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Phytochemical profiling and Ovicidal efficacy of *Boswellia sacra* Resin extracts against the filarial vector *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract
Vector control is facing a threat due to emergence of resistance to synthetic insecticides. Insecticides of plant origin may serve as suitable alternative to synthetic insecticides in future. Therefore, the present study was undertaken to evaluate ovicidal activity of solvent extracts of resin of *Boswellia sacra* against vector mosquito *Culex quinquefasciatus*. *B. sacra* extracts of acetone, chloroform and ethanol were tested against the eggs of *Cx. quinquefasciatus* at different concentrations. The data were subjected to statistical analysis and was found to be significant. Results revealed that acetone extract of *B. sacra* possessed strong ovicidal activity. Phytochemical profiling of the extracts showed the presence of many secondary metabolites, which might be reason for its high efficacy. The present investigation lead the path of exploration of *B. sacra* resin for eradication of selected medically important human vector mosquitoes, thereby gaining a real momentum to include this resin for intense vector control programme.

Keywords: Plant extracts, resin, mosquito, Ovicidal activity, vector control, phytochemicals

1. Introduction
Mosquitoes being vector for many tropical and subtropical diseases are the most important single group of insect well known for their public health importance [1]. Mosquito borne diseases are still a major problem in the world particularly in tropical and subtropical regions and WHO has declared the mosquitoes as "Public enemy number one" [2]. They are still representing the world's number one vector of human and domestic animals comprising approximately 3500 species. They are distributed globally and most female mosquitoes take blood meals from vertebrates to obtain the necessary nutrition to produce their eggs, injecting saliva (which may contain pathogens) into host animal. Mosquitoes breed in water, occasionally depositing eggs directly on water, but generally using a variety of moist surfaces, tree holes and containers [3, 4].
Synthetic insecticides have created a number of ecological problems such as the development of the resistant insect strains, ecological imbalance and are harmful to mammals. Hence there is a constant need for developing biologically active plant materials such as ovicides, which are expected to reduce the hazards to human and other organisms by minimizing the residue accumulation in the environment. Natural products are generally preferred because of their less harmful nature to non target organisms and their innate biodegrability [5]. A survey conducted on 344 plant species, revealed that certain phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction, or act on the olfactory receptors, eliciting responses of attractancy or repellency [6].
Several plants have been used in folklore medicine. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on human body. Natural products, which come out from medicinal plants are important for pharmaceutical research and for drug development as a sources of therapeutic agents. At present the demand for herbal or medicinal plant products has increased significantly [7]. Secondary metabolites are present in plant as key candidate with insecticidal properties and

can be explored to develop the natural compounds to control mosquito population [8].

Botanical extracts with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, ovicidal and adulticidal properties [9]. Plants could be an alternative source for mosquitocide because they constitute a potential source of bioactive chemicals and typically are free from harmful effects [10]. More than 2000 phytochemicals have been identified from plants. The amount of phytochemical substances varies considerably from species to species and even from plant to plant, depending on the age and various ecological climatic factors [11]. The present study was carried out to evaluate the ovicidal activity of solvent extracts of *Boswellia sacra* resin against eggs of the vector mosquito *Culex quinquefasciatus*.

2. Materials and Methods

2.1 Origin and laboratory maintenance of the mosquito colonies

Mosquitoes used in study were *Culex quinquefasciatus*. Individuals were reared for several generations under laboratory conditions by Hay infusion method.

2.1.1 Collection of test materials

Resins of the selected tree namely *B. sacra* were collected from natural habitat of Thrissur locale, Kerala, India.

2.1.2 Preparation of resin powder

Resins were collected and dried under shade at room temperature for 2 to 3 weeks and were powdered using an electric pulverizer. Fine powder was obtained by sieving.

2.1.3 Preparation of extracts

10g of the resin powder was weighed using an electronic balance and were subjected to extraction [12, 13]. Chloroform extraction was followed by acetone and ethanol extraction, so that powders were subjected to extraction with solvents in the order of increasing polarity. The resin extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further bioassays.

