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Research Article

Larvicidal Activity of *Tetrapleura tetraptera* (Schum and Thonn) Taubert (Mimosaceae) extracts against *Anopheles gambiae*

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I N F O

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A B S T R A C T

This study investigated the effects of hot water and ethanolic extracts of *Tetrapleura tetraptera* fruit against the larvae of *Anopheles gambiae*. The larvae of *Anopheles gambiae* used for this bioassay was obtained from the wild. The mosquito was identified and the bioassay was carried out following standard processes. The larvae of *Anopheles gambiae* was exposed to hot water and ethanolic extracts of *Tetrapleura tetraptera* fruit for 24 hours. The results showed a statistical increase at $p<0.05$ in mortality rate in a concentration dependant pattern. Furthermore, the overall LC50 values were 99.57 ppm and 143.07 ppm for ethanolic and hot water extracts of *Tetrapleura tetraptera* fruit. The values also showed that the ethanolic extracts confer superior larvicidal activity as compared with the hot water extract. From the observations of this study, there is need for further research to be carried out to show the compounds responsible for the larvicidal activities of *Tetrapleura tetraptera*.

Keywords: Insects, Malaria, Public Health, *Tetrapleura tetraptera*, Vector Control

Introduction

Mosquitoes are important vectors of several diseases that affect humans. Some of the diseases that mosquitoes transmit include chikungunya, lymphatic filariasis, Japanese encephalitis, malaria and dengue fever.[1-7] Mosquitoes belong to the Class Insecta, Order Diptera and Family Culicidae. There two major subfamilies viz: *Anophelinae* (the sub-family includes Anopheles species and some other important genus) and Culicinae (includes other important genus of mosquito including *Aedes, Culex, Coquillettidia, Culiseta, Orthopodomyia, Psorophora*, etc.).[3,4,8] The other sub-family is *Toxorhynchitinae*. Human malaria is transmitted only by females of the genus *Anopheles*.[9]

Plasmodium species is the parasite that causes malaria in humans. The parasites are transmitted through the human population by the female *Anopheles* mosquito. There are 5 species of Plasmodium that cause malaria in humans. Among the 5 species, *Plasmodium falciparum* and *Plasmodium* vivax pose the greatest threat to humans. For instance, in 2018, *Plasmodium falciparum* accounted for about 99.7%, 50%, 71% and 65% of malaria cases in Sub-Saharan Africa, South-East Asia, Eastern Mediterranean and Western Pacific World Health Organization (WHO) region, respectively, while 75% of malaria cases in America WHO region was caused by Plasmodium vivax.[10]

In many regions that malaria is endemic, the genus *Anopheles* is the most important iniquitous dipteran. According to the World Health Organization, there were approximately 228 million and 405 000 cases and deaths, respectively resulting from malaria worldwide in 2018.[10] Of

these deaths, 67% were children under the age of 5 making children the most vulnerable group to malaria infection in the endemic areas.

A significant proportion of malaria cases and deaths occur in sub-Saharan Africa. For instance, 93% and 94% of cases and deaths, respectively occurred in Africa in 2018.[10] Six countries accounted for over 50% of global malaria cases in 2018 including Nigeria (25%), Democratic Republic of Congo (12%), Uganda (5%), Côte d'Ivoire, Mozambique and Niger (4% each).[10] This makes Nigeria the most endemic region of malaria infection in the world.

