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Evaluation of Acute Toxicity, Antioxidant and Antibacterial Potential of Leaves Extracts from Two Anacardiaceae's Species: *Lannea microcarpa* Engl. & K. Krause and *Mangifera indica* L.

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Abstract

Background: Leaves of *Mangifera indica* L or *Lannea microcarpa* Engl. & K. Krause are used in traditional medicine in Burkina Faso to treat bacterial, parasitic or metabolic diseases. **Objective:** The aim of this study is to evaluate the acute general toxicity, antioxidant potential and antibacterial activitiy of leave's aqueous extracts from *Lannea microcarpa* Engl. and K. Krause and *Mangifera indica* L. The use of these plants in traditional medicine motivated our choice to lead scientific studies. **Methods:** The aqueous decoction of the leaves is the form of use recommended by traditional healers. We used the same type of extracts for studies. Acute toxicity was studied in NMRI strain mice, with the dose of 2000 mg/kg body weight, for each plant species. The antioxidant activity is evaluated by the method of reduction of radical DPPH. The phytochemical compounds were detected with specific reagent: Alcaloids with Dragendorff's reagent, Flavonoids with ammonia (NH_4OH), Polyphenols and tannins are revelated by ferric chloride ($FeCl_3$). Saponosides were revelated by their foaming power property. Bacterial inhibiting activity is tested by measuring the diameters' inhibition of extracts on reference strains. **Results:** The aqueous extracts were not toxic at the maximum dose of 2000 mg/kg body weight, for each plant species. The extracts showed an antioxidant activity with an IC_{50}, 20 µg/ml for *Lannea microcarpa* and 18 µg/ml for *Mangifera indica*. The extracts showed no bacterial activity on three strains of bacteria tested: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococ-*

cus aureus. The phytochemicals we have identified are for *L. microcarpa*: tannins and phenolics compounds, triterpenes, saponosides. We identified in *M. indica*'s extracts: tannins and phenolics compounds, triterpenes, saponosides and flavonoids.

Keywords

Lannea microcarpa, *Mangifera indica*, Toxicity, Antioxidant Activity, DPPH, Antibacterial Activity, Phytochemical Compounds

1. Introduction

Traditional healers in Burkina Faso use the leaves of *Lannea microcarpa* Engl. and K. Krause diarrhea, bloody stools, febrile body aches, hypertension, gastroenteritis and malaria.

According to Nacoulma [1] different parts (roots, barks, and leaves) are used for several therapeutic purposes. Indeed, in internal use, Leaves are used for the following cases: diarrhea, bloody stools, febrile body aches, hypertension, gastroenteritis, malaria, renal colic, generalized edema. They have an astringent, hypotensive, antibacterial, stimulant, diuretic effect.

Mangifera indica is used to treat Diarrhea, dysentery, asthenia, fever, bladder inflammation, asthma, gonorrhea, bronchitis, tonsillitis, retinopathy of hypertension, diabetes, venous disorders and capillary fragility, purpurea, cirrhosis, prevention of haemorrhagic accidents, mental disorders, old diarrhea, giardiasis, acute or chronic tracheitis cough, sinusitis, etc [1].

We have studied the toxicity of aqueous leaf extracts of *Lannea microcarpa* and *Mangifera indica*, to find out if the population that uses it is not exposed to the toxic effects of leaf decoctions of these plants.

Also, we studied the antioxidant activity, the antibacterial activity and the phytochemical composition of *Lannea microcarpa* and *Mangifera indica*.

The use of antioxidants is related to their ability to reduce tissue damage from free radicals in several diseases such as cardiovascular diseases, cancers, inflammatory diseases, diseases, skin, malaria, immune deficiency diseases, etc. Scientific research of secondary plant metabolites should be encouraged for their antioxidant effect to combat the effects of free radicals in several diseases [2] [3]. Scientific research of secondary plant metabolites should be encouraged for their antioxidant effect to combat the effects of free radicals in several diseases [4] [5] [6].

We have studied the toxicity of aqueous leaf extracts of *Lannea microcarpa* and *Mangifera indica*, to find out if the population that uses it is not exposed to the toxics effects of leaf decoctions of these plants. Also, we studied the antioxidant activity, the antibacterial activity and the phytochemical composition of *Lannea microcarpa* and *Mangifera indica*.

