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Research Article

Phytochemical and anti-termite efficiency study of bark extracts of *Guibourtia Tessmanii* (kevazingo) from Gabon

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ABSTRACT

Our work focused on the study of the extract rates, phytochemical and anti-termite tests of the bark of *Guibourtia tessmannii* from Gabon commonly called Kévazingo on two levels, the buttress and at a height of 6 m. Extraction of the bark powders was carried out using the cold maceration method with tricholroethylene, acetone, ethanol and water. The phytochemical screening made it possible to highlight groups of chemical families present in the extracts. Anti-termite activity was evaluated in wild termites of the genus Isoptera. The following extracts yields were obtained: 17.11% for the buttress and 13.42% for the height at 6 m. Phytochemical tests revealed in the extracts the presence of alkaloids, polyphenols, sterols, tannins, reducing compounds, flavonoids, saponins, anthraquinones. The results obtained indicated that the anti-termite activity varies with the different parts of the bark studied, the extraction solvent and the concentration (50/50) and (25/75) of the extracts used. The extracts at the concentrations (50/50) showed a slightly better anti-termite activity compared to (25/75) and the buttress kevazingo showed the strongest anti-termite activity for the aqueous extract with a survival rate of 0 % after 2 days.

Keywords: Extracts, Anti-termite activity, *Guibourtia tessmannii*, Phytochemical screening

INTRODUCTION

Guibourtia tessmannii commonly called Kévazingo is one of these famous species from Central African countries (Gabon or Cameroon). The abandonment in forests of waste of *Guibourtia tessmannii* in the form of a crown rich in bark and branches of variable diameters, or of slabs, chips [1]. Sawdust in waste reception centers constitutes a considerable financial loss and a risk of losing a deposit [2]. Biomolecules of interest from this essence widely used in the treatment of several diseases [3]. It has been observed that in Gabon, a large number of potentially recoverable waste is left in the openings after felling and during shaping in the yard. In addition, the literature reports that 400 million m^3 of woodwasteis produced in Gabon Nze Nguéma (2010) [4]. These wastes are often thrown away in the form of debris or used by the population as firewood, artisanal charcoal making or sometimes by the boilers of wood processing units. Thus, the issue of recycling tropical wood waste from logging and industrial processing aimed at the sustainable development of the forest economy in the countries of the Congo Basin is the subject of numerous studies.

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Wood chemicals, among others, offer new perspectives in this area and allow access to new markets [5]. Indeed, bioactive molecules or extractable molecules from renewable resources such as wood are subject to a great deal of research and development in the pharmaceutical, agrifood and cosmetic fields. Likewise, certain wood species are naturally resistant to termite attacks due to their high content of extractable compounds which are part of their natural defense systems. These studies are generally limited to extracts from temperate species. However, tropical woods have higher levels of extractable molecules, which further reinforce the interest in upgrading them. This valuation of wood represents a major opportunity on the economic level, by the creation of new jobs constituting a factor of growth. In this context, it seemed interesting to us to explore the diversity of chemical compounds present in the bark of *Guibourtia tessmannii* and to study some of their valuable properties in the fine chemicals industry as sources for the development of new animal products termite, etc. For this we determined (i) the extractable content, then (ii) the phytochemical screening and finally (iii) study the anti-termite activity of these extractables on the wild termites of Gabon of the genus Isoptera [6].

MATERIALS AND METHODS

Termites

Handling and harvesting of wild termites of the genus Isoptera was done on the day of the experiments so that removal from their natural habitat would not have a significant influence on their mortality. The termites are put in a small empty butter pot with the clods of earth and its lid perforated with a heated needle. The termites are immediately taken to the laboratory of the Iphamétra (Pharmacopoeia Institute of Traditional Medicine) in Libreville (Gabon) [7].

Plant Material

The bark sampling phase was carried out according to a previously established procedure. The mature kevazingo bark was collected using a machete on April 8, 2020. The kévazingo bark collection in Kango, in the province of the Estuary (N 00 ° 02'44.5 " and E 010 ° 17'23.7 ") was carried out at two heights: at the level of the buttress and six meters from the ground. The bark was cut into small pieces using a pair of scissors [8]. They were then sent to the Multidisciplinary Science Laboratory (LaPluS) of the Ecole Normale Supérieure de Libreville where they were dried in the open air for three weeks.

