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Research Article

Evaluation of Acute Toxicity, Antioxidant and Antibacterial Activities of Aqueous Extracts of *Leaves of Eucalyptus camaldulensis Dehnhardt* (MYRTACEAE) Synonym: *Eucalyptus rostrata Schelcht*

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Abstract: The aim of this study is to evaluate by scientific studies, the *Eucalyptus camaldulensis* potentialities used in traditional medicine in Burkina Faso to treat malaria. Then, acute general toxicity, antioxidant and antibacterial activities phytochemical composition were evaluated. The leaves of *Eucalyptus camaldulensis* Dehnhardt (Myrtaceae) are used in Burkina Faso for the treatment of malaria and respiratory diseases. Aqueous extracts of the leaves are the form of use recommended by traditional healers. We studied the acute toxicity antioxidant activity, the antibacterial activity, the phytochemical composition of aqueous extracts of *Eucalyptus camaldulensis*, used everywhere in Burkina Faso. Acute toxicity was studied in NMRI strain mice. The aqueous extracts were not toxic at the maximum dose of 2000 mg/kg body weight. The extracts presented antioxidant activity with an IC_{50} which is 12.5 μg/mL. The extracts showed no bacterial activity on three strains of bacteria tested: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Phytochemicals compounds that we have identified are alkaloids, flavonoids, tannins and phenolics compounds, triterpenoids and steroids, saponosides.

Keywords: Acute toxicity, antibacterial activity, antioxidant activity, *Eucalyptus camaldulensis* dehnhardt, phytochemicals compounds

INTRODUCTION

Eucalyptus camaldulensis Dehnhardt is evergreen. The plant is not native to Burkina Faso but Tasmania in Australia. This plant is grown in warm regions, especially marshy. The plant is used in Burkina Faso to treat malaria. Its essential oils are more and more used in this country in cosmetics and to repel mosquitoes. According to Nacoulma (1996), the organs used in traditional medicine are the leaves. In internal use, leaves are used in the following cases: respiratory diseases, bronchitis, asthma, phthisis, laryngeal, abdominal pain, dysmenorrhoea, infectious diseases of the genitourinary system, diabetes, hepatitis, chronic gonorrhea, fevers, laryngitis, intestinal worms, urethritis, vaginitis, asthenia; cough, leucorrhea, colibacillosis, rheumatism.

Traditional cures and plant-based remedies remain the main solution to health problems in many developing countries (Azaizeh *et al*., 2003). WHO has adopted a strategy to integrate the good use of traditional medicine with the health care of populations, (WHO (World Health Organization), 2002).

We studied the toxicity of aqueous extracts of *Eucalyptus camaldulensis* to determine if the population that uses it is exposed or no by the harmful effects. Also, we investigated antioxidant activity, antibacterial activity and the phytochemical composition of *Eucalyptus camaldulensis*t.

We have studied acute toxicity to make available the toxicity studies of plants that are still predominantly used in Africa.

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, 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 (Atawodi, 2005; Katalini *et al*., 2006; Lamien-Meda *et al*., 2010; Ouattara *et al*., 2011). So, we evaluated the antibacterial activity of

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aqueous extracts of leaves from *Eucalyptus camaldulensis*.

MATERIALS AND METHODS

Biological 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 *Eucalyptus camaldulensis* Dehnhardt were harvested in Ouagadougou on the site of the University Ouaga1 Pr Joseph KZ.

Aqueous extraction: 50 g 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 of eucalyptus camaldulensis: The method is that described by Lompo *et al*. (1998). Female NMRI strain mice, approximately 10 weeks old, weighing between 25-35 g were used for testing. Three concentrations of dry extracts diluted in water (5, 30 and 200 mg/mL, respectively) are prepared for the respective doses of 50, 300 and 2000 mg/kg, respectively to be administered to each mouse. The test mice and the control group of mice are fasted 12 h before the test. Five batches of mice are made as homogeneous as possible. The administration of the extracts is done by gavage according to the doses 50, 300 and 2000 mg/kg respectively. The evaluation of the LD_{50} lethal dose is done between 72 h and 14 days in order to draw a straight line of dose-mortality regression to know if the extracts are 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, 1 Diphenyl 2 Pycril Hydrazil) according to the method of Sharma *et al*., 2008. The extracts to be tested are diluted in ethanol 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 min at 517 nm. Each test is realized three times.

