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EFFICACY OF *NICOTIANA PLUMBAGINIFOLIA* (SOLANACEAE) LEAF EXTRACTS AS LARVICIDE AGAINST MALARIAL VECTOR *ANOPHELES STEPHENSI* LISTON 1901

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ABSTRACT

The best way to control mosquito borne diseases is to control the mosquito population. The present study was executed to evaluate the mosquito larvicidal activity of *Nicotiana plumbaginifolia* leaves against malarial vector *Anopheles stephensi.*Crude extracts and three different solvent extracts were bio-assayed against *An. stephensi* larvae. LC_{50} and LC_{90} values were determined appropriately. Qualitative phytochemical tests were also done. Statistical justifications were drawn through Log-probit, regression and ANOVA analyses. Effects of bio-active fractions on some of the non-target water fauna were also examined. Crude extract was considerably mosquito larvicidal. Only ethyl acetate extractives exhibited larvicidal activity amongst all solvent extractives. Cent percent mortality of 1^{st}, 2^{nd} and 3^{rd} instars larvae was noticed within 72 h at 50 ppm concentration. Leaves contained tannins, steroids and flavonoids. Non-target organisms were non-responsive to bioactive fractions. *N. plumbaginifolia* leaves bear the potentiality to control *An. stephensi* larvae in a target specific way.

KEYWORDS: ***Anopheles stephensi, Nicotiana plumbaginifolia,*** **larvicide, Non-target organism, Phytochemicals**

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INTRODUCTION

Mosquitoes, medically the most important group of insects, are well-known for active transmission of pathogens resulting public health hazards. They primarily act as major vectors of many diseases like malaria, filariasis, yellow fever, dengue hemorrhagic fever, different types of encephalitis including Japanese encephalitis and so on. Though its socio-economic impacts are chiefly restricted to tropical and subtropical countries, the vector borne diseases are expanding worldwide rapidly [1]. Malaria is one of the most distressing mosquito borne diseases, transmitted by *Anopheline* mosquitoes like the Indian urban vector *An. stephensi*. The disease is prevalent in tropical and sub-tropical regions, including Latin America, Asia and Sub-Saharan Africa [2]. An colossal cause of malaria is poverty and leaves a major negative impression on economic development. A demises of 6.6 million resulted due to malaria alone out of 219 million cases in 2010 [3.] In 2012, 207 million malarial cases and 6.3 million cases of deaths were estimated by WHO [4]. Currently there is no effective precautional remedy for malaria and meanwhile *Plasmodium* is exhibiting a strong resistance to chloroquine and other chemotherapeutic agents. Being the definitive host of malarial parasites, it is indispensable to control target mosquito species to avoid the increasing mortality. Several chemical measures were applied to reduce mosquito populations to check disease incidences. Even it is useful, constant uses of chemical insecticides result failure of mosquito control in long time, interruption among natural biological control systems [5] and likely to cause resistant species of insect. Besides, it has some undesirable effect on non-target organisms and also on human health [6]. Accordingly, with the appearances of lots of terrible incidences of pesticide resistance [7], a new option is to use selectively toxic, biodegradable and green insecticides especially from natural derivation [8, 9, 10, 11]. The insecticides from botanical source can initiate a new era of more efficient insect management without disturbing the ecological balances [12]. The annual herb *Nicotiana plumbaginifolia*, usually called as wild tobacco plant, belongs to solanaceae family. 1-3 ft tall plant has basal oblanceolate, stalkless 15-23 cm long leaves. Rapid production of F1 progeny and transgenic flowering shoots from *N. plumbaginifolia* were reported [13]. The transgenic plant is known to exhibit nitrate reductase activity [14]. This plant also exhibit radiosensitivity to regenerating protoplasts [15]. Principle of the present study was to examine the larvicidal activity of *N. plumbaginifolia* leaves against the target species *An. stephensi*. This is the first ever report of this plant as source of target specific larvicidal agent under laboratory conditions.

MATERIALS AND METHODS

2.1. Collection of plant Materials

Mature, fresh green leaves of *N. plumbaginifolia* Viv. (Solanaceae) were harvested randomly during April 2013-May 2013 from the plants around the outskirts of Burdwan (23°16´N, 87°54´E), West Bengal, India. The plant was identified accurately and a voucher specimen (Voucher No. GCZAS-04) was submitted at the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan.

