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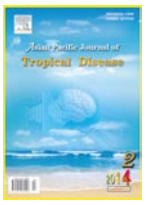

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Insecticidal and growth regulating activity of crude leaf extracts of *Cassia occidentalis* L. (Caesalpinaceae) against the urban malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae)

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ABSTRACT

Objective: To investigate insecticidal and growth regulating activity of crude leaf extracts of *Cassia occidentalis* L. (Caesalpinaceae) against the urban malaria vector, *Anopheles stephensi* Liston. (Diptera: Culicidae).

Methods: Larvicidal activity was studied against third instar larvae for 24 h at concentrations of 62.5, 125.0, 250.0, 500.0, 1000.0, 2000.0, 4000.0 and 8000.0 mg/L. The effect on development and growth of immature mosquitoes was studied at concentrations of 125.0, 250.0 and 500.0 mg/L and was assessed by growth index. Adulticidal activity on topical application was studied on test dosages of 0.01, 0.05, 0.10, 0.25 and 0.50 μg per newly emerged unfed adult female mosquito.

Results: Larvicidal activity was poor, not proportional to concentration and LC_{50} values were above the acceptable dose of 10 mg/L. Potential growth regulating activity was observed and the growth index was 277.5, 111.0 and 27.5 times less than control, respectively. Average larval pupal transformation was 5.8, 4.3 and 96.0 times greater than pupal adult transformation in hexane, 5.0, 4.3 and 48.4 in ethyl acetate, and 4.8, 4.2 and 10.2 in methanol extract, respectively. Adulticidal activity was the highest in ethyl acetate, followed by hexane and methanol extract with LD_{50} values of 0.23, 0.32 and 0.64 μg/female mosquito, respectively.

Conclusions: The crude leaf extracts of *Cassia occidentalis* studied showed poor larvicidal, potential growth regulating and a moderate level of adulticidal activity.

1. Introduction

Insecticides remain the foremost choice for control of vector mosquitoes. Problems associated with use of insecticides such as environmental contamination, toxicity to non-target organisms and insecticide resistance among target population have led to search for alternate means for control of mosquitoes. Plant based derivatives possess insecticidal activity[1-4]. A plethora of plants including medicinal plants have been screened for their insecticidal activity against juvenile and adult mosquitoes[5-7]. *Cassia occidentalis* L. (*C. occidentalis*) (Caesalpinaceae), a medicinal plant used in traditional medicines world-wide[8], is distributed throughout the tropics and subtropical regions of the world[9]. In India, this plant is widely distributed from Jammu and Kashmir to Kanyakumari, and can be found up to an altitude of 1 500 m[10]. The phytochemical compound present in *C. occidentalis* has been reviewed and the presence of insecticidal compounds such as emodin and aloe-emodin has been reported[11]. The insecticidal activity of the extracts of this plant on vector mosquitoes is not sufficiently reported. In the present study, the crude extracts of leaves of *C. occidentalis* was screened for larvicidal activity, effect on development and growth of immatures, and adulticidal activity against the urban malaria vector, *Anopheles stephensi* (*An. stephensi*).

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2. Materials and methods

2.1. Preparation of plant extract

C. occidentalis was collected from foothill regions of Javadhu Hills, Tiruvannamalai District, Tamil Nadu, India and the taxonomical identity was confirmed at the Department of Plant Biology and Biotechnology, Loyola College, Chennai, Tamil Nadu, India. The leaves were removed from the plants, washed with tap water, shade dried at room temperature and powdered with an electric blender. The powdered leaves were macerated with hexane, ethyl acetate and methanol sequentially for a period of 72 h in each solvent and then filtered. The filtered content was concentrated by a rotary vacuum evaporator. Crude extracts thus obtained were weighed and stored at 4 °C in sterilized bottles.

