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Evaluation of Acute Toxicity, Antioxidant and Antibacterial Potentials of Leaves from Two Combretaceae, *Combretum micranthum* G. Don. and *Terminalia mantaly* H. Perr.

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Abstract: Aqueous extracts of the leaves from *Combretum micranthum* G. Don. (Combretaceae), *Terminalia mantaly* H. Perr. (Combretaceae) are used by traditional healers in Burkina Faso. We studied the acute toxicity, antioxidant, antibacterial potential activity and phytochemical composition of these plants. Acute toxicity was studied in NMRI strain mice. The aqueous extracts were not toxic at the maximum dose of 2000mg/kg body weight. Extracts showed an antioxidant activity with an IC_{50} = 20µg/ml and 25µg/ml for *C. micranthum* and *T. mantaly* by the method of reduction of DPPH° radical. The extracts showed no bacterial activity on three strains of bacteria tested: *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The phytochemicals we have identified are for *C. micranthum* as for *T. mantaly* were: flavonoids, tannins and phenolics compounds, triterpenes, saponosides.

Key words: *C. micranthum* · *T. mantaly* · Acute toxicity · Antioxidant Activity · Phytochemical Compounds

INTRODUCTION

Traditional healers in Burkina Faso use the leaves of *Combretum micranthum* G. Don. (Combretaceae) for the treatment of hepatitis B and hypertension. In the bibliography, *C. micranthum*is listed among plants used for treat diabetes in Togo [1]. According to Nacoulma [2] leaves of *C. micranthum* are used to treat the following diseases: diarrhea, dysentery, gastrointestinal bleeding, dysentery syndromes, colic, stomach disorders, nausea, digestive disorders, malaria, beriberi, intestinal parasites, asthenia, cough, bronchitis, fever, lumbago, hematuric bilious fever, epistaxis, infant diarrhea, gall bladder disease, anemia, gonorrhea, enuresis, metrorrhagia, antileaty, hypertension, weaning (Nanny), masmites. The work of Nacoulma [2] showed that the leafy stems of *Terminalia mantaly* H. Perr. (Combretaceae) are used to treat diabetes or hypertension. Scientific research concerned plants used to treat diabete [3-5].

We have studied acute toxicity to make available the toxicity studies of plants that are still predominantly used in traditional medicine in Burkina Faso or Africa. Scientific research of secondary plant metabolites should be encouraged for their antioxidant effect to combat the effects of free radicals in several diseases [6-8]. The antibacterial activity of several plant species is studied by several authors [9, 10].

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MATERIALS AND METHODS

Materials: The studies were conducted at University Ouaga 1 Pr Joseph KI-ZERBO, (Burkina Faso), UFR/SVT, Department of Biochemistry-Microbiology, in the Laboratory of Biochemistry and Applied Chemistry, specializing in medicinal plants. The leaves of *Combretum micranthum* G. Don. and *Terminalia mantaly* H. Perr. were harvested in Ouagadougou.

Aqueous Extraction: About 50g of vegetable powdered was extracted with 500 ml of distilled water during one hour at 100°C. Then the mixture was filtered on Wattman paper after cooling. The decoction is lyophilized and kept in a box, for studies.

Evaluation of Acute Toxicity: The method was described by Done [11] and Lompo *et al*. [12]. Female NMRI strain mice, approximately 10 weeks old, weighing between 25-35g were used for testing. A concentration of dry extracts diluted in water (200mg/ml) was prepared for the dose of 2000 mg/kg to be administered to each mouse. The test mice and the control group of mice were fasted 12 hours before the test. Two batches of mice are made as homogeneous as possible. The administration of the extracts was done by gavage according to the dose of 2000mg/Kg. The evaluation of the LD_{50} lethal dose was done at 72hours. Mices were observed during 14 days. A curve drawn of dose-mortality regression help to know if the extracts were an extremely toxic substance, a very toxic substance or a weakly toxic substance.

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, 1Diphenyl 2 PycrilHydrazil) according to the method of Sharma and Bhat [13]. The extracts to be tested were diluted in ethanol from 100 µg/ml by the limit dilution technique. In an Ependorf 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 was read every 15 minutes at 517nm. Each test was realised three times.

Antibacterial Activity: Aqueous extracts of *Combretum micranthum* G. Don. And *Terminalia mantaly* H. Perr. leaves were 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, Penicillin. The bacterial inhibiting activity was researched by the method of Ezoubeiri *et al*. [14]. Minimal inhibition concentration (MIC) was determinated by the method of Ellof [15].

Phytochemical Studies: Methods of Ciulei [16] were used to detect Alkaloids with Dragendorff's reagent, Flavonoids with ammonia (NH_4OH), Polyphenols and tannins with ferric chloride ($FeCl_3$), Triterpenes and / or free steroids with sulfuric acid (H_2SO_4). The property of saponosides was their foaming power. They were soluble in water. Then, poured 2ml of extract (Dissolved in water) into a test tube that was vigorously stirred. The appearance and persistence of a foam column of at least 1 cm for 15 minutes indicates the presence of saponosides.

Statistical Analyzes: All experiments are performed in triplicate and the results are expressed in means +/- standard deviation using Microsft excel 2013.

RESULTS AND DISCUSSION

Acute Toxicity: The results of the toxicity tests for *C. micranthum* were shown in Table 1, for the two batches of mice: controls and 2000mg/Kg.

The results indicate that there were no dead animals in any group of mice: controls or 2000mg/kg. The mortality rate was 0%. For body weight, controls were increased from 34g to 36g. Mice receiving 2000 mg/kg aqueous extracts leaves from *C. micranthum* increased from 33g to 34g in 14 days. This result indicates that the aqueous extracts from *C. micranthum* were not toxic.