2.2. Collection of larvae and colony set up

Larvae of *An. stephensi* were taken from a well maintained mosquito colony of the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan. The colony was kept free from all kinds of pathogens, repellents and insecticides and maintained at 34 ± 2° C temperature and 85% relative humidity under 14:10 light and dark cycles in a day. The larvae were fed with a powdered mixture of dog biscuits and dried Brewer's yeast powder in the ratio of 3:1.

2.3. Preparation of Crude extract

At first the collected fresh and green leaves were rinsed well in tap water followed by distilled water and soaked on a paper towel. Mature leaves were crushed with mechanical grinder and the juice was filtered using Whatman No. 1 filter paper and the filtrate was used as a stock solution (100% concentration) for further bioassay experiments. The concentrations (0.06%, 0.07%, 0.08%, 0.09% and 0.1%) were prepared with addition of distilled water with the stock solution.

2.4. Preparation of solvent extract

The leaves of *N. plumbaginifolia* were rinsed well in distilled water and socked on paper towel. Leaves were dried for few days in shed at room temperature. The dried leaves were cut in small pieces and put (200 g) into the thimble of Soxhlet apparatus. 2000 ml of each solvent was loaded on still pot. 72 hours of extraction period for each solvent (8 hours maximum in a day) was fixed. Three different solvents namely petroleum ether, ethyl acetate and acetone were passed through the same thimble with the same material one after another. Extracts were accumulated from the still pot and kept on separate beakers. The extractives were intensified through rotary evaporator. The concentrated extractives were preserved at 4° C in a refrigerator.

2.5. Dose-response Larvicidal Bioassay

The larvicidal bioassays were done according to the standard protocol of WHO [16]. Each larval instar of *An. stephensi* was tested against previously prepared concentrations of crude and solvent extracts. Twenty five larvae of different instars (1^{st}, 2^{nd}, 3^{rd} and 4^{th}) were transferred into sterilized glass Petri dishes filled with 100 ml of distilled water. Different concentrations of crude extracts viz. 0.06% to 0.1% were applied in different Petri dishes. Likewise, graded concentrations of solvent extracts (from 20 ppm to 60 ppm) were prepared and tested for their larvicidal potentiality against each instar. Each experiment was done in triplicate (n = 9) with 2 replicates of control. Petri dishes were placed at room temperature (30 ± 2° C) and 88 ± 2 % relative humidity for 72 h of total observation. The percent mortality was recorded after 24 h, 48 h and 72 h of post exposure. The larvae were assumed dead when they failed to move after probing with needle in the siphon or cervical area of it or when they were unable to reach the water surface [17].

2.6. Phytochemical analyses of plant extracts

Aqueous leaf extract was tested to identify secondary metabolites like alkaloids, flavonoids, terpenoids, tannins, steroids, and saponins following standard protocols of Trease and Evans [18], Sofowara [19] and Harborne [20] with slight alterations as follows.

2.6.1. Tests for Alkaloid detection

Presence of alkaloid was tested through Mayer's test, Wagner's test [18-20]. Aqueous extract was firstly acidified with glacial acetic acid. Mayer's reagent (1.36 g of $HgCl_2$ and 5 g of KI in 100 ml distilled water) was applied to 1 ml of acidified aqueous extract. Formation of pale yellow precipitate indicates the presence of alkaloids (Mayer's test). Very few drops of Wagner's reagent (Iodine solution in potassium iodide), was given to 1 ml of acidified aqueous extract. Emergence of a reddish brown impetuous, indicate the presence of alkaloids (Wagner's test).

2.6.2. Tests for Flavonoid detection

10 drops of diluted HCl (0.5 N) were added in 1 ml of aqueous extract, followed by the addition of a small piece of zinc. Establishment of pink or reddish pink colour precipitate indicates the presence of flavonoids (Zinc hydrochloride test) [19]. In 1 ml of aqueous extract very few magnesium turnings were specified and then concentrated HCl (2 N) was loaded drop by drop. Creation of pink scarlet or green colour specifies the existence of flavonoids (Shinoda test) [19]. 1.5 ml methanol (50%) solution was assorted with 4 ml of aqueous extract and then the mixture was warmed, 5-6 drops of concentrated HCl (2 N) with few metal magnesium turnings were added. The manifestation of red colour indicates the presence of flavonoids [21, 22].