2.2 Ovicidal Bioassay

Ovicidal activity was assessed by the slightly modified method of Su and Mulla [14]. The egg raft/eggs of *Cx. quinquefasciatus* were collected. The *B. sacra* resin extracts were diluted in the appropriate solvents to achieve various concentrations ranging from 100 to 300 ppm. Eggs of the mosquito species (100 nos.) were exposed to each concentration of *B. sacra* resin extracts. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope.

Each experiment was replicated three times along with appropriate control. The hatch rates were assessed 48h after treatment and counts were made every 24 hours after exposure until the test was terminated. The hatch rates were assessed by the following formula.

$$\% \text{ of egg mortality} = \frac{\text{Mortality at treatment} - \text{Mortality at control}}{100 - \text{Mortality at control}} \times 100$$

2.3 Statistical analysis

The data of bioassay studies were also subjected to One Way Analysis of Variance (ANOVA) as described by Panse and Sukhatme [15].

2.4 Phytochemical screening

Preliminary phytochemical screening of leaf extract of selected plant was carried out using the standard procedures.

A. Test for Alkaloids

- **Mayer's test [16]:** A fraction of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test [17]:** A fraction of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test [18]:** A few ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

B. Test for Tannins

- **Ferric chloride test [19]:** About 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

C. Test for Phenols

- **Ferric chloride test [20]:** The extract (50mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour
- **Lead acetate test [21, 22]:** The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

D. Test for Flavonoids

- **NaOH test [19]:** Few quantity of the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test [21, 22]:** Test extract (50 mg) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

F. Test for Sterols

- **Liebermann-Burchard test [23]:** The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this one or two drop of Conc. H_2SO_4 is added along the side of the test tube and observed for an array of colour changes.

G. Test for Terpenoids

- **Liebermann-Burchard test [24]:** A little of extract (50 mg) was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids.

H. Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The suspension is vigorously shaken in a graduated cylinder for 15 minutes and observed for the formation of 2 cm layer thick foam.

I. Test for Anthraquinones

- **Borntrager's test [24]:** About 0.2 g of extract to be tested was shaken with 10 ml of benzene and then filtered. 5 ml of the 10% ammonia solution was then added to the filtrate and thereafter shaken and observed for the appearance of a pink, red or violet colour in the ammoniacal (lower) phase.

J. Test for Proteins

- **Ninhydrin test [25]:** Two drops of ninhydrin solution (10 mg of ninhydrine in 200 ml of acetone) are added to 2 ml of aqueous filtrate and observed for the present of characteristic purple colour.
- **Biuret test [26]:** An aliquot of 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

K. Test for Quinones

- **H_2SO_4 test [22]:** To 1 ml of extract add 1 ml of Conc. H_2SO_4 and observed for the formation of red colour.
- **HCl test [27, 28]:** To 1 ml of the extract 5 ml of HCl and observed for the presence of yellow colour precipitate.

3. Results

3.1 Egg hatchability

The maximum ovicidal activity was noted in the acetone extract of *Boswellia sacra* resin. The egg hatchability was totally inhibited in the concentrations ranging from 150 ppm to 300 ppm (Table 1). At 150 ppm to 300 ppm zero percentage egg hatchability was recorded at 48, 72 and 96 h. At lower concentration of 100 ppm 20% and 23.33% egg hatchability was reported in 48 h and 72 h respectively.

However, the moderate ovicidal activity was exhibited by chloroform extract which showed zero percentage egg hatchability at concentrations ranging from 250 ppm to 300 ppm at 48, 72 and 96 h. When concentration was lowered egg hatchability percentage was reported to increase. That is at 250, 150 and 100 ppm total egg hatchability was recorded as 8.33, 26.66 and 65% respectively. The minimum ovicidal activity was noted in ethanol extract, in which zero percentage egg hatchability was reported to be higher concentration of 300 ppm throughout the study. The egg hatchability was found to be inversely proportional to the concentration of extract.

