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ACARICIDAL EFFICACY OF *ANANCARDIUM OCCIDENTALE L.* (CASHEW) LEAF AND STEM BARK METHANOL EXTRACTS ON *AMBLYOMMA VARIEGATUM* F. (TICKS)

[*1]Yusuf, A., [2]Wada, Y., [2]Wada Y. A. and [1]Ekpo, Z.
[1]Department of Biology, Ahmadu Bello University, Zara, Nigeria
[2]Department of Zoology, Ahmadu Bello University, Zara, Nigeria
For correspondence: yusufmayowa@gmail.com

Abstract

Anancardium occidentale L. (cashew) methanol leaves and stem bark extracts were evaluated for their acaricidal efficacy against *Amblyomma variegatum* F. (ticks). Phyochemical analysis (qualitative and quantitative) was carried out, and four different concentrations were evaluated (25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml) and controls using distilled water (negative control) and synthetic acaricide (Amitrax) used as standard control. Three hundred ticks were used, 10 ticks per petri dish in three replicate were exposed to different concentrations. Two-way analysis of variance was used to compare the mean mortality of ticks exposed to the different concentrations. The lethal concentration (LC_{50}) was determined using probit analysis. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols, while anthraquinones was absent. There was significant difference in mean mortality of ticks at different concentrations; Mortality was concentration and time dependent. 75 mg/ml 100 mg/ml concentration of the leaves extracts gave the highest mortality (10.00 ± 0.00) and for the stem bark extract 100% mortality of the ticks were observed at 100 mg/ml concentration of the extracts (10.00 ± 0.00). There was significant difference in *A. variegatum* mortality with respect to the leaf and stem back extract, however acaricidal effect of leaf extract had greater efficacy compared to stem bark extract.

Keywords: *Anacardium occidentale*, *Amblyomma variegatum*, Acaricidal effects and Acarology

Introduction

Amblyomma variegatum, also known as the "Tropical bont tick" is a three host tick that originated in Africa (Yonow, 1995). It has since spread to several countries, including the Caribbean islands, where it is known as the Senegalese tick (Carib Vet, 2011a) and the Antigua gold tick (Pegram *et al*., 2004). Ticks are one of the best known groups of parasites. They have accompanied humans and their domestic animals throughout recorded history (Hoogstraal, 1970) and have become a major focus of medical and veterinary research, not only because of their direct pathogenic influence on hosts, such as blood loss and tick-induced paralysis (Pfaffle *et al*., 2009), but more importantly because of their role as vectors of a very wide range of viral, bacterial and protozoan diseases (Nicholson *et al*., 2009). Indeed, ticks are of considerable economic importance as a constraint to animal production in most of the countries where they occur (Jongejan and Uilenberg, 2004). Despite this sinister background, ticks are a fascinating, highly successful group, manifesting a wide variety of adaptations to their hosts and the environments in which they live. The tropical bont tick has had a huge effect on the livestock industry, primarily through its transmission of heartwater disease, *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*) and their association with dermatophilosis, *Dermatophilus congolensis* (Barré and Garris., 1990; Allan *et al*., 1998; Parola *et al*., 1999; Carib Vet 2011b). The tropical bont tick has also been implicated as a vector or potential vector for several diseases which include Crimean-Congo hemorrhagic fever virus, Dugbe virus, yellow fever virus,

Rickettsia africae (African tick bite fever) and Jos virus (Merck, 2011). In addition ticks borne diseases continue to be a serious animal health problem, causing major economic loss to farmers. Chemical acaricides such as organophosphate compounds, synthetic pyrethroids, and amitrax are used for control of ticks. The continuous application of these chemical acaricides results in acaricidal resistance, environmental pollution, residues in food and toxicity to workers (Graf *et al.*, 2004).