2. Materials and Methods

2.1. Material

The studies were conducted at University Joseph KI-ZERBO, (Burkina Faso), at UFR/SVT, in the Department of Biochemistry-Microbiology, in the Laboratory of Biochemistry and Applied Chemistry, specializing in medicinal plants. The leaves of *Lannea microcarpa* Engl. and K. Krause and leaves of *Mangifera indica* L. were harvested in Ouagadougou from the University Joseph KZ site in November 2021. Studies were conducted from November 2021 to March 2022.

2.2. Aqueous Extracts

We proceeded to the extraction by decoction of 50 g of powder of *Lannea microcarpa* Engl. and K. Krause or *Mangifera indica* L., boiling under reflux in 500 ml of water for one hour, then the mixture was filtered on Wattman paper after cooling. The decoction is lyophilized and kept in a box, for studies.

2.3. Evaluation of Acute General Toxicity of Leaves Extracts from *L. Microcarpa* and *M. indica*

The method is that described by Lompo [7]. Female NMRI strain mice, approximately 10 weeks old, weighing between 25 - 35 g were used for testing. A total of 12 mice were used for the tests. A concentration of dry extracts diluted in water (200 mg/ml) is prepared for the dose of 2000 mg/kg to be administered to each mouse. The test mice and the control group of mice are fasted 12 hours before the test. Two batches of mice are made as homogeneous as possible. The administration of the extracts is done by gavage according to the dose of 2000 mg/Kg. The evaluation of the LD_{50} lethal dose is done at 72 hours. Mice are observed during 14 days. A curve drawn of dose-mortality regression help to know if the extracts are an extremely toxic substance, a very toxic substance or a weakly toxic substance.

2.4. Antioxidant Activity by the Reduction of the DPPH

The antioxidant activity of the extracts was evaluated *in vitro* by the capacity of reduction of the radical DPPH (1,1 Diphenyl-2-Pycril Hydrazil) according to the method of Sharma [8]. The extracts to be tested are diluted in methanol from 100 µg/ml by the limit dilution technique. In an Eppendorf tube, we put 250 µl of extract diluted in methanol and then 500 µl of the DPPH solution (2 mg/ml). The white consists of 250 µl of methanol and 500 µl of DPPH (2 mg/l). Zero is made up of 750 ml of Methanol. The absorbance is read every 15 minutes at 517 nm. Each test is realised three times.

2.5. Research of the Bacterial Inhibiting Activity

Aqueous extracts of *L. microcarpa* leaves or *M. indica* used to determine their antibacterial activity. Reference strains from ATCC (American Type Culture Collection, Rockville): *Staphylococcus aureus* ATCC 6538, *Escherichia coli*

ATCC 25922, and a wild strain of *Pseudomonas aeruginosa*. The following reference antibiotics were used: Ampicillin, Bactrim, Erythromycin and Penicillin.

Preparation of inoculate: The inoculate of bacterial strains were adjusted to 10^6 bacteria/ml, [9]. In each Petri plate containing solid medium, put 3 mL of the suspension, 10^6 colony forming units (cfu) per millilitre. Eliminate excess from inoculate. Incubate 24 hours. Make wells and put it 50 µL of the extracts (50, 100, 200, 500 µg/ml) or antibiotics of reference. Incubate during 24 hours. Measure the diameters of inhibition. Each test is carried out three times.

2.6. Minimal Inhibition Concentration (MIC)

Micro-well dilution assay: Minimum inhibition concentration (MIC) was determined by the microdilution method in culture broth as recommended by Ellof [10] and the National Committee for Clinical Laboratory Standard (NCCLS, 2001). The 96-well micro-plate (NUNC, Danemark) containing 100 µL of Mueller Hinton (MH) broth were used. For each bacteria strain, three columns of eight wells to the microplate were used. Each well has getting: the culture medium + extract + inoculums (10 µL of inoculate) and INT (50 µl; 0.2 mg/mL). The plate were covered and incubated overnight at 37˚C and at 44˚C for *Escherichia coli* for 24 h. Each MIC experiment was repeated three times. Inhibition of bacterial growth was judged by rose or yellow colour. The MIC is defined as a lowest concentration of the extract at which the bacteria does not demonstrate the visible growth.

