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# Vector Control and Isolation of Larvicidal Compounds from *Melia Azedarach* (A. Juss) Leaf Using *Anopheles Gambiae* Larva

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## Abstract

The aim of this present investigation is to evaluate and isolate larvicidal principles in leaves of Melia azedarach using the fourth instar larvae of An. gambiae. Larvicidal activity was evaluated by exposing 4th instar larvae of Anopheles gambiae to serial concentrations (0.625-10 mg/mL) of extracts, fractions and compounds of Melia azedarach. Larval mortality was recorded after 24h of exposure and 50% lethal concentration (LC50) values determined. L Results were compared to those of larvae exposed to N, Ndiethyl-m-toluamid (DEET), the reference insecticides and untreated groups. Larvicidal activity of the crude methanol extract of both leaves and stem bark of M. azedarach were 5.80 and 7.59mg/mL respectively. The hexane soluble fraction of M. azedarach leaf displayed highly lethality at 0.625 mg/mL with LC50 of 0.025 than the remaining fractions. Similarly, larvicidal activity of DEET was significantly low (LC50=1.09mg/mL) than the hexane fractions ($p<0.05$). Fractionation and purification of the active fractions led to isolation of three compounds. Fraction 35-49 (designated as 'F') of M. azedarach showed larvicidal activity on larvae with LC50 of 0.017 mg/mL. Two compounds were isolated from fraction 'F' using preparative thin layer chromatographic. Compound 2 and 3 had Rf values of 0.75 and 0.63 respectively on silica gel G using CH3Cl3-CH3OH (9.5:0.5) and CH3Cl3-Acetone (9:1) respectively. Compounds 2 and 3 showed positive test for the presence of triterpenoids after spraying with anisaldehyde/H2SO4. The presence of triterpenoids may be responsible for the insecticidal activity observed in the two compounds isolated from the leaves of M. azedarach. The leaves of M. azedarach showed potent anti larvicidal activity. Compounds from this plant may serve as sources for the development of malaria vector control compounds.

**Keywords:** Melia azedarach; β-sitosterol; Triterpenoids; Larvicidal activity

## Introduction

The increase in occurrence of malaria in Africa is becoming alarming. Despite all effort being put together to reduce tis deadly diseases. Statistics from the World Health Organisation (WHO) shows sub–Saharan Africa were more affected. In 2006, there were 216 million cases of malaria, up from 211 million cases in 2015. The estimated number of malaria death stood at 445,000 in 2016, a similar number to the previous year, 446,000 [1]. Among the cases infant, children, and pregnant women were prone to this infection disease. Control of malaria is becoming increasingly difficult because of development of resistance of mosquitoes to pesticides [2]. Hence, a marked increase in malaria infection has necessitated the needs to control the vector which is a major delivery system that harbor the parasite, since parasites were resistant to malaria drugs. Control of mosquito is essential as many species of mosquitoes are vectors to prevalent and infection disease including malaria, filariais, and many arboviral diseases and they constitute an intolerable biting nuisance [3,4]. Co-evolution has equipped plants with a plethora of chemical defenses against insect predators. According to Feinstein [5], more than 2,000 species of plants representing 170 families are said to have insecticide properties. The Meliaceae plant family is known to contain a variety of compounds that show insecticidal, antifeedant, growth regulating and development-modifying properties [6,7]. Melia azedarach commonly known as Chinaberry or Persian lilac tree is a deciduous tree that is native to northwestern India and has long been recognized for their insecticidal properties. Fruit extracts of M. azedarach and Azadirachta indica elicit a variety of effects in insects such as antifeedant, growth retardation, reduced fecundity, moulting disorder, morphogenetic defects, and changes of behavior [8,9]. They are commonly laid between the pages of books and in folded woolen clotting as protection against insect attack [10]. The bark, flowers and leaves of M. azedarach have been shown to be toxic, but they are less toxic than fruits. The present study aim to isolate the vector control compounds from the leaf of Melia azadarach.