The use of natural products mainly the botanical acaricides for the control of ticks has been the focus of research in many countries, principally to withstand the noticeable increasing frequency of acaricides resistance tick strains. The spread of such ticks population will inhibit the efforts of improving livestock and animal intensive industry due to the impact of ticks and tick-borne diseases. Acaricides resistance has been documented in literatures as a result of continuous uses or abuses of acaricides, this necessitated urgent need of an alternative safe method for tick control because bites from ticks cause skin damage and feeding adults predispose livestock to dermatophilosis (Hall and Papierok, 1982).

Pereira and Famadas (2004) evaluated the action of the ethanolic root extract of *Dahlstedtia pentaphylla* (Leguminosae) against two strains of tick: one acaricide-sensitive Mozo strain, and one from the field. The plant was less efficient against the field strain. The efficiency was close to 100% at a concentration of 20%: LC50 1:34.94 mL (≈2.86%) against engorged females and 1:231.337 mL (≈0.43%) against larvae. The LC50 and LC99 of the oleoresinous extract of *Copaifera reticulata* (Leguminosae) against larvae were 1.579 ppm (≈0.16%) and 3.491 ppm (≈ 0.35%), respectively (Fernandes and Freitas, 2007).

Hexanic, ethyl acetate and ethanolic extracts from leaves of *Piper aduncum* (Piperaceae) were tested against engorged females in increasing, double concentrations from 5 to 100 mg.mL (≈0.5 to 10%). For all extracts, even at the highest concentration, the reproductive control was no higher than 62%. Larvae mortality was evaluated at concentrations of 1 to 20 mg.mL1 (≈0.1 to 2%), and was found to be 70.42, 40.5 and 17.2% in the hexanic, ethanolic and ethyl acetate extracts, respectively, at the highest concentration. Hydrodistillation of the hexanic extract produced 6.8% essential oil, 94.84% consisting on the sesquiterpene dill apiol, which caused 100% larval mortality at 0.1 mg.mL1 (≈0.01%). The essential oil of *Piper mikanianum* (LC50 2.33µ L.mL1; ≈0.233%) was more active against larvae than that of *Piper xylosteoides* (LC50 6.15 µL.mL1;≈0.615%), while the oil of *Piper amalago* was inactive. The main compounds were phenylpropanoids, monoterpenes and sesquiterpene hydrocarbons (Ferraz *et al*., 2010).

Hexanic and methanolic extracts from stems and leaves of *Hypericum polyanthemum* (Clusiaceae) were tested at concentrations of 6.25 to 50 mg.mL$_1$ (≈ 0.625 to 5%). The effect against engorged cattle tick *Rhipicephalus* (*Boophilus*) *microplus* females was low (19.2%) at the highest concentration of the hexanic extract, but on other hand, it killed all larvae in all concentrations (Ribeiro *et al*., 2008). Similar effects were observed with the hexanic extracts of stems and leaves of *Calea serrata* (Asteraceae) at the same concentrations (Ribeiro *et al*., 2007). *Anacardium occidental*e commonly called cashew in English, 'Kashu' in Hausa, 'Okpokpo' in Ibo and Kaju in Yoruba .It is a multipurpose tree whose leaves, stems and bark extracts are used extensively for the treatment of diarrhea, dysentery and colonic pain. It has also been reported to possess anti-diabetic, anti-inflammatory and anti-ulcerogenic properties (Akinpelu, 2001).

Torres *et al.* (2015) reported hexane and ethanol extracts of *A. occidentale* showed promising potential as an alternative source of a more sustainable, non-toxic, and environmentally friendly

solution for the control Aedes *aegypti, however, the acaricidal efficacy of A. occidentale* leaf and stem bark methanol extracts on ticks have not been documented, this necessitated this study to evaluate the *acaricidal efficacy of* anancardium *occidentale* leaf and stem bark methanol extracts on *Amblyomma variegatum* (ticks).

