

Original Article

Laboratory Evaluations of the Fractions Efficacy of *Annona senegalensis* (Annonaceae) Leaf Extract on Immature Stage Development of Malarial and Filarial Mosquito Vectors

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Abstract

Background: Within the framework to control mosquitoes, ovicidal, larvicidal and pupicidal activity of *Annona senegalensis* leaf extract and its 4 fractions against *Anopheles gambiae* and *Culex quinquefasciatus* were evaluated in the laboratory conditions.

Methods: Ovicidal test was performed by submitting at least 100 eggs of mosquitoes to 125, 250, 500, 1000 and 2000 ppm concentrations, while larvicidal and pupicidal effects were assessed by submitting 25 larvae or pupae to the concentrations of 2500, 1250, 625 and 312.5 ppm of plant extract or fractions of *A. senegalensis*.

Results: The eggs of *An. gambiae* were most affected by N-hexane (0.00% hatchability) and chloroform (03.67% hatchability) fractions compared to *Cx. quinquefasciatus* where at least 25 % hatchability were recorded at 2000 ppm. For larvicidal test, N-hexane (LC₅₀= 298.8 ppm) and chloroform (LC₅₀= 418.3 ppm) fractions were more effective than other fractions on *An. gambiae* larvae while, a moderate effectiveness was also observed with N-hexane (LC₅₀= 2087.6 ppm), chloroform (LC₅₀= 9010.1 ppm) fractions on *Cx. quinquefasciatus* larvae. The highest mortality percent of the pupae were also recorded with N-hexane and chloroform fractions on *An. gambiae* at 2500 ppm. As for *Cx. quinquefasciatus* only 50 % and 36 % mortality were recorded with N-hexane and chloroform fractions respectively.

Conclusion: The extract of *A. senegalensis* was toxic on immature stage of mosquito species tested. By splitting methanolic crude extract, only N-hexane and chloroform fractions were revealed to possess a mosquitocidal effects and could be considered and utilized for future immature mosquito vectors control.

Keywords: Fractions, Pupicidal, *Annona senegalensis*, *Anopheles gambiae*, *Culex quinquefasciatus*

Introduction

Apart nuisances they inflict to human beings, mosquitoes are responsible of dreadful diseases such as malaria, filariasis, dengue haemorrhagic fever, etc widespread in the world (Murugan et al. 2007, Kamaraj et al. 2009). Most of Sub-Saharan Africa countries have stable endemic malaria because climatic conditions, which are ideal for the transmission, coincide with the range of *An. gambiae*, *An. arabiensis* and *An. funestus*, the most efficient vector mosquitoes in the world (Foko et al. 2011). In Cameroon *An.*

gambiae is the principal vector of malaria in rural and urban areas (Foko et al. 2011). Filariasis, a disease affecting the arms, legs and genitals, is much prevalent in the world. Filariasis caused by *Wuchereria bancrofti* is transmitted by *Cx. quinquefasciatus*, mosquitoes widespread in the countries now and lymphatic filariasis infects 80 million people annually of which 30 million cases exist in chronic infection (Samidurai et al. 2009).

Synthetic pesticides have been extensively used for vector control by either killing, pre-

venting adult mosquitoes to bite human beings or by killing mosquito immature stages at the breeding sites of the vectors (Joshep et al. 2004). Development of insect resistance to synthetic pesticides such as Malathion, DDT, Deltamethrin and even biopesticides such as *Bacillus thuringiensis* (Tabashnik 1994), added to high operational cost, environmental pollution and deleterious effects on non-target organisms are the problems people are facing to control vector borne diseases (Mittal et al. 2003).

These problems have highlighted and need for the development of new strategies for selective mosquito control. With these problems in focus, it becomes increasingly necessary to search for an alternative in the development of environmentally safe, biodegradable, low cost, target specific insecticides for mosquito control and which can be used with minimum care by individuals and communities in specific situations (Dua et al. 2010). Plants may be a source of alternative agent to replace the synthetic insecticides for mosquito control. Toxicity of phytochemicals in mosquitoes was first reported by Campbell et al. (1933). Review papers from all over the world have documented the toxic effect of plant extracts on mosquito eggs, larvae and pupae (Tare et al. 2004, De Lima et al. 2006, Promsiri et al. 2006, Govindarajan et al. 2012, Dhivya and Manimegalai 2013). Earlier, the plant extracts belonging to the family of Annonaceae such as *Annona muricata*, *A. cherimolia*, *A. squamosa*, *Cananga odorata*, *Ferula hormonis*, etc have shown larvicidal effect against *Anopheles sp* and *Culex quinquefasciatus* (Saxena et al. 1993, Bodadilla-Alvarez et al. 2002, Moore and Lenglet 2004, Isman 2006). Das et al. (2007) reported that the ethanol leaf extract of *Annona squamosa* was found to have the most promising larvicidal activity against *Cx. quinquefasciatus* larvae. Ovicidal, larvicidal and pupicidal effects of *Hyptis suaveolens*, *Calotropis gigantea* and *Delonix elata* against *An. gambiae* and

Cx. quinquefasciatus were also reported (Ivoke et al. 2009, Govindarajan et al. 2012, Dhivya and Manimegalai 2013). Gueye et al. (2011) reported insecticidal activity of extract *A. senegalensis* extract on eggs and adults groundnut weevils *Caryedon serratus*. In northern part of Cameroon, the leaves of this plant are used locally to protect maize, millet and sorghum against weevils' attacks (Ngamo et al. 2007). In ethno-medicine, the leaves of *A. senegalensis* are used as antidiarrheic and antitrypanosomic (Ogbadoyi 2007), antidiarrheic (Suleiman 2008), cures snake bite, generalized edemes, aches and constipation (Akoegninou et al. 2006).