2.7. Phytochemical Studies

Methods of Ciulei [11] were used.

Alkaloids are revelated with Dragendorff's reagent: Appearance of a yellowish-white precipitate shows the presence of alkaloid bases or salts depending on the type of extract used. **Flavonoids** can be revelated with ammonia (NH_4OH). The observation of a yellow color indicates the presence of flavonoids.

Polyphenols and tannins are revelated by ferric chloride ($FeCl_3$). The appearance of a blue-black or blackish-green color respectively indicates the presence of gallic tannins and catechin tannins.

The property of **saponosides** is their foaming power. They are soluble in water. It poured 2 ml of extract (dissolved in water) into a test tube that is vigorously stirred. The appearance and persistence of a foam column of at least 1 cm for 15 minutes indicates the presence of saponosides.

Triterpenes and/or free steroids are revelated with concentrated sulfuric acid. We slowly poured 2 ml of on the tube wall. The appearance of a purplish red ring at the interface of the two liquids indicates the presence of terpenes while the appearance of a blue-green color indicates the presence of steroids.

2.8. Statistical Analyzes

All experiments are performed in triplicate and the results are expressed in

means +/− standard deviation using Microsft excel 2013.

3. Results

3.1. Acute Toxicity

The results of the toxicity tests for *L. microcarpa* are shown in Table 1, for the two batches of mice: controls and 2000 mg/Kg. The results indicate that there were no dead animals in any group of mice: controls or 2000 mg/kg. **The mortality rate is 0%**. For body weight, **controls were increased from 34 g to 36 g**. **Mice receiving 2000 mg/kg** aqueous extracts leaves from *L. microcarpa* increased from **35 g to 37 g** in 14 days. This result indicates that the aqueous extracts of *L. microcarpa* are not toxic.

The results of the toxicity tests for *M. indica* are shown in Table 2, for the two batches of mice: controls and 2000 mg/Kg. The results indicate that there were no dead animals in any group of mice: controls or 2000 mg/kg. **The mortality rate is 0%**. For body weight, **controls were increased from 26 g to 34 g**. **Mice receiving 2000 mg/kg** aqueous extracts leaves from *M. indica* increased from 27 **g to 34 g** in 14 days. This result indicates that the aqueous extracts of *M. indica* are not toxic.

3.2. Antioxidant Activity

The ability of extracts to reduce DPPH has been tested. The reduction of DPPH

Table 1. Acute toxicity tests performed with aqueous extracts of leaves from *Lannea microcarpa*.

Mice	Weight (g)	Administered volume (ml) With Solution 200 mg/ml	Number of dead animals				Weight (g)			
			D1	D2	D3	D14	D1	D2	D3	D14
Controles mice										
1	34.58	0	0	0	0	0	34.47	34.92	35.51	36.89
2	35.62	0	0	0	0	0	36.48	36.30	37.10	37.95
3	32.11	0	0	0	0	0	33.33	33.10	33.42	34.25
Averages	**34.10 ± 1.80**		**0**	**0**	**0**	**0**	**34.76 ± 1.59**	**34.77 ± 1.61**	**35.34 ± 1.85**	**36.36 ± 1.76**
Results after 14 days for 2000 mg/Kg of *Lannea microcarpa*										
1	34.93	0.35	0	0	0	0	35.84	35.99	35.44	36.57
2	35.40	0.35	0	0	0	0	35.15	35.47	35.42	36.57
3	34.56	0.35	0	0	0	0	35.49	35.32	36.25	38.80
Averages	**34.96 ± 0.42**		**0**	**0**	**0**	**0**	**35.49 ± 0.35**	**35.59 ± 0.35**	**35.70 ± 0.47**	**37.31 ± 1.29**

Table 2. Acute toxicity tests performed with aqueous extracts of leaves from *M. indica*.

