Anchor University Journal of Science and Technology (AUJST)
A publication of the Faculty of Science and Science Education, Anchor University Lagos
URL: journal.aul.edu.ng
Vol. 1 No 2, December 2020, Pp. 35 - 43
ISSN: 2736-0059 (Print); 2736-0067 (Online)

Activity of *Trichilia megalantha* Harms and *Trichilia welwitschii* CDC Extracts and Fractions on *Anopheles gambiae* Larvae.

[1]Fadare, D. A., [2]Ajaiyeoba E. O.

[1]Department of Chemical Sciences, Anchor University, Lagos.

[2]Department of Pharmacognosy, University of Ibadan, Nigeria.

. **Corresponding author:**

E-mail: e.ajaiyeoba@mail.ui.edu.ng; edajaiye@gmail.com

Phone: +234 802 3222 796

Submitted 21 July, 2020

Accepted 1 September, 2020

Competing Interests: The authors declare no competing interests.

ABSTRACT

Background: Lately, there is an increasing shift towards the use of environmentally friendly and biodegradable natural vector control of plant origin as agent for disease vector control.
Objectives: To determine the toxicity of extracts and fractions of *T. megalantha* and *T. welwitschii* on *Anopheles gambiae* larvae.
Methods: The larvicidal activity of methanol extracts of leaf, stem bark and root bark of *Trichilia megalantha* and *Trichilia welwitschii* (Meliaceae) and fractions of stem bark of *T. megalantha* and root of *T. welwitschii* were evaluated on early 4th instar *Anopheles gambiae* larvae. Larvae were exposed to various concentration of plant extracts and fractions. Dead larvae were counted after 24 h of exposure. The most active extracts of both plants were partitioned into hexane, dichloromethane (DCM), ethyl acetate and methanol and subjected to the same assay. Ethanol (5%) was included as a negative control. The experiments were done in triplicate. Results were compared to those of larvae exposed to N,N-diethyl-3-methylbenzamide (LC_{50} =1000.09 µg.mL^{-1}), the reference insecticide.
Results: All tested extracts and fractions showed larvae mortality. Of the six crude extracts screened, *T. megalantha* stem bark showed the highest activity with LC_{50} of 15.6 µg.mL^{-1} while the leaf (LC_{50} =496.1 µg.mL^{-1}) showed the least activity. The root of *T. welwitschii* was more toxic to the larvae (LC_{50} =65.0 µg.mL^{-1}) while the leaf (LC_{50} =232.0 µg.mL^{-1}) showed the least larvae toxicity.
Conclusion: The results showed that the extracts and fractions of *T. megalantha* and *T. welwitschii* were significantly toxic to *Anopheles gambiae* larvae. The promising activity demonstrated by the two plants in mosquito vector control could contribute significantly to malaria control.

Keywords: Plant extract, Meliaceae, larvicidal, vector control

INTRODUCTION

Insects are major vectors of infectious diseases resulting in ill health and death worldwide. Mosquitoes transmit the disease to more than 700 million people annually (WHO, 2013; Caraballo and King, 2014; WHO, 2017; MDMBD, 2019). Malaria by caused parasite of the genus *Plasmodium* is spread through the bite of the female *Anopheles* mosquitoes (Adams, 2006). According to the World Health Organization (WHO, 2016), malaria is prevalent in over 100 countries. Presently, there are no drugs to combat the disease completely while the only official vaccine**,** RTS,S, has shown a relatively low efficacy (WHO, 2016). Low results obtained in the control programmes of malaria is due to the increased resistance of malaria to readily available drugs, and resistance of mosquitoes to insecticides because of their prolong use (Adams, 2006; Rajpai and Camboj, 2018). The disadvantageous effect of continuous use of insecticides is the accumulation of chemicals being hazardous to the biological environment (Mahmood *et al*, 2016). Plants and their extracts are considered to be safe, less harmful to nature and non-target organisms and biodegradable. Hence, an important alternative strategy for the control of mosquito vector (Rakhshan, 2018; Pavela *et al*, 2019). Control programme by elimination of mosquitoes at the larval stage is preferred because the larvae cannot escape from their breeding sites until the adult stage and also reduces the overall pesticide use in control of adult mosquitoes by aerial application of adulticidal chemicals (Walker and Lynch, 2007).