On the global perspective, Southeast Asia contributes about 2.5 million to the total malaria cases, and of this, India contributes approximately 77% million cases.[11] According to Kumar et al.,[11] approximately 2 million malaria cases and 1,000 deaths occur annually in India. Reports have shown that malaria incidence is on the decreasing trend in India.[12] For instance, between 2001-2013, a total of 20,728,060 and 12,760 confirmed cases and deaths associated with malaria were recorded in India, respectively. The death cases translate to approximately 981 per annum.[12] The analysis of malaria cases between 2001-2013, showed that in Delhi, Bihar, Himachal Pradesh, Kerala, Jammu & Kashmir, Lakshadweep, Sikkim, Punjab, Puducherry and Uttarakhand (subset A), Chandigarh, Manipur, Tamil Nadu, Uttar Pradesh, Andhra Pradesh (Subset B), Karnataka, Rajasthan and Maharashtra and Daman & Diu (Subset C), West Bengal, Haryana, Gujarat, Madhya Pradesh, Assam, Nagaland and Goa (Subset D), Tripura (Subset E), Chhattisgarh, Jharkhand and Andaman & Nicobar Islands (Subset F), Orissa (Subset G), and Mizoram, Dadar & Nagar Haveli, Arunachal Pradesh and Meghalaya (Subset H) there is no statistical deviation between states with a particular subset, and states within different subsets differed significantly viz Subset A < B < C < D < E < F < G and H.[12] Authors have reported disability adjusted life lost due to malaria to be 1.86 million years, and cost–benefit analysis have indicated that for every Rupee invested by the National Malaria Control Program a dividend of 19.7 Rupees is paid.[11,12]

Several attempts have been made to eradicate mosquitoes at the different stages of development (oval, larvae, pupa and adults) using insecticides in diverse habitats including their breeding ground and homestead. But due to the poor sanitation and the cosmopolitan nature of the fly in its control breeding areas, it has not been quite successful especially in coastal areas of the endemic region. Due to the possible effects of chemical based insecticides on the environment and associated non-target organisms, researches have been focused on possible alternatives to chemical based insecticides, and plants have emerged as credible candidates.

Tetrapleura tetraptera (Schum and Thonn) Taubert (Mimosaceae) is also known as Aridan tree in the South West region of Nigeria.[13-15] The plant is known to possess several therapeutic potentials for the treatment of numerous diseases including inflammation, arthritis, hypertension, diabetes, schistosomiasis, epilepsy and it's also used as an antioxidant and analgesic.[14,16] Other studies have also shown that it acts as an anti-convulsant, anti-ulcerative and anti-microbial while also having trypanocidal, hirudinicidal, molluscicidal and ectoxicity properties.[16,17] *Tetrapleura tetraptera* also has uses as a birth control, schistosomiasis control, and the control of intestinal parasites whilst also having neuromuscular, hypotensive, cardio-vascular and hypoglycemic effects.[16,17] It is also used as an antihypertensive,[14,16,17] cytoprotective, piscicidal, antigonadotropic, antimutagenic, antimalarial,[18] aphrodisiac agent[19] and an anti-convulsant agent.[20]

The fruit *Tetrapleura tetraptera* is commonly used after birth by breastfeeding mothers to prevent postpartum contraction and for the treatment of inflammations.[14] The fruit of *Tetrapleura tetraptera* possesses a pungent aromatic odour which has been attributed to its insect repellent properties.[16] Aina et al.[9] studied the activities of extracts of T*etrapleura tetraptera, Delonix regia* and *Raphia vinifera* on the larvae of *Anopheles gambiae*. Oniya and Idowu[21] reported that *Tetrapleura tetraptera* extract is highly toxic to tadpoles with LC50 value of 0.189ml/L at 24 hours of exposure. But information about the larvicidal activities of Tetrapleura tetraptera is limited in the literature. Hence, this paper is focused on the larvicidal activity of *Tetrapleura tetraptera* extract against the larvae of *Anopheles gambiae*.

Materials and Methods

Plant Collection and Preparation

The dried fruit of *Tetrapleura tetraptera* used for this study was purchased from Swali Market in Yenagoa metropolis, Nigeria. The *Tetrapleura tetraptera* was further shade dried and subsequently blended into powder using an electronic blender.

Plant Extracts

The *Tetrapleura tetraptera* powder was extracted by weighing 500g into 1000ml of the solvent (ethanol and hot water). It was allowed to soak for 72 hours. The mixture was filtered using a double layered muslin cloth.[4] The filtrate was then concentrated using a rotary evaporator, and the resultant residue was reconstituted with distilled water to different concentrations (0.00ppm, 40.00ppm, 80.00ppm, 120ppm and 160ppm and 200ppm).