With a large scale of activity as insecticide and medicinal plant, it should be advisable to extent biological properties of this plant on mosquitoes. This study was aimed to evaluate ovicidal, larvicidal and pupacidal activity of *A. senegalensis* methanolic crude extract and its fractions (N-hexane, chloroform, ethyl-acetate and methanol fractions) on *An. gambiae* and *Cx. quinquefasciatus* mosquito species.

Materials and Methods

Collection and processing the plant material

The fresh leaves of *A. senegalensis* were collected at Dang, a village of Ngaoundere in the Adamaoua region (latitude 7°24.949'N, longitude 13°32.870'E and altitude 1093 masl), Cameroon in December 2011. The plant was identified by the herbalist, Pr. Mapongmetsem Pierre-Marie, Department of Biological Science, University of Ngaoundere, Cameroon and then confirmed at the National Herbarium in Yaoundé, where voucher sample was deposited. The leaves are then dried at room temperature, then grounded with an electric grinder and then stored at 4 °C in the refrigerator until use.

Extraction and fractionation

The method of Gueye et al. (2011) was followed for the purpose. Indeed, five hundred (500) grams of powder were macerated in 2500 ml of methanol for 72 h at room temperature and then the maceration was filtrated using filter paper Whatman No.1. The filtrate was summited to Rotary Evaporator apparatus to obtain a residue called crude extract. Part of this crude extract was separated successively by the method of differential solubility in four solvents of different polarity: n-hexane, chloroform, ethyl-acetate and methanol. The crude extract was mixed with silica gel (70–260 mesh size) and macerated in N-hexane, then filtered with Whatman No. 1 filter paper after phase separation. N-hexane fraction and marceration (1) are recovered. Marceration (1) is dried in the open air and then soaked in chloroform, phase chloroform fraction filtrated and marceration (2) are also recovered. Marceration (2) after dried in open air is soaked in ethyl-acetate; phase ethyl-acetate fraction filtered and marceration (3) are also recovered. Marceration (3) is finally taken up in methanol to recover the polar compounds in the methanol fraction after filtration. Each fraction has been concentrated using Rotary evaporator and the solid fractions gotten were stored at -4 °C until bioassays.

Mosquito breeding

The larvae of *Cx. quinquefasciatus* were collected from the laboratory of National Arbovirus Research Center (NAVRC), Enugu, Nigeria in February 2013 while *An. gambiae* larvae were collected from stagnant water in the gutter at Awka market, Anambra State, Nigeria (06°12'23"N, 07°03'23"E) and identified also in NAVRC in January 2013. Larvae were kept in plastic trays containing tap water. Larvae were fed a diet containing crayfish and biscuit in a ratio of 3: 1, respectively. Pupae were transferred from the trays to a cup containing tap water and were main-

tained in our insectary cage (45×45×40 cm), where adults emerged. Adults were maintained in cages and were continuously provided with 10 % sucrose solution in a jar with a cotton wick. On day five, blood meal was given to the female adults for eggs maturation. Beaker with 100 ml of tap water lined with filter paper was kept inside the cage for oviposition (Kamaraj et al. 2008). All the experiments were carried out, at 27±2 °C and 75–85 % relative humidity under 12: 12 light and dark cycles.

Ovicidal activity

For ovicidal activity, the method of Kumar et al. (2012) was followed for the purpose. The freshly laid eggs were collected by providing ovitraps in mosquito cages kept 2 days after the female mosquitoes were given a blood meal. Indeed, 100 gravids female mosquito were placed in a screen cage where ten oviposition cups were introduced for oviposition, 30 min before the start of the dusk period. The eggs were laid on filter paper No. 1 provided in the ovitrap. Out of these ten cups, nine were filled with test solution of 125, 250, 500, 1000 and 2000 ppm concentration of plant extract/ fractions of the leaves of *A. senegalensis* while one was filled with 99 ml of distilled water mixed with 1 ml of Tween-80 used as a control. A minimum of 100 eggs was used for each treatment, and the experiment was replicated four times. After treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with tap water for hatching assessment after counting the eggs under microscope (Su and Mulla 1998). The percent of egg mortality was calculated on the basis of nonhatchability of eggs with unopened opercula (Chenniappan and Kadarkarai 2008).