Antibacterial activity: Aqueous extracts of *Eucalyptus camaldulensis* 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 and Penicillin.

Research of the bacterial inhibiting activity:

Preparation of inoculating: The inoculum of bacterial strains was adjusted to 106 bacteria/mL (Ezoubeiri *et al*., 2005). In each Petri plate containing a solid medium, put 3 mL of the suspension, 10^6 Colony Forming Units (CFU) per milliliter (Ezoubeiri *et al*., 2005). Eliminate excess from inoculating. Incubate 24 h. Make wells and put it 50 µL of the extracts (50, 100, 200, 500 µg/mL, respectively) or antibiotics of reference. Incubate for 24 h. Measure the diameters of inhibition. Each test is carried out three times.

Minimal Inhibition Concentration (MIC):

Micro-well dilution assay: Minimum Inhibition Concentration (MIC) was determined by the microdilution method in culture broth as recommended by Eloff (1998) 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 was 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 a rose or yellow color. The MIC is defined as the lowest concentration of the extract at which the bacteria does not demonstrate the visible growth.

Phytochemical studies: Methods of Ciulei 1982 are 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. We 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 min indicate the presence of saponosides (Ciulei, 1982).

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. (Ciulei, 1982), while the appearance of a blue-green color indicates the presence of steroids.

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 *Eucalyptus camaldulensis* are shown in Table 1, for the five batches of mice: controls, 5, 50, 300 and 2000 mg/mL respectively.

The results indicate that there were no dead animals in any group of mice up to a dose of 2000 mg/kg. The mortality rate is 0%. For body weight, controls were increased from 30 g to 32 g. Mice receiving 2000 mg/kg aqueous extracts of *Eucalyptus camaldulensis* leaves increased from 25 g to 29 g in 14

Table 1: Results for acute toxicity tests performed with aqueous extracts of leaves of *E. camaldulensis*

		Administered volume (mL)	Number of of dead animals				
Mice	Weight (g)	Day (D) 0	D1	D2	D3	D11	D14
	Controles mice						
1	31.90	0	0	0	0	0	0
2	27.05	0	0	0	0	0	0
3	31.74	0	0	0	0	0	0
Averages	30.23±2.76						
	Results after 14 days for 5 mg/kg						
1	23.90	0.24	0	0	0	0	0
2	29.13	0.30	0	0	0	0	0
3	28.60	0.29	0	0	0	0	0
Averages	27.21±2.88						
	Results after 14 days for 50 mg/kg						
1	26.32	0.27	0	0	0	0	0
2	30.79	0.31	0	0	0	0	0
3	27.16	0.27	0	0	0	0	0
Averages	28.09±2.38						
	Results after 14 days for 300 mg/kg						
1	24.53	0.25	0	0	0	0	0
2	26.69	0.27	0	0	0	0	0
3	27.61	0.28	0	0	0	0	0
Averages	26.28±1.58						
	Results after 14 days for 2000 mg/kg						
1	26.19	0.26	0	0	0	0	0
2	23.09	0.23	0	0	0	0	0
3	27.13	0.27	0	0	0	0	0
Averages	25.47±2.11						