The results of the toxicity tests for *T. mantaly* were shown in Table 2, for the two batches of mice: controls and 2000mg/Kg.

The results indicate that there were no dead animals in any group of mice: controls or 2000mg/kg. The mortality rate is 0%. For body weight, controls were increased from 27g to 36g. Mice receiving 2000 mg/kg aqueous extracts leaves from *T. mantaly* increased from 38g to 38g in 14 days. This result indicates that the aqueous extracts of *T. mantaly* were not toxic.

Table 1: Acute toxicity tests performed with aqueous extracts of leaves of *C. micranthum*

			Number of dead animals				Weight (g)			
Mice	Weight (g)	Administered volume	D1	D2	D3	D14	D1	D2	D3	D14
	Control 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
	After 14 days for 2000mg/Kg of *C. micranthum*									
1	32.43		0	0	0	0	32.19	32.73	33.61	34.26
2	32.60		0	0	0	0	31.85	31.50	32.74	33.64
3	33.22		0	0	0	0	32.75	33.03	33.74	34.57
Averages	32.75±0.42		0	0	0	0	32.26±0.45	32.42±0.81	33.36±0.54	34.16±0.47

Table 2: Acute toxicity tests performed with aqueous extracts of leaves of *T. mantaly*

			Number of deadanimals				Weight (g)			
Mice	Weight (g)	Administered volume	D1	D2	D3	D14	D1	D2	D3	D14
	Control mice									
1	29.28	0	0	0	0	0	29.81	31.21	31.37	37.01
2	24.96	0	0	0	0	0	25.70	26.52	27.92	33.67
3	27.90	0	0	0	0	0	28.14	29.28	30.11	37.61
Averages	27.38±2.21		0	0	0	0	27.88±2.07	29.00±2.36	29.80±1.75	36.10±2.12
	After 14 days for 2000mg/Kg of *Terminalia mantaly*									
1	29.15	0	0	0	0	0	32.57	34.75	35.90	39.64
2	29.21	0	0	0	0	0	31.33	32.60	33.39	37.22
3	26.37	0	0	0	0	0	28.65	30.07	30.53	36.00
Averages	28.24±1.62		0	0	0	0	30.85±2.00	32.47±2.34	33.27±2.69	37.62±1.85

Table 3: Reduction (%) of DPPH ° obtained with Aqueous Extracts of Leaves of *Combretum micranthum* and *Terminalia mantaly*

Concentrations µg/ml	3.125	6.25	12.5	25	50	100
Percentage of DPPH° reduction (%) *C. micranthum*	24.2±0.17	36.70 ±0.46	47.00±0.31	53.97±2.05	58.78±0.77	65.81±7.15
Percentage of DPPH° reduction (%) *T. mantaly*	30.96±0.48	40.99±0.68	47.16±1.17	50.93±0.96	58.35±0.68	63.20±1.15

Table 4: Phytochemicals identified in aqueous extracts from *C. micranthum and T. mantaly* leaves

	Alkaloids	Flavonoids	Tannins and phenolics compounds	Saponosides	Triterpènes et stéroïdes
C. micranthum	-	+	+++	++	+++
T. mantaly	-	++	+++	-	+++

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

Antioxidant Activity: The ability of extracts to reduce DPPH has been tested. The reduction of DPPH by the extracts reduces the initial violet coloration. The first parameter determined was the percentage reduction (Pr) of the DPPH by the extracts, which was calculated according to the formula:

$$Pr = \frac{(Absorbanc\ of\ Controle - Absorbance\ Extract)}{Absorbance\ Controle} x100$$

These Pr values, Table 3, allowed us to determine the IC_{50}. IC_{50} was the concentration of antioxidant required to inhibit or reduce the initial concentration of DPPH by 50%. The aqueous extracts from *C. micranthum* and *T. mantaly* have an IC_{50} which was 20 µg/ml and 25 µg/ml respectively, determined from the Pr=f (Extracted concentration).

Flavonoids and tannins are known for their potential oxidants [4, 5].

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 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 which measured around

the wells for each bacterial strain were of the order of 11 to12 mm, which was very insignificant. In the 2nd test, it can be used the 96-well plates where distributed the extracts at different concentrations, then the bacterial strains. After incubation and addition of INT, all wells were 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, which means that their growth was not inhibited in the presence of extracts. In the wells where the study used conventional antibiotics, there was no pink staining, depending on the bacterial strain and the antibiotic used.

The aqueous extracts of *C. micranthum* and *T. mantaly* did not have any antibacterial activity.

Phytochemical Studies: The phytochemicals which identified were for *C. micranthum*: flavonoids, tannins and phenolic compounds, triterpenes, saponosides. The phytochemicals identified were for *T. mantaly*: Flavonoids, triterpénoids, stéroids, tannins (Table 4).

CONCLUSION

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 obtained data, aqueous extracts of leaves *of C. micranthum* and *T. mantaly* which found to be no toxic.

Also, the research evaluated other potentialities of these extracts. Aqueous extracts of *C. micranthum* and *T. mantaly* leaves showed no anti-bacterial activity on three strains of bacteria used.

The IC_{50} was 20 µg/ml for *C. micranthum*. The IC_{50} was 25µg/ml for *T. mantaly*.

The phytochemicals identified were for *C. micranthum*: flavonoids, tannins and phenolic compounds, triterpenes, saponosides. The phytochemicals we have identified were for *T. mantaly*: Flavonoids, triterpénoids, stéroids, tannins.

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