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Evaluation of *Combretum micranthum* G. Don (Combretaceae) as a Biopesticide Against Pest Termite

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1. Introduction

In tropical agroforestry, although expensive and complex, chemical inputs are most widely used by farmers to control pest termites (1). Despite the many chemical treatments used, the damage and losses caused by termites remain high (2). With the intensification of agriculture and restrictions in the use of pesticides for their toxic effects on the environment and humans (3), we wanted to develop the local knowledge of plants with pesticidal properties. We have focused our work to search for biopesticides. Important prerequisites required before the use of a natural substance termiticide are: dose, lethal concentration, action on pest population, ways of action, transmission, assignment to the task of termites foraging and persistence of efficiency.

We chose the kinkeliba, *Combretum micranthum* G. Don (Combretaceae).

In West African Sahel, this shrub has shown potential in many medicines, construction, handicrafts, energy and agroforestry.

The virtues in traditional medicine and modern pharmacopoeia of Kinkeliba are well known. It possesses tonic, diuretic, antidiarrhea, antibiotic, antigonorrheal, antirheumatic and vermifuge (4, 5, 6). The leaves in decoction and chewed fresh leaves are used to treat coughs, bronchitis, hepatobiliary diseases, hepatitis and malaria (7). In combination with other plants, the leaves of Kinkeliba treat gonorrhoea. A leaf infusion is prescribed to prevent the contamination of leprosy (8). The root is prescribed in the diet of infertile women as energy medicine. The decoction of the root is anthelmintic and bactericidal for wounds.

Wood obtained from *Combretum* was in demand as it is mostly used as building materials for habitats and granaries in Sahelian country (9). Young stems, hard and soft, are used in basketry and carpentry (9). In the energy sector, the stems are used as firewood and charcoal

(10). In agroforestry, this shrub is highly resistant to fires and drought, is often found on abandoned agricultural land and is considered as a sterile or nearly sterile soil indicator (5). In biotoxicity, in-vitro research has shown that decoction and alcohol extract of leaf exhibits strong antimalarial properties against strains of *Plasmodium falciparum* (11). The methanol extract of the leaf showed potent acaricidal activity against the spider mite *Tetranychus urticae* (12). However, its pesticides properties and its use in protection against the attacks of insects and especially termites are less accurate. The wood of *C. micranthum* is sought as building material for its strength and its resistance to borers and termites in particular (9). However, other studies indicate that the roots of this shrub are very susceptible to termites' attack (10).

Also, we checked the basis of traditional use of this plant in the country for anti-termite and its mode of action on termites. The choice of this study is based on the following observation: Traditional methods from herbal products against insect pests are more and more abandoned to the profit of chemical inputs. The overall objective of this study is to propose an efficient, more accessible and cheaper alternative to farmers, without threat to the environment and human-being in order to reduce the use of chemical inputs.

This study aims to examine the question of the effectiveness of Kinkeliba as a termiticide, by a series of bioassays in the laboratory.

The working hypothesis is that the Kinkeliba *C. micranthum* can provide effective protection against attack by termite pests and be an alternative to the use of chemical inputs.

More specifically, it is to study:

- The direct toxicity by contact of various total aqueous, alcoholic and hexane leaf extracts of *C. micranthum* on adult workers of termite species, *Macrotermes bellicosus* Rambur;
- Whether the termite mortality may be the result or not of the consumption of the extract (consumption test);
- Whether the extract is toxic without being in contact with the termite (inhalation test);
- Whether the workers when are able to detect the product and avoid it (test of choice);
- Assess the lethal dose 50 (LD50) in 24 hours;
- Determine the active extracts of this plant's persistence of efficiency;
- Identify the main classes of secondary metabolites contained in the most active extracts of this plant.

2. Materials and methods

2.1. Study site

The work took place in Côte d'Ivoire, West Africa. The biological tests, extractions and chemical analysis of extracts were performed at the University of Cocody in Abidjan.

2.2. Plant material

Combretum comprises about 370 species, with approximately 300 species in Africa alone. We chose, in particular, the Kinkeliba *Combretum micranthum* G. Don (Combretaceae) (Figure 1).

