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Full Length Research Paper

The termite controlling capabilities of extracts of *Thevetia peruviana* (Pers.) K. Schum in Ghana

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There is an increasing interest in using natural products in pest control because of their low mammalian toxicity and environmental safety. Some local plant materials have been identified as potential alternative. The objective of this work was to determine the antitermitic efficacy of *Thevetiaperuviana* K Schum. The termite controlling properties of petroleum ether, ethanol and water extracts obtained from the roots of *T. peruviana* (pers) K. Schum roots were studied. To do this stakes were impregnated with extacts obtained from T. peruviana and burried in the field for a period of 5 months. The repellency/attractancy tendencies of ethanol extract of *T. peruviana* in different solvent media were also determined as well as Brine shrimp lethality tests to determine the efficacy of four fractions obtained from the ethanol extract. Phytochemical investigation was carried out on all extact to determine types of secondary metabolites in them. Results obtained from the field tests indicated that stakes impregnated with ethanol extract of *T. peruviana* offered significant protection against subterranean termites and this compares well with results obtained for Chlorpyrifos (Dursban). Repellency/attractancy tendencies results showed that ethanol extract enhanced attrantancy of subterranean termites. Brine shrimp lethality tests results gave an indication that fraction 1 was most efficacious suggesting obvious cytotoxicity. Phytochemical investigation revealed the presence of glycosides in the ethanol extract. The results suggest that ethanol extract of *T. peruviana* and its components could serve as a useful option in the control of subterranean termites.

Keywords: *Thevetia peruviana*, extracts, termites, brine shrimp

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INTRODUCTION

Insects are a class of animals within the Arthropod i.e a type of invertebrate animal that lack a backbone. They have external exoskeleton and their body is composed of three sections, head, thorax and abdomen. Insects are the earth's most diverse organisms and account for about half of the described species of living things and about three quarters of all known animals. Being the most diverse group of organisms, they play a major role in

ecosystem diversity and sustainability. For example, several insect species are predators or parasitoids on other harmful pests, others are pollinators, decomposers of organic matter or producers of valuable products such as honey or silk. Some can be used to produce pharmacologically active compounds such as venoms or antibodies. However a number of the known insect species are considered pests, inflicting damage to humans, farm animals and crops. Depending on the structure of the ecosystem in a given area and man's view point, a certain insect might or might not be considered a pest (Kremen and Chaplin-Kramer, 2007; Sallam, 2008). Some insects can constitute a major threat to entire countries or a group of nations. An example of this is the tsetse fly that puts about 100 million people and 60 million head of cattle at risk in sub-Saharan Africa due to the transmission of trypanosomiasis (ICIPE, 1997).

Termites are social insects and belong to the order *Isoptera*, a common and important insect order in the warmer regions of the world including West Africa (Engel and Krishna, 2004).Termites feed chiefly on wood from which they obtain cellulose. They are very important organisms ecologically as they significantly contribute to the organic decomposition process either by direct consumption of decomposing plant materials or by physical and chemical conditioning of the soil they inhabit. Despite the beneficial role played by termites, a number of species are destructive and are a serious threat to agriculture, forest and infrastructure (including buildings, dams, railway lines, roads, utility poles, and underground cables and pipes) (UNEP, 2000).

Termites have been controlled mostly by conventional methods which rely on the use of synthetic termiticides**.** Various synthetic insecticides offer reasonable protection against termites (Spooner and Priest, 1999; Smith *et al.,* 2002). The use of these synthetic insecticides is however saddled with problems such as toxicity to non-target organisms, development of termite resistance to the substances used and health hazards due to resistance of these synthetic substances in the environment (Kamble *et al.,* 1992; Gamo *et al.,* 1995; Chen *et al*., 2000). These problems have led to increasing legal restrictions to their applications and efficacy necessitating the need to develop alternative approach to the control of termites.

In our previous study the termite controlling properties of some local plants were reported. Pulverizedmaterials obtained from the roots of *T. peruviana* was found under field conditions to be most effective in controlling the destructive effects of subterranean termites (Tagbor and Twumasi, 2009). In this study the termite controlling properties of petroleum ether, ethanol and waterextracts obtained from *T. peruviana* roots are reported.

