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Phytochemical Screening and Evaluation of Insecticidal Effect of Hydro-Ethanolic Extracts of Leaves and Bark of *Nauclea latifolia* (Rubiaceae) on Workers of *Macrotermes bellicosus* (Rambur, Isoptera) in the Laboratory

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Abstract **Original Research Article**

Pesticides are increasingly criticized for their harmful effects on the environment and the health of farmers by environmental protection organizations and farmers themselves. Faced with this, new strategies including the use of plant-based extracts are proposed. The objective of this study is to evaluate the insecticidal effect of two plant extracts on *Macrotermes bellicosus* workers. Thus, through phytochemical screening and direct toxicity test, the secondary compounds and the insecticidal effect of hydro-ethanolic extracts of the bark and leaves of *Nauclea latifolia* were respectively evaluated. Phytochemical screening revealed the presence of sterols, polyphenols, saponins, flavonoids and alkaloids in the hydro-ethanolic extracts of the bark and leaves of *N. latifolia.* On the other hand, it revealed an absence of tannins in both extracts. The direct toxicity test showed the efficacy of both extracts on *M. bellicosus* workers. The LD_{50} of the hydro-ethanolic extract of the bark of *N. latifolia* (49.5 mg/L) was 2.6 times lower than that of the hydro-ethanolic extract of the leaves (129.55 mg/L). Total mortality with the hydro-ethanolic extract of the bark with doses of 50 and 100 µl was obtained 72 hours after treatment, while with the hydro-ethanolic extract of the leaves, it was obtained 96 hours after treatment. Efficacy is raised with the hydro-ethanolic extract of the bark. The consumption test revealed the presence of veneer in both treated and control samples. Filter papers were consumed for both extracts in both treated and control samples extracts of the bark and leaves of *N. latifolia* are effective against *M. bellicosus* workers. They can be recommended for the control of termite pests after evaluation of their acute toxicity to mammals.
Keywords: Insecticide, *Nauclea latifolia,* Hydro-ethanolic, *Macrotermes bellicosus,* Phytochemical screening.

INTRODUCTION

After its independence, Côte d'Ivoire focused its economy on agriculture. This sector, with a diversity of crops, has enabled the country to rise to the rank of agricultural and middle-income countries. The diversification of agricultural products in rural Ivorian areas is one of the main strategies adopted by farmers to cope with food and agro-ecological constraints. Indeed, producers hope to improve their economic level while having enough to satisfy their food needs. However, agricultural losses due to harmful insects in Africa are around 25 to 30% [1]. Termites alone cause losses of around 20 to 45% depending on the crops [2]. According to [3] in Côte d'Ivoire, termites, especially the genus Macrotermes, cause enormous damage to crops such as rubber trees in the regions of Agboville and Daoukro [4] mentions termite damage to cocoa trees. As for [5] they report damage to mango trees. These losses severely penalize the market value of production and stored products, creating deficits that are largely filled by expensive imports of foodstuffs [6]. To reduce them to an economically acceptable level, certain substances are used, such as chemical pesticides, which include a range of services (insecticides, herbicides, fungicides). Since these pesticides are mostly composed of chemical substances, generally toxic, they have repercussions on producers, consumers and the increasingly threatened environment. The use of chemical insecticides also leads to an ecotoxicological disorder that is accompanied by a spectacular increase in the number of species that have become resistant [7]. The abuse of fertilizers and pesticides in cultivated fields in Côte d'Ivoire is damaging the water quality of Lake Buyo and its watershed [8]. Studies conducted in recent years have implicated pesticides in many diseases. In Burkina Faso, in a sample of 100 producers in the cotton-growing area of Gourma, 92% of those surveyed suffered from severe headaches [9]. Currently, plant-based insecticides are being studied to replace synthetic chemical insecticides in the field of phytoprotection. This interest has prompted our study and the following working hypothesis: Does *Nauclea latifolia,* which is well known in African pharmacopoeia, have insecticidal properties? The general objective of this study is to evaluate the insecticidal effect of hydro-ethanolic extracts of *Nauclea latifolia,* on the termite pest, *Macrotermes bellicosus.*

2. MATERIALS AND METHODS

2.1. Material

2.1.1. Study area

The study was conducted at the Félix HOUPHOUËT-BOIGNY University in Abidjan, Ivory Coast. The various tests were carried out at the former Endocrinology and Reproductive Biology Laboratory. The extractions were carried out at the former Animal Physiology Laboratory.