It is a bushy shrub or creeper, top 2 to 5 m, but up to 10 m if hugs trees (6). Widely spread throughout Africa, the local plant is particularly dense on the cuirasses (6). This species is socio-economically important and frequently used in the Sahelian countries. It produces good firewood and charcoal (10). A study shows the operation impact of this wood in building homes and granaries in the Sahel region (9).



Figure 1. Stems with leaves of Kinkeliba *Combretum micranthum* G. Don

C. micranthum is highly ranked among the sahelians preferred plants for several basis criteria. Its wood is strong, durable, it does not rot, is resistant to termites and borers, the port is straight and easy to work. It is also appreciated in wicker basketry. Yet, production following natural seeding is most of the time compromised by climatic factors. This species breeds in very little by seeding, but so abundant by stump and natural layering, especially west of the Niger and southern Burkina Faso (13).

This medical plant has tonic, diuretic, antimalarial, antibacterial, antidiarrhea, antibiotic, antigonorrhoeal, antirheumatic and vermifuge properties (5, 7). It is more used in West Africa as an antimalarial drug and this antimalarial activity has been demonstrated by several authors. In-vitro research has shown that aqueous extract of leaf showing high antiplasmodial properties with an IC₅₀ inferior to 5 g/ml (7, 13) and against strains of *Plasmodium falciparum* resistant to chloroquine, at IC₅₀ values lower than that of *Azadirachta indica* (12). Alcoholic extract of leaf exhibits also strong antimalarial properties against strains of *Plasmodium falciparum* (11).

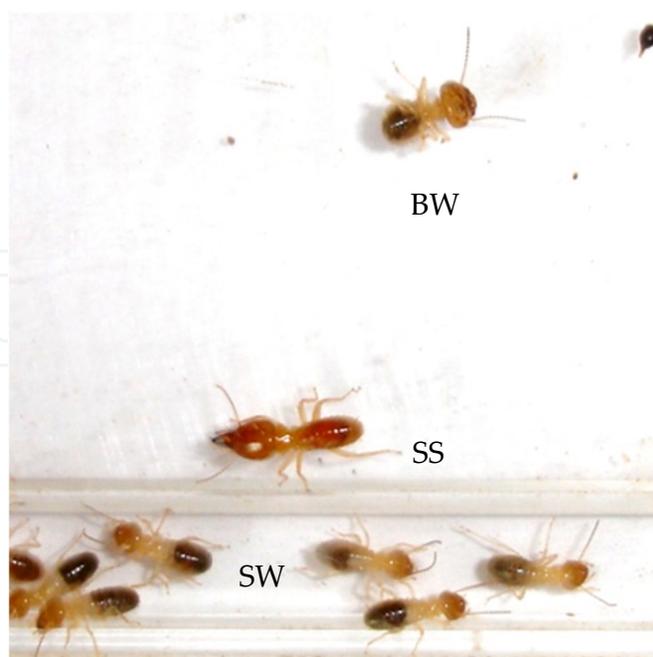
By cons, kinkeliba has not been systematically studied to determine its pesticides properties. The methanol extract of the leaf showed potent acaricidal activity against the spider mite *Tetranychus urticae* (12). Apart from its known particular resistance to termite attacks, we have no data about its anti-termite activities.

The leaves of the plant tested in its work come from Ouagadougou, in Burkina Faso.

2.3. Animal material

The termite is one of the greatest scourges of tropical agroforestry. Ten percent of termite species described to date are pests (13). Termites can cause damages to crops, buildings, pastures, forests and even non-cellulose materials such as electric cables. In many African countries, with the intensification of agriculture and restrictions in the use of pesticides, termite attacks are considerable. The most important damages are caused by species belonging to the subfamily of fungus-growing termites, the Macrotermitinae. Over 60% of destructive species belong to this subfamily (1). *Macrotermes bellicosus* Rambur 1842, formerly *Bellicositermes natalensis*, belongs to this Macrotermitinae. It was chosen for biotesting because of its impact on many crops in various countries of Africa and Côte d'Ivoire in particular. The attacks by this species involve very different cultures including groundnuts, maize, millet, sorghum, sugar cane, tobacco, tea, coffee, coconut and eucalyptus (1, 14). This termite is responsible of external attacks on the plants it covers by veneer of soil. It also penetrates the roots and stems of plants (14). The fungus of the genus *Termitomyces* with whom it develops a symbiotic relationship allows it to assimilate easily complex compounds such as wood cellulose or lignin, by predigested food on wheels.