Larvicidal test

The larvicidal activity of extract/fractions

of *A. senegalensis* was evaluated against *An. gambiae* and *Cx. quinquefasciatus* according to the method described by WHO (1996). The extract/fractions were dissolved in 0.5 ml of Tween-80. The concentrations of 2500, 1250, 625 and 312.5 ppm of extract/fractions were prepared in the volume of 100 ml with tap water in the 250 ml beakers. Twenty five fourth instar larvae were transferred into the used and four replicates were maintained for each concentration. Mortality was recorded after 24 h of exposure, during which no food was given to the larvae. Larvae were considered dead if appendages did not move when probed with needle in the siphon or cervical region. Larvae incapable of rising to the surface or not showing the characteristic diving reaction when water was disturbed, were considered moribund and added to the dead larvae for calculating percentage of mortality. Data were adjusted for control mortality using Abbott's formula (Abbott 1925), if mortality in the control sets exceeded 5 %.

Pupicidal effect

Pupicidal effect was assessed according to the method applied by Ashfaq and Ashfaq (2012). Twenty five freshly emerged pupae of each mosquito species were transferred into beakers of 250 ml volume, containing 75 ml of tap water. The extract/fractions of the plant used were dissolved in Tween-80 and then added with tap water to make up to 100 ml corresponding to the concentrations of 2500, 1250, 625 and 312.5 ppm of extract/fractions of *A. senegalensis* were made. Each treatment was replicated four times and the number of emerged adults for each replication was recorded after 48 h.

Phytochemical screening

The qualitative phytochemical analyses of the components responsible of toxicity on insects were carried out according to the methods of Harborne (1973) and Trease and Evans (1989). These methods are founded on de-

tection the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids, terpenoids, oil and fats which possess insecticidal properties in the extract and fractions of *A. senegalensis*.

Statistical analysis

The values recorded from toxicity essays were transformed in percentage of mortality, hatchability using Microsoft Excel 2010 and were corrected using Abbot's formula whenever required. The percentage of mortality, hatchability data were subjected to the ANOVA procedure using the Statistical Package for the Social Science (SPSS 16.0). Duncan test at $P=0.05$ was applied for mean separation. Probit analysis (Finney 1971, SPSS 16.0) was applied to determine lethal concentrations causing 50 % (LC_{50}) and 90 % (LC_{90}) mortality of larvae and pupae 24h after treatment application.

Results

The results of ovicidal activity of *A. senegalensis* extracts against against *An. gambiae* and *Cx. quinquefasciatus* are presented in Table1. A significant ($P<0.001$) variation of percentage of eggs hatchability is observed in mosquito species assessed. The rate of hatchability has significantly ($P<0.001$) reduced with the increasing of concentration. In comparison with the methanolic crude extract, the fractionation process pointed out the effectiveness of N-hexane followed by chloroform fractions on mosquito eggs. At the highest concentration (2000 ppm), less than 5 % rate of hatchability of *An. gambiae* were recorded with N-hexane (0.00%), Methanolic crude extract (04.67%), chloroform fraction (03.67%) contrary to ethyl-acetate and methanol fractions where, the high values of 22.67 % and 60.33% were recorded respectively. The eggs of *Cx.*

quiquefasciatus were less affected by the extracts used. The percentage of hatchability was 100 % with Methanol fraction with all concentrations tested and at least, 25 % of eggs hatched were observed in others extract or fractions of *A. senegalensis*.

The toxicities of the fractions of *A. senegalensis* extract on early four instar *An. gambiae* and *Cx. quinquefasciatus* larvae were noted, and the LC₅₀, LC₉₀, 95 % confidence limits of LCL and UCL and chi-square were also calculated (Table 2). At 2500 ppm and 1250 ppm, 100 % mortality of the larvae of *An. gambiae* was recorded with N-hexane and chloroform fractions. Among the fractions, N-hexane (LC₅₀= 298.8 ppm, LC₉₀= 572.9 ppm) and chloroform (LC₅₀= 418.3 ppm, LC₉₀= 822.0 ppm) fractions were found to be more effective than other fractions against *An. gambiae* larvae. As for *Cx. quinquefasciatus*, a moderate effectiveness was also observed with the mortality percent of 48% for N-hexane fraction, 29.33 % for methanolic crude Extract and 13.33 % for chloroform fraction recorded at 2500 ppm. In comparison of fractions, N-hexane fraction (LC₅₀= 2087.6 ppm) was most toxic followed by methanolic crude extract (LC₅₀= 5884.1 ppm) and chloroform fraction (LC₅₀= 9010.1 ppm) while, no mortality was recorded with Ethyl-acetate and methanol fractions. Apart ethyl-acetate and methanol fractions

of *A. senegalensis*, others fractions especially N- hexane and chloroform fractions possessed a significant (P< 0.001) efficacy against pupae of mosquito species assessed. At the highest concentration (2500 ppm), 80 %, 70 % and 62 % mortality of *An. gambiae* pupae were recorded with N- hexane, chloroform fractions and methanolic crude extract respectively (Fig. 1). A moderate mortality of *Cx. quinquefasciatus* pupae were noted with N-hexane fraction (50%) chloroform fraction (36%) and methanolic crude extract (34%) at the highest concentration (2500 ppm) (Fig. 2). At all concentrations were tested and no mortality of the pupae of *An. gambiae* and *Cx. quinquefasciatus* was recorded with ethyl-acetate and methanol fractions of *A. senegalensis*.