## MATERIALS AND METHODS

### Plant material

The leaves of M. azedarach were collected from Botanical garden of University Ibadan, Nigerian, in July 2010. Plants materials were authenticated by Mr. Oluseun Osiyemi of the Forest Research Institute of Nigeria (FRIN, Ibadan, and voucher specimens were deposited under FHI 108966, in FRIN.

### Preparation of plant extract

Dried powdered leaf of M. azedarach was exhaustively extracted with methanol by cold maceration for 72 h at room temperature (RT. Extracts were filtered and the solvents evaporated to dryness using a rotary evaporator at 40°C. The resulting extracts were stored in the refrigerator at 4°C until needed for analysis.

### Larvae

Larvae were collected at Ojoo area, Ibadan, Oyo state, Nigeria, from tyre-print breeding sites and reared in plastic bowels containing clean well water. They were fed with dog biscuit.

### Larval toxicity assay

Stock solutions of each extract were prepared at 20 mg/mL with ethanol. Test solutions of concentration of 10, 5, 2.5, 1.25 and 0.625 mg/mL were prepared by serial dilution of the stock solution with ethanol. Sterile disposable cups (250 mL) were used for the study. Dilution of extracts consisting of 1 mL was transferred into a cup containing 99 mL of well water (99 mL). Twenty fourth-instar larvae were released into each cup containing 100 mL solution of each test concentration. After 24 h, the number of dead larvae in each cup was counted (assessed by inability of larva to get to the surface when agitated). The experiment was extended to 48h. Control experiments carried out with DEET and with 1% ethanol were run in parallel. Experiments were done in duplicates.

### Statistical analysis

Results were expressed as mean ± SEM of two independent experiments. Larval toxicities were reported as LC50 obtained from Graph Pad Prism statistical software, analysis.

## Discussion

One of the aims of this study was to interfere with their life cycle by blocking the metamorphosis of larvae to pupa form using plant extracts. In this study, plant extracts (leaf and stem bark) of*Melia azedarach* from Meliaceae family were subjected to larvicidal activity. The results of preliminary larvicidal activity of the leaf and stem-bark methanol extracts of*M. azedarach* are shown in Table 1.

This was carried out to ascertain if M. *azedarach* had any lethality effect on the *Anophele* mosquitoes. The methanolic leaf extract displayed higher toxicity on mosquitoes larval than stem bark of M. azedarzch. Activity was not concentration dependent; probably extracts displayed rather a repellant effect on the mosquitoes at higher concentrations. The LC50 of the crude extracts were presented in the Table 2 & Table 3, the leaf extract was more active than that of stem bark extract and the reference drug, DEET is 1.09 mg/mL. The results of screening fractions obtained by partitioning the crude methanol extracts into hexane, chloroform and ethyl acetate showed that the non-polar fractions were the most active. The hexane soluble fraction of M. azedarach leaf exhibited the highest toxicity among the fractions although chloroform and ethyl acetate also displaced toxicity but not as active as hexane fraction.

**Table 1:** Showing % mortalities of crude extracts.

| Concentration (mg/ml) | Mean % Mortality ( ± SEM) | | |
|---|---|---|---|
| | MAL | MAS | DEET |
| 10 | 100 ± 0.0 | 100 ± 25.0 | 100 ± 0.0 |
| 5 | 100 ± 0.0 | 87.5 ± 12.5 | 45.0 ± 1.0 |
| 2.5 | 77.5 ± 2.5 | 25.0 ± 12.1 | 37.5 ± 2.0 |
| 1.25 | 82.5 ± 2.5 | 25.0 ± 0.0 | 30.0 ± 2.5 |
| 0.625 | 75.0 ± 5.0 | 12.5 ± 12.5 | 20.0 ± 0.5 |
| 1% Ethanol | 0.0 ± 0.0 | 0.0 ± 0.0 | |