MATERIALS AND METHODS

Extraction of *T. peruviana*

Roots of *Thevetiaperuviana* (Pers) K Shum (yellow oleander) were obtained from bungalow No, Ridge Road, Kwame Nkrumah University of Science and Technology and pulverized. The pulverized roots of *T. peruviana* (1kg) was filled in a thimble and extracted exhaustively and sequentially using 1L each of petroleum ether (20-40^{o}C), ethanol (96%) and distilled water in a soxhlet apparatus. The three different extracts obtained were concentrated in vacuum and stored at 5^{o} C in a refrigerator.

Termite Identification and collection

Termites were identified, collected and cultured in metal cans according to methods described by Tamashiro *et al.* (1973) and at the time of assay had been held in the laboratory for up to 14 days. Termite identification was done using keys provided by Wagner *et al*. (2008). Termites were maintained in the dark at 25^{o}C in metal trash can (113 litres) containing 7-by 3-by -1 cm wood blocks of obeche. Undifferentiated termite workers were used for bioassays. They were sorted out using a soft bird feather.

Phytochemical screening of extracts

Basic phytochemical screening was carried out on all the three extracts to determine the phytochemicals present in them (Pavia *et al*., 1999; Carey, 2003).

Stock solutions were prepared by dissolving 2 g of the dried extract in 100 ml each of the three solvents (petroleum ether, ethanol and water). Parts of these stock solutions were taken and diluted with the various solvents (petroleum ether, ethanol and water) to prepare 2 mg/ml of test solutions.

Laboratory testing of extracts (evaluation of extract toxicity in a force-feed environment)

Test solution (1ml each containing 2 mg of extract) was topically applied to cover the whole of 5 cm filter paper. The solvent was allowed to evaporate and the filter papers were then moistened with 1ml portions of distilled water. These were placed in Petri dishes of 5 cm diameter and 25 termite workers were counted onto the filter paper and covered with a mesh. Filter papers that

had been treated with solvent alone served as controls. Filter paper treated with only distilled water served a dual purpose of a blank as well as control for water extract. Termite mortality rates were monitored and recorded for 48 hours.

Repellency Test (assayed termite attractancy or repellency to extract)

For the repellency/attractancy test concentrated ethanol extract was partitioned between methanol and cyclohexane and the methanol soluble fraction was subsequently partitioned with Chloroform and distilled water. The respective fractions were evaporated to dryness in vacuum to give residues as methanol, cyclohexane, chloroform and water soluble fractions. Stock solutions were prepared and parts of these were diluted with the respective solvents to prepare 2mg/l of test solutions.

The testing procedure for repellency was modified from Lewis *et al*. (1978).

Cellulose pad halves were treated with 0.5 ml aliquots of methanol, chloroform and water solutions of *T. peruviana* extracts. These treated halves were placed beside untreated pad halves into the bottom of cylindrical plastic containers 4.0 cm high by 5.3 cm in diameter. Ten termites were added to each. The locations of the termites were noted at eight time intervals: 15, 30, 45, 60, 90, 120, 180, and 240 minutes. Based on the number of termites which chose to stay on the extract-treated pad halves, each extract was designated to be an attractant or a repellent. A treatment concentration was considered as repellent when 21 or more of the termites (sum of three replicates) were observed on untreated pad halves (Lewis *et al*., 1978). The average number of termites on the untreated half of disc was converted to percentage repellency (PR) using the formula: [PR = 2(C – 50)] (Talukder and Howse 1993; Talukder and Howse 1995) where C is the percentage of termite on the untreated half of the disc

Field testing of extracts

The field tests to assess the termite controlling properties of the extracts of *T. peruviana* were carried out by adopting the 'Graveyard Test' method. The method exposes the plant materials to termite species in their natural habitat (Edwin and Ashraf, 2006; Antwi-Boasiako and Allotey, 2010). The test site was an old termite testing site of the Materials Research Division of the Council for Scientific and Industrial Research-Building and Road Research Institute at Fumesua. Previous studies have shown that termite species from the following families are present at the site; Termitidae (sub-family; Macrotermitinae and Nasutermitinae) and Rhinotermidae (sub-family Coptotermitinae). Some soil feeding termites were also reported to be present (Ocloo, 1975).