The fractions of *A. senegalensis* leaves extract were screened for the presence of major phytochemical groups responsible of insecticidal activity. The preliminary phytochemical screening of the crude extract revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids, terpenoids, oil and fats except steroids (Table 3). By splitting the crude extract, the same phytochemicals were found in N-hexane fraction excepted saponins, steroids and terpenoids. Alkaloids, flavonoids tannins and phenolic compounds were also found in chloroform fraction.

Table 1. Hatchability percent of *Anopheles gambiae* and *Culex quinquefasciatus* eggs treated with extract/fractions of *Annona senegalensis*

| Mosquito species | Conc (ppm) | Percentage of egg hatch ability | | | | | |
|-------------------------------|------------|---------------------------------|--------------|--------------|--------------|--------------|-----------|
| | | Fractions | | | | | |
| | | MCE | NHF | CHF | EAF | MTF | F |
| <i>Anopheles gambiae</i> | 0 | 100±0.00fA | 100±0.00fA | 100±0.00fA | 100±0.00eA | 100±0.00eA | 0.00ns |
| | 125 | 69.67±1.53eB | 62.67±2.08eA | 79.00±2.00eC | 96.33±1.53eD | 98.67±0.58eD | 285.59*** |
| | 250 | 52.00±2.00dB | 39.67±2.08dA | 58.33±2.08dC | 81.33±2.08dD | 95.67±1.15dE | 421.70*** |
| | 500 | 34.00±1.00cB | 19.67±3.21cA | 29.67±1.53cB | 65.0±3.61cC | 88.67±1.53cD | 423.60*** |
| | 1000 | 17.67±1.53bC | 04.00±2.00bA | 10.00±2.00bB | 41.33±2.08bD | 77.00±2.00bE | 716.29*** |
| | 2000 | 04.67±1.53aA | 00.00±0.00aA | 03.67±1.15aA | 22.67±3.06aB | 60.33±2.08aC | 545.37*** |
| | F | 1850*** | 1153*** | 1689*** | 516.18*** | 348.42*** | |
| <i>Culex quinquefasciatus</i> | 0 | 100±0.00eA | 100±0.00fA | 100±0.00fA | 100±0.00eA | 100±0.00aA | 0.00ns |
| | 125 | 98.00±1.00eB | 88.00±2.00eA | 96.67±1.53eB | 100.0±0.00eC | 100.0±0.00aC | 50.63*** |
| | 250 | 92.67±1.53dC | 74.00±2.00dA | 84.67±1.53dB | 98.00±1.00dD | 100.0±0.00aD | 176.69*** |

Table 1. Continued...

| | | | | | | |
|------|--------------|--------------|--------------|--------------|--------------|-----------|
| 500 | 81.67±3.51cC | 58.33±1.53cA | 69.67±1.53cB | 95.67±0.58cD | 100.0±0.00aD | 263.67*** |
| 1000 | 66.67±2.08bC | 42.67±3.06bA | 55.67±0.58bB | 90.33±0.58bD | 100.0±0.00aE | 594.27*** |
| 2000 | 57.00±3.00aC | 25.33±2.08aA | 40.67±2.52aB | 84.67±1.53aD | 100.0±0.00aE | 644.53*** |
| F | 192.67*** | 592.68*** | 729.05*** | 168.93*** | 0.00ns | |

MCE: Methanolic Crude Extract, NHF: N-hexane fraction, CHF: Chloroform fraction, EAF: Ethyl-Acetate fraction, MTF: Methanol fraction

Means ± SE in the same column for the same category of concentration, followed by the same small letter and in the same row for the same category of extract, followed by the same capital letter do not differ significantly at P= 0.05 (Duncan's test). Each datum represents the mean of three replicates of 100 eggs each. ns= P> 0.05, ***= P< 0.001

Table 2. LC₅₀ and LC₉₀ values [ppm (95% fiducial limits)] at 24 h of fractions of *Annona senegalensis* extract against fourth instar larvae of *Anopheles gambiae* and *Culex quinquefasciatus*

| Mosquito species | Extracts | Slope±SE | R ² | LC ₅₀ (95% FL) | LC ₉₀ (95% FL) | ² |
|-----------------------------|----------|-----------|----------------|-----------------------------------|--------------------------------------|---------------------|
| <i>An. gambiae</i> | MCE | 2.69±0.14 | 0.98 | 973.3 (840.9-1132.7) | 2914.8 (2290.3-4130.2) | 33.44* |
| | NHF | 4.54±0.38 | 0.77 | 298.8 (274.6-320.1) | 572.9 (530.6-629.0) | 8.63 ^{NS} |
| | CHF | 4.37±0.27 | 0.85 | 418.3 (394.3-441.9) | 822.0 (760.6-902.6) | 16.19 ^{NS} |
| | EAF | 2.38±0.18 | 0.97 | 2789.3 (2464.3-3256.2) | 9662.1 (7427.4-13697.9) | 17.31 ^{NS} |
| | MTF | 2.45±0.46 | 0.70 | 8511.4 (5608.7-20304.7) | 28360.3 (13685.4-133304.7) | 15.37 ^{NS} |
| <i>Cx. quinquefasciatus</i> | MCE | 1.64±0.17 | 0.94 | 5884.1 (3888.6-12610.7) | 35659.9 (15583.6-175387.5) | 19.36* |
| | NHF | 1.68±0.14 | 0.98 | 2807.6 (2382.6-3467.3) | 16240.9 (11105.9-27263.4) | 14.01 ^{NS} |
| | CHF | 1.95±0.28 | 0.88 | 9010.1 (6024.9-18200.9) | 40915.2 (19789.1-149218.8) | 16.05 ^{NS} |
| | EAF | - | - | - | - | - |
| | MTF | - | - | - | - | - |