This specie is also abundant in the study area: 1.85 nests per ha has been counted in the south and 1.7 in Lamto in the center of Côte d'Ivoire (15); 2.7 live nests per ha in Booro Borotou in the north west of Côte d'Ivoire (16). Density can reach 37.5 nests per hectare in the north-eastern Côte d'Ivoire (17). It epigeous slender and shaped cathedral nest, described by several authors whose (17), allows a large number of workers capture.



Big worker (BW); Small worker (SW) and Small soldier (SS)

Figure 2. Population sample of *Macrotermes bellicosus* Rambur

The workers studied in this work (Figure 2) come from the same colonies from the same nest on the campus of Cocody (Figure 3). Dimorphism between the size of the head of small and large workers in this specie has allowed separating in experiences.



Figure 3. Nest of *Macrotermes bellicosus*

2.4. Preparation of total extracts

The leaves of the plant are harvested in September 2009. They are spread out on sheets of paper, dried in the shade and then sprayed with an electric grinder. The total aqueous, alcoholic and hexane leaves extracts are produced by the method of extraction with successive conventional solvents of different polarity. This protocol allows a selective separation of the components of the organ. The collected solutions were evaporated by a rotary evaporator to obtain total extracts hexane (oil), methanol and aqueous dried under vacuum (18).

2.5. Calculate the rate (in %) performance in the extraction

The performance of each extraction fraction (R) is defined as the ratio of the amount of the extract (E) on the dry matter of the product (DM):

$$R = E / DM \times 100$$

2.6. Preparation of formulations

A stock solution of 10% was prepared from each dried extract with the corresponding solvent before being tested on 1 gram fresh weight of young workers (PO) of the adult termite *M. bellicosus* (or on 136 workers) at doses of 10, 20, 50 and 100 µl per box, so at four levels: 1, 2, 5 and 10 mg of extract / l.

2.7. The biological tests

Four bioassays were performed according to protocols described in (19):

1. The direct toxicity test measures the response of termites in soil treated with the insecticide;
2. The consumption toxicity test is to determine whether mortality of small workers is the result or not of insecticide consumption and specifies the importance of consumption of the product in this mortality;
3. The inhalation toxicity test is to investigate whether the fumes insecticide are toxic;
4. The avoidance test of the extract is to determine whether the workers, when a choice is given, are able to detect the product and to avoid it.

The tests are performed at the laboratory room temperature between 27 and 28 °C. Direct Toxicity and consumption test are made in a small rectangular plexiglass box, with 95 x 65 x 20 mm high, containing 7 grams of soil moistened with 2 ml of distilled water. Tests of inhalation and avoidance are performed in a large box of plexiglass 180 x 120 x 70 mm high, containing 17 grams of sieved soil and moistened with 5 ml of distilled water.

Using a micropipette, the doses are deposited either on land (for direct toxicity), or on pieces of Whatman paper No. 1 of 4 cm² (for tests of consumption, inhalation and avoidance). After deposition, the boxes are dried in the open air for 1 hour. Small workers of *M. bellicosus* are then introduced into these devices that are closed and do not allow air circulation. Each extract solution is tested with four doses previously mentioned. Each dose is repeated ten times for all tests. Each control box is treated with the corresponding solvent. The mortality of young workers was determined 24 hours after treatment. The LD50 is calculated. The surface of each Whatman paper covered by veneer and consume (mm²/worker) were measured daily with an ocular micrometer adapted to a magnifying glass. The amount of extract ingested per worker per day is calculated in ppm.

2.8. Persistence of efficiency (in days) of the extract the most active

In a small box of plexiglass 95 x 65 x 20 mm high containing 7 grams of soil moistened with 2 ml of distilled water, the soil is treated in the most effective dose of the extract. Small workers are removed and replaced by new every 24 hours for 7 days. Control box were treated with the corresponding solvent. Small dead workers are counted until the amount in the boxes in the testing and control is not significantly different. The mortality rate is calculated.