Natural insecticides have been increasingly used for mosquito control because of their efficacy and absence of toxicity on non-target organisms (Al- Sharook *et al*, 1991; Senthil Nathan, 2006; Gleiser and Zygaldo, 2007; Senthiill Kumar *et al*, 2008; Rajkumar and Jebanesan, 2005; 2009; Kovendan *et al*, 2013; Andrew and Shad, 2013; Rathy *et al*, 2015). The development of insect growth regulators has drawn much attention as selective control of insects of agriculture, medical and veterinary importance and has produced mortality due to their neurotoxic effects (Al-Zahrani *et al*, 2019; Bouazzis *et al.*, 2015; Ashwini and Asha, 2017, Senthil Nathan *et al.*, 2005, 2006 a,b). Studies on insecticidal properties of chemicals derived from plant material showed that they are environmentally safe, degradable and target specific. Several plant extracts have shown larvae toxicity against different species of mosquito (Borges *et al.*, 2003; Prabhu *et al*, 2011; Tennyson *et al.*, 2012).

Various plants such as *Azadirachta indica*, Eucalyptus spp., *Lantana camara*, *Vitex negundo*, *Cymbopogon spp.*, *Menthapiperita*, *Tagetesminuta* have been reported to possess repellent activity against mosquitoes. (Karunamoorthi *et al.*, 2009a). The smoke produced by burning of dried leaves of various plants has been used for protection against mosquitoes since ancient times (Mittal and Subbarao, 2003; Karunamoorthi *et al.*, 2009b; Alayo *et al*, 2015; Remia *et al*, 2017; Afolabi *et al.*, 2018). The major advantages of plant-based traditional repellents are that they are inexpensive, easily available, locally known, and culturally acceptable (Karunamoorthi *et al.*, 2008).

Trichilia (Meliaceae) is a genus of trees, rarely shrubs. Surveys of the Meliaceae family have identified the genus *Trichilia* as a potential source of substances with insecticidal action similar to *Azadirachta indica*, the best known species within the family (Wheeler *et al.*, 2001; Matos *et al.*, 2009; Garcia-Gomez *et al*, 2018; Liu *et al*, 2017; Liu *et al*, 2018).

Genus *Trichilia* is essential because of it's medicinally significant tetranortriterpenoids or limonoids (Vieira *et al.*, 2014). Root bark extracts of five Meliaceae species (*Turraea abyssinica*, *Turraea wakefeldii*, *Turraea mombassana*, *Trichilia roka* and *Melia volkensii*) and different fractions were compared for their immediate toxicity and long-term effects on *An. gambiae.* (Ndung'u *et al.*, 2004).

Trichilia megalantha is a medium-sized tree of 30 m high and above found in the moist semi-deciduous forest in Ivory Coast and Nigeria. The bark is grey or reddish, rough and scaly. (Burkil, 1985). *Trichilia welwitschii* is small to medium-sized tree 10 –20 m high, found in the deciduous forest and secondary jungle; in the South East of Nigeria. The stem bark is used medicinally as treatment for dropsy, swellings, oedema and gout. In DR Congo, a bark decoction is administered as enema to treat haemorrhoids and other abdominal disorders and as an abortifacient whereas pounded young leaves are applied to sores from syphilis. (Louppe *et al.*, 2008). The seed of *T. welwitschii* have been found to contain limonoids such as dregeanin, rohituka and trichilia lactone (Tsamo *et.al*, 2013).