MAL: M. azedarach leaf; MAS: M. azedarach stem bark

**Table 2:** Larvicidal activity of hexane, chloroform and ethyl acetate fraction of *M. azedarach* leaf on *An. Gambiae* .

| Concentration (mg/ml) | Mean % Mortality ( ± SEM) | | | |
|---|---|---|---|---|
| | Hexane | Chloroform | Ethyl acetate | DEET |
| 10 | 100 ± 0.0 | 100 ± 0.0 | 97.5 ± 2.5 | 100 ± 0.0 |
| 5 | 100 ± 0.0 | 94.7 ± 0.0 | 96.5 ± 0.0 | 45.0 ± 1.0 |
| 2.5 | 100 ± 0.0 | 86.8 ± 2.6 | 95.0 ± 5.0 | 37.5 ± 2.0 |
| 1.25 | 100 ± 0.0 | 76.3 ± 2.6 | 95.0 ± 5.0 | 30.0 ± 2.5 |
| 0.625 | 96.0 ± 0.0 | 71.1 ± 2.6 | 95.0 ± 5.0 | 20.0 ± 0.5 |
| 1% Ethanol | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | |

**Table 3:** $LC_{50}$ value of crude methanol extract of *M. azedarach* leaf and stem bark.

| Plant extracts | $LC_{50}$ |
|---|---|
| M. azedarach leaf | 5.808 |
| M. azedarach stem bark | 7.594 |

The *M. azedarach* leaf hexane fraction produced 100% mortality at 2.5 mg/ml (Figure 1), while as chloroform, ethyl acetate produced 94.7%, 96.5% at 5 mg/ml respectively. The hexane soluble fraction of *M. azedarach* (10 g) was chromatographed on silica gel using column chromatographic technique and eluted with Hex:$CHCl_3$, $CHCl_3$:EtoAc and then EtoAc:$CH_3OH$ mixture of increasing polarity (going from 100% Hex to 100% $CHCl_3$ and then 100% EtoAc to 20% $CH_3OH$ ). All 50 fractions collected were monitored by TLC. Fraction 21 that were eluted with 100% $CHCl_3$ crystallized to obtain compound 1, 0.4 g, $R_f$=0.4 (silica gel, 100% $CHCl_3$). The compound was compared with standard reference of β-sitosterol ($R_f$=0.4) to ascertain the name of the compound above. It was found that the compound have similar $R_f$ with standard reference of β-sitosterol. Thereafter, the compound gave a deep brown colour after spraying the TLC plate with vanillin/sulphuric acid. However, this fraction has no larvicidal activity when tested on *Anopheles gambiae* but could be used for commercial purpose in future.