2.6.3. Tests for Tannin detection

5-10 drops of $FeCl_3$ were implied to 2 ml of aqueous extract. The formation of bluish black colour indicates the presence of tannins (Ferric chloride test) [18, 20]. Few drops of bromine solution were added to 1 ml of

aqueous extract. Decolourization of the bromine water signifies the presence of tannins (Bromine water test) [18]. 4-6 drops NaOH (1 N) solution was added to 1 ml of aqueous extract. The quick appearances of yellow to red impetuous identify the presence of tannins (Alkaline reagent test) [19].

2.6.4. Tests for terpenoid and steroid detection

Standard protocol of Kantamreddi *et al.,* 2010 [23] was followed to detect the presence of terpenoid and steroid. 1 ml of aqueous extract was acidified with 1 ml of glacial acetic acid (1 N) and 1 ml of concentrated sulphuric acid (4 N) was added through the wall of the test tubes in ice chamber. Development of brown or green colour indicates the presence of terpenoids and steroids respectively.

2.7. Effect on non-target organism

The species who share the same aquatic habitat with mosquito larvae are the most vulnerable group when exposure to larvicide is concerned. Impact of the plant extractives on non-target group was examined on *Daphnia* and *Chironomus circumdatus* larvae. They were exposed to the LC_{50} value of 3rd instar larvae for 72 h [24, 25]. The mortality or other abnormalities like sluggishness and/or reduced swimming activity were observed upto 72 h of post exposure.

2.8. Statistical analyses

The observed percent mortality (%M) was précised by using Abbott's formula [26]. Determination of LC_{50} and LC_{90} values of crude and solvent extracts were carried out through Log-probit and regression analyses (Y = mortality; X = concentration). Further statistical justifications were established through three way ANOVA analyses using different instars, hours and different concentrations as three random variables to validate the significance between the above parameters and larval mortality.

RESULTS

N. plumbaginifolia was found to have good mosquitocidal property against *An. stephensi* in our laboratory observations. Crude extracts of *N. plumbaginifolia* leaves brought about cent percent mortality with 0.09% concentration against the 1st instar within 48 h of post exposure (Table 1). Among the three solvent media, only ethyl acetate extractives were noticed for larvicidal activity against *An. stephensi* larvae. 1st instar larvae exhibited 100% mortality after 48 h of post exposure at 60 ppm concentration with ethyl acetate extractives. The mortality rate gradually increased in all instars with every lethal concentration with the increase of post-exposure time. It was highest at 60 ppm after 72 h of post-exposure and lowest at 20 ppm after 24 h of post-exposure (Table- 2) in all the instars. The results of regression analyses revealed that the mortality (Y) was positively correlated with the concentration of exposure (X) with a regression coefficient (R^2) close to 1 in each case (Table 3). Log probit analyses (95% confidence level) revealed that LC_{50} and LC_{90} values gradually decreased with the increase of exposure periods in each instar. The lowest LC_{50} and LC_{90} values were recorded after 72 h of exposure. LC_{50} and LC_{90} value of 1st instars larvae after 72 h of exposure was 9.25 and 19.42 ppm respectively (Table- 3). Three-way factorial ANOVA established statistical significance of larval mortality ($p<0.05$) (Table 4) in terms of concentrations, instars and time of exposure collectively. The preliminary qualitative phytochemical analyses revealed that the leaves of *N. plumbaginifolia* enclosed tannin, steroid and flavonoid (Table 5).

