three phases. Solvent A: 50 mM ammonium phosphate ($NH_4H_2PO_4$) at pH 2.6 (adjusted with ortho phosphoric acid), solvent B: (80/20 (v/v)) acetonitrile/solvent A, and solvent C: 200 Mm ortho phosphoric acid (H_3PO_4) at pH 1.5 (pH was adjusted with 0.1 M NaOH). After preparation, the solvents were put in an ultrasonic device for 10 min for homogenization. The profile of the gradient used for 60 min is presented in Table 1. The elution flow rate was 1 mL/min and the injection loop capacity 20 µL. Detection was performed at 280 and 320 nm. Standard phenolic

compounds (9 standards) were prepared by dissolving 2 mg/mL. In each sample, the phenolic compound was identified by the retention time of the corresponding standard and the concentration of the phenolic compound was calculated by comparing the peak areas. The samples were analyzed at least three times. After each cycle, the system was reconditioned 10 minutes before a new analysis.

Table 1. Profile of the gradient used for 60 min.

T (mn)	A%	B%	C%
0 - 4	100	0	0
4 - 10	92	8	0
10 - 22.5	0	14	86
22.5 - 27.5	0	16	84
27.5 - 50	0	25	75
50 - 55	0	20	80
55 - 60	100	0	0

2.2.5. Antioxidant Activity Assay

1) Scavenging capacity of ABTS radicals

The method developed by Kim *et al.* [17] slightly modified has been used in this experiment. 1.0 mM AAPH was mixed with 2.5 mM ABTS using buffer. The buffer solution consists of 100 mM potassium phosphate (pH 7.4) containing 150 Mm NaCl. The mixture was heated in a water bath at 68˚C for 20 minutes until the concentration of the blue-green ABTS radical complex gives an absorbance of between 0.65 ± 0.02 at 734 nm. To 60 µL of the sample has been added 2.94 mL of the radical blue-green solution of ABTS. The mixture was incubated in a water bath at 37˚C for 20 minutes. The control consists of 60 µL of methanol and 2.94 mL of ABTS and was checked for each series of samples. The absorbance decay was measured at 734 nm. Stable radical scavenging activity in the ABTS test of phenolic compounds was expressed in mg equivalent vitamin C (mg EVC/100 (DM). The radical solution was prepared daily. All samples were analyzed at least three times

2) Scavenging capacity of DPPH radicals

The DPPH radical scavenging activity was determined using the method of Hoste *et al.* [18] with some modification. To 2.90 ml of an aqueous solution of 50% methanol (100 mM of DPPH), 100 µL of plant extract were added. The mixture has been heated in a water bath at 20˚C away from light for 40 min. The blank was prepared with (100 µL of 50% methanol and 2.90 mL of the DPPH solution) and checked for each series of samples. The decrease in absorbance was measured at 517 nm 40 minutes later. The free radical scavenging activity in the DPPH test of total phenolic compounds was expressed in mg equivalents vitamin C (mg EVC/100 g (DM). The radical solution has been prepared daily. All samples were analyzed at least three times.

2.2.6. Statistical Analysis

The results were processed with software such as: Excel version 2019 and Minitab 18.1, for analysis of variance (ANOVA) was used to compare the mean values of these varieties with the Fischer test at the probability threshold P = 0.05.

3. Results and Discussion

3.1. Content of Total Polyphenolic Compounds

Numerous studies have shown that several metabolites are involved in the antioxidant activities of plant extracts. Among these metabolites, total polyphenols and flavonoids play an important role [19] [20]. This is why we wanted to evaluate their content in our samples. The results of quantitative analyses of phenolic compounds in extracts of *L. microcarpa* trunk bark are reported in **Figure 1**. These results indicate that these extracts are mainly composed of flavonoids. Its composition in polyphenols, tannins and anthocyanins are very low (TFC > TPC > TTC > TAC). Many phenolic compounds have been isolated from different parts of the plant [21] [22] and [23]. The role of phenolic compounds is widely shown in protection against certain diseases due to their possible interaction with numerous enzymes and their antioxidant properties [24]. This may explain the uses of our plant in traditional medicine.