Samples of *Triplochitonscleroxylon* (Obeche) were impregnated with extracts obtained from pet ether, ethanol and water in triplicate (45 stakes in all). Solutions were prepared by dissolving 15g of each of the extract in the corresponding 1000ml solvent that was used for the extraction i.e. pet ether, ethanol and water. Controls consisted of 45 stakes treated with only pet ether, ethanol and water. The wood samples were heated in the solution for 2 hours and left standing for 24 hours and then allowed to dry in an oven at 40^{o}C and weighed periodically till they attained constant weights. Five test plots were selected and the stakes were randomly assigned to each plot of 3 rows of 6 stakes. Dursban containing Chlorpyriphos as active ingredient, a conventional termiticide popularly used in Ghana at the time was used to treat stakes (15 in all) at 1.0% dilution and these were also assigned to 5 plots about 50 m away from the main test plots (Ocloo 1975). The graveyard test lasted for 5 months and was inspected 5 times at 1-month interval. Assessment was done by visual inspection.

Column Chromatography

The crude ethanol extract was further fractionated using column chromatography (silica gel) Merck 70, ASTM 70-230. Fractions were collected and pooled on the basis of similar thin layer chromatography results. Four fractions were collected in all and the solvent were removed using the rotary evaporator. They were then dried on silica gel and weighed. Parts of these were later used for the brine shrimp lethality test to determine the most active fraction.

Bioassay of fractions: Brine shrimp lethality test

`The brine shrimp, Artemiasalina toxicity test was conducted according to methods described by McLaughlin and colleagues (McLaughlin et al., 1991) and the assessment of toxicity was done by methods described elsewhere (Lieberman, 1999; Milhem et al., 2008). The artificial seawater was prepared by adding a quarter teaspoon of sea salt, 9.5g to 250 cm3 of distilled water. The seawater was put in a small tank of 1000cm3 and a teaspoon of brine shrimp eggs added to one side of the tank, which was covered. The other side was not covered so as to allow light that would attract the hatched shrimps. The tank containing the brine shrimp eggs was left at room temperature for 48 hours to allow for the eggs to hatch.

Test tubes used were washed and dried in an autoclave. Different concentrations of ethanol extract were prepared, using dimethyl sulfoxide (DMSO). For

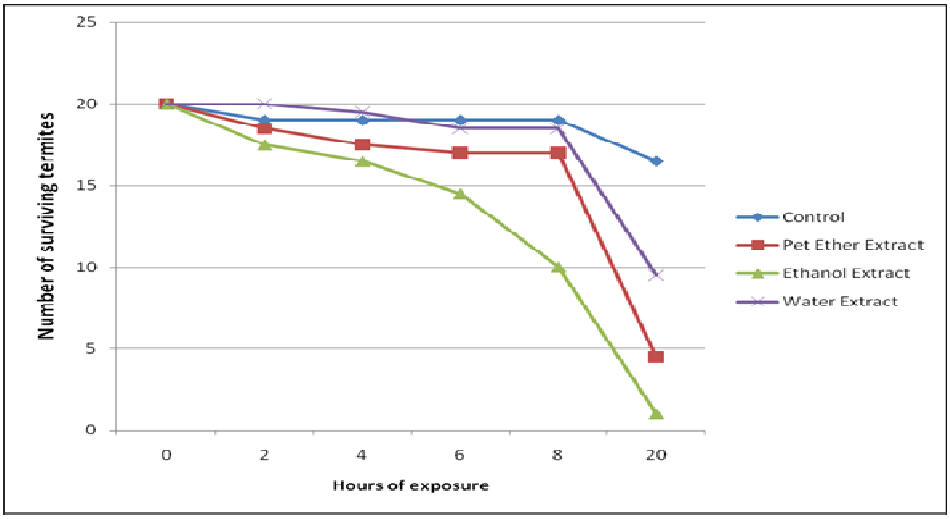

Figure 1. Survival of termites over 20hr period of exposure to *T. peruviana extracts*

each of the test extracts, 20 mg was weighed in a test tube and 2 ml of DMSO was added. This served as a stock solution of concentration 10,000 ppm. For ethanol extract, lower concentrations were prepared by using a micro pipette 0.005, 0.05 and 0.5 ml of the stock diluted with artificial sea water to make concentrations 10, 100 and 1000 ppm respectively. Each test solution was replicated three times and 10 Brine Shrimp larvae (nauplii) were added to each test tube. The brine-shrimp tests were left for 24 hours, after which the number of deaths out of the 30 shrimps per dose was recorded, with the aid of a hand-lens.