Culture of Anopheles Gambiae and Larvicidal Bioassay

The larvae of *Anopheles gambiae* used for this study was obtained from the wild following the methods previously

described by Izah,[4] Youkparigha and Izah[5] and Izah and Youkparigha.[6] Some of the larvae were allowed to develop into adults and the characteristics possessed were compared with the ones presented by Gimba and Idris,[22] Ahmed and Ahmed[23] and Izah.[4]

The activity of hot water and ethanolic extracts of *Tetrapleura tetraptera* against the larvae of *Anopheles gambiae* was done in line with the World Health Organization's protocol[24,25] with slight modifications. Varying concentrations of hot water and ethanolic extracts were made viz: 0.00ppm, 40.00ppm, 80.00ppm, 120ppm and 160ppm and 200ppm with distilled water. 10 larvae of *Anopheles gambiae* were introduced to the various concentrations made in triplicates. Mortality was determined when the larvae did not respond to repeated prodding with a soft brush after 24 hours of exposure.[4]

Statistical Analysis

Statistical Package for Social Sciences, Graph pad prism 5 and Microsoft excel were used for the data analysis. One-way analysis of variance at $p<0.05$ and Duncan statistics was used to show significant variation across the percentage mortality of the different concentrations. The data was expressed as mean ± standard error in a chart format. The LC50 values were calculated using Finney Table – Microsoft excel regression method previously applied by Izah.[4] T-test statistics was used to compare the LC50 values of ethanolic and hot water extracts.

Results and Discussion

Figure 1 and 2, presents the mortality rates of the larvae of *Anopheles gambiae* exposed to hot water and ethanolic extracts of *Tetrapleura tetraptera* fruit. The mortality rate at 0.00ppm, 40.00ppm, 80.00ppm, 120.00ppm, 160.00ppm and 200.00ppm were 0.00%, 16.67%, 33.33%, 46.67%, 73.33% and 86.67%, respectively (for ethanolic extract) (Figure 1) and 0.00%, 10.00%, 16.67%, 33.33%, 53.33% and 73.33%, respectively (for hot water extract) (Figure 2). Statistically, there was deviation at $p<0.05$ across the various concentrations in a dose dependent pattern for both extracts (hot water and ethanolic). The trends observed in the results of this study had some similarity with findings of related works.[4-6,9]

Figure 3 and Table 1, presents the larvicidal activities of hot water and ethanolic extracts of Tetrapleura tetraptera against Anopheles gambiae. The triplicate values of the extracts of Tetrapleura tetraptera against the mosquito had LC50 values of 103.99 ppm, 97.72ppm and 97.05 ppm, respectively (for ethanol extracts) and 140.92 ppm, 145.21 ppm and 154.52 ppm, respectively (for hot water extracts). Furthermore, t-test revealed (t-test value =-13.293; p-value =0.001) that there is significant deviation in the mean LC50 value of mortality induced by ethanolic and hot water extracts of *Tetrapleura tetraptera* fruit against *Anopheles gambiae*. The results further showed that the ethanolic extract of *Tetrapleura tetraptera* is more potent against *Anopheles gambiae* as compared to the hot water extracts. This is in accordance with the results of previous studies which indicated that solvents such as ethanol and acetone extracts of plants tend to be more potent against mosquito larvae.[4-6,9] The superiority of the ethanolic extract may be due to the polarity of the solvent and its physical and chemical characteristics. The values reported in this study have some similarity with the values previously reported in other natural food species against mosquitoes. For instance, Izah and Youkparigha[5] reported LC50 values of 161.01 ppm and 104.47 ppm for *Anopheles gambiae* larva induced by aqueous and ethanolic extracts of *Cymbopogon citratus*, respectively. Izah[4] reported LC50 values of 115.24ppm, 173.16ppm and 265.19ppm for *Anopheles gambiae* larvae induced by ethanolic, acetone and crude extracts *Capsicum frutescens* var. minima fruit.

