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CHEMICAL COMPOSITION AND TERMITICIDAL ACTIVITY OF *Khaya ivorensis* STEM BARK EXTRACTS ON WOODS

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In memoriam of Dr. Thomas C. MANNES

ABSTRACT

The genus *Khaya* is extractives rich, but the extractives in the stem bark are among the most abundant, accessible and utilised materials in Nigeria. These extractives are of considerable interest for wood protection because of their pesticidal properties. In this study, the chemical constituents of *Khaya ivorensis* stem bark and their termiticidal activity were investigated on *Triplochiton scleroxylon* and *Vitex doniana* woods. Spectrophotometric and Forlin-Ciocalteu analyses showed that *Khaya ivorensis* stem bark contained total: alkaloids 38,98±0,02 mg/g, flavonoids 0,37±0,02 mg/g, phenol 50,62±0,11 mg/g, saponins 156,31±0,76 mg/g, and tannins 59,95±0,54 mg/g. Field tests demonstrated that the extract from *Khaya ivorensis* stem bark had significant termiticidal activity at tested concentrations compared to the control. The results indicated that *Khaya ivorensis* stem bark has potential as a natural agent for termite control.

Keywords: Bioactive compounds, chemical analysis, phytochemical extracts, *Triplochiton scleroxylon*, *Vitex doniana*, wood treatability.

INTRODUCTION

The genus *Khaya* (Family: Meliaceae) is rich in extractives throughout the plant, but these extractives are primarily concentrated in the stem bark (Olayinka *et al.* 1992, Iwu 1993). These extractives are of considerable interest for wood protection because of their indigenously known pesticidal properties (Ademola *et al.* 2004, Agbedahunsi *et al.* 2004). *Khaya ivorensis* (Oganwo, Yoruba Nigeria) and its congeners have a long traditional history of pesticidal uses including control of human intestinal roundworm infestations (anthelmintics) (Stephen *et al.* 2009, Lawal *et al.* 2010, Olusola and Oyeleke 2015) and protective activities against skin diseases usually caused by fungi and bacteria (Olusola and Oyeleke 2015, Adedeji 2016). They have also been used to protect stored cassava/yam chips against pests (Babajide *et al.* 2007, Loko *et al.* 2013).

Leaf extracts from *K. ivorensis* showed excellent antifungal activities against stored foods spoilage fungi (Babajide *et al.* 2008, Oladimeji *et al.* 2013). The root has been reported to cure sexually transmitted diseases (Kayode *et al.* 2009). Limonoids isolated from *K. senegalensis* fruits, and seed oil exhibited strong biological activity against the plant pathogenic fungus *Botrytis cinerea* Pers (Abdelgaleil *et al.* 2004) and cattle ticks (*Boophilus decoloratus*) (Choudhury and Boshe 2013). Specifically, limonoids from *K. ivorensis* seed/fruit and stem bark exerted antifeeding, antifungal, antibacterial, antitrypanosomal and antitumor activities (Vanucci *et al.* 1992, Abdelgaleil and El-Aswad 2005, Abdelgaleil *et al.* 2005, Zhang *et al.* 2009, Ji *et al.* 2014). Ewete and Bamigbola

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(1998) reported that a crude extract of *K. ivorensis* stem bark at 500-1000ppm significantly reduced *Callosobruchus maculatus* emergence on Bambara groundnut over several generations. However, very little is known about the pesticidal activity of *K. ivorensis* against wood-degrading agents. In this study, the chemical composition of *K. ivorensis* stem bark extractives was quantified and termiticidal activity on *Triplochiton scleroxylon* and *Vitex doniana* wood test blocks was investigated under field conditions.