	Weight (g)				
Mice	D1	D2	D3	D11	D14
	Controles mice				
1	31.82	32.23	32.25	32.08	34.32
2	28.08	27.68	27.13	27.38	29.57
3	32.34	32.34	31.67	31.41	31.73
Averages	30.75±2.32	30.75±2.66	30.35±2.80	30.29±2.54	31.87±2.38
	Results after 14 days for 5 mg/kg				
1	25.43	26.94	26.64	26.92	27.18
2	29.29	29.30	29.08	28.88	29.25
3	29.48	30.07	30.56	30.96	30.76
Averages	28.07±2.29	28.77±1.63	28.76±1.98	28.92±2.02	29.06±1.80
	Results after 14 days for 50 mg/kg				
1	28.62	29.55	29.31	29.51	29.46
2	32.07	35.42	34.19	34.72	35.32
3	27.10	27.62	27.20	27.42	27.10
Averages	29.26±2.58	30.86±4.06	30.23±3.59	30.55±3.76	30.63±4.23
	Results after 14 days for 300 mg/kg				
1	26.80	27.94	28.24	28.92	29.04
2	28.58	30.59	30.46	30.42	31.24
3	28.40	30.26	29.90	29.98	30.37
Averages	27.93±0.98	29.60±1.44	29.53±1.15	29.77±0.77	30.22±1.11
	Results after 14 days for 2000 mg/kg				
1	27.05	27.72	28.34	28.73	30.28
2	23.82	25.41	25.04	25.9	25.99
3	28.64	28.34	28.71	29.09	29.89
Averages	26.50±2.46	27.16±1.54	27.36±2.02	27.91±1.75	28.72±2.37

Table 2: Percentage reduction of DPPH° obtained with aqueous extracts of leaves

Concentrations µg/ml	Percentage of reduction of DPPH° (%)
3.125	34.70
6.25	44.23
12.5	49.80
25	61,86
50	62.64
100	70.67

Table 3: Phytochemicals identified in aqueous extracts of *Eucalyptus camaldulensis* leaves

Phytochemical compounds	Alkaloids	Flavonoids	Tannins and phenolic compounds	Saponosides	Triterpenes et stéroïdes
Presence	+ + +	+	+++	++	+++

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

days. This result indicates that the aqueous extracts of *Eucalyptus camaldulensis* are not toxic.

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 is the Percentage reduction (Pr) of the DPPH by the extracts, which is calculated according to the formula:

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

These Pr values (Table 2) allowed us to determine the IC_{50}. IC_{50} is the concentration of antioxidant required to reduce the initial concentration of DPPH by 50%. The aqueous extracts of *Eucalyptus camaldulensis* have an EC_{50} which is 12.5 µg/ml, determined from the Pr = f (extracted concentration).

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 11 to 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 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 *Eucalyptus camaldulensis* did not have any antibacterial activity.

Phytochemical studies: The phytochemicals identified by the simple characterization tests are shown in Table 3. The phytochemicals that we have identified are alkaloids, flavonoids, tannins and phenolics compounds, triterpenoids and steroids.

Discussion: Our studies have shown that aqueous extracts of *Eucalyptus camaldulensis* are not toxic to NMRI strain mice. The essential oils of *E. camaldulensis* have been the subject of some studies. The essential oils of *E. camaldulensis* are toxic to insects. The plant is used in this case as a bioinsecticide, (Negahban *et al*., 2007). The essential oils of *E. camaldulensis* were also toxic to fungi that hinder the production of Sorghum (Somda *et al*., 2007).

The antioxidant activity of *E. camaldulensis* has been measured. IC_{50} is 12.5 µg/mL. Studies have assessed and established that extracts that have an EC50 less than 50 µg/mL are considered to have significant antioxidant activity (Abdel-Hameed, 2009). We can compare the results of our extracts to those of the positive control, quercetin, who's IC_{50} was 3.6 µg/mL. Antioxidant activity was appreciable with IC_{50} is 12.5 µg/mL for *Eucalyptus camaldulensis*, that can be put to use. Aqueous extracts of *Eucalyptus camaldulensis* leaves showed no anti-bacterial activity on three strains of bacteria used.

The phytochemicals we have identified in *Eucalyptus camaldulensis* are alkaloids, flavonoids, tannins and polyphenols, steroids and triterpenes, saponosides. The antioxidant activity is related to phenolic compounds (Ahmed *et al*., 2014). The activity we observed in extracts of E. c. is probably due to flavonoids, tannins and other identified phenolic compounds.

CONCLUSION

The main objective of our study was to know if *Eucalyptus camaldulensis* used in traditional medicine in Burkina Faso to treat malaria is toxic or not.

According to our studies, aqueous extracts of leaves of *Eucalyptus camaldulensis* which are recommended for use by patients, are not toxic. We then evaluated other potentialities of these extracts.

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