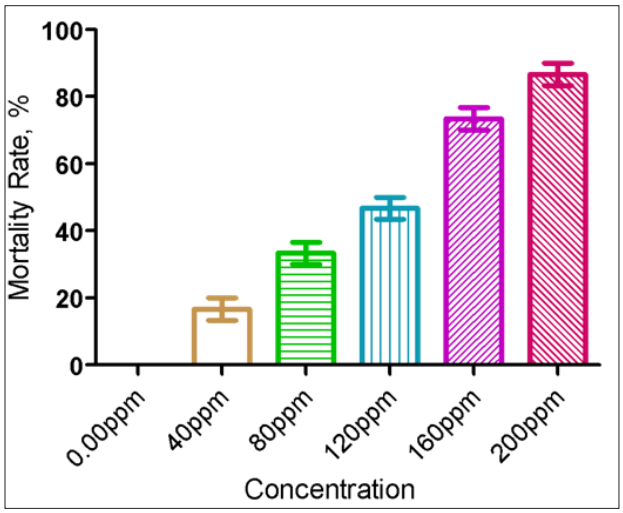

Figure 1.Mortality rate of ethanolic extracts of Tetrapleura tetraptera against Anopheles gambiae

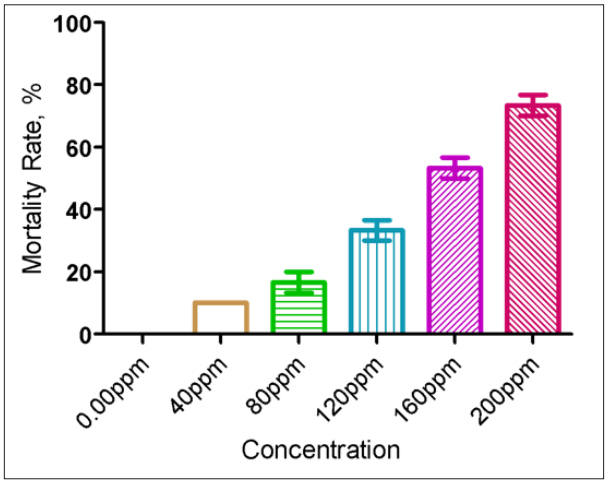

Figure 2.Mortality rate of hot water extracts of Tetrapleura tetraptera against Anopheles gambiae

The larvicidal activities of *Tetrapleura tetraptera* against *Anopheles gambiae* may be due to the bioactive and phytochemical constituents of the plant. Studies have shown that oil from the of leaf of *Tetrapleura tetraptera* possess 31 compounds including esters, alkanes, alkenes, terpenoids, fatty acids and vitamins, and these compounds account for the antibacterial, antimicrobial, antioxidant, cytotoxic, anti-inflammatory, anticancer, anxiolytic, antidepressant, hypotensive, insecticide, cytoprotective, antidiabetic, anti-cardiovascular, anti-dementia, anticonvulsant, antiadipose tissue, anticoagulant, antidepressant and antistress potentials of *Tetrapleura tetraptera*.[14] Enema et al.[14] showed that trans-geranylgeraniol found in the leaf of *Tetrapleura tetraptera* makes the plant confer insecticidal activities. Okwute et al.[13] studied *Tetrapleura tetraptera* and reported that alkaloids, phlobatanins, volatile oil, tannins and flavonoids are found in the leaf and stem-bark, steroids and saponins are found in only stem-bark, and terpenoids, resins and balsams are found only in the leaf. Akin-Idowu et al.[26] reported the presence of polyphenols, flavonoids, saponins, tannins and phytate in the fruit of Tetrapleura tetraptera. Nwoba[27] reported the presence of flavonoids, alkaloids, saponins, tannins, phenol and glycoside in *Tetrapleura tetraptera*. Adusei et al.[28] reported the presence of flavonoids, alkaloids, saponins and phenol in pulp, seed and fruits of *Tetrapleura tetraptera* using water and ethanol as solvents. Tannins and steroids were only detected in the pulp and fruit using water and ethanol as extraction solvents and terpernoids were only present in the ethanol extract of Tetrapleura tetraptera's pulp and fruit. Enema et al.[15] reported that phenol, alkaloids, terpenoids, tannins, flavonoids and saponins were found in *Tetrapleura tetraptera* fruit using different solvent as extracts. The authors further reported that different solvents such as n-hexane, dichloromethane, ethyl acetate and methanol possessed varying effects on the phytochemical constituents of *Tetrapleura tetraptera* fruits. Agu and Thomas[29] reported that plants containing alkaloids may be able to ward off pests including insects. According to David et al.[30,31] and Izah[4] polyphenols found in plants confer insecticidal activity. This further indicates that the phenolic compounds and its derivatives found in the plant could also contribute to its toxicity to the mosquito larvae. The *Tetrapleura tetraptera* fruit extracts may have caused an alteration in the midgut of the mosquito's epithelium thereby leading to death.[5,6,32,33]