Analysis of Results

The principal analyses of data generated involved first the descriptive analysis of the antitermitic properties of the extracts and statistical analysis of the efficacy of the extracts. The analyses of the antitermitic properties involved the determination of contact toxicity to termites, repellency and attractancy, protection against damage. Positive values expressed repellency and negative values attractancy. Regression analysis using STATA version 10 used to compare the mean weight losses of stakes buried in treated soil following exposure to termites adjusting for soil samples. All statistical tests performed were two-sided.

RESULTS AND DISCUSSION

Toxicity of *T. peruviana* extracts to termites

Ethanol extract of *T. peruviana* caused the highest mortality of termite (i.e 95%) followed by the petroleum ether (75%) and water extracts (50%) in that order. In the control, termite mortality was under 15% (Figure 1). A total of 175 termite mortality occurred when they were exposed to extracts of *T. peruviana*; 15, 51, 81 and 28 in the control, petroleum extract, ethanol extract and water extract respectively (Figure 1). The differences in mortality regardless of exposure duration as compared by symmetry and marginal homogeneity tests showed statistically significant differences ($P < 0.0001$) between the extracts.

Visual Assessment of Termite attack and damage

Ethanol enhanced the termite resistance property of *T. peruviana* roots on the field as shown in Table 1. As indicated in the table, *T. peruviana* compares very well with Chlorpyrifos after 5 months of field exposure ($F = 2855.9$ df = 6, 98, $p = 0.0001$).

Repellency/attractancy

There was significant increase in the numbers of termites in contact with the ethanol extract, methanol fraction and methanol control discs in comparison to the numbers of termites on the corresponding untreated pads. In contrast, there was significantly less numbers of termites in contact with the chloroform fraction and water fraction discs in comparison to the numbers of termites on the corresponding untreated pads (Figure 2). There was no significant difference in the average number of termites present on all untreated (Table 2). But there were differences in the average number of termites present on the treated pads. The current study shows that methanol and ethanol enhanced attractancy of *T.*

Table 1. Termite damage on *T.scleroxylon* stakes impregnated with extracts of *T. Peruviana* after five months

Sample	Mean Percentage Damage/Sig
Water only	74.67/a
Pet ether extract	60.00/a
Pet ether only	58.67/a
Ethanol only	54.67/a
Water extract	53.33/a
Ethanol extract	0.00/a
Chlorpyrifos	0.00/b

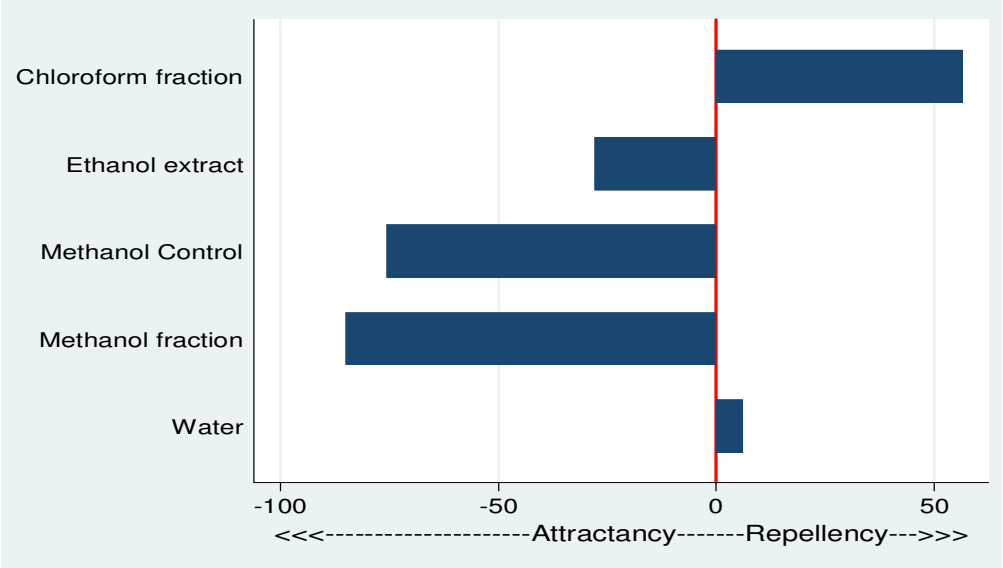

Figure 2. Attratancy or repellency of termites to extracts of *T. peruviana* in different solvent media.