Extraction

The extraction of the chemical elements began with the fragmentation and grinding of the bark. The bark was first cut with scissors to facilitate crushing; the fragments obtained were then introduced in small quantities into a Retsch-type mill, with Iphametra. This is necessary to obtain a suitable grain size with a fraction between 1 and 2 mm, thus corresponding to the requirements of ASTM No. 1105 (1996) in terms of grain size to quantify the rate of wood extract. Finally, the powder collected was sieved and stored in the dark in glass jars, closed until the time of chemical tests. The chemical tests began with the determination of the extract levels. We opted for the successive extraction by maceration of the bark powders using solvents of increasing polarities (Table 1).

Table 1. Maceration of the bark powders.

Solvents	Dielectric constant
Trichlorethylene	3,4
Acetone	20,7
Ethanol	24,5
Distilled water	78,5

The bark powder was placed in a 1/10 ratio (10 g of dry matter in 100 ml of solvent) in a 250 ml glass Erlenmeyer flask, closed with a rubber stopper covered with aluminum foil [9]. The aluminum thus prevents the solvent from coming into contact with the rubber, which prevents possible contamination. In addition, the Erlenmeyer flasks were covered with aluminum foil to prevent degradation of photosensitive molecules. The mixtures were subséquent placed under stirring on a stirrer (of the PIERRON type) for 24 h. After 24 h, the mixtures were separated by vacuum filtration through a Whatman filter in a Buchner type funnel. With regard to the extraction in aqueous medium, the water is evaporated by lyophilization. The extracts were then dried in a flask (pre-weighed) on a rotary evaporator, in a 40°C bath and were then placed in an oven at 40°C for about 24h, until that a constant mass is measured. The extracts are then stored in the refrigerator in closed bottles and covered with aluminum foil for the next tests. We can thus calculate the percentage of extracts relative to the initial mass of bark powder using the following equation (1):

R("%")="Mext"/"Mech"×100

Where:

R: Yield of extracts in%

M_{ext}: Mass of the extract after evaporation in grams

M_{ech}: Anhydrous mass of the bark powder sample in grams.

Phytochemical Screening

The reagents used to carry out the phytochemical screening of the extracts were prepared and used according to the protocols described by. All the different tests were carried out in triplicate. For alkaloids, 10 ml of extract was placed in a test tube and then a few drops of Dragendorff's reagent solution were added. The appearance of a red-orange colored precipitate indicated the presence of the alkaloids. For polyphenols, 2 ml of extract was placed in a test tube and then a few drops of the 2% ethanolic ferric chloride solution were added. The appearance of a blue-blackish color indicates the presence of polyphenols. Sterols and terpenes were demonstrated by placing 2 ml of the extract in a test tube and then adding a few drops of concentrated sulfuric acid [10]. The appearance of a purple coloration indicates the presence of terpenes and a green coloration indicates the presence of sterols. The presence of tannins was demonstrated by adding to 1 ml of extract, 1 ml of distilled water and 1 to 2 drops of $FeCl_3$ solution (iron perchloride or iron (III) chloride) diluted to 1%. The appearance of a dark green color indicates the presence of tannins. For the reducing compounds introduced, 2 ml of the extract in a test tube, then 2 ml of Fehling's liquor is added. The whole was then put in a boiling water bath for 8 minutes [11]. The appearance of a brick red precipitate indicated the presence of reducing compounds. For flavonoids, 1 ml of extract was placed in a test tube, then 1 ml of hydrochloric acid, 1 ml of isoamyl alcohol was added and then some magnesium shavings were added. The appearance of a pinkish-orange color indicates the presence of flavonoids. The saponosides were identified by introducing 10 ml of each extract into a test tube and then vigorously shaking with a vortex for 15 seconds. The tube is left to stand for 15 minutes. The appearance of persistent foam indicates the presence of saponins. For anthraquinones, to 2 ml of each extract is added 1 ml of 10% NH_4OH (basic aqueous ammonia solution). After shaking, the appearance of a purple color indicates a positive test [12].

