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Toxicological and Phytochemical Studies of an Aqueous Extract of *Lophira lanceolata* (Ochnaceae) Leaves in Mus Musculus (Muridae) Mice of the Swiss Homogeneous Parent Stock

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Abstract **Original Research Article**

Lophira lanceolata is a plant commonly used in traditional African medicine to treat several diseases. The qualitative phytochemical study, carried out using the methods described in the work of Méa *et al* (2017), using an aqueous extract of dried *Lophira lanceolata* leaves, revealed the presence of sterols, polyphenols, flavonoids, saponosides, quinone compounds, alkaloids and gallic tannins, which are believed to be responsible for certain pharmacological effects on diseases. The study of the acute toxicity of this extract in mice, at doses ranging from 150 to 1000 mg/kg b.w., determined the LD50 values to be 375 and 439 mg/kg b.w., obtained respectively by the Dragstedt and Lang calculation method and the Miller and Tainter graphical method. According to Diezi's classification (1989), this substance is highly toxic when administered intraperitoneally.

Keywords: *Lophira lanceolata*, acute toxicity, intraperitoneal, phytochemical screening.

INTRODUCTION

Lophira lanceolata is a species belonging to the Ochnaceae family and is commonly found in West and Central African countries [1]. In Côte d'Ivoire, Lophira lanceolata is found in the western mountainous region: Lampleu, 33 km from Danané; in the Zro forest between Guiglo and Tai; and in Pinhou between Douékoué and Buyo. The plant also grows in the lower Sassandra and southern Comoé regions [2]. Its inflorescence is a terminal, pyramidal, loose panicle 15 to 20 cm long. The fruits are conical in shape. The seeds are ovoid, chestnut-coloured and glabrous [3].

The roots and leaves of the tree are mainly used to treat yellow fever, malaria and stomach ache. The sap is mainly used to promote wound healing. The fruits are mainly used for oil production [4].

However, as the traditional use of plant extracts does not guarantee their safety [4], it is essential to determine their toxicity [5]. In Côte d'Ivoire, more than 1,421 species of medicinal plants have been identified for the treatment of various diseases [6]. Medicinal plants play an important role in the treatment of diseases, but they are often the cause of certain accidents due to self-medication and ignorance of dosages. It is therefore necessary to conduct scientific studies in order to understand and control the action of the various active compounds contained in these plants and their toxicity.

The objective of this study is to determine the main chemical compounds in the aqueous extract of *Lophira lanceolata* leaves through a phytochemical study and to evaluate the acute toxicity of this extract in mice.

I-MATERIALS AND METHODS

I-Materials

I-1-Plant material

The leaves of Lophira lanceolata were harvested in June 2023 in Korhogo, a town in northern Ivory Coast. They were identified at the National Centre for Floristics (CNF) by comparison with sample No. UCJ013145 held at the centre.

I-2-Animal material

I-2-1-Mice

The mice used for acute toxicity testing were of the species Mus Musculus (Muridae) and of the homogeneous Swiss parental strain, weighing between 20 and 29 g. They were raised in the vivarium of the École Normale Supérieure d'Abidjan (ENS) at room temperature (28°C±3°C), with free access to food and water.

I-Study methods

II-1-Preparation of the aqueous extract of *Lophira lanceolata* leaves

Freshly harvested Lophira lanceolata leaves were dried in the shade and ground in the pharmacy department laboratory using a grinder. Next, 200 g of this leaf powder was boiled at 100°C in two litres of distilled water for 30 minutes. The resulting decoction was filtered successively through cotton wool and Wattman No. 4 paper. The filtrate obtained was dried in an oven at 60°C for 72 hours. The result is a perfectly water-soluble powder that constitutes the aqueous extract of *Lophira lanceolata* leaves (EALl).

II-2-Phytochemical study

This study consists of identifying the major chemical groups that have high pharmacological potential, namely sterols, polyterpenes, polyphenols, flavonoids, tannins, quinone compounds, alkaloids and saponosides. The detection of these chemical compounds is based on the principle that they induce chemical reactions in the presence of appropriate reagents [7].

These tests were carried out using the analytical techniques described in the work of [8]. For these tests, an EALl solution is prepared by dissolving 5 g of the extract in 50 ml of distilled water.

II-3- Toxicological study

II-3-1- Acute toxicity study in mice via intraperitoneal route

II-3-1-1- Experimental protocol

Cages labelled 1 to 9 were used to form eight (9) groups of six (6) mice. Each group consisted of an equal number of males and females. The tests were carried out on a control group (group 1) and the eight other treated groups. The extract was diluted in 9‰ NaCl.