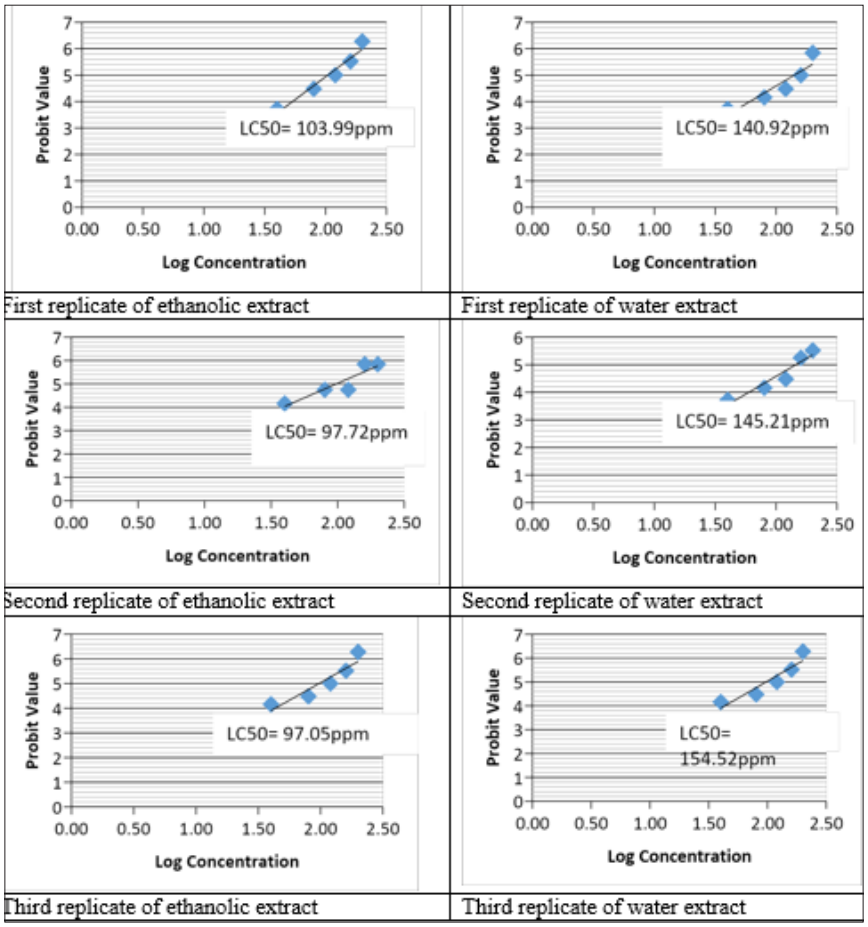

Figure 3.LC50 values of mortality rate of *Anopheles gambiae* induced by hot water and ethanolic extracts of *Tetrapleura tetraptera*

Table 1.Comparative LC50 values of the mortality rate of *Anopheles gambiae* induced by hot water and ethanolic extracts of *Tetrapleura tetraptera*

Ethanolic extract	Water extract	t-test	p-value
99.59±2.21	143.07±2.15	-13.293	0.001

Conclusion

The activities of ethanolic and hot water extracts of *Tetrapleura tetraptera* fruit against the larvae of *Anopheles gambiae* showed that the plant possessed larvicidal potentials. The study further indicated that the ethanolic extracts of *Tetrapleura tetraptera* fruit had a superior effect as compared to the hot water extracts against the mosquito. Based on the findings of this study, there is the need for further studies to be carried out with respect to identifying the compounds responsible for the larvicidal activities of the plant, as well as determining the potential toxicity of the plant extracts on non-target organisms and the potential effect of the residues on humans, animals, and on different ecosystems. The effect of environmental components such as pH and temperature on the mortality rate of *Anopheles gambiae* larvae induced by *Tetrapleura tetraptera* fruit extracts also needs to be studied.

Conflict of Interest: None

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