Table 1: Ovicidal activity of extracts of *Boswellia sacra* resin against *Cx. quinquefasciatus*

Solvents used	Conc. in ppm	Egg hatchability %			Total egg hatchability	Total Egg mortality
		48h	72h	96h		
Acetone	Control	30	40	30	100	0
	100	20	23.33	15	58.3	41.66
	150	0	0	0	0	100
	200	0	0	0	0	100
	250	0	0	0	0	100
	300	0	0	0	0	100
	F	63.6000	63.7600	28.3500	107.7027	8.2952
	SED	0.4714	0.6086	0.6667	1.1706	4.023
	CD (0.05)	1.0271**	1.3260**	1.4526**	2.5506**	8.7656**
	CD (0.01)	1.4400**	1.8590**	2.0365**	3.5759**	12.2893**
Chloroform	Control	40	31.66	28.33	100	0
	100	30	21.66	13.33	65	35
	150	13.33	8.33	5	26.66	73.33
	200	3.33	1.66	3.33	8.33	90
	250	0	0	0	0	100
	300	0	0	0	0	100
	F	23.8769	23.6500	21.6000	72.0480	72.8041
	SED	0.9813	0.7698	0.6667	1.3608	1.3472
	CD (0.05)	2.1381**	1.6773**	1.4526**	2.9650**	2.9352**
	CD (0.01)	2.9976**	2.3515**	2.0365**	4.1569**	4.1151**
Ethanol	Control	36.66	31.66	31.66	100	0
	100	26.66	26.66	20	73.33	26.66
	150	10	13.33	6.66	30	70
	200	5	3.33	3.33	11.66	88.33
	250	5	3.33	1.66	10	90
	300	0	0	0	0	100
	F	22.6400	27.5000	21.4000	49.1408	49.1408
	SED	0.8607	0.7201	0.7698	1.6216	1.6216
	CD (0.05)	1.8752**	1.5689**	1.6773**	3.5332**	3.5332**
	CD (0.01)	2.6291**	2.1996**	2.3515**	4.9535**	4.9535**

** - Significant at p = 0.01 SED = Standard Error Deviation CD = Critical Difference

3.2 Egg Mortality

Maximum egg mortality was exhibited by acetone extract of *B. sacra* resin in which 100% mortality was reported in higher concentrations of 150-300 ppm. In the lowest concentration of 100 ppm egg mortality was 41.66%. The activity of acetone extract was followed by chloroform extract which recorded 100% mortality in concentrations of 250-300 ppm. When concentration was lowered to 200 ppm the egg mortality percentage was also seen to fall down, i.e. egg mortality percentage is directionally proportional to the concentration of extract. Lowest egg mortality was found in ethanol extract which shows 100% mortality only in 300 ppm.

3.3 Phytochemical Analysis

The results of the phytochemical analysis reported the presence of different secondary metabolites. The results of phytochemical screening of different solvent extracts of *B. sacra* are depicted in Table 2. The acetone extract of *B. sacra* which showed maximum ovicidal activity (zero % egg hatchability at 150-300 ppm), when tested for its phytochemicals revealed the presence of phytocompounds like alkaloid, flavonoids sterols, terpenoids, anthroquinone, saponins and proteins.