MATERIAL AND METHODS

Plant collection and extraction

The stem bark of *K. ivorensis* was collected from Masanwo/Adedeji Village via Imeko, Ogun State, Nigeria and air-dried for three months. The air-dried materials were milled before 500 g samples (n=4) were separately extracted in 2,5 L of 70% ethanol with constant shaking for five days. The ethanol extracts were filtered and concentrated on a rotary evaporator to dryness. The percentage yield was evaluated following the formula (Equation 1) thus:

$$\text{Percentage yield} = \frac{\text{Average dry weight of concentrated extracts}}{\text{Average weight of the air-dried milled samples}} \times 100 \quad (1)$$

Quantifications of chemicals in *Khaya ivorensis* stem bark (KISB) extract

Concentrations of alkaloids, flavonoids, phenol, saponins and tannins in the KISB extract were quantitatively analysed by using Spectrophotometric and Forlin-Ciocalteu methods (Hiai *et al.* 1976, Padmaja 1989, Singh *et al.* 2004, Chan *et al.* 2007, Kale *et al.* 2010). All the quantifications were performed in duplicate and presented as mean values (n = 2) ± Standard Deviation (SD) in mg/g.

Procedures

Briefly, 25 mL of methanol was added to 0,5 g of sample contained in a covered 50 mL centrifuge tube which was shaken continuously for 1 hour at room temperature (Chan *et al.* 2007). The mixture was centrifuged at 3000 rpm for 10 min, and then the supernatant was collected and stored at -20°C until the analysis was done.

Quantification of total alkaloids content (TAC)

The total alkaloid contents in the samples were measured using the 1, 10-phenanthroline method described by Singh *et al.* (2004) with slight modifications. A 100 mg sample powder was extracted in 10 mL of 80% ethanol. This was centrifuged at 5000 rpm for 10 min. The supernatant was collected and used for further estimation of total alkaloids. The reaction mixture contained 1 mL plant extract, 1 mL of 0,025M FeCl₃ in 0,5M HCl and 1 mL of 0,05M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in a hot water bath at 70±2°C. Absorbance of the red coloured complex was measured at 510 nm against a reagent blank. Alkaloid contents were estimated against a standard curve of quinine (0,1 mg/mL, 10 mg dissolved in 10 mL ethanol and diluted to 100 mL with distilled water). The values were expressed as g.100g⁻¹ of dry weight.

Quantification of total flavonoids content (TFC)

TFC was determined by the aluminium chloride method as reported by Kale *et al.* (2010). A sample (0,5 mL) of the extract was dispensed into a test tube along with 1,5 mL of methanol 0,1 mL of aluminium chloride (10%) 0,1 mL of 1M potassium acetate and 2,8 mL of distilled water. The reaction mixture was mixed and allowed to stand at room temperature for 30 minutes before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g material.

Quantification of total phenolic content (TPC)

The total phenolic content of the extracts was determined according to the Folin–Ciocalteu method (Chan *et al.* 2007). Briefly, 300 μ L of extract was dispensed into a test tube (in triplicates) along with 1,5 mL of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1,2 mL of Na CO solution (7,5w/v). The reaction mixture was mixed and allowed to stand for 30 min at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 μ L of distilled instead of sample extract. TPC was expressed as Gallic acid equivalent (GAE) in mg/g material.

Quantification of total saponins content (TSC)

Total saponins (TS) were determined using a modification of the method of Hiai *et al.* (1976) as described by Makkar *et al.* (2007). The sample (0,5 g) was extracted with 25 mL of 80% aqueous methanol on a mechanical shaker for 2 hour, after which the contents of the tubes were centrifuged for 10 min at 3000 rpm. An aliquot (0,25 mL) of the supernatant was added to 0,25 mL vanillin reagent (8% vanillin in ethanol) and 2,5 mL of 72% aqueous H SO in a test tube. The reaction mixtures were heated in a water bath at 60 °C for 10 min. The tubes were cooled on ice for 4 min and then acclimatized to room temperature. Absorbance was measured in a Uv/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

Quantification of total tannin content (TTC)

Tannin content of samples was determined according to the method of Padmaja (1989). Sample (0,1 g) was extracted with 5 mL of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3000 rpm for 20 minutes, 0.1 mL of the supernatant was added to 7,5 mL of distilled water 0,5 mL of Folin-Denis reagent, 1 mL of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 760 nm. A blank was prepared with water instead of the extract. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material.