Materials and Methods

Study Area and Collection of Plant Materials

The study was carried out in Ahmadu Bello University, Zaria, Nigeria. Zaria lies within the Guinea savanna with a latitude of 11^0 3□ N and longitude $7^0$42□ E. (Hore, 1970)

Fresh leaves and stem bark of *Anacardium occidentale* were collected within the botanical garden, Department of Biology, Ahmadu Bello University, Zaria. The leaves and stem bark were taken to the herbarium for verification and authentication in the Department of Biological Sciences A.B.U. Zaria.

Processing of Plant Materials

The plant leaves and stem bark were washed thoroughly to remove soil debris and shade dried at room temperature to get rid of moisture content (Mulungu *et al*., 2007) after drying, they were pounded into powder using a clean pestle and mortar (Muktar and Tukur, 2000). Extraction of Plant Materials was carried out using Soxhlet Extraction Standard Method of Joseph and Ranjit (2008), phytochemical screening was carried out using Standard Method of Trease and Evans (2009), using Bontragers, Dragendorff, Sodium hydroxide, Frothing, Ferric chloride test for Anthraquinone, Flavonoids, Saponins, Phenols and Tannins respectively.

Collection and identification *Amblyomma variegatum*

Three hundred adult ticks of *Amblyomma variegatum* were collected from cattle at Zango cattle market, Zaria, they were carefully picked using laboratory forceps off the body of the cattle after restraining them. The adult ticks were placed in a plastic bowl and lid perforated all over for proper ventilation and were taken immediately to Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for proper identification.

Experimental Design

Three hundred (300) *Amblyomma variegatum with an average weight of 0.lg* were subjected to different treatment levels of the leaf and stem bark extracts. The ticks were placed on a chilling table which is an equipment used to demobilize the ticks to make counting easy and the ticks were weighed individually. Adult ticks of (*A. variegatum*) were placed in 10 experimental groups that is (1,2,3,4,) for the leaves, (5,6,7,8) for the stem bark and group 9 used for the negative control using distilled water while group 10 used as the standard control using synthetic acaricides (Amitrax) and each experimental group was replicated thrice. Various concentrations of the leaves and stem bark (25mg/ml 100mg/ml) were tested on the ticks. 2mls of each concentration was measured using a syringe and poured into a spray bottle and sprayed on each experimental group with their replicate immediately and after every 24hours. The mortality of ticks was observed at 24h, 48h, 72h and 96h after spraying. A magnifying hand lens was used in counting the number of mortality.

Statistical analyses

The data obtained was analyzed using Two-Way Analysis of Variance (ANOVA) to compare the effects of the plant parts. Where there was significant difference Duncan Multiple Range test (DMRT) was used to separate the means using SPSS Version 20. Probit Analysis was carried out to determine the lethal concentrations (LC_{50}) using Minitab 17 statistical software.

Results

Qualitative Phytochemical Screening of the Leaves and Stem Bark of *Anacardium occidentale* (Table 1), Alkaloids, Flavonoids, Saponins, Phenols and Tannins were present while Anthraquinones was absent.

Quantitative Phytochemical Composition of the Leaves and Stem Bark Extract of *Anacardium occidentale* (Table 1), indicated all the phytochemicals present were higher in the leaf than in stem bark of plant. Flavonoids have 20% and 12% for the leaf and bark, followed by Alkaloids with 16% and 10% for the leaf and stem bark respectively, also the percentage composition of phenols was found to be 15.2% for the leaf and 7.7% for the bark, Saponins 14% and 10% for leaf and bark and Tannins with 12.4% and 10.1% for the leaf and bark.

Mean and Percentage Mortality of *Amblyomma variegatum* exposed to Leaf Methanol extracts of *Anacardium occidentale* (Table 2), at 24h there was no significant difference between the effect of the concentrations (25mg/ml -100mg/ml) and the negative control but there was significant difference when compared with the synthetic acaricide which had the highest mortality rate. At 48h of exposure of the ticks to the leaves extract there was significant difference between all the concentrations 25mg/ml-100mg/ml and the control groups while at 72h there was also significant difference in the rate of mortality at 25 75 mg/ml and water but there was no significant difference between the effect of the concentration at 100mg/ml and the synthetic acaricide and at 96h there was significant difference between the concentrations at 25mg/ml 50mg/ml and the negative control group but there was no significant difference between the effect of the concentrations at 75mg/ml 100gm/ml and the synthetic acaricides.