MCE= Methanolic crude extract, NHF= N-hexane fraction, CHF= Chloroform fraction, EAF= Ethyl-Acetate fraction, MTF: Methanol fraction, FL= Fiducial Limit, LC= Lethal concentration, NS= No significant.

*= P< 0.01, variances and covariances have been multiplied by the heterogeneity factor in computing fiducial limits of lethal concentrations because the probability of ² value P< 0.05, - = undetermined values because of low or no mortality

Table 3. Qualitative phytochemical screening of some components of extract/fractions of *Annona senegalensis*

| Photochemical Components | <i>Annona senegalensis</i> | | | | |
|--------------------------|----------------------------|-----|-----|-----|-----|
| | MCE | NHF | CHF | EAF | MTF |
| Alkaloids | + | + | + | - | - |
| Flavonoids | + | + | + | + | - |
| Saponins | + | - | - | + | + |
| Tannins | + | + | + | + | + |
| Phenolic compounds | + | + | + | - | - |
| Steroids | - | - | - | - | - |
| Fats and oils | + | + | - | - | + |
| Terpenoids | + | - | - | + | + |

MCE= Methanolic crude extract, NHF= N-hexane fraction, CHF= Chloroform fraction, EAF= Ethyl-acetate fraction, MTF= Methanol fraction, +=present, - = absent

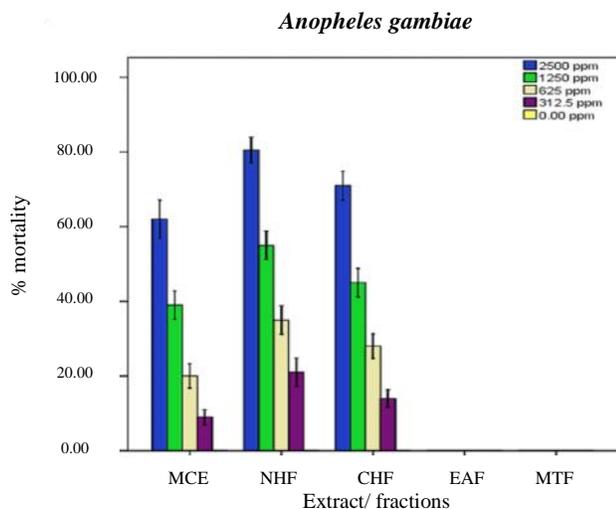


Fig. 1. Pupicidal activity of extract/fractions of *Annona senegalensis* against *Anopheles gambiae*

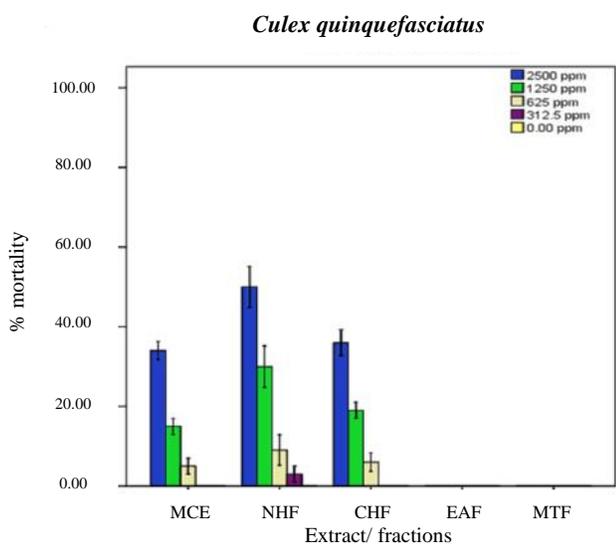


Fig. 2. Pupicidal activity of extract/fractions of *Annona senegalensis* against *Culex quinquefasciatus*

Discussion

Overall, ovicidal, larvicidal and pupicidal activity of various extract or fractions of the leaves of *A. senegalensis* were demonstrated on *An. gambiae* and *Cx. quinquefasciatus* mosquito species. Thus, toxicity of fractions varied with solvent used. N-hexane and Chloroform fractions showed promising mosquitocidal activity.

