PHYTOCHEMICALS AND ANTITERMITE ACTIVITY OF THE STEM BARK AND LEAVES OF *ZANTHOXYLUM ZANTHOXYLOIDES*

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Abstract
Zanthoxylum zanthoxyloides is a plant that has been found to possess various uses. Since there is increased awareness about the use of Z. zanthoxyloides for the control of termites, it has become an interesting area for scientific investigation. The crude methanol extracts and solvent soluble fractions of the stem bark and the leaves were screened for their phytochemical properties and their antitermite activities. The antitermite activity revealed the stem bark to be the most efficacious extract and the n-hexane fraction to be the most active fraction with the highest activity (96.67%). The phytochemical screening of the stem bark and the leaves of this plant revealed the presence of tannins, flavonoids, cardiac glycosides, alkaloids and glycosides. Thus, these results show that the plant possess antitermite effect.

Keywords: *Zanthoxylum zanthoxyloides*; antitermite activity; leaf extract; stem bark extract, fraction

Introduction

Nigeria is blessed with abundance of natural resources. These natural resources especially plants, have served as source of insecticides and pesticides. Termite is highly destructive polyphagous insect pest, which lives in huge mounds, feeds on paper and on everything that is cellulosic in nature. They cause damage to commercial wood, fibers, cellulose sheets, papers, clothes, woolens and mats, and woody building material, millet, sugarcane, and infest green standing foliages, cereals in the place of storage [1]. Both worker and soldier termites harm non seasoned commercial wood and finished products of wood. The nuisance caused by termite is greatly felt everywhere it attacks both the rural and urban areas. Conversely, different means have been employed in combating them especially in the field and areas of infestation. The most popular is the use of synthetic pesticides such as cyclodiene [2], cypermethrin [3], hydroquinone and indoxocarb [4]. These chemicals are very effective when applied correctly, but they have associated problems of toxicity to non- target organisms, high cost of purchase, development of resistance to pesticide, in fact their effect is very harmful as they are not easily cleared from the environment as a result of their long residual time [1]. These led to the banning of their usage in year 2000 by Environmental Protection Agency (EPA) and sourcing for other alternatives from plant origin. Various parts of plants and plant extracts are known to be either toxic or repellent to agricultural pests and are widely used in rural areas. Some of these extracts have been investigated in the laboratory and proven effective against termites. Plant extracts, such as those of neem, wild tobacco and dried chilli, have been used to control termites in the field and storage warehouses [5]. Termiticidal extractives from natural resources are very attractive and may have great market potential as future preservatives if it can economically be produced on a large scale. Various plant-derived products and parts are known to possess antitermitic properties, including termiticidal activity, repellency, antifeedancy, toxicant and insect growth regulation. The antitermitic activity exhibited depends on the type of plant and which products or parts such as essential oils, seeds, bark, leaves, fruits, roots, wood and resins that are being applied against termites [6-8]. The extracted chemicals may act as feeding deterrent, oviposition deterrents, repellent and toxicant. Secondary metabolites are often found to exhibit termiticidal activity. Many kinds of chemicals are found to be termiticidal due to different functional groups present. These chemicals include 2-furfuraldehyde [9], 2-bromomethylanthraquinone [9], ferruginol and manool [10], nootkatone [11-14], chamaecynone [15], 2-phenoxyethanol [16], eugenol

[17]; 3, 13E-clerodien-15-oic acid; 4(18), 13E-clerodien-15oic acid; 18-oxo-3,13E-clerodien-15-oic acid; and 2-oxo-3,13Eclerodien-15-oic acid [18]. Terpene [19], α-terpineol, torreyol (δ-cadinol), α-cadinol [20], 7-methyljuglone [21], cedrol, α -cadinol [22], α-pinene, p-cymene and 1, 8- cineole [23] and aphthalene [24]. These compositions may have different effects on different species of termites. *Zanthoxylum zanthoxyloides* (Lam.) Zepernick & Timler is a plant that belongs to the family *Rutaceae* (Synonym: *Fagara zanthoxyloides*. Lam.). It is commonly called African satinwood and Kosonkori in Nupe, Fasakuwari in Hausa and Ata in Yoruba. It is commonly found in the savannah and dry forest vegetation of Nigeria [5]. The plant possesses antioxidative, antiinflammatory, antisickling, antibacterial, antiviral, anti hepatotoxicity, anti allergic, antitumoral and antihypertensive properties [5]. It is used as an internal and external parasiticide. Root or stem bark macerations, decoctions or infusions are widely taken to treat malaria, fever, sickle cell anaemia, tuberculosis, paralysis, oedema and general body weakness [5, 26, 27]. It is also widely taken to treat intestinal problems, including colic, dysentery, intestinal worms, gonorrhoea and urethritis, but also as an emmenagogue, stimulant and to treat pain during childbirth, migraine and neuralgia [5, 26, 27]. Also, roots are externally applied to ulcers, swellings, haemorrhoids, abscesses, snake bites, yaws, wounds leprosy and syphilitic sores as well as rheumatic and arthritic pain and hernia [5, 26, 27]. The roots and stem bark give a warm, pungent and benumbing effect on the palate when chewed, and are widely used in the treatment of sore gums, toothache and dental caries [26]. The roots, young shoots and twigs are used commonly as chewing-sticks [5, 25-27]. Various parts of this plant had been ethnobotanically reported to be resistant to termites and commonly used as such in North Central Nigeria (Mann's personal communication). Literature search done on the antitermite activity of this plant shows no report on the metabolites of the stem bark and leaves of *Z. zanthoxyloides*.