First, the toxicological study was conducted by intraperitoneally injecting different doses of EALl into the mice in groups 2 to 9. A single dose was administered to all mice in each group. The mice in the control batch receive a solution of 9‰ NaCl intraperitoneally. Mortality rates are determined after a 24-hour observation period. At the end of this first stage, the two limit doses of the substance are determined; those causing 0% and 100% mortality, respectively. During the second stage, a series of dilutions is carried out between these two doses in order to determine the dose that causes lethality or mortality in half of a given population of mice, or the 50% lethal dose (LD50).

II-3-1-1- 1-Determination of the 50% lethal dose (LD_{50})

The lethal dose 50% is the dose of a substance that causes the death of 50% of the mouse population studied. It is an essential parameter in any toxicological study. It allows the short-term toxic potential or immediate or acute toxicity of a given substance to be assessed and the physiological concentration range to be selected. It is determined by graphical and calculation methods.

II-3-1-1- 1-1-Graphical method or Miller and Tainter method

In this method [9], the percentages of dead mice are used to plot the mortality curve as a function of the logarithm of the product concentration, expressed in mg/kg of body weight. The curve is obtained using the STATISTICA7 programme.

On the linear curve, the LD_{50} is the abscissa of the point corresponding to 50% mortality.

II-3-1-1- 1-2-Calculation method or Dragstedt and Lang method

The [10] is also used to determine the LD_{50}.

This method is based on the following assumption:

- Any animal that survives a given dose of a substance administered to it will survive any other dose lower than that given dose;
- Similarly, any animal that dies from a given dose of a substance administered to it will also die from any other dose higher than that given dose.

Thus, the mortality percentage (M %) for a given dose of the substance administered is given by the number of specimens that died (Nm) at that dose, divided by the number of specimens that died plus the number of survivors (Nv):

M % = Nm x 100 / Nm + Nv

The LD_{50} is calculated using the Dragstedt and Lang method by extrapolation, i.e. by finding the approximate value of the dose that corresponds to 50% mortality in an interval (X1 –X2).

LD50 = [50(X2-X1) + (X1Y2-X2Y1)] / (Y2-Y1)

- X1: lower dose framing the LD_{50};

- X2: upper dose framing the LD_{50};
- Y1: percentage of mortality corresponding to X1;
- Y2: percentage of mortality corresponding to X2.

II-4-Processing of results

Statistical analysis of the values and graphical representation of the data were performed using GraphPad Prism 8.4 software (San Diego, California, USA). The statistical difference between the results was determined using analysis of variance (ANOVA). All values are represented as mean ± SEM (standard error of the mean)

III-RESULTS AND DISCUSSIONS

III-1-Results

III-1-1- Phytochemical study of the aqueous extract of dried leaves of Lophira lanceolata

The qualitative phytochemical study, carried out using the aqueous extract of dried Lophira lanceolata leaves, revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, saponosides, quinone compounds, alkaloids and gallic tannins. However, the presence of catechin tannins was not detected in the extract (Table I).

III-1-2-Toxicity of the aqueous extract of dry leaves of Lophira lanceolata in mice.

After administration of doses ranging from 150 to 300 mg/kg b.w., the animals moved around the cage dragging their hindquarters and then curled up for twenty (20) minutes. They practically stopped eating and drinking. After about 1 hour, the mice in these groups regained their appetite, drank and began to eat properly. However, in animals that received doses ranging from 350 to 1000 mg/kg b.w., jerky breathing and decreased motor activity were observed, with the mice remaining huddled in the corner of the cage. They no longer feed and show signs of fatigue. Death occurs between 3 and 24 hours after administration of EALl. Table II shows the mortality of mice as a percentage and in probits, depending on the dose of EALl injected. For doses less than or equal to 300 mg/kg b.w., the mortality rate is zero (0%). However, for doses greater than 300 mg/kg b.w., the mortality rate increases with dose. At a dose of 1000 mg/kg b.w., the mortality rate is 100%.

Acute toxicity allowed us to obtain an LD_{50} of 439 mg/kg bw using the graphical method (Figure 1) of Miller and Tainter (1944) and 365 mg/kg bw using the calculation method of Dragstedt and Lang (1957).