Wood block preparation

Small wood samples (2 cm x 2 cm x 6 cm) were processed from unblemished 22 year old *Triplochiton scleroxylon* and 28 year old *Vitex doniana* trees' bolts. Wood samples were oven dried at 103 ± 2 °C to constant weight (ASTM D-1413-2007) and weighed (W_0). The samples were then kept in air-tight bags prior to use. Thereby mix samples of each wood species containing mixtures of sapwood and heartwood were selected for tests.

Test formulations development and wood treatments

The extract was diluted in 1 L of 70% ethanol to 5%, 10%, 15%, and 20% of (26,7g or 5,3% yield) along with solvent alone and a control was included. The oven dried wood samples (18 replicates) were then soaked in their respective formulations for 3 days. Then weight gain (W_2) was used to calculate the net absorption and retention.

Field exposure test

Termite resistance of the treated and untreated wood samples was evaluated according to procedures used by Lenz *et al.* (2003), Lenz *et al.* (2011), Asamoah *et al.* (2014). The termite field test was carried out at Latitude 7° 44' 29" and 7° 44' 31" N and Longitude 3° 89' 75" and 3° 89' 77" E within the University of Ibadan, Nigeria. The area is a humid agro-ecological zone (Ajayi *et al.* 2012) and is known to be perennially infested with variety of termites. The area experiences high relative humidity and generally two rainfall maxima regimes during the rainfall period of March to October with an average of 1230 mm per annum. The mean annual temperatures range between 22°C and 31°C

and relative humidity ranges between 57% and 99% (Oshunsanya 2013). The soil type is an Alfisol formed from the basement complex rocks (Oshunsanya 2013). Termite's infestation in the area was very severe, essentially subterranean types (such as *Amitermes evuncifer*, *Macrotermes bellicosus*, and *Odontotermes* spp (Adedeji 2016)) which have variable foraging specificity ranging from damp to dry woods.

From eighteen treated blocks each of *T. scleroxylon* (TS) and *V. doniana* (VD) samples per treatment, six replicates were used. The TS and VD samples treated with the same extract concentration were placed side by side horizontally and buried on the topsoil level spaced at 1 m apart from each different treatment wood blocks. After six months, the test samples were carefully exhumed from the field, renumbered, scrubbed and washed to remove all the soil particles, dried at room temperature and finally oven dried for 22 hours at $103\pm 2^{\circ}\text{C}$. From the oven dry weight after the treatment and final oven-dried weight after the field test, the weight loss % and termiticidal activity of the formulations were calculated with the reference to the control samples using the standard formula (Equation 2).

$$\text{Wood block weight loss \%} = \frac{[(W_2 - W_3)]}{W_2} \times 100 \quad (2)$$

Where

W_2 = is the oven dry weight before attack exposure test,

W_3 = is the oven dry weight after attack test.

Statistical data analysis

Data from the experiments were analysed using analysis of variance (ANOVA) and Least Significant Difference (LSD) at $p < 0,05$. All results were expressed as means \pm Standard Deviation (SD).

RESULTS AND DISCUSSION

Percentages extractive yield

Khaya ivorensis stem bark extract ($5,33\pm 0,02\%$) was obtained as very fine glassy/shinning reddish-brown pellets. Phytochemicals, including phenolic content are known to influence the rate of wood degradation by termites (Syofuna *et al.* 2012, Kadir 2017). The extracts contained appreciable amounts of: saponins ($156,31\pm 0,76$ mg/g), tannins ($59,95\pm 0,54$ mg/g), phenol ($50,62\pm 0,11$ mg/g), alkaloids ($38,98\pm 0,02$ mg/g), and flavonoids ($0,37\pm 0,02$ mg/g). KISB extract yield was much lower than 13% obtained using hot water (Tepongning *et al.* 2011, Tepongning *et al.* 2013) in Cameroon. High extract yields were also obtained from *K. senegalensis* leave with methanol (20,5%) or aqueous extraction (17,4%) in Sudan (Satti and Elamin 2012). This variability may reflect the variability in solvents used and potential the moisture levels at which the yields were determined. The glassy/shining appearance of the extract suggested that KISB might contain silica. The KISB extract colour correlated well with the previous studied (Taiwo and Ogunbodede 1995, Falodun *et al.* 2009, Stephen *et al.* 2009, Tepongning *et al.* 2011).