Anti-termite Activity

The protocol described for the anti-termite tests was inspired by those described by. Only the acetone, ethanolic and aqueous extracts were tested. Two concentrations were tested against wild termites for the screening tests, mass ratios of (50:50) and (25:75) (extract: extraction solvent) in mg. 70 µl of solutions were impregnated on Whatman filter papers before being exposed to termites. The papers impregnated with the various solutions to be tested were dried in the open air, (27°C/75% relative humidity (RH) for 2 hours [13]. The tests were carried out in Petri dishes (9 cm in diameter) where 15 g of wet sand (1 volume of water for 4 volumes of sand) were placed at the periphery. The Whatman extracts soaked papers were placed on a plastic rack in the middle of the petri dish. Puis 20 termites sauvages ouvriers ont été ajoutés à chaque dispositif de test sans aucune possibilité d'alimentation

pour vérifier la survie des termites. Pour chaque concentration testée, nous avons préparé trois boîtes de Pétri avec extrait et trois boîtes de Pétri avec solvant d'extraction sans extrait comme témoin. Petri dishes were stored in the dark at 27°C, 75% relative humidity for 12 days. At the end, the samples were cleaned and air dried. These devices were monitored regularly throughout the trial. The test is then stopped when all of the termites in the boxes tested have died. Motality and daily survival rates were respectively calculated according to equation (2) and deduced according to equation (3). The results of the screening tests were presented in the form of curves of survival rates as a function of day and time of incubation in order to facilitate interpretations [14]. "Mortality rate (%) = "Number of termites alive at the end of the test"/"Number of termites used for the test" × 100 (2)" Survival rate (%)=100%-rate mortality (%) (3)

Statistical Analysis

XLSTAT 2019 was used, and the results were known as the main values representing the mean of the repetitions ± the standard deviations. The values are statistically significant at $\rho<0.05$ [14,15].

RESULTS

The Extracts Contents

Table 2. Extracts contents obtained by maceration.

Extracts contents (average of three tests ± standard deviation)		
	Extracts contents (%)	
Solvant	E_1KC	E_2KC
Trichloroéthylène	0.79 ± 0.11	1.0 ± 0.11
Acetone	9.92 ± 0.14	6.82 ± 0.13
Ethanol	6.23 ± 0.07	5.39 ± 0.12
Water	0.17 ± 0.07	0.21 ± 0.01
Total	17,11	13,42

E1KC: sample 1 Kévazingo from Kango collected at the buttress and E2KC: sample 2 Kévazingo from Kango collected at 6 m [16,17].

In general, the results obtained in this study concerning the extraction yields indicate that the rate of extractables vary from one solvent to another, in the two samples of kevazingo bark, the base and therefore the buttress up to 6 m [18]. Acetone extraction rates are higher in samples E1KC, E2KC. The rates of extracts obtained with trichlorethylene and with water are the lowest regardless of the type of sample [19]. Indeed, for trichlorethylene, the first solvent used during the successive extraction is the least polar solvent and therefore dissolves the phenolic compounds less. Non-polar solvents mainly extracted from nonpolar substances while polar solvents dissolve polar compounds such as polyphenols [20]. The successive extraction, which combines non-polar and polar solvents, allows the extractables to be partitioned into different fractions, facilitating subsequent analyzes and the sum of the extracts with each solvent gives an idea of the overall extract content of the bark [21]. The overall extract rates of the different parts studied vary from 17.11% to 13.42% respectively for the buttress and the height at 6m (Table 2). The results showed that the content of total extracts varies depending on the diameter and height of the trees (the level of extractables decreases with height and increases with diameter) [22]. These results do not differ from most of those obtained by other authors. Indeed, rather obtained significantly higher extraction yields for Eucalyptus in the wood at the base of the trunk compared to the rest of the tree [23]. In addition, numerous studies carried out on several tropical species have shown very variable extract rates, sometimes with high levels of 20 to 22% for certain woods (Tables 3,4) [24].

Table 3. Phytochemical screening (E1KC).

Compounds	**Solvents**			
	Trichloroethylene	**Acetone**	**Ethanol**	**Water**
Alcaloïdes	+++	+++	+++	++
Polyphenols	++	+++	+++	++
Sterols	+	-	-	-
Tannins	-	+++	+++	+++
Reducing compounds	-	+++	+++	-
Flavonoïdes	-	+++	+++	-
Saponines	-	-	-	+++
Anthraquinones	-	+++	+++	+++
Coloring: +++: very intense, ++: moderately intense, +: not very intense, -: absence				

Table 4: Phytochemical screening (E2KC).