2.1.2. Vegetal material

The plant material consists of the leaves and bark of *N. latifolia,* harvested in the department of Tanda in August 2017. The choice of this plant is explained by the presence of secondary metabolites [10]. It is common in Côte d'Ivoire, therefore easily accessible and widely used in African pharmacopoeia. The leaves and bark were identified at the National Floristic Center (CNF).

2.1.3. Animal material

The animal material used in this study consists of *Macrotermes bellicosus workers.* They were used in this study because of their impact on many crops in Côte d'Ivoire [11]. They were collected at the University of Cocody, specifically at the National Floristic Center (CNF).

2.2. Methods

2.2.1. Preparation of hydro-ethanolic extracts of the leaves and bark of *Nauclea latifolia*

The leaves and bark of *N. latifolia* were dried for two and three weeks respectively for the leaves and barks, then ground by an electric grinder before being used for extractions. The powders obtained were used to prepare the extracts following the method of [12]. Thus, five grams (5g) of each powder obtained was dissolved in 100 ml of a hydro-alcoholic solution (70 ml of ethanol + 30 ml of distilled water) for 10 to 15 minutes in a mixer. Using a separating funnel, the hydroalcoholic phase and the deposit were separated. The hydroalcoholic phase was dried in a Venticell oven at 60 ° C for three days. The powders obtained constituted the hydro-ethanolic extracts.

2.2.2. Preparation of mother solutions of hydro-ethanolic extracts of the bark and leaves of *N. latifolia*

A 30% stock solution was prepared by mixing 30 grams of hydroethanolic extracts in 100 ml of distilled water. From this solution, doses of 10, 20, 50 and 100 µl were used for the different biological tests.

2.2.3. Phytochemical study of hydro-ethanolic extracts of the bark and leaves of *N. latifolia*

A qualitative phytochemical screening was carried out on the leaves and barks of *Nauclea latifolia*, in order to highlight the major chemical groups. The tests were carried out according to the technique of [13]. For each secondary metabolite, the strongly positive presence or not was assessed by the quality of the color.

The alkaloids were identified based on their solubility in water. Thus, their detection was made respectively by the tests with Dragendorff and Bouchardat reagents (iodine-iodine reagent) on the hydro-ethanolic extracts. The appearance of a red-orange precipitate (for Dragendorff reagents) and brown, red or black for Bouchardat reagent shows the presence of the alkaloids.

For saponins, the tests with potassium dichromate (1%) acidified with sulfuric acid (98%) and the foam made on the extract were used to identify them. On the one hand, the appearance of the dirty green color for the first test and the persistent foam following vigorous shaking for the second test testify to the presence of saponins.

Flavonoids were detected by the caustic soda test (1%) and hydrochloric acid test (1%) carried out on the extract. The formation of blue-greenish coloration indicates the presence of flavonoids.

Steroids were also identified by the Lieberman-Burchard reagent test (acetic acid + sulfuric acid) performed on the extracts. The appearance of purple and green coloration indicates the presence of steroids.

Tannins were detected using the Stiasny reagent test (1% ferric chloride). The formation of blue, blue-green, dark blue or green coloration indicates the presence of tannins.

Quinones were identified by the ammonia test (1%) performed on the organic benzene extract. The appearance of the pinkish-red color indicates the presence of quinones.

Phenols were detected by the ferric chloride ($FeCl3$ at 1%) and sulfuric acid (98%) test conducted on the ethanolic organic extract. The formation of the dark red color indicates the presence of phenols.