Chemical analyses showed with total saponins content being most copious. Previously, many studies on *K. ivorensis* chemicals have been documented (Adesogan and Taylor 1970, Adesida *et al.* 1971, Taylor 1977, Vanucci *et al.* 1992, Abdelgaleil *et al.* 2005, Zhang *et al.* 2009, Ji *et al.* 2014). These studies were however focused on specific compounds mainly limonoids. Taiwo and Ogunbodede (1995) documented an increasing extractable tannin (%) contents from reducing *K. ivorensis* bark ground particle sizes for development of adhesives. Recently, Adeyemi *et al.* (2014) qualitatively documented among others, the presence of saponins, tannins and flavonoids in *K. ivorensis* stem bark. The highest total amounts of saponins found in this study agreed with the findings of Ejikeme *et al.* (2014) that *K.*

ivorensis wood heavily contained saponins. While plants produce varying chemical compounds as part of their normal routine metabolic activities, the high quantities of total saponins > tannins > phenol > alkaloids contents found in this study confirmed the extractives richness of KISB.

Treatability

The two tropical test wood species showed no significant variability at the level of treatability in terms of KISB extractives uptake (absorption) and retention (Table 1). VD absorbed and retained extractives more than TS wood samples. The slight differences were likely the variations in chemical composition, cell wall organisation and density (Khazaei 2008). Previous studies have documented absorption of 24,9 kg/m³ (Omole and Onilude 2000) 54,86 - 64,90 kg/m³ (Olajuyigbe *et al.* 2010) 70,37 – 117,13 kg/m³ (Ogunsanwo and Adedeji 2010) and 190,1 – 206,8 kg/m³ (Emerhi *et al.* 2015) for *T. scleroxylon* wood.

Table 1. Means comparison for treatability of woods with extract formulations.

Absorption		Retention	
Parameters	Mean (kg/m ³)	Parameters	Mean (kg/m ³)
Wood blocks species		Wood block species	
<i>Vitex doniana</i>	32,15±15,84 ^a	<i>Vitex doniana</i>	1,79±1,56 ^a
<i>Triplochiton scleroxylon</i>	28,88±18,08 ^a	<i>Triplochiton scleroxylon</i>	1,66±1,71 ^a
Extract concentrations		Extract concentrations	
15%	41,69±12,62 ^a	20%	3,94±0,40 ^a
5%	40,19±8,28 ^a	15%	3,34±1,01 ^b
10%	37,95±14,29 ^a	10%	2,02±0,76 ^c
20%	36,95±5,09 ^a	5%	1,07±0,22 ^d
0%	26,33±5,60 ^b	0%	0,00±0,00 ^e
Control	0,00±0,00 ^b	Control	0,00±0,00 ^e

Means with the same alphabet within parameter are not significantly different from each other at $\alpha = 0,05$

Termiticidal activity of KISB extract

Termiticidal activity of KISB extract formulations against field termites on treated and untreated two wood species test samples was assessed and their effectiveness was quantitatively evaluated by the corresponding wood weight loss (WWL) values as presented in Table 2 and Figure 1. In line with the global trend of going back to nature, this study evaluated termiticidal activity of KISB crude extract using established below-ground contact approach on TS and VD woods. Generally, VD wood was more heavily attacked than TS wood while blocks treated with 10%, 15% and 5% concentrations of the extract showed moderate effectiveness (27%-31,5% wood weight loss (WWL)) according BS EN 252 2014 (Table 3) compared to 84,4% WWL for 20% concentration and 100% WWL for both controls. The significant interaction effect showed the severity levels of WWL among the formulations (Figure 1) but 15%, 10%, and 5% indicated moderately effectiveness on both test wood species. The percentage mean WWL (ranges of 27 % to 31,5%) for 15%, 10% and 5% were reflections of remarkably moderate termiticidal activity and such indicated better protection of wood against termites than 54,5% saving (45,5% weight loss) of stored Sorghum grains treated *Khaya senegalensis* leaf extract against beetles (*Trogoderma granarium*) reported in Sudan (Satti and Elamin 2012). This termiticidal property agreed with the reported biological activity of *K. ivorensis* stem bark at 250-1000 ppm against Bambara groundnut weevil, *Callosobruchus maculatus* (Ewete and Bamigbola 1998). This trend of termiticidal result has a promising conservation implication as little will effect better protection. *Amitermes evuncifer*, *Macrotermes bellicosus*, and *Odontotermes* spp. were the common termites species identified in the study area. However, *Amitermes evuncifer* was found to be the dominant species degrading the testing blocks under the soil. Termites' attacks were negligible in some but the high numbers of the test blocks were attacked by some soil-inhabiting fungi notable one being strand-forming, like *Coniophora olivacea* fungus. The additional WWL caused by fungi was likely occasioned by early downpour of rainfall (precipitation) between February and April in the year 2014.