The emergence of resistance in mosquitoes to conventional synthetic insecticides like temephos, fenthion, cypermethrin, cypermethrin and cyhalothrin; suggests the search for better alternative means which will be safer and effective (Tikar *et al*, 2008).

In continuation of the study on plants from family Meliaceae as malaria vector control (Ojo *et al*, 2013), the present study investigated the toxicity of morphological parts of *T. megalantha* and *T. welwitschii* crude methanol extracts and fractions on malarial vector, *Anopheles gambiae.*

MATERIALS AND METHOD

Preparation of extracts

Plant parts were collected in March, 2014 from their natural habitats in Ibadan, Nigeria and were identified and authenticated at Forestry Research Institute of Nigeria, Ibadan, voucher specimens were deposited with FHI numbers 109556 and 109557 for *T. mega-*

lantha and *T. welwitschii* respectively in the Herbarium. Preliminary phytochemical screening were carried out on plants using standard methods (Sofowora, 1993; Harborne, 1973).

Air dried powdered plant specimens (500 g) were extracted into distilled methanol by maceration for 72 h. The filtrate was concentrated and extracts were stored at 4°C before use.

Determination of larvicidal activity

Stock solutions of each extract were prepared at 2000 μg.mL^{-1} with ethanol. The stock solution was serially diluted to prepare the test solutions of 15.65, 31.25, 62.5,125, 250, 500 and 1000 μg.mL^{-1}.

Larvae were collected in May, 2014 from mosquito breeding sites in Ibadan, Nigeria. Collected larvae were washed in clean water before assay. Standard methods for assaying larvicidal activity as recommended by the World Health Organisation were followed in all experiments. Preliminary bioassays were performed with early 4th instar larvae of *An. gambiae* in triplicate using 20 larvae. The larvae were placed in disposable plastic cups containing 100 mL of different concentrations of the test solution at ambient temperature of 28-32°C. Larvae were considered dead when they were unable to reach the surface of the solution when pricked with needle. The number of dead larvae was determined after 24 h of exposure. An aqueous solution of ethanol (5%) was employed as the negative control while N,N-diethyl-3-methylbenzamide (DEET) was included as a positive control. Fractionation of the most active crude methanol extracts of *T. megalantha* stem bark and *T. welwitschii* root was done by solvent-solvent partitioning into hexane, DCM, ethyl acetate and methanol. Appropriate concentrations of the fractions were made and used in the experiments.

1.2 Data Analysis

The data obtained were statistically assessed for mean and standard error of the mean (M ± SEM). Statistical significance was determined by Graphpad[4] Prism software. One way ANOVA was used to compare parameters within groups. All the data were analysed at a 95% confidence interval ($P<0.05$). The concentration required to kill 50% (LC_{50}) of the larvae present was determined.

RESULTS

Preliminary phytochemical screening result indicated the presence of alkaloids and terpenoids in all the plant parts investigated; flavonoids were detected only in the leaf of *T. megalantha* and saponin was abundant in *T. welwitschii* stem and root (Table 1).

Assessment of the larvicidal effect of extracts of the leaves, stem bark and root (15.63 –1000.00 μg.mL^{-1}) of *T. megalantha* and *T. welwitschii* against early 4th instar larvae of *Anopheles gambiae* showed various larval toxicities. Activity observed was concentration- dependent (Figure 1). At the highest concentration tested (1000 μg.mL^{-1}), *T. megalantha* stem bark killed the entire larvae. The LC_{50} showed that the stem bark extract of *T. megalantha* was the most toxic, with an LC_{50} of 15.6 μg.mL^{-1} followed by the root extract with LC_{50} of 22.1 μg.mL^{-1}. Among fractions tested, the hexane soluble fraction of *T. megalantha* exhibited the highest toxicity; $LC_{50,}$ 22.2 μg.mL^{-1} (100% mortality at 250 and 500 μg.mL^{-1}) (Figure 2) while the methanol soluble fraction was the least toxic (LC_{50} of 625.1 μg.mL^{-1}).