2.2. Bioassay for larvicidal activity

Bioassays were performed with laboratory reared third instar *An. stephensi* larvae following World Health Organization standard protocol[12]. The larvicidal activity of hexane, ethyl acetate and methanol crude leaf extracts was studied at test concentrations of 62.5, 125.0, 250.0, 500.0, 1 000.0, 2 000.0, 4 000.0 and 8 000.0 mg/L. Twenty larvae were introduced into each bowl with 250 mL of test solution and was checked for mortality after 24 h of continuous exposure. All larvae during the course of observation were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1). Untreated water was kept as control. A total of three trials were carried out with three replicates per trial. Mortality was carried out using the following formula.

Mortality(%)=(Number of deaths/Number treated)×100

2.3. Bioassay for effect on the development and growth of immature vector mosquitoes

The effect of crude leaf extracts on the development, growth and survival of immature mosquitoes was studied at concentrations of 125.0, 250.0 and 500.0 mg/L. Bioassays were performed with early first instar larvae from laboratory reared *An. stephensi* in 500 mL beakers with 250 mL of test solutions. Three replicates were run for each concentration. Twenty five early first instar larvae were introduced in test solutions and were fed daily on larval food. Twenty percent of the immature larvae were monitored daily for instar progression. All immature larvae found dead were removed and the number noted. Growth index (GI) was calculated using the following formula[13].

$$GI=\frac{\text{Transformation of larvae into adult (\%)}}{\text{Average developmental period in days}}$$

2.4. Bioassay for adulticidal activity

Adulticidal activity of the crude leaf extracts was determined on topical application to newly emerged unfed female *An. stephensi* mosquitoes. Standard World Health Organization protocol was followed[12]. The hexane, ethyl acetate and methanol crude leaf extracts were dissolved in acetone to yield a graded series of test concentration of the respective crude leaf extracts. The test dosages to study the activity were 0.01, 0.05, 0.10, 0.25 and 0.50 μg/female mosquito. Control included acetone treated individuals. Prior to treatment, the mosquitoes were briefly anaesthetized using anaesthetic ether and 0.1 μL of desired test dosage was applied to the pronotum using a 0.1 μL micropipette. Thereafter, the treated and control mosquitoes were grouped on the basis of dosages and were maintained at a room temperature of (27±2) °C and relative humidity of 75%-85% in one feet mosquito cages. Ten percent glucose solution soaked in cotton was given as feed for the recovering mosquitoes. At the end of 24 h period, the number of dead mosquitoes was noted. A total of 20 mosquitoes were used at each dosage and three trials carried out.

2.5. Statistical analysis

Bioassay tests showing more than 20% control mortality were discarded and repeated. When control mortality ranged from 5% to 20%, the mortality was corrected using Abbott's formula[14]. The LC_{50} and LC_{90} values for larval bioassays and LD_{50} for adulticidal bioassays with their fiducial limits was determined using log probit analysis test. Results with $P<0.05$ were considered to be statistically significant. One-way ANOVA F-text statistic and Duncan multiple range test were performed to test the differences in mortality at different concentrations. SPSS version 11.5 statistical software was used for statistical analysis.

3. Results

Results of the study to determine the effect of treatment of hexane, ethyl acetate and methanol leaf crude extracts on the larval stages of *An. stephensi* indicated deleterious effect resulting in larval mortality. The larval mortality was not proportional to the dosage and it ranged from 12.2% to 66.5%, 25.5% to 72.5% and 20.0% to 62.2% respectively. The larval mortality at the lowest (62.5 mg/L) and the highest (8 000.0 mg/L) concentration was 13.8% and 66.5%, 28.0%, 72.5%, 23.3% and 62.2%, respectively. There was no mortality in control. The LC_{50} and LC_{90} values with respective confidence intervals for 24 h exposure period was 5 303.4 (4 065.6-7 578.3) and 11 076.0 (8 488.6-66 624.8), 3 792.3 (2 459.2-6 608.1) and 10 223.8 (7 152.4-19 612.4) and 4 432.6 (3 039.1-7 465.3) and 11 014.9 (7 820.6-20 232.5) mg/L, respectively.