Indeed, plants are extensively reported to

possess toxic effect on the mosquitoes and can be utilized as a potent source of mosquito control (Kumar et al. 2012). The secondary metabolites are known to be effective against a wide range of insect pests as well as mosquito vectors (Sriwattanarungsee et al. 2008). These compounds may jointly or independently prove its efficacy against the mosquito targets by its ovicidal, larvicidal, pupicidal, adulticidal and by inhibition of growth activity (Borah et al. 2010). Earlier, some plants such as *Annona squamosa* L., *Gloriosa superba* L., *Millingtonia hortensis*, *Abuta grandifolia*, *Mintostachys setose*, *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens*, etc have been reported to control mosquito population (Ciccia et al. 2000, Kaushik and Saini 2008, Bagavan et al. 2009, Okigbo et al. 2010).

The ovicidal effects were generally dose dependent. Rajkumar and Jebanesan (2004) recorded similar observations in their study of ovicidal activity of *Moschosma polystachyum* leaf extract against *Cx. quinquefasciatus*. Compared to the present study, a complete inhibition of egg hatching of *An. gambiae* with methanolic extract of *Hyptis suaveolens* was reported by Ivoke et al. (2009). The ethanolic flower extract *Calotropis gigantea* has exhibited toxic effect on egg rafts of *Cx. quinquefasciatus* with 100 percent mortality at 200 ppm (Dhivya and Manimegalai 2013). Govindarajan et al. (2011) recorded similar findings in which the methanol leaf extract of *Coccinia indica* exerted zero hatchability (100% mortality) at 150 ppm for *Cx. quinquefasciatus*. No hatchability eggs of *Ae. aegypti* were also recorded with methanol, benzene and acetone extracts of *Cassia fistula* at 160 mg/l (Govindarajan 2009). On the contrary of this study, ovicidal effect of *A. senegalensis* was obtained with ethyl-acetate and methanol fractions on the eggs of *Caryedon serratus* (Gueye et al. 2011). This difference can be explained by the difference on insect species used and the phytochemical constituents of

plant extracts and extraction solvent used (Shalaby et al. 1998).

In comparison to this study, the chloroform soluble fractions of *Tagetes erecta* showed the highest toxicity than the other samples and consequently, the lowest LC₅₀ values (75.48 mg/L) in fourth instar larvae of *Cx. quinquefasciatus* (Farjana et al. 2011). The hexane extracts of *Cleistanthus collinus* and *Murraya koeingii* plants showed 100 percent mortality at 24 h bioassay against the third instar larvae of *Cx. quinquefasciatus* at 1000 ppm concentration (Tennyson et al. 2012). A significant mortality of four instar larvae of *Cx. quinquefasciatus* was also recorded with methanol extract of *Cliona celata* with LC₅₀= 95.63 ppm and LC₉₀= 242.16 ppm (Appadurai et al. 2013). The results are also comparable with an earlier report by Fred-Jaiyesimi and Anthony (2011) reported that the effects of the methanol extract, petroleum ether and chloroform fractions of *Paullinia pinnata* leaf have been investigated against the third and fourth instar larvae of *An. gambiae*. Govindarajan et al. (2008) reported also that methanolic leaf extract of *Acalypha indica* was more lethal to the egg and larvae of *Cx. quinquefasciatus* and *An. stephensi*. Kamaraj et al. 2011 reported values at 1000 ppm of 78, 73, 75 and 100 % mortality larvae of *An. subpictus* with N-hexane, chloroform, ethyl-acetate and methanol bark extracts of *Annona squamosa* respectively. Anupam et al. (2012) reported that N-hexane is the most non polar (polarity index of 0.1) that mainly extracts essential oil, chloroform or ethyl acetate are moderately polar (polarity index of 4.1) that mainly extracts steroids, alkaloids, etc. The presence of these phytochemicals distributed in each fraction confers to the fraction, its larvicidal property.

Prabhu and Murugan (2011) observed a significant pupicidal effect of plant extracts of *Moringa oleifera* against *An. stephensi* and pupal mortality of greater than 70 % was

encountered, similar to the results of the present study with *An. gambiae*. The pupae of *Ae. aegypti* were found greatly susceptible to higher dose (230 ppm) of plant extract *Catharanthus roseus* which caused mortality of 79 % (Remia and Logaswamy 2010) confirms the findings of the present study with N-hexane fraction on *An. gambiae*. The effectiveness of the extract or fractions of *A. senegalensis* could be explained by the presence of alkaloids and others compounds which are toxics for eggs, larvae and pupae of mosquito species. Earlier, Jolad et al. (1984) reported the presence of alkaloids, carbohydrate, lipids, amino acids, polyphenols, essential oils terpenoids the genus *Annona sp* plant.

Conclusion

Annona senegalensis contained active ovicidal, larvicidal and pupicidal compound in its leaves. However, these phytochemical compounds found in N-hexane and chloroform fractions could be the key candidates in insecticide values of this plant. This makes it a more suitable candidate for the development of new potential eco-friendly insecticides. Further investigation is needed to identify the active compounds of N-hexane and chloroform fractions responsible for its activity.