In the case of *T. welwitschii* (Figure 1) the crude methanol root extract was the most active, exhibiting 100% larvae mortality at 1000 μg.mL^{-1} with LC_{50} of 35.2 μg.mL^{-1}, followed by the stem bark while the leaf displayed the lowest activity. The dichloromethane fraction of *T. welwitschii* root (Figure 2) demonstrated the highest larval toxicity (LC_{50} 43.1 μg.mL^{-1}) followed by the hexane fraction. Methanol fraction had the least toxicity to the larvae.

DISCUSSION

The efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts used, age of plant parts (young, mature or senescent), solvent used during extraction as well as upon the available vector species.(Ghosh *et al*., 2012; Bincy *et al*., 2017).

Table 1: Preliminary Phytochemical Screening of *T. megalantha* and *T. welwitschii*

Plant species	Part used	Alk	Tan	Sap	Ster	Terp	Flav	Gly
T. megalantha	Leaf	+	-	-	+	+	-	-
	stem	+	+	+	-	+	+	+
	root	+	+	-	+	+	-	-
T. welwitschii	leaf	-	-	-	+	+	-	+
	stem	+	-	+	-	+	-	-
	root	+	+	+	-	+	-	-

Key: + = positive; - = not detected; Alk: Alkaloids; Tan: Tannins; Sap: Saponins; Ster: Sterols; Terp: Terpenoids; Flav: Flavonoids; Gly: Glycosides