Table 2. Mean number of termites on untreated pads after adjusting for those on treated pads.

Sample	Mean (%) contact	SD	Coefficient of regression	[95% Conf. Interval]	p-value
Chloroform fraction	78.2	11.3	1.0		
Ethanol extract	36.1	8.8	1.3	(-13.3 - 15.5)	0.858
Methanol control	12.2	8.1	0.1	(-19.1 - 19.3)	0.993
Methanol fraction	7.5	6.1	2.2	(-18.7 - 23.0)	0.834
Water	53.1	27.8	7.2	(-5.2 - 19.6)	0.244

peruviana extract to subterranean termites.

Bioassay of fractions

Brine shrimp lethality test

The results of the brine shrimp toxicity tests are shown in Figures 3 and 4. Figure 3 shows the percentage of viable brine shrimp larvae left after exposure to crude ethanolic extracts of *T. peruviana* at varying concentrations. At both 12 and 24 hours of observation, the extract applied at lower concentrations was the least toxic and so toxicity increased with increased concentration of the extract. Figure 4 shows the percentage of viable brine shrimp larvae exposed to column chromatography fractions of the ethanolic extracts of *T. peruviana* surviving over time. Fraction 1 was most lethal at 12 and 24 hours respectively.

Results of phytochemical screening of the extract in

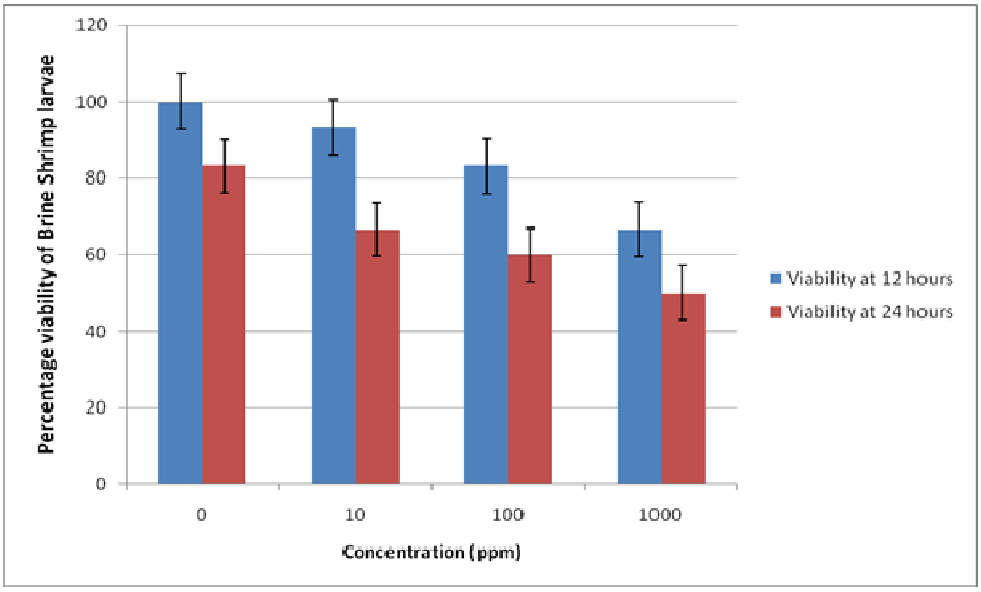

Figure 3. Percentage of viable brine shrimp larvae (Mean ± SD) exposed to the ethanolic extracts of *T. peruviana*