Mean and Percentage Mortality of *Amblyomma variegatum* exposed to Stem Bark Methanol extracts of *Anacardium occidentale* (Table 3), showed that at 24h exposure of the ticks to the stem bark extracts there was significant difference between the concentrations (25mg/ml 100mg/ml) and control groups where at 48h exposure of the ticks there was no significant difference between the concentrations and negative control but there was significant difference when compared with the positive control also at 72h there was also significant difference between the concentrations and control groups, at 96h exposure there was significant difference between the concentrations and negative control group but at 100mg/ml there was no significant difference between the effect of extract and synthetic acaricide.

The mean and percentage mortality of the different parts of the plant and its interaction with time is presented in Table 4, at 24h there was a significant difference between the extracts (leaf and stem bark) and control groups (water and synthetic acaricide), also at 48h and 72h there was also significant difference between the extract and control groups, however at 96h there was no significant difference between the methanol leaf extract and the synthetic acaricide at 96h exposure, but compared to the methanol stem bark extract and water that was significant difference.

The lethal concentrations (Lc_{50}) of the methanolic extracts of the leaves and stem bark at various time in mg/ml of *Anacardium occidentale* is presented in fig 1 and 2, The results clearly indicate that little quantity of the leaves concentration is needed to kill 50% of the ticks which is 16.34 mg/ml of the extract compare to 24.56mg/ml for the stem bark. So also as the time of exposure increases the lower the concentration and the more effective it is on the ticks.

Discussion

The use of natural products mainly the botanical acaricides for the control of ticks has been the focus of research in many countries, principally to withstand the noticeable increasing frequency of acaricides resistance tick strains, this situation has encouraged efforts that should be undertaken to address the emergence of acaricides resistance ticks of veterinary importance, hence the present study was conducted to evaluate the acaricidal efficacy of methanol extracts of leaf and stem bark of *Anacardium occidentale* against *Amblyomma variegatum*. Control of ticks with chemical acaricides has become difficult due to resistance development. Toxicity and resistance problems of insecticides (Nolan, 1987) have encouraged the use of alternative control using plants as acaricides. The chemical also cause environmental pollution due to prolonged time for biodegradability and health hazards owing to residue in milk and meat products.

The phytochemical screening of the leafy stem's powder of *Tephrosia vogelii* revealed the presence of catechol tannins, saponins, sugars, leuco-anthocyanins, polyterpenes, and sterols and ethanolic extract of the leafy stems of *T. vongelii* against *Rhipicephalus sanguineus* larvae (Dougnon *et al*., 2015), In vitro acaricidal activity of ethanol extract of leafy stem of *Tephrosia vogelii* act as larvacidal potential due to the chemical composition of *T. vogelii*. The phytochemical screening revealed the presence of catechol tannins, saponins, reducing compounds (sugars), leuco-anthocyanins, and sterols-polyterpenes which have biocidal properties (Dougnon *et al*., 2015). The combined action of tannin and saponin may be responsible for the larvicidal activity on Rhipicephalus (Boophilus) microplus which observed in vitro (Fernández-Salas *et al*., 2011). The phytochemicals in leaf and stem back in this present study also showed the presence of tannins and saponins as reported by Dougnon *et al*. (2015) for *T. Vongelii.*

The phytochemical screening of *Anacardium occidentale* leaf and stem bark revealed the presence of alkaloids that acts as antimalaria, anti-amoebic agents, Tannins which are reported to have various physiological effects like anti-irritant, antibacterial and antiparasitic effects (Trease and Evans, 2009).