2.9. Calculation of mortality percentage

The mortality rate (Pc) is calculated using the ratio of individual dead observed on the total number of termites.

$$Pc = \text{observed mortality} / \text{total termites} \times 100$$

2.10. Chemical characterization tests for the secondary metabolites extracted from the plant

A phytochemical screening was performed on the leaves of *C. micranthum* to highlight the major chemical groups. The tests were performed using the technique described in (20). These chemical characterization tests, for the alkaloids from disclosure by the Dragendorff reagent, flavonoids with the perchloride test, saponins by the index of foam; tannins by reaction with ferric chloride at 1%, terpenoids by reaction with acetic anhydride - sulfuric acid.

2.11. Statistical analysis

Data collected during the biological tests are processed using the software Statistica (2001). The box plot, the estimated bootstrap, the nonparametric tests of Newman-Keuls and Kruskal-Wallis (at 5%) and correlation tests were implemented here. The LD50 is calculated by Probit analysis based on the mortalities obtained after 24 hours in different doses.

3. Results

3.1. Yield of successive extractions of the leaves of *Combretum micranthum*

The successive quantities extracted by each of the three solvents, expressed as a percentage of the total amount extracted, are higher in aqueous and alcohol solvents (respectively 9 and 8%) compared to the hexane solvent (3%).

3.2. Direct toxicity of extracts of *Combretum micranthum*

Three classes of extracts were prepared from the most efficient to the less active according to their toxicity (LD50) of the termite *M. bellicosus* stand. Topping the list is to find the alcoholic extract of leaves. This extract has greater toxicity than all other extracted plants. Secondly, we have to find the aqueous extract of leaves. However, the extracted hexane is less toxic (Table 1). The mortality of small workers total is obtained between 48 and 96 hours after treatment with the different extracts. When the toxicity of kinkeliba is compared to other plants having termiticides properties such as neem and papaya, tested under the same experimental conditions and the same species of *M. bellicosus*, the LD50 of alcoholic extract of kinkeliba leaves is 2.5 to 2.7 times lower than that of the alcoholic extract of neem and of papaya leaves (Table 2).

Extract	LD50 (mg/l)
Alcoholic leaf	3.69 ± 0.03 a
Aqueous leaf	6.49 ± 0.00 b
Hexane leaf	10.22 ± 0.03 c

Within a column values followed by same letters are not significantly different at the 5% according to the Kruskal-Wallis ($p < 0.05$, ANOVA - analysis of variance). LD50: 50 lethal dose, quantitative indicator of the toxicity of a substance.

Table 1. LD50 values of *Combretum micranthum* extracts tested on small workers of *M. bellicosus*.

Alcoholic leaf extract	LD50 (mg/l)
<i>C. micranthum</i>	3.69 ± 0.03 a
<i>A. indica</i>	9.52 ± 0.68 b
<i>C. papaya</i>	10.32 ± 0.03 c

Within a column values followed by same letters are not significantly different at the 5% according to the Kruskal-Wallis ($p < 0.05$, ANOVA - analysis of variance). LD50: 50 lethal dose, quantitative indicator of the toxicity of a substance.

Table 2. LD50 values compared of different plants tested on small workers of *M. bellicosus*.

3.3. Persistence of efficacy (in days) of different extracts of *Combretum micranthum*

The persistence of efficacy is the duration of the residual activity which generates a sample rate of higher mortality in the control at 5%. Leaf extracts of kinkeliba (aqueous and alcoholic) have persistent efficacy among the lowest (between 3.4 and 3.6 days). The hexane extracted from the leaves is the best (4 days).

3.4. Action of the most active alcoholic extract of leaves of *Combretum micranthum*

a. Toxicity consumption

Papers witnesses and papers treated with the extract are visited by the termite, as shown by the veneer of soil. By cons, the termite does not consume treated papers. In contrast, the average area of paper consumed in the control is 1.6 mm² or 0.012 mm² / worker (Table 3).