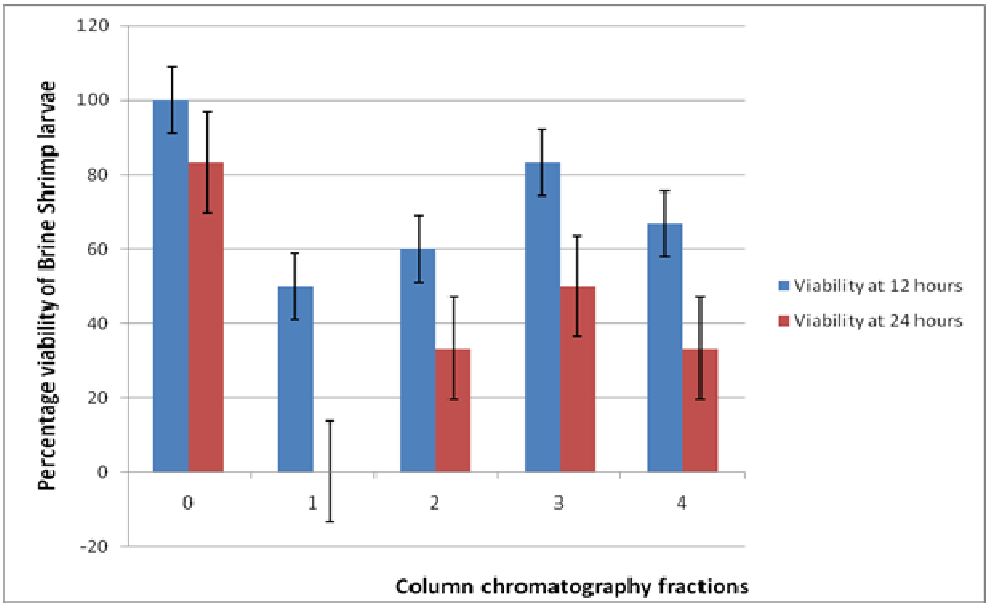

Figure 4. Percentage of viable brine shrimp larvae (Mean ± SD) after exposure to fractions obtained from column chromatography of ethanolic extracts of *T. peruviana*

Table 3 showed that ethanol extract of *T. peruviana* which was most efficacious in controlling termite infestation, contains glycosides. In the attractancy/repellency tests, the ethanol extract was partitioned in methanol, cyclohexane, chloroform and water. The relatively non-polar fractions cyclohexane and chloroform fractions showed repellency whilst the polar fractions ethanol and methanol fractions showed attrantancy with water fraction showing just borderline repellency. Thus the observed attractancy and toxicity of the ethanol extract of *T. peruviana* may be due to the sugar moiety attached to the poisonous genin and the fact that the genin part may be soluble in non-polar solvents whilst the sugar moiety may be soluble in the polar solvents. The attractancy and repellent properties may be employed in the formulation of antitermitic agents.

This study has confirmed reports of botanical and

Table 3: Phytoconstituents of extracts of *T.peruviana*

Plant Extract	Class Of Phytochemical Identified	Functional Group Identified
Petroleum Ether	Terpenoids/steroids	
Ethanol Extract	General glycosides	Aliphatic aldehyde
Water Extract	General glycosides	Aliphatic aldehyde

anthropological studies which have shown that plants or material extracted from them have both insect and microbial-resistant properties and found useful for preventing and controlling insect pests (Apantaku, 1999; Cobbinah *et al.,* 1999) However at the time of this work there was no reported work on the anti-termite control properties of extract from T. peruviana.

CONCLUSION

This work has shown that the potential for the use of ethanol extract of *T. peruviana* as an anti-termite agent is promising. The ethanol extract of *T. peruviana*may be used as termite barrier under people's residences and to impregnate and protect wood used in new housing or other wood construction. Ethanol extract of *T. peruviana* could be formulated into bait. General glycosides found in *T. peruviana* may be useful as natural termite repelling agent and the structures if identified could be used as lead compounds for the development of termite repelling and other agents for the protection of crops, trees and other wood products against termites' damage.

T. peruviana is a common plant that grows freely in most communities and its origin from local plant makes the supply reliable and inexpensive. Thus this finding is of great economic significance especially in Ghana and other tropical countries where individuals mostly affected by termite infestation are poor and unable to afford expensive imported synthetic termiticides for the protection of their properties.

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