Mice	Weight (g)	Administered volume (ml) With Solution 200 mg/ml	Number of dead animals				Weight (g)			
			D1	D2	D3	D14	D1	D2	D3	D14
Controles mice										
1	25.43	0	0	0	0	0	27.80	27.59	28.60	34.13
2	25.74	0	0	0	0	0	30.30	28.64	29.65	35.08
3	26.25	0	0	0	0	0	28.14	28.68	29.01	33.55
Averages	**25.81 ± 0.41**		**0**	**0**	**0**	**0**	**28.75 ± 1.36**	**28.30 ± 0.62**	**29.09 ± 0.53**	**34.25 ± 0.77**
Results after 14 days for 2000 mg/Kg of *M. indica*										
1	26.52	0.27	0	0	0	0	27.41	25.92	27.64	32.81
2	26.31	0.26	0	0	0	0	24.88	27.80	30.47	36.09
3	26.80	0.27	0	0	0	0	26.94	27.69	27.22	33.42
Averages	**26.54 ± 0.25**		**0**	**0**	**0**	**0**	**26.41 ± 1.35**	**27.14 ± 1.06**	**28.44 ± 1.77**	**34.11 ± 1.74**

by the extracts reduces the initial violet coloration. The first parameter determined is the percentage reduction (Pr) of the DPPH by the extracts, which is calculated according to the formula:

$$\text{Pr} = \frac{\text{Absorbance of Controle} - \text{Absorbance Extract}}{\text{Absorbance controle}} \times 100 .$$

These Pr values (Table 3), allowed us to determine the IC_{50}. IC_{50} is the concentration of antioxidant required to inhibit or reduce the initial concentration of DPPH by 50%. The aqueous extracts of *L. microcarpa* have an IC_{50} which is 20 µg/ml, determined from the Pr = f (extracted concentration). IC_{50} which is 18 µg/ml for *M. indica*.

3.3. Antibacterial Activity

Two different tests were performed to determine whether the extracts inhibit bacterial growth or not. In the first test, in Petri dishes where a bacterial strain was seeded, the extracts were distributed in the wells. Compared with the positive controls in which we observed a growth inhibition, the wells where there were the extracts at 500 µg/ml did not inhibit the growth of the bacteria. The inhibition diameters that we measured around the wells for each bacterial strain were of the order of 12 mm, which is very insignificant. In the 2nd test, we used the 96-well plates where we distributed the extracts at different concentrations, then the bacterial strains. After incubation and addition of INT, all wells were

Table 3. Percentage reduction of DPPH obtained with aqueous extracts of leaves of *Lannea microcarpa* and *Mangifera indica*.

Concentrations µg/ml	3.125	6.25	12.5	25	50	100
L. microcarpa Percentage of DPPH reduction (%)	27.8 ± 0.12	35.63 ± 24	47.80 ± 0.31	54.75 ± 0.36	60.87 ± 0.54	71.10 ± 0.68
M. indica Percentage of DPPH reduction (%)	19.54 ± 0.52	32.72 ± 2.18	45.71 ± 1.04	53.39 ± 0.69	57.04 ± 1.27	66.20 ± 1.23

stained purple, regardless of the concentrations of extracts used, ranging from 62.5 µg/ml to 500 µg/ml. The pink color indicating the presence of the bacteria, that means that their growth was not inhibited in the presence of extracts. In the wells where we used conventional antibiotics, there was no pink staining, depending on the bacterial strain and the antibiotic used. **The aqueous extracts of** *L. microcarpa* or *M. indica* **did not have any antibacterial activity.**

3.4. Phytochemical Studies

The phytochemical compounds (**Table 4**) identified were, for *L. microcarpa*: tannins and phenolics compounds, triterpenes, saponosides. We identified in *M. indica*'s extracts: tannins and phenolics compounds, triterpenes, saponosides and flavonoids.

4. Discussion

Medicinal plants can be toxic. A study led to Morocco showed that on 123 listed healing plants, 83 were known by the populations as being toxic, [12]. It turns out to be necessary to lead scientific studies to classify a plant medicinal as being toxic or not. In our case, the aqueous extracts of the leaves of *L. microcarpa or M. indica* were not toxic, by scientific tests.