Results of the study on the development and growth of immature *An. stephensi* indicated potential growth regulating activity as observed by the prolonged duration and mortality caused in immature stages. In control, the average larval period was 8 d, the pupal period 1 d

Table 1

Effect of leaf extracts of *C. occidentalis* on development and growth of immature *An. stephensi*.

Extracts	Concentration (mg/L)	Average larval period (d)	Transformation of larvae into pupae (%)	Average pupal period (d)	Transformation of pupae into adult (%)	Average development (d)	Transformation of larvae in adult (%)	GI
Hexane	125.0	9	87.6	9	15.1	18	13.2	0.70
	250.0	10	91.6	8	7.9	18	7.2	0.40
	500.0	11	86.4	7	0.9	18	0.8	0.04
Ethyl acetate	125.0	9	91.6	9	18.3	18	16.8	0.90
	250.0	9	80.4	9	18.9	18	15.2	0.80
	500.0	10	87.2	8	1.8	18	1.6	0.10
Methanol	125.0	9	96.4	8	19.9	17	19.2	1.10
	250.0	9	74.8	9	17.9	18	15.2	1.30
	500.0	9	94.8	9	9.3	18	8.8	0.40
Control	0.0	8	100.0	1	100.0	9	100.0	11.10

Twenty five first instar larvae were introduced in each concentration and trial.

Table 2

Adulticidal activity on topical application of crude extracts of leaves of *C. occidentalis* on *An. stephensi*.

Extracts	Percent mortality (mean±SD) at different dosages						*F*-test	LD_{50}
	Control	0.01	0.05	0.10	0.25	0.50		
Hexane	0^{a}	30.0 ± 17.3^{b}	36.7 ± 15.3^{bc}	43.3 ± 15.3^{bc}	46.7 ± 41.1^{bc}	60.0 ± 36.1^{c}	11.3^{a}	0.32 (0.2-2.2)
Ethyl acetate	0	20.0±26.5	30.0±36.1	53.3±25.1	63.3±30.6	73.0±30.6	1.9	0.23 (0.1-0.5)
Methanol	0^{a}	13.3 ± 15.3^{ab}	16.7 ± 20.8^{abc}	23.3 ± 25.2^{bc}	23.3 ± 25.2^{bc}	36.7 ± 25.2^{c}	3.1^{a}	0.64 (0.4-22.4)

[a]: Significantly different at $P<0.05$ level; Superscripts in italics denote differences among mortality observed at different concentrations in each extract by Duncan Multiple Range test; Values in parenthesis denote 95% confidence interval.

and the GI was 11.1. In hexane and ethyl acetate treated bowls, the average larval period in all concentrations (125.0, 250.0 and 500.0 mg/L) was 1 to 3 d and 1 to 2 d more than control. In methanol treated bowls, it was 1 d more than all concentrations. The pupal period was 6 to 8 more days in hexane and 7 to 8 more days in ethyl acetate and methanol treated bowls. GI varied at different concentrations and it reached the lowest index at the highest concentration in each of the studied extracts. The lowest GI of 0.04 was noted at concentration of 500.0 mg/L in hexane treated bowls. When compared to control, the GI was 277.5, 111.0 and 27.5 times less in hexane, ethyl acetate and methanol treated bowls.

Mortality was noticed in both larval and pupal stages in hexane, ethyl acetate and methanol treated bowls. No mortality was observed in control (Table 1). During the developmental period, the number of larvae that transformed into pupae (larval-pupal transformation) was more than the number that was transformed from pupae to adult (pupal-adult transformation). At concentration of 125.0, 250.0 and 500.0 mg/L, the average larval-pupal transformation was 5.8, 4.3 and 96.0 times greater than pupal-adult transformation in hexane, 5.0, 4.3 and 48.4 in ethyl acetate and 4.8, 4.2 and 10.2 in methanol extracts, respectively. The number of larvae that successfully emerged as adults varied in different concentrations and extracts. Adult emergence in treatments were always lesser than control. Adult emergence noted at the lowest and highest concentration in hexane, ethyl acetate and methanol treated bowls were 13.2% and 0.8%, 16.8% and 1.6% and 19.2% and 8.8%. In control bowls it was 100.0%.