Materials and Methods

Collection and Identification of the Plant Material

The stem bark and leaves of *Zanthoxylum zanthoxyloides* were collected from behind Male hostel, Federal Polytechnic Bida, Niger State, Nigeria in the month of March, 2010. The plant was duly identified by Plant Taxonomist, Umar S Gallah and deposited with Voucher No. 68033 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Extraction Procedure

Sixty grams (60 g) and five hundred grams (500 g) of air-dried pulverized leaves and stem bark were separately macerated with 3×100 ml of methanol for 72 hr respectively. The resulting solutions were concentrated *in vacuo* using a rotary evaporator and the dried extracts were labelled CMLE for Crude methanol leaf extract (greenish black in colour, 25 g) and CSBE for crude methanol stem bark extract (brownish black in colour, 47 g).

Phytochemical Screening of the Extracts

The obtained extracts (CMLE and CSBE) were subjected to phytochemical screening to determine the presence of various secondary metabolites using standard protocols [28-30].

Fractionation of Methanolic Extracts of the Leaf and Stem Bark

Crude methanolic extracts (CMLE and CSBE) were further fractionated successively with 3×100ml portions of n-hexane, chloroform, ethyl acetate and acetone respectively to obtain the leaf and stem bark extracts.

Screening of the Extracts for Antitermite Activity

Termites used for the screening were collected from a termitarium in front of Federal University of Technology Minna. Fifteen worker termites were transferred using a brush into each Petri-dish containing the filter paper impregnated with the extracts and then covered with a net in order to aerate the termites. It was left to stand for 24 hr in order to observe the mortality rate. After 24 hr the numbers of termites that died was recorded. It was left for another 24 hr and the numbers of dead termites were determined. This was carried out for stem-bark and leaves extracts.

Statistical analysis

Values are means of duplicates. Means, standard deviation, t-test, percentage were calculated using Analysis of variance (ANOVA) was carried out for the multiples of mean at p≤0.05 probability level.

Results and Discussion

Table 1: Phytochemical constituents of leaf and stem bark extracts of *Z. zanthoxyloides*

Secondary metabolites	Test	Observation	CMLE	CSBE

Saponins	Frothing test	Persistent frothing	—	—
Glycosides	Fehling's test	Orange-red ppt	++	++
Alkaloids	Mayer's reagent: Wagner's reagent	Orange-red ppt White to buff ppt	— —	— —
Tannins	Ferric Chloride	Blue, black or green ppt	++	++
Terpenoids	Shinoda's reagent	Reddish ppt	++	++
Phlobatannins	Filtrate + HCl	Red ppt	—	—
Cardiac glycosides	Salkowski	Reddish brown ring at interface	+++	+++
Flavonoids	Ferric Chloride	Yellow ppt	++	++
Anthraquinones	Borntrager's test	Pink, red or violet colour	—	—

Key: +++= Highly present; ++= moderately present; ;- = absent; CMLE= Crude methanol Leaf extract; CSBE= crude methanol stem bark extract

Table 2: Comparison of mortality of stem bark and leaf of the crude extract/fractions Of extract/fractions of *Z. zanthoxyloides*

Extract/ Fractions	Stem bark		Leaf	
	Days			
	1	2	1	2
CMLE	$11.00^{ab}\pm0.71$	$3.00^{bc}\pm0.71$	$12.00^{a}\pm1.41$	$3.00^{bc}\pm1.41$
n-hexane	$14.00^{a}\pm0.71$	$1.00^{c}\pm0.00$	$14.00^{a}\pm1.41$	$2.00^{c}\pm0.00$
Chloroform	$11.00^{ab}\pm1.41$	$4.00^{b}\pm1.41$	$9.00^{a}\pm1.41$	$6.00^{bc}\pm1.41$
Ethyl acetate	$13.00^{b}\pm2.83$	$4.00^{b}\pm0.00$	$11.00^{ab}\pm2.12$	$4.00^{ab}\pm1.41$
Acetone	$8.00^{c}\pm9.43$	$7.00^{a}\pm1.41$	$3.00^{bc}\pm1.41$	$7.00^{a}\pm0.71$
Control	$4.00^{c}\pm0.71$	$3.00^{b}\pm1.41$	$2.00^{c}\pm0.71$	$4.00^{bc}\pm1.41$

Key: Values are means of duplicates. Values on the same column with different superscripts are significantly different from each other ($p\leq0.05$).