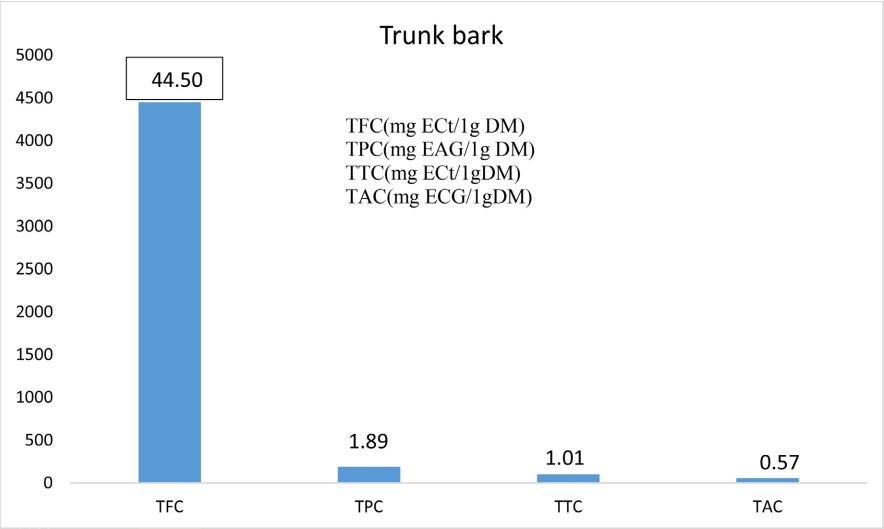

Figure 1. Quantitative composition of phenolic compounds in *L. microcarpa* extracts.

3.2. HPLC Analysis

The results of qualitative and quantitative analyses of the identified phenolic compounds are shown in **Table 2**. Analysis of these results shows that the extract of the trunk bark is moderately rich in caffeic acid (37.51 µg/mL). Chlorogenic acid has the power to inhibit the enzymes responsible for the regeneration of ROS (reactive oxygen species) in living cells [25]. Flavonoids are the most characteristic secondary metabolites of the genus Fabaceae [26]. Rutin is reputed to be a powerful antioxidant that is used in Chinese medicine to treat high blood pressure and

to inhibit damage induced by the oxidative effects of UV radiation [27]. It is also known for its antiinflammatory, hepato-protective and antioxidant properties [28]. Caffeic acid has been shown to be very effective against viruses, bacteria and fungi [29].

Table 2. HLPC Analysis results of the Three *Lannea microcarpa* extracts.

Names of compound	Rt (min)	Trunk Bark µg/mL
Gallic Acid	09.65	Nd
Protocatechic Acid	11.85	Nd
Chlorogenic Acid	16.72	Nd
Caffeic Acid	19.55	37.51 ± 01.51
Lawsone acid	25.98	Nd
P-Cumaric Acid	33.90	Nd
Rutin	35.50	Nd
Quercetin	39.82	Nd
Apigenin	41.40	Nd
Kaempferol	42.52	Nd

Results reported are means of triplicate samples ± standard deviation. Rt: Retention time, Nd: no detected.

3.3. Antioxidant Activity

The results of the antioxidant activity AOA) are reported in **Table 3**. The extract of the trunk bark of *L. microcarpa* exhibited the highest value of antioxidant activity in both tests (ABTS and DPPH). The study of the antioxidant activity of our extracts was carried out using two tests: ABTS test and DPPH test. The results of antioxidant activity are recorded in **Table 3**. The extracts of the trunk bark of our plant reduced DPPH and ABTS with 4.24 mgVCE and 2.7 mgVCE respectively. Our results are in agreement with those of Diallo [30], who also demonstrated the high (AOA) of the ethanolic extract of the trunk bark of *L. microcarpa* with an IC50 = 11.2 ±2 µg/ml. This could explain the richness of the plant in flavonoids. Flavonoids prevent tissue infiltration and strengthen capillary walls; which may justify the use of *L. microcarpa* in the treatment of eye ailments [31]. Furthermore, according to Bossokpi *et al.* [32], flavonoids are antioxidant substances active in maintaining good blood circulation. They contribute to increasing the production of nitric oxide in blood platelets, which limits the formation of clots by preventing platelets from clumping together (therefore helping to prevent atherosclerosis). This property supports the traditional use of *L. microcarpa* in the treatment of heaviness in the legs, myalgia and hemorrhoid [31]. This anti-radical activity is linked to a high content of total phenolic compounds [31]. The high antioxidant capacity of our extracts confirmed the results of HPLC analysis (**Table 2**), which identified Caffeic acid in the studied extracts. According to Bossokpi *et al.* [32], these phenolic acids have antioxidant and antiradical activities.

Table 3. ABTS and DPPH radical scavenging activities of the Trunk bark extract of *Lannea macrocarpa*.

Samples	ABTS-Test (g EVC/100 g (DM))	DPPH-Test (g EVC/100 g (DM))
TRUNK BARK	2.70 ± 0.01	4.24 ± 0.02

Results reported are means of triplicate samples ± standard deviation.

4. Conclusion

The results of this study revealed the presence flavonoids and Caffeic acid with considerable quantity in the extracts of our studied plant. These chemical constituents are known to have the ability to scavenge free radicals from ABTS and DPPH. This explains the best antioxidant activities of our plant extracts. In sum, the results of phytochemistry and antioxidant activity analysis of *Lannea microcarpa* extracts revealed that it is a medicinal plant.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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