The potential role of flavonoid in modulating the reproductive function of ticks has already been reported by (Sanis *et al*., 2012). Goncalues *et al*. (2005) had earlier reported the presence of tannins, alkaloid, saponins and flavonoid in *A. occidentale*. The acaricidal activity of the extracts on the test organism may be due to the presence of the above phytochemical constituents. Harbone (1984) reported that the methanol extract of the leaf of *A. occidentale* was more active against all test bacteria than the stem bark extract, the leaf methanolic extracts was also observed to be more active than the stem bark methanolic extract, similar trend was obtained from this study which shows that the methanolic leaf extract was more effective on the ticks than the methanolic stem bark extract and this may be due to the high concentrations of the phytochemicals present in the leaves than the stem bark. Umeh *et al*. (2005) reported that many of the phytochemical constituents properties in plants are concentrated in the leaves than the stem bark. Extracts from *Tephrosia vogelii* plant has toxic effect against various genera of ticks using petroleum esther, chloroform, methanol and water. Methanol extract has the highest mortality

rate than any two of the solvents and water. All extracts killed 100% of the exposed ticks but variations was noted in the time taken to achieve 100% of exposed ticks dead, Petroleum ether, chloroform, methanol and water killled 100% of the ticks in an average mortality of 8.3, 9.7, 10.3 and 1.3 respectively (Matovu *et al*., 2007).

Hexanic and methanolic extracts from stems and leaves of *Hypericum polyanthemum* (Clusiaceae) were tested at concentrations of 6.25 to 50 mg.mL1 (≈0.625 to 5%), against engorged cattle tick *Rhipicephalus* (*Boophilus*) *microplus* females was low (19.2%) at the highest concentration of the hexanic extract, but on other hand, it killed all larvae in all concentrations (Ribeiro *et al*., 2008). Similar effects were observed with the hexanic extracts of stems and leaves of *Calea serrata* (Asteraceae) at the same concentrations (Ribeiro *et al*., 2007). The LC_{50} and LC_{99} of the ethanolic extracts of stems of *Magonia pubescens* (Sapindaceae) were 365 ppm (≈ 0.036%) and 4,000 ppm (≈0.4%) against larvae (Fernandes *et al*., 2008). Over 95% larvae mortality was obtained with the essential oil of *Drimys brasiliensis (Winteraceae) at* concentrations of 3.125 to 25 µ L.mL1 (≈0.3125 to 2.5%). The oil was characterized by sesquiterpenoids, cyclocolorenone, bicyclogermacrene and alpha-gurjunene (Ribeiro *et al*., 2008). The ethanolic extract of seeds of *Annona muricata* L. (Annonaceae) at a concentration of 2% had 100% efficacy against engorged cattle tick *Rhipicephalus* (*Boophilus*) *microplus* females (Borges *et al*., 2011).

The mean mortality of the methanolic extracts of the leaves and stem bark of *A.occidentale* in this study were 9.00 for the leaves and 8.00 for the stem bark is in line with the result of the methanolic extract of *Tephrosia vogelii.* Variation was also noted in the time taken to achieve 100% mortality of the ticks exposed to various concentrations. There are many factors that affect the acaricidal activity of the extract including ; solvent, extraction time, extract concentration, extracted plant age, target organism (ticks) and exposure time (Khudrathulla and Jagannath, 2000). (John, 2001) reported that the methanol extract of the leaves of *A. occidentale* was more active against all the test bacteria than the aqueous extract. This may be due to the ability of methanol to extract a wider range of antibacterial principles than the aqueous solvent, The leaves methanolic extract was also observed to be more active than the stem bark methanolic extract. The methanolic extracts of the leaves and stem bark of *A.occidentale* have strong acaricidal activity but the leaves been more effective than the stem bark.This finding is in agreement with the report of Sukumar *et al*. (1991), They established that the activity of phytochemicals on targeted species varies with respect to plant parts from which they were extracted; solvent of extraction, geographical origin of the plant and photosensitivity of some of the compounds in the extract. The results obtained from this study suggest a potent acaricidal activity, it may be an alternative agent to chemical acaricides.