	Solvents			
Compounds	**Trichloroethylene**	**Acetone**	**Ethanol**	**Water**

Alcaloïdes	+++	++	+++	++
Polyphenols	-	+++	+++	++
Sterols	+	-	++	-
Tannins	-	+++	+++	+++
Reducing compounds	-	+++	+++	++
Flavonoïdes	-	+	+++	-
Saponines	-	-	-	+++
Anthraquinones	-	+	++	+++
Coloring: +++: very intense, ++: moderately intense, +: not very intense, -: absence				

The set of phytochemical tests (Tables 3 and 4) that we performed on the different parts of the bark indicates the presence of alkaloids, tannins, polyphenols, reducing compounds, flavonoids, saponins and anthraquinones as the predominant compounds [25]. Conversely, sterols are the least common. These compounds have multiple therapeutic properties. These results support the use of Guibourtea tessmanii in traditional medicine, in the treatment of several pathologies [26]. Our phytochemical screening results are in line with those obtained by Sima obiang et which demonstrated the presence of saponines, tannins, phenols and flavonoids, alkaloids, reducing compounds, anthraquinone, sterols in the bark of barks of Coula edulis Baill, Pseudospondias longifolia Engl and Carapa klaineana Pierre from Gabon [26]. The presence of all classes of compounds in tropical timber has been reported in the literature, when studying extractables from some tropical species, found lipophilic compounds, mainly fatty acids and hydrophilic compounds (phenolic acids, flavonoids, sterols (Figure 1,2) [27].

Anti-termite Activity

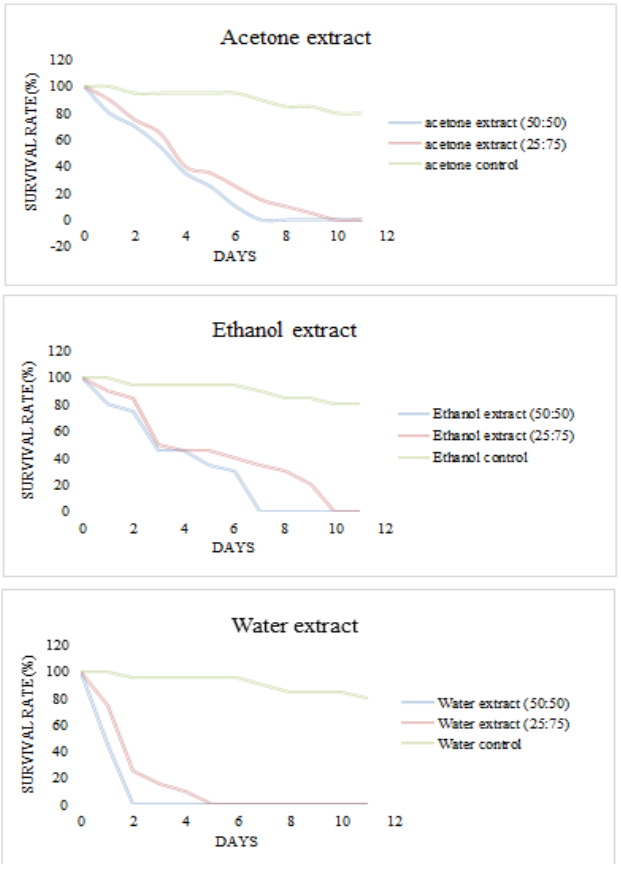

Figure 1. Effect of extracts from the E1KC sample on the daily survival rate of termites.