Table 2: Phytochemicals present in the extracts of *B. sacra* resin

Sl No.	Constituents	*Boswellia sacra* resin		
		Acetone extract	Chloroform extract	Ethanol extract
1	Alkaloids	+	+	-
2	Flavonoids	+	+	+
3	Sterols	+	+	+
4	Terpenoids	+	+	+
5	Anthroquinones	+	-	-
6	Phenols	-	+	-
7	Saponins	+	-	-
8	Tannins	-	-	+
9	Proteins	+	+	-
10	Quinones	-	-	+

'+' Detected '-'Not Detected

Moderately active chloroform extracts (zero % egg hatchability at 250-300 ppm) of *B. sacra* resin, reported the presence of secondary metabolites viz., alkaloids, flavanoids, sterols, terpenoids, phenols and proteins. Ethanol extract which exhibited minimum ovicidal rate when screened for the phytochemicals tabulated the presence of flavonoids, sterols, terpenoids, tannins and quinones. The presence of these secondary metabolites may be the reason for the ovicidal efficacy of the resin extracts.

4. Discussion

Due to indiscriminate use of synthetic chemicals to control the mosquitoes in the natural habitats, they have developed strong resistance to almost all the chemicals. Moreover, chemicals pesticides gradually altered the behavior of non-target organisms. Thus, in the context, the world scientific community is intensively searching for the alternative mosquitocidal agent preferably from plants available in nature. Today the environmental safety of an insecticide is considered to be of important milestone in the field of pest control in general and vector control programme in particular. An insecticide must not cause high mortality in target organisms in order to be acceptable [29].

In the present study, as the acetone extract showed decreasing egg hatchability at all ppm, it was compared with the efficacy of methanolic crude leaf extracts of *Pamphis acidula* which exhibited 100% ovicidal activity against *Cx. quinquefasciatus* at 500 ppm [30]. Similarly Rajkumar and Jebanesan [31] studied ovicidal activity of *Moschosma polystachyum* leaf extract against *Cx. quinquefasciatus* and observed 100% egg mortality at 100 ml/l.

At the present study in chloroform extract of *B. sacra* 100% mortality was found at 250-300 ppm, which is comparable with similar work of Mullai and Jebnesan [32] who reported complete ovicidal activity at 300 ppm for methanol, benzene, petroleum ether, and ethyl acetate extracts of *Citrullus pubescens* against *Cx. quinquefasciatus*. Broadbent and Pree [33] reported that when eggs were directly exposed to higher concentrations of the compounds, more chemicals entered the egg shell, which affected the embryogenesis and exposure time also has a crucial role in causing toxicity.

In the present investigation among the three extracts of *B. sacra* which were subjected to phytochemical screening, acetone extract showed maximum number of phytoconstituents when compared to chloroform and ethanol extracts. These variations may due to number of environmental factors such as climate, altitude, rainfall etc. It has been previously recorded by Kokate *et al* [34] that the plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and has protective or disease preventive properties.

Flavonoids, sterols and terpenoids were present in the three extracts namely acetone, chloroform and ethanol in the present study. It is said that sterols and terpenoids have antimicrobial activity as stated by Sneha *et al* [35]. In general plants contain flavonoids that can either occur as glycones or as o-or c-glycosides. Recently, flavonoids have attracted interest due to the discovery of their pharmacological activity. Phytochemicals may serve as suitable alterations to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available throughout the world as recorded by Bowers *et al* [36].

Phytochemicals such as alkaloids, flavanoids, steroids, terpenoids, anthroquinones, saponins and proteins were present in acetone extract and the ovicidal activity was found to be more in this extract. In accordance to the results of present study Rajkumar *et al* [37] has reported that the methanolic extract of *Coccinia indica* treated eggs exhibited

an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion.

Alkaloids and proteins were observed in acetone and chloroform extract of *B. sacra*. Alkaloids and proteins are generally known to be important sources of potent insecticides, fungicides, bactericides and herbicides for pest control as reported by Gbolade [38]. In the present flavonoids, sterols, terpenoids, tannins and quinones were present in ethanol extract of *B. sacra* resin. Similar observation were noted in Gopieshkhanna and Kannabiran [39] in which phytochemicals such as saponins, carbohydrates, phytosterols, phenols, flavonoids and tannins were present in the plant extracts.