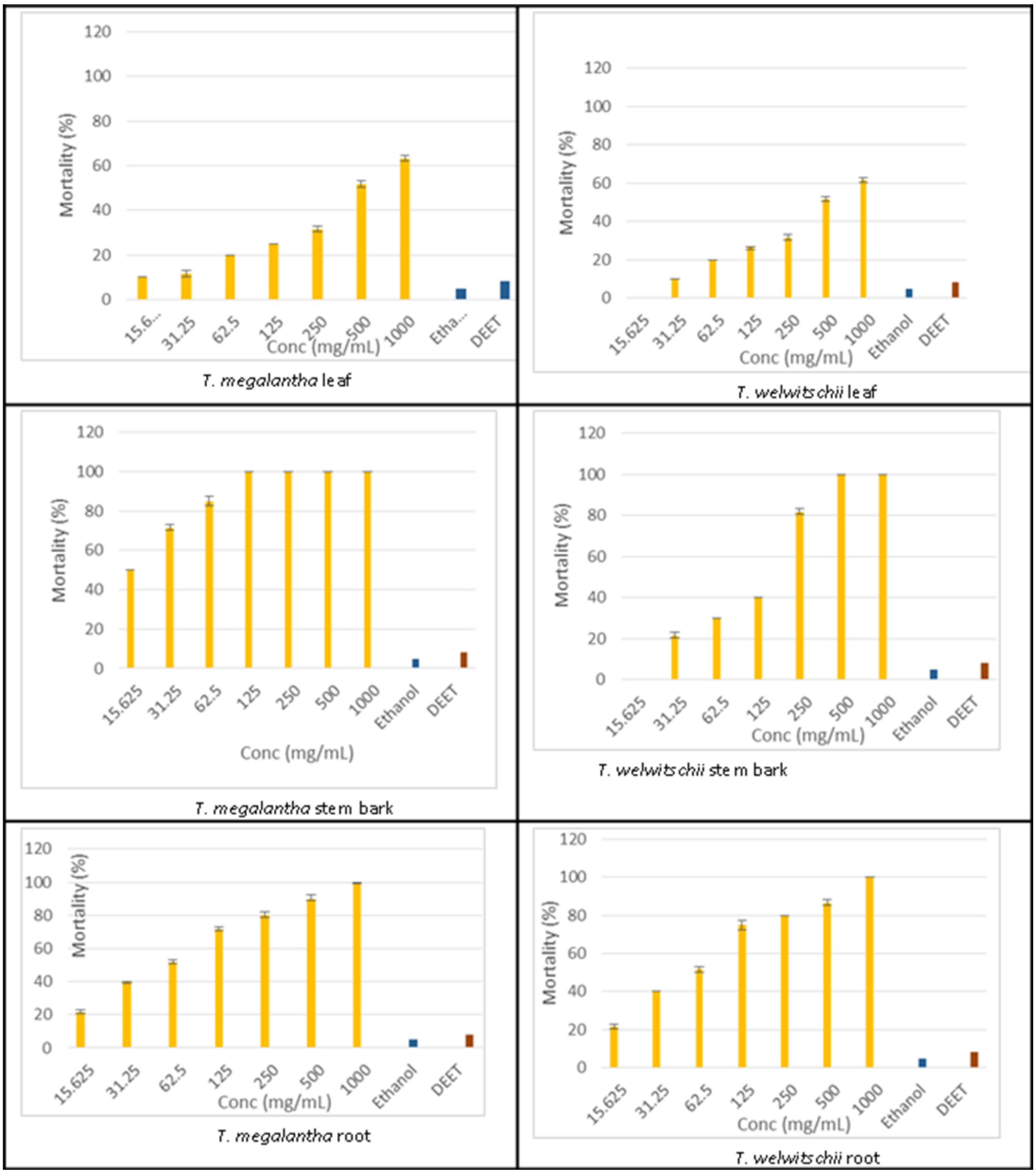

Figure 1: Larva Toxicity Activity of *T. megalantha* and *T. welwitchii* crude extracts against 4^{th} instar *An. gambiae* larvae