2.2.4. Direct toxicity tests

The direct toxicity test was carried out according to the protocol of [14]. This test measures the responses of termites to soil treated with the insecticide. Using a micropipette, doses of 10, 20, 50 and 100 µl of each solution were placed on the soil. After deposition and homogenization, the boxes were dried in the open air for one hour. Fifty (50) *Macrotermes bellicosus workers* were then introduced into this device. The extract solution dosed at 30 g/ml of distilled water was tested at the above doses. Each dose was repeated 4 times with 50 workers. A control is treated with distilled water. Worker mortality was determined every 2 hours after treatment for 24 hours. After 24 hours, mortality is determined in days.

2.2.5. Consumption test

The consumption test was carried out on termites according to the protocol of [14]. In this test, the effective dose of each extract is poured onto 4 cm2 pieces of Whatman® No. 4 filter paper using a micropipette. After the deposits, the pieces of paper are dried in the open air in Petri dishes for 1 hour. Then, the treated filter papers are placed in a Petri dish containing 3.5 g of soil previously moistened with 1 ml of distilled water. Fifty (50) *M. bellicosus* workers kept fasting for 24 hours are then introduced into this device. Each dose is repeated 4 times for each extract. Dead workers are counted after 24 hours. The surfaces of the filter paper consumed (mm2 / worker) are measured using Mesurim software version 7.1. The control boxes are treated with distilled water.

2.2.6. Data processing

2.2.6.1. Calculation of the mortality percentage

The mortality percentage (Pc) is calculated according to the ratio of the number of dead individuals observed to the total number of termites.

Pc = (observed mortality / total number of termites) x 100

2.2.6.2. Calculation of DL50

SAS Probit analysis was used using XLSTAT 2016 software to calculate LD50 values after 24 hours of treatment by direct toxicity.

2.2.6.3. Statistical analysis

For statistical analyses, XLSTAT 2016 software was used. Cumulative mortality (mean ± standard deviation) was calculated for each treatment. One-way ANOVA analysis of variance at the 5% threshold was used to perform statistical analyses. A p value < 0.05 indicates a significant difference. The Tukey multiple comparison test was used to compare means with each other.

3. RESULTS

3.1. Secondary metabolites of hydro-ethanolic extracts of leaves and bark of *Nauclea latifolia*

Phytochemical screening performed with the different extracts (leaf and bark extracts of *N. latifolia)* revealed an absence of tannin (catechuic and gallic) and a presence of sterol, polyphenol, saponoside and flavonoid in both *N. latifolia* extracts. These chemical compounds were more abundant in the hydro-ethanolic extract of the bark than in the leaf extract. Quinones were present only in the hydro-ethanolic extract of the leaves of *Nauclea latifolia* (Table I).

Table I: The different chemical compounds of the leaves and bark of *Nauclea latifolia*

Secondary compounds		Leaves	Bark
Sterols		+	+++
Polyphenols		+	+++
Quinones		+	-
Flavonoids		+	+++
Saponins		++	+++
Alkaloids	Dragendorf reagent	++	+++
	Bouchard reagent	++	+++
Tannins	gallic	-	-
	catechists	-	-

Very positive reaction: +++; Positive reaction: ++ Negative reaction: -

3.2. Insecticidal effect of hydro-ethanolic extract of *Nauclea latifolia* leaves on *Macrotermes bellicosus* workers

Four increasing doses (10, 20, 50 and 100 µl) of hydro-ethanolic extracts of *N. latifolia* leaves were tested on *M. bellicosus* workers. After 24 hours and 48 hours of treatment, the mortality rates obtained with the 50µL and 100µL doses were significantly higher than those obtained with the 10µl, 20µl and control doses. More than 50% of mortality was obtained after 72 hours of treatment with the 4 doses. Total mortality was reached 96 hours after treatment regardless of the dose (Table II).

Table II: Mortality rate of *M. bellicosus workers* as a function of doses of the hydro-ethanolic extract of *N. latifolia leaves*

Doses (µl)	Mean mortality (% ±Standard deviation)			
	24 hours	48 hours	72 hours	96 hours
Control	1.00± 0.58a	6.5± 1.50 h	17.5± 2.63 g	32±2.16i
10	2.5± 1.89 a	25± 3.317 b	81.5± 2.87c	100± 0d
20	8± 4.69h	23.5± 2.18 b	85.5± 2.38 c	100± 0d
50	19.5± 2.315g	39.5± 3.22 i	86± 1.72 c	100± 0d
100	20± 2,218g	40.25± 2.16 i	90.5± 1.84 c	100± 0d

Data are presented as mean ± SD. Tukey's test was used to make comparisons with controls. In the same column, means ± SD assigned the same letter are not significantly different at the 5% level.

