

## 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 (LC<sub>50</sub>= 298.8 ppm) and chloroform (LC<sub>50</sub>= 418.3 ppm) fractions were more effective than other fractions on *An. gambiae* larvae while, a moderate effectiveness was also observed with N-hexane (LC<sub>50</sub>= 2087.6 ppm), chloroform (LC<sub>50</sub>= 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 antitypanosomic (Ogbadoyi 2007), antidiarrheic (Suleiman 2008), cures snake bite, generalized eodemes, 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 LC<sub>50</sub>, LC<sub>90</sub>, 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 (LC<sub>50</sub>= 298.8 ppm, LC<sub>90</sub>= 572.9 ppm) and chloroform (LC<sub>50</sub>= 418.3 ppm, LC<sub>90</sub>= 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 (LC<sub>50</sub>= 2087.6 ppm) was most toxic followed by methanolic crude extract (LC<sub>50</sub>= 5884.1 ppm) and chloroform fraction (LC<sub>50</sub>= 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.** LC<sub>50</sub> and LC<sub>90</sub> 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 <sup>2</sup>	LC <sub>50</sub> (95% FL)	LC <sub>90</sub> (95% FL)	<sup>2</sup>
<i>An. gambiae</i>	MCE	2.69±0.14	0.98	<b>973.3</b> (840.9-1132.7)	<b>2914.8</b> (2290.3-4130.2)	33.44*
	NHF	4.54±0.38	0.77	<b>298.8</b> (274.6-320.1)	<b>572.9</b> (530.6-629.0)	8.63 <sup>NS</sup>
	CHF	4.37±0.27	0.85	<b>418.3</b> (394.3-441.9)	<b>822.0</b> (760.6-902.6)	16.19 <sup>NS</sup>
	EAF	2.38±0.18	0.97	<b>2789.3</b> (2464.3-3256.2)	<b>9662.1</b> (7427.4-13697.9)	17.31 <sup>NS</sup>
	MTF	2.45±0.46	0.70	<b>8511.4</b> (5608.7-20304.7)	<b>28360.3</b> (13685.4-133304.7)	15.37 <sup>NS</sup>
<i>Cx. quinquefasciatus</i>	MCE	1.64±0.17	0.94	<b>5884.1</b> (3888.6-12610.7)	<b>35659.9</b> (15583.6-175387.5)	19.36*
	NHF	1.68±0.14	0.98	<b>2807.6</b> (2382.6-3467.3)	<b>16240.9</b> (11105.9-27263.4)	14.01 <sup>NS</sup>
	CHF	1.95±0.28	0.88	<b>9010.1</b> (6024.9-18200.9)	<b>40915.2</b> (19789.1-149218.8)	16.05 <sup>NS</sup>
	EAF	-	-	-	-	-
	MTF	-	-	-	-	-

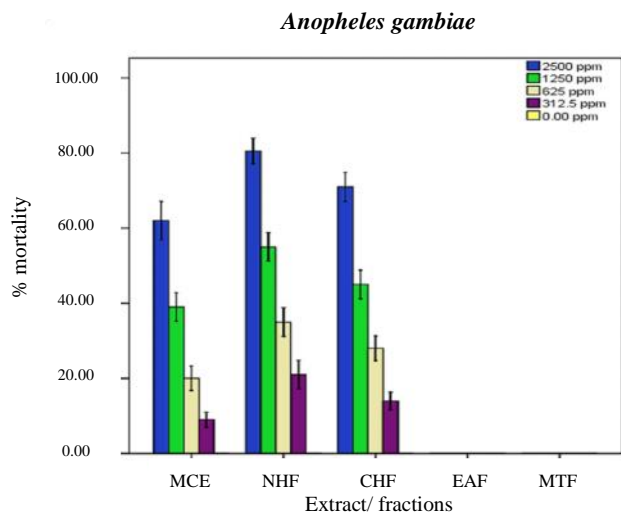
MCE= Methanolic crude extract, NHF= N-hexane fraction, CHF= Chloroform fraction, EAF= Ethyl-Acetate fraction, MTF: Methanol fraction, FL= Fiducial Limit, LC= Lethal concentration, NS= No significant.

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

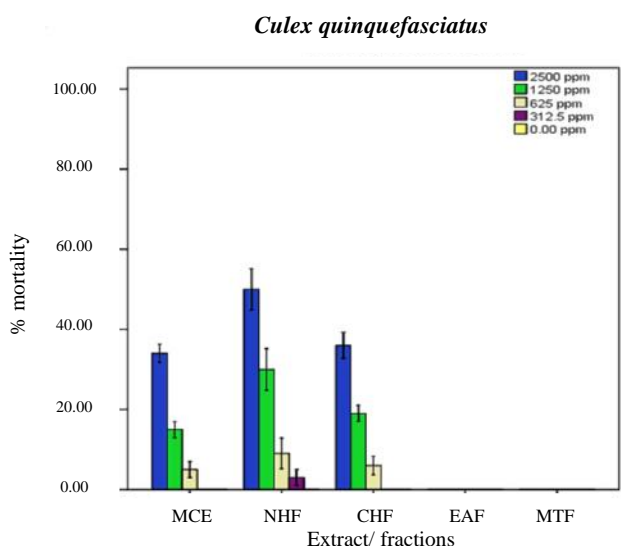
**Table 3.** Qualitative phytochemical screening of some components of extract/fractions of *Annona senegalensis*

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 (Sriwattananarungsee 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 LC<sub>50</sub> 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 LC<sub>50</sub>= 95.63 ppm and LC<sub>90</sub>= 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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