5. Conclusion

The finding of the present investigation revealed that the extracts of *B. sacra* possessed remarkable ovicidal activity against *Cx. quinquefasciatus*. A number of phytochemicals are identified from the *B. sacra* resin extracts in the present study. Further analysis is required to isolate the active principles and for purification and evaluation of these compounds. The trees are well known and available in many areas of South India. Therefore, the resin extract might be used as natural biocides, for three main reasons; firstly: ecologically acceptable (natural products), secondly: economical (less cost), thirdly: easily available. The results of this study demonstrated the potential of new alternative ovicides from botanical sources, as they are free of adverse effects and are also safe. The study could encourage the search for new active natural compound from other medicinal plants, to replace synthetic insecticides. Further studies are needed to investigate the toxicity of the resin extract against wide range of other insects and to identify the active compounds of the extracts responsible for ovicidal activity against *Cx. quinquefasciatus*.

6. References

1. Klempner S, Unnash V. Larvicidal activities of leaf extracts of *Adansonia digitata* L. (Malvales: Malvaceace) and *Ficus sur forssk* (Rosales: Moraceae) against *Cx. quinquefasciatus* mosquito (Diptera: Culicidae). Journal of Mosquito Research. 2007; 7(15):115-124.
2. WHO. Report of the WHO informal consultation on the testing of insecticides, WHO, Geneva, 1996.
3. Reiter P. Climate change and Mosquito borne disease. Environmental Health Perspectives. 2001; 109(1):141-161.
4. WHO. World Health Organization, Expert Committee on Malaria. Technical Report Series 892, Geneva, 2000.
5. Govindarajan M, Karuppannan P. Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (Asteraceae) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). Asian Pacific Journal of Tropical Medicine. 2011; 4(1):24-28.
6. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control. Journal of the American Mosquito Control Association. 1991; 7(2):210-237.
7. Dhivya R, Manimegalai K. Comparitive ovicidal potential of the flower and leaf extracts of *Calotropis gigantea* against the filarial vector Cx. quinquefasciatus (Diptera: Culicidae). International Journal of Recent Scientific Research. 2013; 4(6):735-737.
8. Philosia A, Dhivya R. Phytochemical composition and ovicidal efficacy of *Catharanthus roseus* leaf extract against the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). Journal of Entomology and Zoology Studies. 2017; 5(3):44-49.
9. Babu R, Murugan K. Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* say. Neem News Letters. 1998; 15(2):9-11.
10. Isman MB. Botanical insecticides, deterrent and repellent in modern agriculture and increasingly regulated world. Annual Reviews of Entomology. 2006; 51:45-66.
11. Baquar SR. Medicinal and poisonous plants of Pakistan (print at Karachi, Pakistan). 1989; 23(3):32-48.
12. Harbourne JB. Phytochemical Methods. Chapman and Hall, Ltd, London. 1973; 49-188.
13. Vogel AI. Text book of practical organic chemistry. The English language book society and Longman, London, 1978, 1368.
14. Panse VG, Sukhatme PV. Statistical methods for agricultural workers, 4th edition, ICAR, New Delhi, 1985, 359.
15. Su T, Mulla MS. Ovicidal activity of neem products (*Azadirachtin*) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). Journal of American Mosquito Control Association. 1998; 14:201-209.
16. Evans WC. Trease and Evans Pharmacology. 14th edn. Harcourt Brace and company. Asia. Pvt. Ltd. Singapore, 1997.
17. Wagner H. Pharmazeutische Biology 5th edn. AUFI. 15 BN 3-437-20 498-X. Gustav fisher Vwelag. Stuttgart. Germany, 1993.
18. Wagner HXS, Bladt Z, Gain EM. Plant drug analysis. Springer Veralag. Berlin. Germany, 1996.
19. Trease GE, Evans WC. Pharmacognosy. 15th Ed. London: Saunders Publishers, 2002; 42–44, 221–229, 246–249, 304–306, 331–332, 391–393.
20. Mace ME. Histochemical localization of phenols in healthy and diseased tomato roots. Phytopathology, 1963; 16:915-925.
21. Brain KR, Turner TD. Wright Scien Technica. 1st Ed. Bristol: Practical evaluation of phyto pharmaceuticals, 1975, 144.
22. Evans WC. Pharmacognosy. 14th ed. WB Saunders Co. Ltd. Singapore, 1996, 713-734.
23. Finar IL. Stereo Chemistry and the chemistry of Natural products. Longman. Singapore, 1986, 2.
24. Sofowora A. Medicinal plants and traditional medicinal in Africa. 2nd Ed. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. Screening Plants for Bioactive Agents, 1993, 134-156.
25. Yasuma A Ichikawa. Ninhydrin-Schiff and 5-Schiff staining. A new histochemical staining method for proteins. Journal of Laboratory and Clinical Medicine. 1953; 41:296-299.
26. Gahan PB. Plant Histochemistry and cytochemistry: An introduction. Academic press, Florida, USA, 1984.
27. Kokate CK. Practical Pharmacognosy, Vallabh