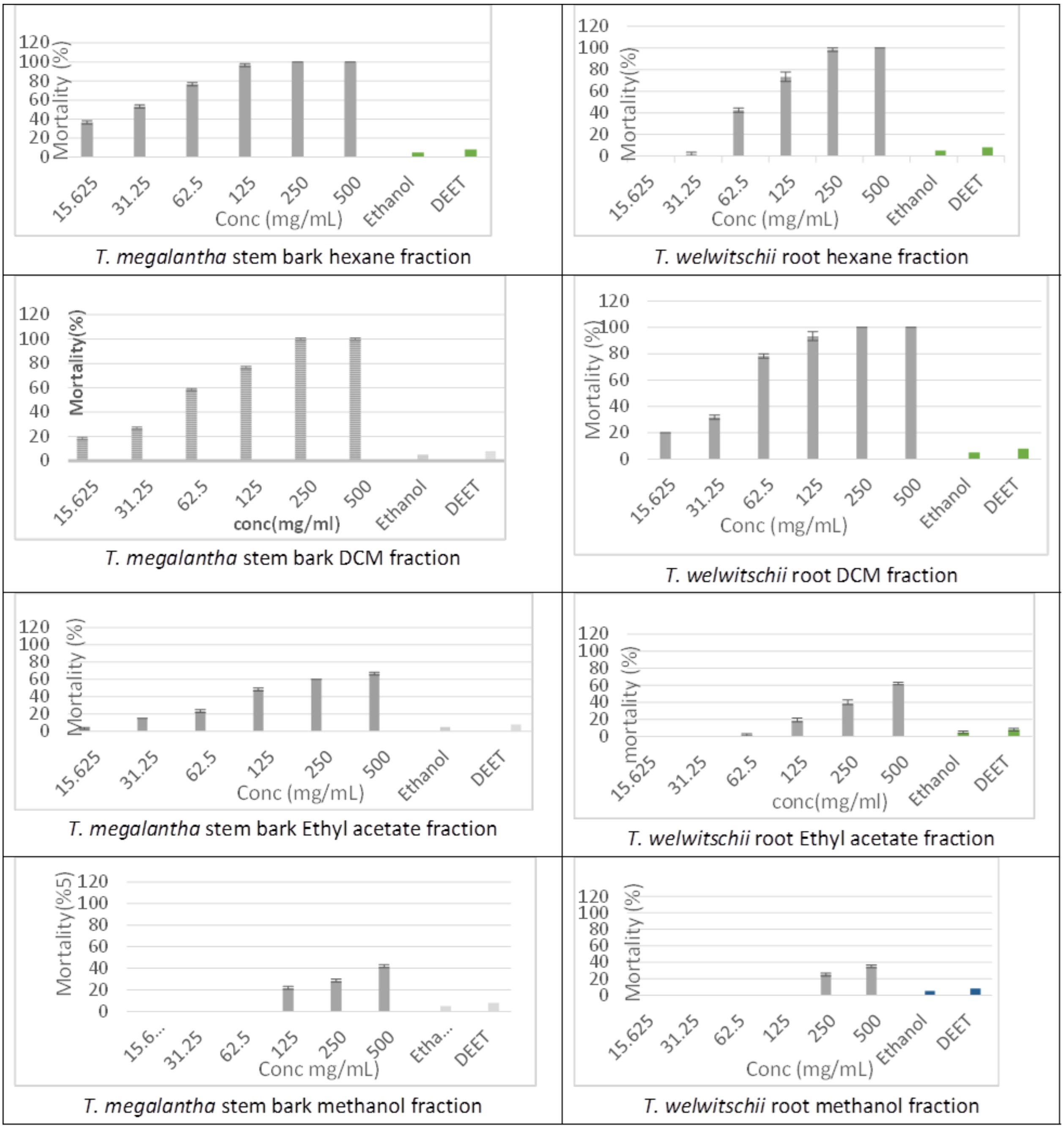

Figure 2: Larva Toxicity Activity of *T. megalantha* and *T. welwitchii* fractions against 4th instar *An. gambiae* larvae

Several active secondary metabolites such as phenolics, terpenoids, and alkaloids have been reported to be present in different parts of plant which are responsible for various activities demonstrated by the plant extracts (Kamaraj *et al.,* 2011). The result of preliminary phytochemical screening of *T. megalantha* and *T. welwitschii* showed the presence of these secondary metabolites which may contribute greatly to the larvicidal activities observed.

Assessment of the mosquito larvicidal effect of *T. megalantha* and *T. welwitschii* indicated that all extracts and fractions were significantly toxic to the larvae. The larvae were more susceptible to *T. megalantha* stem bark extract. Larval mortality was considerably less in leaf extracts of both plants when compared to the root and stem barks. This may be due to several secondary metabolites identified in the stem bark when compared with other parts screened (Table 1).

The results of screening of fractions obtained by partitioning the crude methanol extracts into hexane, DCM and ethyl acetate showed that the non-polar fractions were the most active. Several studies have demonstrated that moderately polar extracts are more toxic to mosquitoes than the polar extracts. Reports of other studies have also shown that the chloroform extract exhibited high toxicity towards larvae of different mosquito species. (Sama *et al*, 2000; Johnson *et al*., 2018; Kumar *et al*., 2018).

Comparing the extracts of the two species, *T. megalantha* extract showed better larvicidal activity than *T. welwitschii.* Of all the metabolites present in Trichilia the limonoids, triterpenes modified with high oxygenation, were observed with high frequency in the genus. Compounds derived from terpenes pathway were more significant, corresponding to about 87.7% of isolated and identified compounds from various Trichilia species (Vieira *et al*, 2014). The plants under study showed similar larvicidal and insecticidal activities to *Azadirachta indica* a member of the family Meliaceae (Anjum *et al*., 2010).

CONCLUSION

The results of this study has shown that *T. megalantha* and *T. welwitschii* exhibited promising larvicidal activities, hence could be explore as alternative environmentally friendly and biodegradable as vector management for malaria control.

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