The adulticidal activity of extracts observed on topical application is given in Table 2. The average weight of unfed female mosquitoes was 2.0 mg. The susceptibility of *An. stephensi* female mosquitoes to graded series of concentration was dose dependent. In hexane, ethyl acetate and methanol extract treated mosquitoes, the mortality observed ranged from (30.0±17.3)% to (60.0±36.1)%, (20.0±26.5)% to (73.0±30.6)% and (13.3±15.3)% to (36.7±25.2)%. No mortality was observed in control mosquitoes. One-way ANOVA performed to find difference in mortality at different dosages indicated significant difference at $P<0.05$ in hexane and methanol extracts. Among the extracts, the highest adulticidal potential was exhibited by ethyl acetate followed by hexane and methanol with LD_{50} values of 0.23, 0.32 and 0.64 μg/female mosquito.

4. Discussion

The genus *Cassia* of the Family Caesalpinaceae comprises about 692 species and some of them such as *C. occidentalis*[15,16], *Cassia holosericea*[17], *Cassia tora*[18], *Cassia siamea*[19], *Cassia fistula* (*C. fistula*)[20,21], *Cassia auriculata*[22,23], *Cassia nigricans*[24], *Cassia obtusifolia*[25], have been studied for their mosquitocidal property. Phytochemicals such as emodin, aloe-emodin which possess insecticidal activity are reported to be present in plants of this genus[11]. Alkaloids, flavonoids, tannins, phlorobatannins, chrysophanol, emodin, physcion, tetrahydroanthracene derivatives, germichrysone and occidentalins A and B are some of the plant metabolites

present in the leaves of *C. occidentalis*[26].

Based on the afore-mentioned literature on the mosquitocidal activity of different species of the genus *Cassia*, good insecticidal and growth regulating activity was expected. The larval bioassays with hexane, ethyl acetate and methanol crude leaf extracts showed poor larvicidal activity. Hundred percent mortality was not obtained in both the lowest and highest concentration. The LC_{50} values obtained were very high making it unfit to be considered as a potential larvicidal agent. Similar poor larvicidal activity was also reported in larval bioassays with ethanolic crude leaf extract of *C. occidentalis* and the LC_{50} value obtained against fourth instar *An. stephensi* larvae was as high as 70.56%[27]. The larvicidal activity of ethanol and methanol crude leaf extracts of other *Cassia* sp., namely *Cassia obtusifolia* and *C. fistula* against *An. stephensi*, however, were very different and showed comparatively higher level of larvicidal activity at lower concentrations. The LC_{50} value for 24 h observation period reported was 52.20 and 17.47 mg/L, respectively[20,25]. A toxic dose of 10 mg/L which causes 100 per cent mortality in third and fourth instar larvae on 24 h is considered to be an effective concentration of the crude extract and further studies was recommended in plants that yielded these results[28]. This not being so in the present bioassay, indicate the absence of any potential larvicidal activity.

The studies on the effect of these extracts on the development and growth of immature stages showed a strong growth regulating mechanism causing prolongation of instar duration and higher rate of mortality among immature mosquito particularly in the pupal instars. Pupal-adult transformation was relatively very less than larval-pupal transformation indicating profound effect on development and metamorphosis in pupal instars. *C. fistula* showed similar growth regulating activity[29]. In mosquitoes, growth and development are controlled by a myriad of hormones, the juvenile and ecdysone hormones being the important ones. These regulate growth and metamorphosis in juvenile stages. Any compound that can affect the production of these hormones or alter the regulatory mechanisms involved can cause delay in growth and lead to mortality. In the present study, the increased duration and higher rate of mortality in pupal instars, a non-feeding stage can be attributed to the activity of phytocompounds in the extracts on prolonged exposure of the active feeding stages of the larvae to extracts. However, a detailed investigation is warranted to understand the possible mode of activity. The role of the crude extract in the inhibition of chitin synthesis is also required to be investigated. Identification of phytocompounds with insect growth regulating activity particularly among the aquatic stages may contribute to develop natural, economical and environmental safe growth regulator for controlling immature mosquito.