The amount of extracts ingested by the workers at the end experience is zero. However, the percentage of mortality obtained from the workers treated are significantly higher than that of the control ($F = 18.455$, $p = 0.000$, $N = 50$). So there is no correlation between the mortality of workers and the consumption of the extract for 48 hours at doses tested ($R = 0.483$, $N = 50$, $p = 0.262$). The toxic effect of the alcoholic extracts of leaves of *C. micranthum* is not related to its ingestion by the termite. Other ways of action of the product will be specified by the following tests.

Dose of alcoholic extract of leaf of <i>C. micranthum</i> (mg/l)	cumulative surface veneer (mm ² /w)	cumulative surface of paper consumed (mm ² /w)	cumulative quantity of extract ingested (ppm/w)
0	0.73 ± 0.07 b	0.01 ± 0.01 b	0 a
1	0.97 ± 0.31 b	0 a	0 a
2	0.44 ± 0.20 a	0 a	0 a
5	0.36 ± 0.21 a	0 a	0 a
10	0.47 ± 0.10 a	0 a	0 a

Measurements made at the time corresponding to 50% mortality of the workers. Average of 10 repetitions ± SD (N = 50). Within a column, values followed by same letters are not significantly different at the 5% according to the Kruskal-Wallis ($p < 0.05$, ANOVA, analysis of variance). Surface Veneer ($F = 13.171$, $p = 0.000$, $N = 50$); surface consumed ($F = 9.486$, $p = 0.000$, $N = 50$); quantity ingested ($F = 0$, $p = 0.000$, $N = 50$). w = worker.

Table 3. Effect of alcoholic extract of leaves of *Combretum micranthum* on the harvesting of *Macrotermes bellicosus* workers (consumption test)

b. Inhalation toxicity

The extract does not act by ingestion; it is interesting to see if it can be toxic without being in contact with the termite. At doses of 2, 5 and 10 mg / l, the inhalation of the extract results in a mortality rate significantly higher than that obtained in the control at 24, 48 and 72 hours after treatment ($F = 22.832$, $p = 0.000$, $N = 50$).

c. Avoidance of the extract

When a choice is presented, control papers and treated papers with the extract are visited by the termite, as shown by the veneer of soil. But no treated paper is consumed by the workers of *M. bellicosus*. By cons, the control paper placed nearby is (Table 4).

Dose of alcoholic extract of leaf of <i>C. micranthum</i> (mg/l)	Cumulative surface veneer (mm ² /w)	Cumulative surface of paper consumed (mm ² /w)
Control	1.12 ± 0,15 c	0.03 ± 0 c
1	0.09 ± 0.02 b	0 a
Control	1.10 ± 0,88 c	0.01 ± 0b
2	0.02 ± 0.02 a	0 a
Control	0.75 ± 0,09 c	0.01 ± 0 b
5	0.02 ± 0.03 a	0 a
Control	0.74 ± 0.58 c	0.01 ± 0 b
10	0.03 ± 0.03 a	0 a

Measurements made at the time corresponding to 50% mortality of the workers. Average of 10 repetitions ± SD (N = 80). Within a column, values followed by same letters are not significantly different at the 5% according to the Kruskal-Wallis ($p < 0.05$, ANOVA - analysis of variance). Surface Veneer ($F = 14.259$, $p = 0.016$, $N = 80$) - surface ingested ($F = 22.563$, $p = 0.000$, $N = 80$).

Table 4. Effect of alcoholic extract of leaves of *Combretum micranthum* on the harvesting of *Macrotermes bellicosus* workers (avoidance test)

3.5. Phytochemical screening of the plant

The leaves of the Combretaceae *C. micranthum* have all the desired compounds (alkaloids, flavonoids, tannins and terpenoids) except saponins. Analyses indicate that the most active alcoholic extract contains phenolic compounds (flavonoids and tannins) and alkaloids. It has no terpenoids (Table 5 and 6).