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References

- Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol.* 18: 265–267.
- Akoègninou A, Van Der Burg WJ, Van Der Maesen LJG (2006) *Flore Analytique du Bénin*, éd. Backhuys Publishers.
- Anupam G, Nandita C, Goutam C (2012) Plant extracts as potential mosquito larvicide. *Indian J Med Res.* 135: 581–598.
- Appadurai DR, Arokia VK, Michael GP, Savarimuthu I (2013) Larvicidal, Ovicidal, and repellent activities of Marine Sponge *Cliona celata* (Grant) extracts against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae). *ISRN Entomol*, pp. 1–9.
- Ashfaq M, Ashfaq U (2012) Evaluation of mosquitocidal activity of water extract of *Moringa oleifera* seeds against *Culex quinquefasciatus* (Diptera: Culicidae) in Pakistan. *Pak Entomol.* 34(1): 21–26.
- Bagavan A, Kamaraj C, Elango G, Zahir, AA, Rahuman AA (2009) Adulticidal and larvicidal efficacy of some medicinal plant extracts against tick, fluke and mosquitoes. *Vet Parasitol.* 166(3–4): 286–292.
- Bobadilla-Alvarez M, Zavaleta-Espejo G, Gil-Franco F, Pollack-Velásquez L, Sisnegas-González M (2002) Efecto bioinsecticida del extracto etanólico de las semillas de *Annona cherimolia* Miller (chirimoya) y *A. muricata* Linnaeus (guanábana) sobre larvas del IV estadio de *Anopheles sp.* *Rev Per Biol.* 9: 64–73.
- Borah R, Kalita MC, Kar A, Talukdar AK (2010) Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. *Afr J Biotechnol.* 9 (16): 2527–2530.
- Campbell FL, Sullivan WW, Smith LN (1933) The relative toxicity of nicotine, anabasine, methyl anabasine and lupinine for Culicinae mosquitoes. *J Econ Entomol.* 26: 500–509.
- Ciccica G, Coussio J, Mongelli E (2000) Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J Ethnopharm.* 72(1–2): 185–189.
- Das NG, Goswami D, Rabha B (2007) Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vector Borne Dis.* 44: 145–148.
- De Lima MGA, Maia ICC, De Sousa BD, De Moraes SM, Freitas SM (2006) Effects of stalk and leaf extracts from Euphorbiaceae species on *Aedes aegypti* (Diptera, Culicidae) larvae. *Rev Inst Med Trop S Paulo.* 48: 211–214.
- Dhivya R and Manimegalai K (2013) Comparative ovicidal potential of the flower and leaf extracts of *Calotropis gigantea* against the filarial vector *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Recent Sci Res.* 4(6): 735–737.
- Dua VK, Pandey AC, Dash AP (2010) Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J Med Res.* 131: 434–439.
- Farjana N, Rowshanul HM, Zahangir AS, Rezaul KM (2011) *Tagetes erecta* Linn. and its mosquitocidal potency against *Culex quinquefasciatus*. *Asian Pac J Trop Biomed:* 186–188.
- Finney DJ (1979) *Probit analysis*. Cambridge University Press, London.
- Foko GA, Tamesse JL, Fekam Boyom F (2011) Adulticidal effects of essential oils extracts from *Capsicum annum* (Solanaceae), *Piper nigrum* (Piperaceae) and *Zingiber officinale* (Zingiberaceae)

- on *Anopheles gambiae* (Diptera-Culicidae), vector of malaria. J Entomol. 8(2): 152–163.
- Fred-Jaiyesimi AA, Anthony O (2011) Larvicidal activities of the extract and fractions of *Paullinia pinnata* Linn leaf. Pharmaco Communi. 1(2): 37–40.
- Govindarajan M (2009) Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). Eur Rev Med Pharm Sci. 13: 99–103
- Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K (2008) Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res. 103: 691–695.
- Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A (2011) Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res. 109(2): 353–367.
- Govindarajan M, Rajeswary M, Sivakumar R (2012) Mosquito larvicidal and ovicidal activity of *Delonix elata* (L.) Gamble against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pac J Trop Dis: 571–573.
- Gueye S, Diop MT, Seck D, Sembene M (2011) Biochemical fractions activity of *Annona senegalensis* Pers. Extract leaves to protect groundnut against the seed-beetle *Caryedon serratus* Ol. (Coleoptera, Chrysomelidea, Bruchinae). Int J Plant Anim Environ Sci. 1(2): 122–130.
- Harborne J (1973) Phytochemical Methods: A guide to modern technique of plant analysis. Chapman and Hall, Thompson Science, London.
- Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and increasingly regulated world. Annu Rev Entomol. 51: 45–66.
- Ivoke N, Okafor FC, Owoicho LO (2009) Evaluation of ovicidal and larvicidal effects of leaf extracts of *Hyptis suaveolens* (L) poit (Lamiaceae) against *Anopheles gambiae* (Diptera: Anophelidae) complex. Anim Res Int. 6(3): 1072–1076.
- Jolad SD, Hoffmann KH, Schram KH, Cole JR, Bates RB, Tempesta MS (1984) A new diterpene from *Cupressus goveniana* var. *abramasiana*: 5 beta-hydroxy-6-oxasugiol (Cupresol). J Nat Prod. 47: 983–987.
- Joshep CC, Ndoils MM, Malima RC, Nkuniya NHM (2004) Larvicidal and mosquitoicidal extracts, a coumrin, isoflavonoids and petrocarpans from *Neorautanenia mitis*. Trans Rev Soc Trop Med Hyg. 98: 451–455.
- Kamaraj C, Bagavan A, Elango G, Abduszahir A, Rajakumar G, Marimuthu S, Santhoshkumar T, Abdul-Rahuman A (2011) Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Indian J Med Res. 134: 101–106.
- Kamaraj C, Bagavan A, Rahuman AA, Zahir AA, Elango G, Pandiyan G (2009) Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). Parasitol Res. 104(5): 1163–1171.
- Kaushik R, Saini P (2008). Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. J Vector Borne Dis. 45: 66–69.
- Kumar G, Karthik L, Bhaskara-Rao1 KV, Kirthi AV, Rahuman AA (2012) Larvicidal, repellent and ovicidal activity of *Calotropis gigantea* against