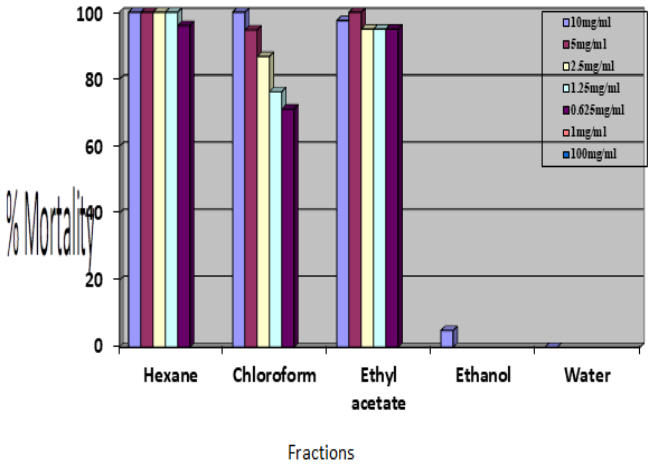


**Figure 1:** Larvicidal activity of fractions obtained from the leaf of *M. azedarach*.

Based on Tlc analysis, 50 fractions collected were pooled together and grouped into 8 groups as a result of similar Rf they have in common. The 8 groups (A-I) are shown in Table 4. Were subjected to larvicidal activity. Out of this groups, fraction F displayed the highest activity at 2 mg/ml against third instar larvae of Anopheles gambiae, and continue to inhibit metamorphosis of larvae to pupa even at 0.5 mg/ml. Comparatively, fraction H was least active of the fractions at LC50 of 703.5 mg/ml compared to LC50 of the highest active fraction at 0.19 mg/ml. Fraction C, D and G displayed moderate LC50 of 1.12, 0.49 and 0.2 mg/ml respectively. Thin layer chromatographic analysis of the fraction F revealed the presence of three compounds which were identified by visualizing the plate under UV light at 254 nm. This fraction Bohnenstengal and co-workers isolated three meliacarpin derivatives from leave of Melia azedarach which was incorporated into artificial diet of larvae of the polyphagous pest of Spodoptera littoralis in a chronic feeding bioassay. Quassinoids, are modified triterpenoids, can thus stand a good chance of being used as insecticides. The result from this study will go a long way in reducing the spread of malarial disease in our environment since the principal host has been eliminated or inhibited by the allelochemicals produced by this fraction. The result is consistent with earlier studies from this plant on other mosquitoes excluding An. gambiae has proof to be an inhibitor was re again subjected to purification column chromatograph in which 59 fractions were collected and analysis on Tlc plates as a result of pooling them together. The three groups were resulted by pooling viz: fraction F1, F2 and F3 were tested on Anopheles gambiae mosquito's larvae and LC50 of the fractions were 5.023, 1.218 and 507.14 mg/ml respectively. Fraction F2 was the most active of the entire sub fraction F and on Tlc plate fraction F2 revealed three compounds which were identified by visualizing the plate under UV light at 254 nm.Preparative thin layer chromatography was finally used to separate the compounds and thereafter spotted on a Tlc plate revealed one spot which was very prominent under UV light at 254 nm. The spot produced after spraying with anisaldehyde/H2SO4 were three spots and all gave dark brown color in daylight and indicated that the compounds belong to triterpenoids. This result is consistent with earlier works by [11], that both leaves and seed extracts of Melia azedarach revealed the presence of triterpenoids and steroids, and both also presented alkaloids and condensed tannins. Compounds present in leaves are different from those in seeds, since the former inhibit mainly egg hatching and the later, larval development. Triterpenoids are known to possess insect antifeedant and growth regulation activity against a variety of agriculture pest [12]. Many triterpenoids present in plants of the Meliaceae family are described as showing insectiaidal activity [13] of emergence of larvae to pupa. The newest research work by Coria [14]. Proved that ethanolic leaf extract of M. azedarach is a strong larvicide on A. aegypti, and all tested larvae died before pupation, and significantly delayed development time, in addition to its inhibition ability of oviposition by the vector females. In comparison with leaf extract the fruit extract showed much weaker effects.

Table 4: LC50 of fractions of MZFL obtained from the column chromatography.

| Fractions | $LC_{50}$ (mg/ml) |
|---|---|
| A | 5.89 |
| B | 25.01 |
| C | 1.12 |
| D | 0.49 |
| E | 8.24 |
| F | 0.19 |
| G | 0.28 |
| H | 703.7 |
| I | 0.82 |

## Conclusion

In this present studies, the very active fractions F2 suggested that the methanol extract might be used directly as larvicidal agents in small volume of aquatic habitats or breeding sites. In comparison with the results of earlier studies, it was noticeable that leaf extract tested in this studies exerted promising mosquito larvicidal potential. The mode of action of this leaf extract on mosquito larvae are not known, but previous studies demonstrated that phytochemical interfered with the proper functioning of mitochondria more specifically at the proton transferring sites [15] and other studies by [16] found that phytochemicals primarily affect the midgut epithelium and secondary affect the gastric caeca and the malpighian tubules in mosquito larvae. Characterization and structural elucidation of the isolated compounds are still ongoing.

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