Conclusions

The methanol extract of the leaf and stem bark of *Anacardium occidentale* have alkaloids, flavonoids, phenols, saponins and tannins. Flavonoids had the highest percentage composition in the leaves than in the stem bark (20% and 12%) and also the percentage composition of each of the phytochemical constituents present is higher in the leaves than the stem bark. The methanolic extracts of the leaves and stem bark of *Anacardium occidentale* have acaricidal effect on *Amblyomma variegatum.* However 75mg/ml 100mg/ml had the best acaricidal efficacy for the leaves extracts than the stem bark.

Recommendations

It is therefore recommended to use 75mg/ml 100mg/ml of the methanolic extract of the leaves of *Anacardium occidentale* for effective acaricidal activity against *Amblyomma variegatum.* Further studies should be carried out to identify the active constituents and mechinism of action

in the leaves and stem bark of *Anacardium occidentale.*

Table 1: Phytochemical Constituents of Leaf and Stem bark Following Various Screening tests

Phytochemical constituent	Test	Leaf	Stem bark	% composition in leaf	% composition in stem bark
Anthraquinone	Bontragers	-	-	-	-
Alkaloids	Dragendorff	+	+	16	10
Flavonoids	Sodium hydroxide	+	+	20	12
Saponins	Frothing	+	+	14	10
Phenols	Ferric chloride	+	+	12.4	10.1
Tannins	Ferric chloride	+	+	15.2	7.7

(+) Constituent present
(-) Constituent absent

Table 2: Mean and Percentage Mortality of *Amblyomma variegatum* Exposed to Methanol extract of *Anacardium occidentale* Leaf

Concentrations	24 hours	48 hours	72 hours	96 hours
25mg/ml	0.00 ± 0.00^{b}(0.00)	2.00 ± 1.00^{d}(20.00)	6.00 ± 0.50^{d}(55.00)	8.00 ± 0.50^{b}(75.00)
50mg/ml	1.00 ± 0.00^{b}(10.00)	4.00 ± 0.00^{c}(40.00)	7.00 ± 0.00^{c}(70.00)	9.00 ± 0.00^{a}(90.00)
75mg/ml	2.00 ± 1.00^{b}(20.00)	5.00 ± 0.00^{bc}(50.00)	8.00 ± 0.00^{b}(80.00)	10.00 ± 0.00^{a}(100.00)
100mg/ml	2.00 ± 1.00^{b}(20.00)	7.00 ± 0.50^{b}(70.00)	10.00 ± 0.00^{a}(100.00)	10.00 ± 0.00^{a}(100.00)
Distilled water	0.00 ± 0.00^{b}(0.00)	1.00 ± 0.50^{d}(10.00)	2.00 ± 0.50^{e}(15.00)	2.00 ± 0.50^{c}(15.00)
Synthetic Acaricidal (2ml/L)	10.00 ± 0.00^{a}(100.00)	10.00 ± 0.00^{a}(100)	10.00 ± 0.00^{a}(100)	10.00 ± 0.00^{a}(100)
P value	0.000	0.000	0.000	0.000

n=3 Means ± standard error with the same superscript (a, b, c, d) along the columns are not significantly different at (P=0.05). Values in bracket represent the percentage mortality.

Table 3: Mean and Percentage of Mortality of *Amblyomma variegatum* Exposed to Methanol Extracts of *Anacardium occidentale* Stem Bark

Concentrations	24 hours	48 hours	72 hours	96 hours
25mg/ml	0.00±0.00^{d}(0.00)	2.00±1.00^{bc}(20.00)	4.00±0.50^{d}(35.00)	6.00±0.50^{c}(55.00)
50mg/ml	1.00±0.50^{cd}(5.00)	3.00±1.00^{bc}(25.00)	5.00±0.00^{c}(50.00)	7.00±0.50^{b}(65.00)
75mg/ml	1.00±0.00^{c}(10.00)	4.00±0.50^{b}(35.00)	6.00±0.00^{c}(60.00)	7.00±0.00^{bc}(70.00)
100mg/ml	3.00±0.00^{b}(30.00)	5.00±0.50^{b}(45.00)	8.00±0.50^{b}(75.00)	10.00±0.50^{a}(100.00)
distilled water	0.00±0.00^{d} (0.00)	1.00±0.50^{c}(10.00)	2.00±0.50^{c}(15.00)	2.00±0.50^{d}(15.00)
synthetic acricidal (2ml/L)	10.00±0.00^{a}(100.00)	10.00±0.00^{a}(100.00)	10.00±0.00^{a}(100.00)	10.00±0.00^{a}(100.00)
P value	0.000	0.002	0.000	0.000