Adulticidal activity on topical application indicate the intrinsic toxicity of the compound against the mosquito species[12]. No reports are available on the effect of topical application of crude leaf extracts or phytocompounds from *Cassia* species on adult mosquitoes. In the present study, the crude leaf extracts of *C. occidentalis* showed toxicity to adult *An. stephensi* mosquitoes. Among the extracts, ethyl acetate extract showed maximum toxicity, the LD_{50} value being 0.23 followed by hexane and methanol with LD_{50} values of 0.32 and 0.64 μg/female mosquito. In similar such study, the LD_{50} values of hexane, ethyl acetate and methanol crude leaf extract of *Ageratum houstonianum* against *An. stephensi* was reported to be 0.18, 0.14 and 0.12 μg/female mosquito[30]. When compared to *Ageratum houstonianum*, the adulticidal activity was found to be low. Synthetic compounds show remarkable activity on topical application. In a study carried out against *Aedes aegypti*, the LD_{50} values of bifenthrin, permethrin and temephos were 0.077, 0.240 and 195 ng/mg female mosquito respectively[31]. Therefore, relatively, the intrinsic toxicity of the leaf extracts of *C. occidentalis* is low in poor adulticidal activity.

The plant *C. occidentalis* is distributed throughout the tropical and subtropical regions around the world and can generally be found in open fields and also in cultivated lands[9]. It has been reported that bioactivity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant part, age of plant part, solvent used in extraction and mosquito species[28]. Screening of these plant extracts from varied geographical regions for growth regulating activity is required. The crude leaf extracts in the present study showed detrimental activity on the growth and development of immature *An. stephensi* mosquitoes. This activity can be potentially exploited against *An. stephensi* immature stages and the extracts can be used in non-potable clean-water storage containers, curing pits and rainwater accumulations in roof tops to control this vector in malaria endemic urban areas. However, more research is required to identify the biologically active phytocompounds affecting growth and development and suitable formulation is to be developed for effective delivery of the compound.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Elimam AM, Elmalik KH, Ali FS. Larvicidal, adult emergence inhibition and oviposition deterrent effects of foliage extract from *Ricinus communis* L. against *Anopheles arabiensis* and *Culex quinquefasciatus* in Sudan. *Trop Biomed* 2009; **26**(2): 130-139.

[2] Govindarajan M. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species. *Asian Pac J Trop Med* 2010; **3**(11): 874-877.

[3] Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res* 2011; **109**(2): 353-367.

[4] Mavundza EJ, Maharaj R, Chukwujekwu JC, Finnie JF, Staden JV. Screening for adulticidal activity against *Anopheles arabiensis* in ten plants used as mosquito repellent in South Africa. *Malaria J* 2014; **13**: 173.

[5] Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitoes viz. *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. *Appl Entomol Zool* 2008; **43**: 57-63.

[6] Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res* 2012; **135**: 581-598.

[7] Tennyson S, Ravindran KJ, Arivoli S. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2012; **2**: S1130-S1134.

[8] Egharevba, Omoregie H, Anselem OC, Abdullahi MS, Sabo M, Okwute, et al. Phytochemical analysis and broad spectrum antimicrobial activity of *Cassia occidentalis* L. (whole plant). *New York Sci J* 2010; **3**(10): 74-81.

[9] Ibrahim MA, Aliyu AB, Sallau AB, Bashir M, Yunusa I, Umar TS. *Senna occidentalis* leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anemia and organ damage. *Pharmacognosy Res* 2010; **2**(3): 175-180.

[10] Khare CP. *Indian medicinal plants*. United States: Springer; 2007, p. 900.

[11] Yadav JP, Arya V, Yadav S, Panghal M, Kumar S, Dhankhar S. *Cassia occidentalis* L.: a review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia* 2010; **81**(4): 223-230.