3.1.3. Insecticidal effect of the hydro-ethanolic extract of the bark of *Nauclea latifolia* on the workers of *Macrotermes bellicosus*

Four increasing doses were applied to *M. bellicosus* workers. The mortality rate of workers after treatment with the hydro-ethanolic extract of the bark of *N. latifolia* was higher than that obtained in the controls. This rate obtained with the dose of 100 µL after 24 hours of treatment was significantly higher than those obtained with the doses 10, 20, and 50 µL which showed no difference with the control. After 48 and 72 hours of treatment, the mortality rates obtained with the doses 50 and 100 µl remained significantly high compared to those obtained with the doses 10 and 20 µl. Total mortality with the doses 50 and 100 µl were obtained 72 hours after treatment against 96 hours with the doses 10 and 20 µl (Table III).

Table III: Mortality rate of *M. bellicosus* workers as a function of doses of hydro-ethanolic extract of *N. latifolia* leaves

Doses (µl)	Mean mortality (% ±Standard deviation)			
	24 hours	48 hours	72 hours	96 hours
Control	7± 1 a	12± 2.4 a	25± 5 b	46.5± 2.75 e
10	11.5± 3.30 a	21± 2.147 b	97± 1,732 c	100± 0 c
20	13.5± 1.5 a	46± 3.89 e	98.5± 2.228 c	100± 0 c
50	14.5± 2.5 a	69.5± 2.36 i	100± 0 c	-
100	31± 2.38 e	85.5± 3,397 i	100± 0 c	-

Data are presented as mean ± SD. Tukey's test was used to make comparisons with controls. In the same column, means ± SD assigned the same letter are not significantly different at the 5% level.

3.1.3. The lethal dose (LD_{50})

The lethal doses (LD_{50}) calculated on the basis of the mortalities obtained after 24 hours of treatment with the hydro-ethanolic extracts of the leaves and bark of *Nauclea latifolia* were respectively 129.55 mg/L and 49.5 mg/L. The lethal dose of the bark extract is 2.6 times lower than that of the leaf extract. The hydro-ethanolic extract of the bark of *N. latifolia* is 2.6 times more effective than that of the leaves.

3.1.4. Consumption test

The mortality rates obtained with the hydro-ethanolic extracts of the leaves and bark of *N. latifolia* were respectively 42.5±4.27 and 31±1.848 while in the different controls this rate was respectively 4±0.5774 and 3±0.2887. The analyses show a significant difference in the mortality rate between the treated and the controls ($p < 0.05$). Soil veneers were observed both in the controls and in the treated with the different extracts. The consumed surface area of the filter papers treated with the hydro-ethanolic extract of the bark of *N. latifolia* is 34.97±2.35 mm^2 compared to 64.97 ±1.50 mm^2 in the control. With the hydro-ethanolic extract of *N. latifolia* leaves, the surface area consumed was 47.56±0.05 mm^2 compared to 82.17±1.21 mm^2 in the controls. The Pearson correlation test gives at the level of the hydro-ethanolic extract of *N. latifolia* bark r = 0.507; N=50; P=0.49 against r = 0.781; N=50/boxes and P=0.21 for the hydro-ethanolic extract of *N. latifolia* leaves. These values show that there is no correlation between worker mortality and product consumption ($p > 0.05$). The mortality of workers exposed to the products is therefore not linked to its consumption.