Extract	Alkaloid Dragendorff	Flavonoid Perchloride	Tannin Chloride Ferric	Terpenoid Acetic anhydride - Sulfuric acid	Saponin Index of foam
Leaf					
Aqueous solvent	+	+	+	+	-
Methanolic solvent	+	+	+	-	-

Table 5. Main classes of secondary metabolites in *Combretum micranthum*

Compound
Proteins
Fat
Carbohydrates
Salts and other minerals
Aliphatic alcohols (C16 au C30)
Phenolic compounds
Alkaloids
Organic acids
Gallic acid
Acid amyrière
Triterpenoid
Beta-sistostérol
Potassium nitrate

(Source: 6, 25, 26)

Table 6. Some chemical data on the leaves of *Combretum micranthum*

4. Discussion

The leaves of the kinkeliba have interest termiticides properties. This result sets a different scope of kinkeliba still underexploited in the context of an overall recovery of natural vegetation. Alcoholic extract of leaves is the most toxic on the termite. The hexane extract (oils) of leaves is less toxic (LD50 2.8 times lower).

The performance of the alcoholic extract of this plant has been mentioned by other authors as potent acaricidal activity against the spider mite *Tetranychus urticae* (12). On insects, we have few other references. In other fields, the alcoholic extract from this plant was successful such as antimalarial drugs (21). According to these authors, this polar solvent is able to extract a wide range of chemicals substances as in traditional mixtures and foster its effectiveness (21). These results confirm also the effectiveness of methanolic leaf extract of medical plants with anti-termite properties and termite-resistant formulations (24).

The extract of leaves, especially the alcoholic extract, is more active in kinkeliba than in neem and papaya. At high doses, this extract has a linear dose-effect. However, these two last plants have other total extracts more efficient on termite than the kinkeliba which are the alcoholic extract of papaya seeds (22) and the aqueous extract of neem seeds (23).

The alcoholic extract constituents of kinkeliba leaves help to explain its potential insecticide action. The extract contains phenolic compounds (flavonoids and tannins) and alkaloids. This result is consistent with the compounds isolated from this plant by other authors (6, 25, 26). These authors state that flavonoids are myricetin glycosides and vitexin; the alkaloids are the stachydrine, 4-hydroxystachydrine and choline (12, 26). A new series of type of piperidine flavan alkaloids, kinkeloids A, B, C, and D have been recently identified (27).

The insecticidal effects of these components were mentioned by several authors. Phenolic compounds have both pesticides and fungicides properties (28). The tannins possess insecticidal, larvicidal and repellent properties (29). Alkaloids induce toxic effects on insects (30, 31). The extracts are total, so several other substances can also induce a toxic effect on the termite.

This kinkeliba alcoholic extract of leaves affect the mortality of the termite, has an extraction yield among the best and persistence of a lower efficiency than the other extracts. This extract is more easily degradable. Its use in field can reduce the potential risk of residual pollution and can be much more environmental friendly as synthetic drugs. The results also show that the contact and inhalation are the two essential ways to the effectiveness of this extract on the termite. Toxic substances from the extract can therefore penetrate through the cuticle and the spiracles of the insect. The alcoholic extract does not act by ingestion and is an inhibitor of food intake by the termite following the experimental doses used. But, inhibition of food intake by the extract is exerted only on the consumption of treated paper and not that of the untreated paper placed nearby. These results indicate that the extract can be efficiently used for soil treatments to protect a food substrate against *M. bellicosus* infestation. However the use of bait in this extract against this insect can cause problems.

C. micranthum leaves could be effective in providing a barrier to prevention and effective control of termite infestation in agronomy cropping systems. With the used doses, the extracts have no effect about 4 days after treatment. The persistence of low efficiency of the extract can be explained. The termite may also be able to detoxify toxic compounds like many other herbivores by producing enzymes that are involved in the mechanisms of metabolic detoxification of organic pollutants (32).

5. Conclusion

Simple techniques for managing termite attacks in the field by leaf extracts like kinkeliba can be more practiced by farmers. The application rates are achievable (in the laboratory, the experimental dose of 1.6 kg of extract per hectare gave satisfactory results). The identification of active compounds on the termite, capabilities of termiticide formulations, the factors that may compromise the effectiveness of kinkeliba on the ground and its impact on soil, insects other than termites are to be assessed in a natural environment.

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