Table 2. Main effect comparison for wood weight loss caused by termites.

Parameters	Mean \pm S.D (%)
Wood blocks species	
<i>Triplochiton scleroxylon</i>	59,41 \pm 33,51 ^a
<i>Vitex doniana</i>	65,34 \pm 35,31 ^b
Extract concentrations	
10%	27,01 \pm 3,42 ^a
15%	31,37 \pm 5,43 ^a
5%	31,49 \pm 3,12 ^a
20%	84,39 \pm 21,8 ^b
0%	100,00 \pm 0,00 ^c
Control	100,00 \pm 0,00 ^c

Means with the same alphabet within parameter are not significantly different from each other at $\alpha = 0,05$

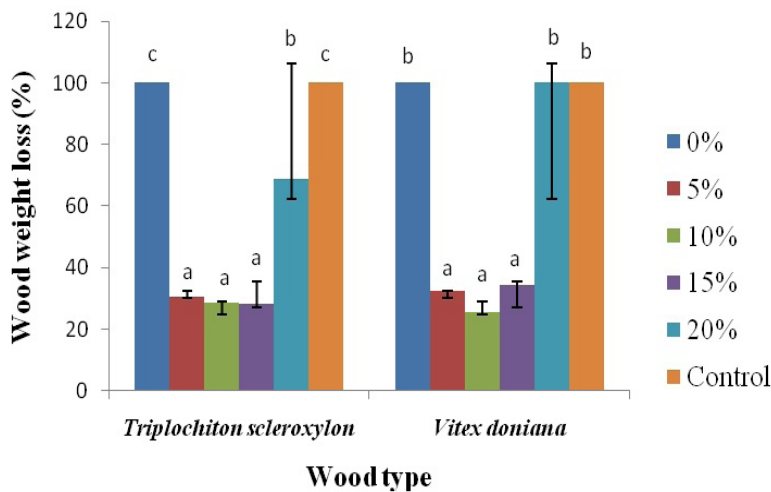


Figure 1. Termiticidal activity of *K. ivorensis* stem bark extracts. Means with the same letter for a wood species are not significantly different from each other at $\alpha = 0,05$.

Table 3. Termite attack protective rating classifications according to BS EN 252 2014.

Weight loss classifications	
Classes	Description
0-5%	Very durable (very effective)
6-10%	Durable (effective)
11-40%	Moderately durable (moderately effective)
41-100%	Non-durable (non-effective)

Source: Antwi-Boasiako *et al.* 2017

CONCLUSIONS

K. ivorensis is an indigenous but suitable plantation species whose stem bark traditionally finds continuous medicinal uses for varying conditions. In this study, termiticidal profile of KISB against underground feeding termites was investigated on woods. The phytochemicals' quantities were in the order of total: saponins > tannins > phenol > alkaloids > flavonoids content. KISB demonstrated moderately effective termiticidal activity in particular 5%, 10% and 15% concentrations (formulations) according to BS EN 252 2014 standard specifications. It was therefore concluded that higher formulations above 15% concentration may not offer protection against termites, hence further biological studies

using smaller quantity of KISB extract for indoor termites or laboratory tests are recommended.

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