Prakashan, Delhi, 2000, 107-111.
28. Harbone JB. Phytochemical Methods, Chapman & Hall, London, 1999, 60-66.
29. Kabaru JM, Gichia L. Insecticidal activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae) Lam. against three arthropods. African Journal of Science and Technology. 2001; 2(2):44-49.
30. Samidurai K, Jebnesan A, Saravanakumar A, Govindarajan M, Pushpananthan T. Larvicidal, ovicidal and repellent activities of *Pemphis acidula* Forst. (Lythraceae) against filarial and dengue vector mosquitoes. Academic Journal Entomology. 2009; 2(2):62-66.
31. Rajkumar S, Jebanesan A. Ovicidal activity of *Moschosma polystachyum* Linn (Lamiaceae) leaf extract against filarial vector *Culex quinquefasciatus* Say. Journal of Tropical Biomedicine. 2004; 21:47-50.
32. Mullai K, Jebnesan A. Larvicidal and ovicidal activity of the leaf extract of two *Cucurbitacious* plants against filarial vector *Culex quinquefasciatus*. Indian Journal of Environmental Ecoplanning. 2006; 12:611-615.
33. Broadbent AB, Pree DJ. Effects of diflubenzuron and bay SIT 8514 on the oriental fruit moth and the oblique banded leaf roller. Journal of Economic Entomolology. 1984; 77:194-197.
34. Kokate CK, Purohit AP, Gokhale SB. Practical pharmacognosy, Second edition. Vallabh Prakashan, New Delhi, 2004, 466-470.
35. Sneha V, Mohan T, Revathy T, Suthindhiran K, Jayasiri MA. Phytochemical evaluation of selected plants. American Journal of Biochemical and Biotechnology. 2013; 9(3):291-299.
36. Bowers WS, Sener B, Evans PH, Bingol F, Erdogan I. Activity of Turkish medicinal plants against mosquitoes *Aedes aegypti* and Anopheles gambiae. Insect Science and its Application. 1995; 16:339-42.
37. Rajkumar S, Jebanesan, A, Nagarajan R. Effect of leaf essential oil of *Coccinia indica* on egg hatchablity and different larval instars of malarial mosquito *Anopheles stephensi*. Asia Pacific Journal of Tropical Medicine. 2011; 4(12):948-951.
38. Gbolade A. Plant derived insecticides in the control of malaria vector. In: Phytomedicine in malaria and sexually transmitted diseases: challenges for new millennium edited by Adewunmi CO and Adesina SK, Drug research and production unit, Faculty of Pharmacy, Obaferni, Awolowo University, IIefe, Nigeria, 2000, 48-50.
39. Gopiesh Khanna V, Kannabiran K. Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and Eclipta prostrata against Culex quinquefaciatus mosquito larvae. African Journal of Biotechnology. 2007; 6:307-311.