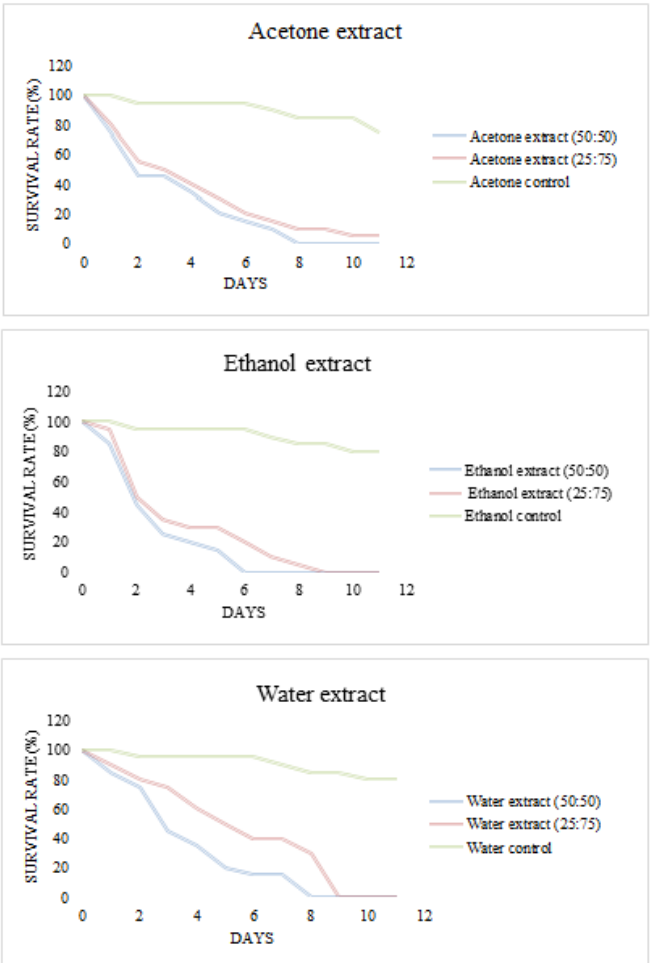

Figure 2. Effect of extracts from the E2KC sample on the daily survival rate of termites.

The results obtained (Figure 1,2) indicate that the anti-termite activity varies with the different parts of the bark studied (heights), the extraction solvent and the concentration of the extracts used as often. Reported in the literature [28]. Overall, the extracts at concentrations (50/50) exhibit slightly better anti-termite activity compared to (25/75). The strongest anti-termite activities were recorded with water extract for the kevazingo Kango buttress with a survival rate of 0% after 2 days, explaining the concentration of the extracts at this level. Diluting the solutions to a concentration of (25/75) increases the survival rate [29]. These results demonstrate that the control paper impregnated only with water or acetone has no effect on the behavior of termites as shown by the survival rates (Ts>12 days with 80%) at the end of the trial. Basically all papers impregnated with extracts showed a protective effect of whatman paper, therefore strong resistance against

termites. For each of these extracts, all the termites died before the end of the test. However, the use of less concentrated solutions still resulted in a 0% survival rate, hence anti-termite activity. Previous analyzes, in particular phytochemical screening, have made it possible to identify secondary metabolites such as polyphenols, tannins, flavonoids and anthraquinones, making it possible to explain the good anti-termite activity of these extracts [30]. The results are in agreement with the literature which explains that tannins are known to be strongly astringent verified that the high mortality of insects treated with condensed and hydrolyzable tannins appears to be due to the toxic properties of these compounds and not to inhibition of digestion. Phytochemical screening revealed the presence of flavonoids in these extracts which are potential termite control agents [31]. Other previous studies have also reported their anti-termite activity and have shown that flavonoids such as catechin interact with the ecdysone receptor of termites. Due to the ability of flavonoids to bind to ecdysone receptors, flavonoids can affect other biological systems in termites [32]. Hypothesized that termites detected and avoided woods containing the extracts rich in antioxidant molecules because they could interfere with the digestion of lignocellulose by termite symbionts. Anthraquinones are very present in the ethanolic and aqueous extracts for the sample at 6 m and very abundant in the acetone, ethanolic and aqueous extracts for the buttress or certain quinones such as 2-methylanthraquinone are repellent against termites, others such as 7-methyljuglone and its derivatives have anti-termite activities. These results explain the importance of phenolic compounds in the resistance of wood to termites [33].

CONCLUSION

This study looked at the variability in the height of the extract rates from the bark of Kévazingo. On the other hand, the elucidation of the families of chemical compounds likely to be active by phytochemical tests and the evaluation of their anti-termite properties. As a result of this work, we have achieved a large number of results. Thus, it turns out that the results showed extractable levels which varied from the buttress to the height of 6 m and according to the type of solvent used. The total extractable level of the samples studied is: E2KC (13.42%) and E1KC (17.11%). Photochemical screening revealed the presence of different groups of molecules such as polyphenols, alkaloids, sterols, tannins, reducing compounds, flavonoids, saponins, anthraquinones. The results obtained indicated that the anti-termite activity varies with the parts of the bark studied (heights), the extraction solvent and the concentration of the extracts used as often reported in the literature. In addition, the extracts at concentrations (50/50) showed slightly better anti-termite activity compared to (25/75) and buttress kevazingo showed the highest anti-termite activity for the aqueous extract with a higher level of anti-termite activity. 0% survival rate after 2 days.

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