- Culex gelidus*, *Culex tritaeniorhynchus* (Diptera: Culicidae). J Agric Entomol. 8(3): 869–880
- Mittal PK, Subbarao SK (2003) Prospects of using herbal products in the control of mosquito vectors. ICMR Bull. 33: 1–10.
- Moore SJ, Lenglet AD (2004) An overview of plants used as insect repellents. In: Wilcox M, Bodeker G, Rasoanavo P (Eds): Traditional medicinal plants and malaria, Vol. 4. CRC Press' Boca Raton, pp. 343–363.
- Murugan K, Murugan P, Noortheen A (2007) Larvicidal and repellent potential of *Albizia amara* Boivin and *Ocimum basilicum* Linn. against dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). Biores Tech. 98: 198–201.
- Ngamo TSL, Ngassoum MB, Mapongmestsem PM, Noudjou WF (2007) Use of essential oils of plants as protectant of grains during storage. Agri J. 2(2): 204–209.
- Ogbadoyi EO, Abdulganiy AO, Adama TZ, Okogun JI (2007). In vivo trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*. J Ethnopharm. 112: 85–89.
- Okigbo RN, Okeke JJ, Madu NC (2010) Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* against mosquito larvae. J Agri Tech. 6(4): 703–719.
- Prabhu K, Murugan A (2011) Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). Asian Pac J Trop Biomed: 124–129.
- Promsiri S, Naksathi A, Kruatrachue M, Thavara U (2006) Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non-target fish. Am J Mosq Control Assoc. 22: 292–295.
- Rajkumar S, Jebanesan A (2004) Ovicidal activity of *Moschosma polystachyum* Linn. (Lamiaceae) leaf extract against filarial vector *Culex quinquefasciatus* Say. Trop Biomed. 21: 47–50.
- Remia KM, Logaswamy S (2010) Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector *Aedes aegypti* (Diptera: Culicidae). Indian J Nat Prod Res. 1(2): 208–212.
- Sahpaz S, Laurens A, Hocquemiller R, Cavé A, Cortés D (1994) Senegalène, une nouvelle acétogénine oléfinique monotetrahydrofurannique des graines d'*Annona senegalensis*. Can J Chem. 72: 1533–1536.
- Samidurai K, Jebanesan A, Saravanakumar A, Govindarajan M, Pushpanathan T (2009) Larvicidal, ovicidal and repellent activities of *Pemphis acidula* Forst. (Lythraceae) against filarial and dengue vector mosquitoes. Acad J Entomol. 2(2): 62–66.
- Saxena RC, Harshan V, Saxena A, Sukumaran P, Sharma MC, Kumar ML (1993) Larvicidal and chemosterilant activity of *Annona squamosa* alkaloids against *Anopheles stephensi*. J Am Mosq Control Assoc. 9: 84–87.
- Shalaby AA, Allam KA, Mostafa AA, Fahmy SM (1998) Insecticidal properties of citrus oils against *Culex pipiens* and *Musca domestica*. J Egypt Sci Parasitol. 28: 595–606.
- Sriwattanarungsee S, Sukontason KL, Olson JK, Chailapakul O, Sukontason K (2008) Efficacy of neem extract against the blowfly and housefly. Parasitol Res. 103: 535–544.
- Suleiman MM, Dzenda T, Sani CA (2008) Antidiarrhoeal activity of the methanol stem-bark extract of *Annona senegalensis* Pers. (Annonaceae). J Ethnopharm. 116: 125–130.
- Tare V, Deshpande S, Sharma RN (2004)

Susceptibility of two different strains of *Aedes aegypti* (Diptera: Culicidae) to plant oils. J Econ Entomol. 97: 1734–1736.

Tennyson S, Ravindran KJ, Arivoli S (2012) Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pac J Trop Biomed.

S1130–S1134.

Trease GE, Evans MC (1989) Text-book of Pharmacognosy, 13th Ed, Baillere Tindall.

WHO (1996) Report of the World Health Organization informal consultation on the evaluation and testing of insecticides. CTD/ WHOPES/IC/96, World Health Organization, Geneva, Switzerland.