The aqueous stem bark of *Lannea microcarpa* (LME) is used traditionally for the treatment of swellings, wounds, rheumatism, pains, and infections. In acute toxicity study, there were no behavioral changes as well as mortality in the LME (300 - 3000 mg/kg) treated rats. Similarly, in the sub-acute studies, LME (300 - 3000 mg kg) treated rats showed no significant differences in the body weight, organ weight, hematological and biochemical parameters that were assessed ($p \leq 0.05$) [13].

For *Mangifera indica*, numerous studies have pointed out the various beneficial effects of mango leaves extract against cancer, diabetes, cardiovascular, and neurodegenerative diseases. These positive effects are due to the presence of a plethora of phytochemicals such as mangiferin followed by phenolic acids, benzophenones, and other antioxidants such as flavonoids, ascorbic acid, carotenoids, and tocopherols [14]. Extracts of leaves from Mangifera indica containing 60% mangiferin was evaluated in Han: Wist male and female rats in a 90 day

Table 4. Phytochemicals componds identified in aqueous extracts from Aqueous Extracts of Leaves of *Lannea microcarpa* and *Mangifera indica*.

	Alcaloïdes	Flavonoids	Tannins and phenolics compounds	Saponosides	Triterpenes et stéroides
L. microcarpa	-	-	+++	++	+++
M.indica	-	+	+++	++	+++

Legend: negative reaction (-), weakly positive reaction (+), moderately positive reaction (++), strongly positive reaction (+++).

study, and it was concluded that ML extracts demonstrated no observed adverse effect at the highest dose tested (2000 mg/kg body weight per day) [15]. Few studies also reported the toxic effects of Mangifera indica's leaves extracts attributed to the presence of allergens. Mango allergy can occur in two ways: either the immediate hypersensitivity reaction as wheezing dyspnoea, anaphylaxis, erythema,urticaria, and angioedema, or the late reaction presenting as contact dermatitis and periorbital oedema [16]. A long-term study (three consecutive months) on Sprague Dawley rats at various doses (100, 300, and 900 mg/kg) of MLs revealed the slight body weight increase and higher fat weight, the serum thyroglobulin and cholesterol levels,and the slight increase in epididymis weight of male rats compared with the control group [17].

IC_{50} which was 20 µg/ml for *L. microcarpa* and was 18 µg/ml for *M. indica*. These values indicate the presence of molecules which neutralize the free radicals implied in the pathologies [18] [19] [20]. It is necessary to us, in perspectives, to estimate the rate of compounds in correlation with observed antioxidant activity.

Medicinal plants are known for their **antibacterial activity** or **antimicrobial activities**, [21] [22]. Usually, we test all our extracts to see if they have an antibacterial activity. **However**, **for these two species**, ***L. microcarpa*** **and** ***M. indica***, **we did not observe antibacterial activity**. The scientific results are essential to verify the uses of plants in traditional medicine.

5. Conclusions

The main objective of our work was to know if the two species used in traditional medicine in Burkina Faso were toxic or not. According to our studies, aqueous extracts of leaves *of Lannea microcarpa* or *Mangifera indica* which are recommended for use by patients, are not toxic.

We then evaluated other potentialities of these extracts. Aqueous extracts of *Lannea microcarpa* or *Mangifera indica* leaves showed no anti-bacterial activity on three strains of bacteria used. Antioxidant activity is appreciable for both species. The IC_{50} is 20 µg/ml for *Lannea microcarpa*. The IC_{50} is 18 µg/ml for *Mangifera indica.* The phytochemicals we have identified in *Lannea microcarpa* and *Mangifera indica* are tannins and phenolics compounds, triterpenes, saponosides.

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Conflicts of Interest

The authors declare no conflict of interests.

Authors Contributions

Initiation and elaboration of protocole: Monique Brigitte OUATTARA, M. KIENDREBEOGO, O. G. NACOULMA, J. H. BATIONO/Acute toxicity tests: OUATTARA Monique Brigitte, J. H. BATIONO/Antibacterial, antioxidant and phytochemicals tests: Monique Brigitte OUATTARA/Phytochemical screening: Monique Brigitte OUATTARA.

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