Table IV: Observed mortality of workers and surfaces of filter papers consumed after the consumption test with extracts of leaves and barks of *N. latifolia* during 24 hours of exposure

Treatment	Mortality (%)	Average surface area consumed	Veneer surface	Average surface area consumed
Barks (100µL)	42.5± 4.27 b	34.97 ±2.35	400 ±0.00	0.699
Control (0µL)	4± 0.5774 a	64.97 ± 1.50	400 ±0.00	1.299
P value	**0.0042**	**0.025**	-	-
Leaves (100µL)	31± 1,848 d	47.56±0.05	400 ± 0.00	0.951
Control (0µL	3± 0.2887 c	82.17 ±1.21	400±0.00	1.643
P value	0.003	0.045	-	-

Values followed by the same letters in the same cell are not significantly different at the 5% threshold (kruskal-wallis, XLSAT 2016). Mean of replicates ± standard deviation. N=50 Workers/boxes; paper surface = 400 mm2; (Pearson correlation r = -0.781; P=0.219 (for leaves) and r =0.507; P=0.497 (for bark). Ov: worker

4. DISCUSSION

Phytochemical screening of hydro-ethanolic extracts of leaves and bark of *N. latifolia* revealed the presence of sterols, polyphenols, saponins, flavonoids and alkaloids in both extracts. However, there was a total absence of quinones in the bark extract and tannins in both extracts. The results obtained from this study corroborate those obtained by [10]. Indeed, this author showed in Mali an absence of tannins in the aqueous extracts of the root bark of *N. latifolia* harvested in Kalouba and Kanadjiguila. On the other hand, the results obtained in this study are contrary to those obtained by [15, 16]. Indeed, these authors observed a high presence of catechic tannins in the aqueous extracts of the bark of the roots of *N. latifolia* respectively in Mali and Burkina. This difference is explained by the pedoclimatic conditions, the solvent, the extraction method used and also the organ of the plant used [17]. Phytochemical screening also revealed a high presence of sterols, polyphenols, saponins, flavonoids and alkaloids in the hydro-ethanolic extracts of the bark than that of the leaves.

The direct toxicity test performed shows the sensitivity of *M. bellicosus* workers to hydro-ethanolic extracts of *N. latifolia* leaves and bark. However, the hydro-ethanolic extract of the bark compared to that of the leaves seems more effective. Indeed, the different lethal doses calculated confirm this effectiveness of the bark. It was 2.6 times higher than that of the leaf extract. Plants represent a rich source of bioactive molecules that can affect growth, development, behavior and act as anti-feedants, toxins or growth regulators [18]. The insecticidal activity of the extracts is induced by the strong presence of secondary metabolites. It has been demonstrated by many authors. The alkaloids have repellent or anti-feedant properties against insect pests [19]. Through tests carried out on *Schistocerca gregaria*, these authors showed that alkaloids extracted from *Zygophyllum gaetulum* and *Peganum harmala* caused significant mortality in young adults. Flavonoids, for their part, alter moulting in insects, which causes their death [20]. These authors showed that exposure of *Callosobruchus chimensis* to flavonoids extracted from *Calotropis procera* (L) for 48 hours resulted in 100% death. The effectiveness of the hydro-ethanolic extract of the bark compared to the hydro-ethanolic extract is explained by the abundance of secondary compounds present in the former.

The results of the consumption test show through the plating observed on the filter paper that the plant extracts are not repellent to insects. This abundance of plating observed on the filter papers is explained by the absence of tannins and terpenoids in both extracts. Indeed, according to [21] Tannins have repellent properties. The filter papers with the extracts were less consumed than the control filter papers. This low consumption could be explained by the combined presence of phenols and alkaloids. According to [22] phenols attract insects and alkaloids have an anti-appetizing effect. These compounds are capable of generating free radicals that can have an influence on the biology of the insect [23].

5. CONCLUSION

Phytochemical screening of hydro-ethanolic extracts of leaves and bark of *Nauclea latifolia* revealed the presence of sterols, polyphenols, saponins, flavonoids and alkaloids, with a much higher intensity of these molecules in the bark than in the leaves. Hydro-ethanolic extracts of leaves and bark of *N. latifolia* were very effective on workers of *M. bellicosus.* However, the hydro-ethanolic extract of the bark was more effective than the hydro-ethanolic extract of the leaves. The LD50 obtained with the bark extract was 2.6 times lower than that obtained with the hydro-ethanolic extract of the leaves. Total mortality was reached for the barks from 72 hours of treatment with the doses of 50 and 100µL while with the leaf extract total mortality was obtained 96 hours after treatment.

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