Table 3: Comparison of percentage mortality of the extract/fractions from the leaf and stem bark of *Zanthoxylum zanthoxyloides*

Extract/Fractions	Days	Plant part		Remark
		Stem	Leaves	
Crude	1	76.67±4.71	80.00±9.43	NS
	2	100.00±0.00	100.00±0.00	NS

n-hexane	1	96.67±4.71	93.33.±9.43	NS
	2	100.00±0.00	100.00±0.00	NS
Ethyl acetate	1	73.33±9.43	60.00±9.43	NS
	2	100.00±0.00	100.00±0.00	NS
Chloroform	1	86.67±18.86	76.67±14.1	*
	2	100.00±0.00	100.00±0.00	NS
Acetone	1	53.33±9.42	20.00±9.42	*
	2	100.00±0.00	63.29±3.45	*
Control	1	30.00±4.71	16.67±4.71	*
	2	28.18±11.57	31.73±9.52	NS

Key: NS not significantly different; *significantly different

Humans have been using plant extracts as termiticides since immoral [31-33]. Antitermitic properties, including termiticidal activity, repellency, antifeedance and insect growth regulation are associated to the metabolites present in the plant organs such as essential oils, seeds, bark, leaves, fruits, roots, wood and resins [6,7]. The phytochemical screening in this study showed that all the experimental plant parts have terpenoids, flavonoids and tannins, and one or more of other phytoconstituents such as general glycosides, steroids and saponins. A relationship may exist between the chemical structure of a phytochemical compound and its antitermitic property [34]. Numerous physiological activities have been attributed to them. Condensed flavonoids, both flavano-tannins and polycyclic flavanoids like peltogynols in various plants are found to be the insecticidal principles [35-38]. Several terpenes exert a repellent action on insects [6, 8]. All the phytochemicals observed in table 1 have been reported in earlier works [2, 7, 39] to possess antitermitic activities.

From the results for day one, the mortality of termite for the crude, *n*-hexane, chloroform fractions of the leaf extract are not significantly different from each other at p≤0.05, but the mortality of termites in these fractions are significantly different from that of the control, acetone and ethyl acetate. The mortality of the termites in the crude extract, n-hexane, chloroform and ethyl acetate fractions of the stem bark extract are not significantly different at p≤0.05, but significantly different from control. From table 2, the stem bark extract shows termite mortality maximum up to ninety seven percent on day one. In addition, the mortality of termites in the ethyl acetate, chloroform and crude fractions are statistically not different from each other, but at p≤0.05 are significantly different from the control, acetone fractions and also significantly different from *n*-hexane, chloroform fractions and crude extract.

On day two, the mortality of termites in the crude extract, *n*-hexane, chloroform and ethyl acetate fractions of the leaf extract are not significantly different from each other as the surviving termites in these fractions were all dead except that in the control and the acetone fraction that proceeded to the third day. For day two, the stem bark extract shows that the mortality rate of the crude extract, *n*-hexane, chloroform, acetone, ethyl acetate fractions were not significantly different from each other as all the surviving termites were dead on day two. The mortality of termites in these fractions is significantly different from the control at p≤0.05. From table 2 above, the *n*-hexane extract of the stem-bark showed the highest activity with the mortality rate of 96.67%. However, the activity of the leaves is comparable to the work done by Tagbor [39] on anti-termite activity of *Thevetia peruviana* plant whose highest mortality rate was 97%. At p≤0.005 the mortality rate of the crude extract of the leaves (76.67±4.71%) was slightly higher that of the stem bark (80.00±9.43%) as shown in table 2 above. However, the mortality rate in that of the stem bark of *n*-hexane fraction (96.67±4.71%) was higher than that in the leaves (93.33.±9.43%). It is therefore recommended that the n-hexane fraction of the stem bark be further investigated to identify the active constituents present.

Conclusion

The anti-termite screening of the stem bark and leaf extracts of *Zanthoxylum zanthoxyloides* showed that the plant is active against termites and that the stem bark is more active than the leaves. The highest activity was observed in the n-hexane fractions of the stem bark. The identified secondary metabolites such as terpenoids, flavonoids, tannins, steroids and saponins in the extracts of *Z. zanthoxyloides* were likely to be responsible for the observed anti-termite properties of this plant, suggesting that the plant could be a promising source of anti-termite substances. Further studies on the isolation and characterization of the biologically active constituents of this plant can help provide cheaper and safer termicidal agents.

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