n=3 Means ± standard error with the same superscript (a, b, c, d) along the columns are not significantly different at (*P*=0.05). Values in bracket represent the percentage mortality.

Table 4: Mean and Percentage Mortality of *Amblyomma variegatum* based on plants part

Parts	24hour	48hour	72hour	96hour
Leaves	2.00^{b} (20.00)	5.00^{b}(50.00)	8.00^{b}(80.00)	9.00^{a} (90.00)
Stem bark	1.00^{bc} (10.00)	4.00^{b} (40.00)	6.00^{b} (60.00)	8.00^{b} (80.00)
Distilled water	0.00^{c} (0.00)	1.00^{c} (10.00)	2.00^{c} (20.00)	2.00^{c} (20.00)
Synthetic acaricide (2ml/L)	10.00^{a} (100.00)	10.00^{a} (100.00)	10.00^{a} (100.00)	10.00^{a}(100.00)
P value	0.000	0.000	0.000	0.000

n=3 Means ± standard error with the same superscript (a, b, c, d) along the columns are not significantly different at (*P*=0.05). Values in bracket represent the percentage mortality.

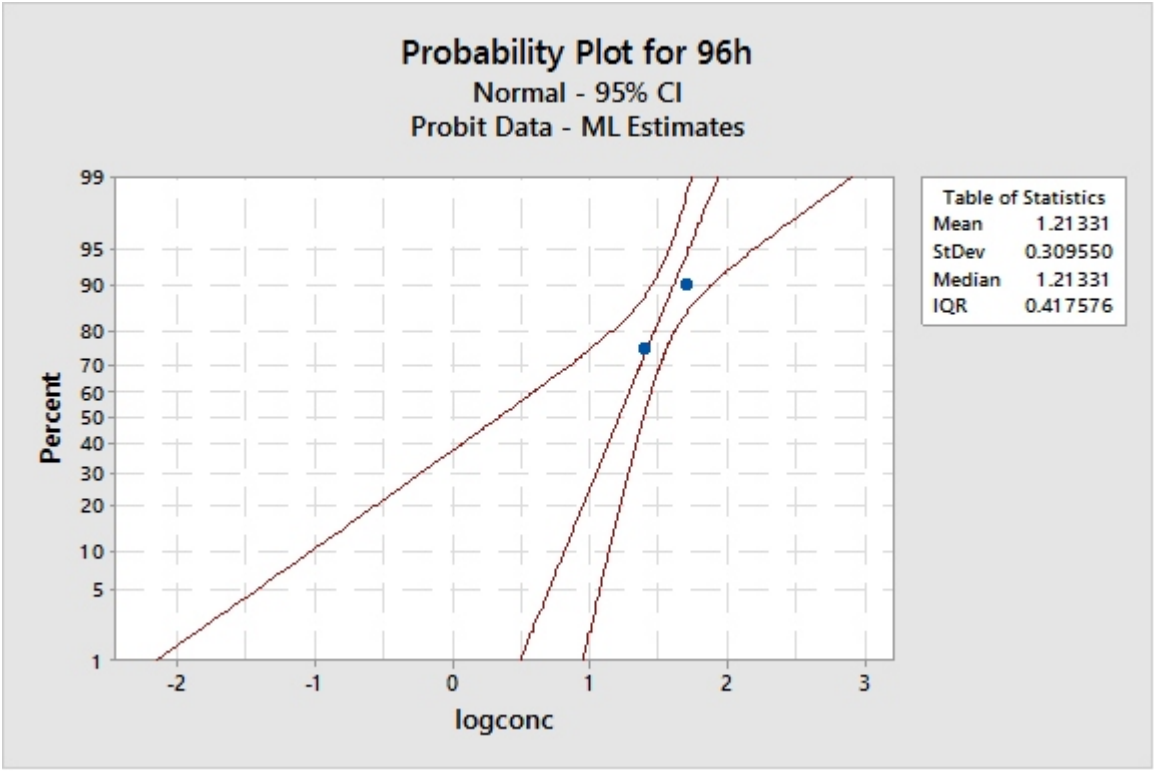

Fig 2: Lethal concentration (LC_{50}) of *Anacardium occidentale* Methanolic Leaf extracts at 96 hours

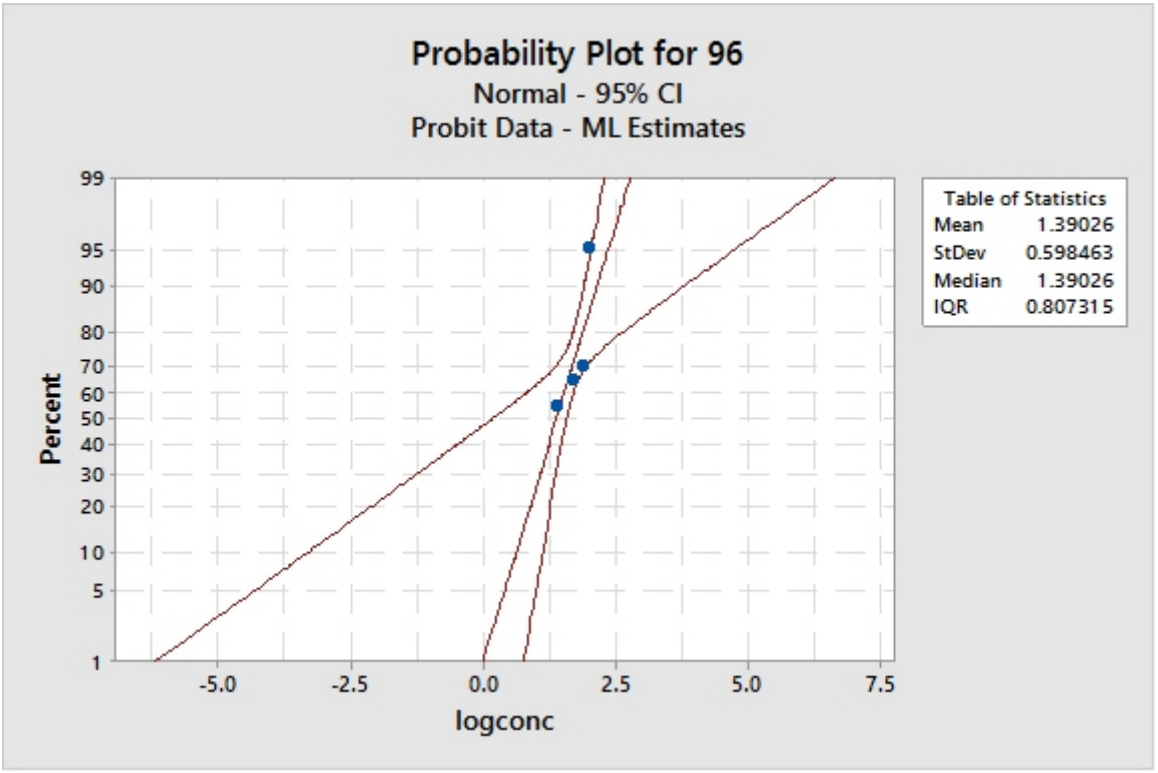

Fig 2: Lethal concentration (LC_{50}) of *Anacardium occidentale* Methanolic Stem Bark Extracts at 96 hours

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