Table I: Chemical composition of the aqueous extract of dry leaves of *Lophira lanceolata*

Compounds sought		Test or reagents	Result
Sterols and polyterpenes		Liebermann	+
Polyphenols		Ferric chloride	+
Flavonoids		Cyanidin	+
Saponosides		Vigorous shaking	+
Quinonic compounds		Borntraeger	+
Alkaloids		Dragendorff	+
		Bouchardat	+
Tannins	Catechins	Stiasny	-
	Gallic	Hydrochloric acid	+

(+): Presence of the compound

(-): Absence of the compound

Table II: Mouse mortality in percentage and probit units as a function of doses of aqueous extract from dry leaves of Lophira lanceolata administered intraperitoneally

Mouse lots	Number of mice tested per batch	Doses administered (mg/kg body weight)	Number of deaths per batch	Mortality (%	Mortality (Probit unit)
1	6	150	0	0	1.90
2	6	250	0	0	1.90
3	6	300	0	0	1.90
4	6	350	2	33,33	4.56
5	6	400	4	66,66	5.42
6	6	500	5	83,33	5.96
7	6	700	5	83,33	5.96
8	6	1000	6	100	8.71

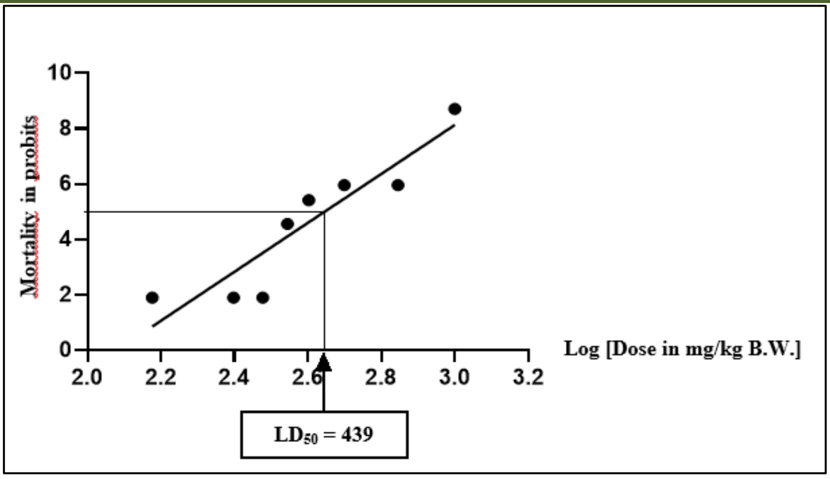

Figure 1: EALl toxicity curve in mice

III-2-DISCUSSION

Phytochemical tests carried out using the analytical techniques described in the work of [8], with the aqueous extract of dried leaves of Lophira lanceolata, revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, saponosides, quinone compounds, alkaloids and gallic tannins. However, catechin tannins were not found in the extract. These results differ from those of [11], who found the presence of the above-mentioned compounds, with the exception of alkaloids, in the dried fruits of Tetrapleura tetraptera. The richness of this aqueous extract in active chemical compounds could explain the use of Lophira lanceolata in traditional medicine to treat many diseases such as malaria, yellow fever, stomach aches, muscle pain and high blood pressure [12].

Indeed, several authors [13-18] have demonstrated the beneficial effects of phenols and flavonoids on the cardiovascular system of laboratory animals through their cardio-inhibitory, vasodilatory and hypertensive activities. According to [19], the hypotensive effects of the aqueous extract of Psidium guajaval L. (Myrtaceae) leaves are linked to the presence of polyphenols, flavonoids and tannins. It is therefore likely that the presence of these compounds in EALl is a serious indicator of pharmacological activities on the cardiovascular system.

Intraperitoneal injection of EALl made it possible to determine LD_{50} values of 375 mg/kg BW and 439 mg/kg BW, respectively, using the Dragstedt and Lang calculation method and the Miller and Tainter graphical method. According to [20], pharmacological substances with an LD_{50} between 50 and 500 mg/kg bw are highly toxic. Those with an LD_{50} between 500 and 5000 mg/kg bw are classified as moderately toxic substances. By this route of administration, EALl is therefore classified as a toxic substance. This is comparable to the aqueous extracts of the roots of Swartzia madagascariensis (Caesalpiniaceae) [21], the bark of the stems of Tamarindus indica (Ceasalpinaceae) bark [22] and Bridelia ferruginea Benth (Euphorbiaceae) bark [23], whose LD_{50} values are 59 mg/kg bw, 377 mg/kg bw and 429.14 mg/kg bw, respectively.

IV. CONCLUSION

The qualitative phytochemical study conducted on the aqueous extract of dried Lophira lanceolata leaves revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, saponosides, alkaloids, gallic tannins and quinone compounds, which are believed to be responsible for pharmacological effects.

Acute intraperitoneal (IP) toxicity in mice revealed that the aqueous extract of dried leaves of Lophira lanceolata (EALl) is highly toxic.

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