[12] WHO. Report of the WHO informal consultation on the "evaluation and testing of insecticides". Geneva: World Health Organization; 1996. [Online] Available from: http://apps.who.int/iris/bitstream/10665/65962/1/CTD_WHOPES_IC_96.1.pdf [Accessed on 16th April, 2014]

[13] Saxena SC, Sumithra L. Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *Anopheles stephensi* Liston Diptera: Culicidae. *Curr Sci* 1985; **54**: 201-202.

[14] Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**: 265-267.

[15] Frodin DG. History and concepts of big plant genera. *Taxon* 2004; **53**(3): 753-776.

[16] Kumar S, Wahab N, Mishra M, Warikoo R. Evaluation of 15 local plant species as larvicidal agents against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Front Physiol* 2012; **3**: 104.

[17] Qureshi SA, Mohiuddin S, Fatima B, Badary Y. Laboratory studies on some plant extracts as mosquito larvicides. *Pak J Sci Ind Res* 1986; **29**: 361-365.

[18] Amerasan D, Murugan K, Kovendan K, Mahesh Kumar P, Panneerselvam C, Subramaniam J, et al. Adulticidal and repellent properties of *Cassia tora* Linn. (Family: Caesalpinaceae) against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. *Parasitol Res* 2012; **111**(5): 1953-1964.

[19] Kamaraj C, Rahuman AA, Bagavan A, Elango G, Zahir AA, Santhoshkumar T. Larvicidal and repellent activity of medicinal plant extracts from Eastern Ghats of South India against malaria and filariasis vectors. *Asian Pac J Trop Med* 2011; **4**(9): 698-705.

[20] Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitol Res* 2008; **102**(2): 289-292.

[21] Govindarajan M. Bioefficacy of *Cassia fistula* Linn. (Leguminosae) leaf extract against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci* 2009; **13**(2): 99-103.

[22] Kamaraj C, Bagavan A, Rahuman AA, Zahir AA, Elango G, Pandiyan G. Larvicidal potential of medicinal plant extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitol Res* 2009; **104**(5): 1163-1171.

[23] Kamaraj C, Abdul Rahman A, Bagavan A, Abduz Zahir A, Elango G, Kandan P, et al. Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). *Trop Biomed* 2010; **27**(2): 211-219.

[24] Georges K, Jayaprakasam B, Dalavoy SS, Nair MG. Pest-managing activities of plant extracts and anthraquinones from *Cassia nigricans* from Burkina Faso. *Bioresour Technol* 2008; **99**(6): 2037-2045.

[25] Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res* 2009; **104**(2): 337-340.

[26] Daniel M. Medicinal plants: chemistry and properties. United States: Science Publishers Inc.; 2005, p. 175.

[27] Dhandapani A, Kadarkarai M. HPTLC quantification of flavonoids, larvicidal and smoke repellent activities of *Cassia occidentalis* L. (Caesalpiniaceae) against malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *J Phytol* 2011; **3**(2): 60-72.

[28] Shaalan EA, Canyon D, Younes MW, Abdel-Wahab H, Mansoura AH. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int* 2005; **31**: 1149-1166.

[29] Mehdi SH, Qamar A, Khan I, Tayyaba PI. Studies on larvicidal and IGR properties of leaf extract of *Cassia fistula* and *Saraca indica* (Family: Leguminosae). *J Herbal Med Toxicol* 2010; **5**: 79-86.

[30] Ravindran J, Samuel T, Alex E, William J. Adulticidal activity of *Ageratum houstonianum* Mill. (Asteraceae) leaf extracts against three vector mosquito species (Diptera: Culicidae). *Asian Pac J Trop Dis* 2012; **2**(3): 177-179.

[31] Corbel V, Duchon S, Zaim M, Hougard JM. Dinotefuran: a potential neonicotinoid insecticide against resistant mosquitoes. *J Med Entomol* 2004; **41**(4): 712-717.