Table 1
Dose response larvicidal bioassay using crude extract of N. plumbaginifolia leaves Against An. stephensi larvae

Larval Instars	Concentration (%)	Percent Mortality (Mean ± SE)		
		24h	48h	72h
First	0.06	62.67 ± 0.33	73.33 ± 0.88	84.00 ± 0.00
	0.07	69.33 ± 0.58	80.00± 1.20	90.67 ± 0.33
	0.08	77.33 ± 0.33	86.67 ± 0.33	94.67 ± 0.89
	0.09	82.67 ± 0.67	93.33 ± 0.67	100.00 ± 0.00
	0.1	89.33 ± 0.33	100.00 ± 0.00	100.00 ± 0.00
Second	0.06	58.67 ± 0.67	65.33 ± 0.33	74.67 ± 0.33
	0.07	66.67 ± 0.58	72.00 ± 0.00	81.33 ± 0.33
	0.08	73.33 ± 1.45	78.67 ± 0.33	89.33 ± 0.33
	0.09	80.00 ± 0.00	85.33 ± 0.88	93.33 ± 1.20
	0.1	86.67 ± 0.88	94.67 ± 0.33	100.00 ± 0.00
Third	0.06	42.67 ± 0.67	53.33± 0.89	61.33 ± 0.54
	0.07	49.33 ± 0.88	60.00 ± 0.33	68.00 ± 0.67
	0.08	56.00 ± 0.00	66.67 ± 0.33	74.67 ± 0.33
	0.09	64.00 ± 0.00	73.33 ± 1.20	81.33 ± 0.88
	0.1	70.67 ± 0.54	80.00 ± 0.33	84.00 ± 0.00
Fourth	0.06	20.00 ± 0.00	26.67 ± 0.33	26.67 ± 0.33
	0.07	22.67 ± 1.20	30.67 ± 0.33	32.00 ± 0.00
	0.08	28.00 ± 0.00	34.67 ± 0.33	37.33 ± 0.67
	0.09	34.67± 0.33	40.00 ± 0.67	45.33 ± 0.33
	0.1	40.00 ± 0.00	45.33 ± 0.88	52.00 ± 0.00

Table 2
Dose response larvicidal bioassays using ethyl acetate extract of N. plumbaginifolia leaves against An. stephensi larvae

Larval Instars	Concentration (ppm)	Percent Mortality (Mean±SE)		
		24h	48h	72h
1st	20	62.67±0.33	82.67±0.33	94.67±0.88
	30	72.00±0.00	88.00±0.33	98.67±0.54
	40	78.67±0.58	94.67±0.33	100.00±0.00
	50	86.67±0.33	100.00±0.00	100.00±0.00
	60	94.67±0.33	100.00±0.00	100.00±0.00
2nd	20	61.33±0.33	73.33±0.33	85.33±0.58
	30	68.00±0.00	80.00±1.20	93.33±0.33
	40	74.67±0.58	85.33±0.58	100.00±0.00
	50	81.33±0.33	93.33±0.33	100.00±0.00
	60	89.33±0.33	100.00±0.00	100.00±0.00
3rd	20	58.67±0.58	66.67±0.58	76.00±0.67
	30	66.67±1.20	74.67±0.33	82.67±0.88
	40	73.33±0.88	80.00±0.33	92.00±0.00
	50	81.33±0.67	86.67±0.67	100.00±0.00
	60	88.00±0.00	100.00±0.00	100.00±0.00
4th	20	28.00±0.00	32.00±0.88	36.00±0.33
	30	33.33±0.33	37.33±0.33	40.00±0.00
	40	38.67±0.57	44.00±0.00	49.33±0.67
	50	45.33±0.33	50.67±0.58	57.33±0.33
	60	52.00±0.00	58.67±0.33	64.00±0.00

Table 3
Assessment of LC_{50} and LC_{90} values through log-probit and regression analyses using ethyl acetate extract of N. plumbaginifolia leaves

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2-value
1st	24	15.49	64.68	0.196x + 11.86	0.99
	48	11.95	29.36	0.116x + 18.60	0.94
	72	8.43	19.42	0.006x + 24.67	0.89
2nd	24	15.93	85.88	0.189x + 10.87	0.99
	48	12.22	41.44	0.166x + 14.93	0.99
	72	9.25	24.14	0.090x + 20.32	0.87
3rd	24	17.07	99.33	0.166x + 11.99	0.99
	48	13.16	51.76	0.196x + 12.53	0.97
	72	12.67	41.75	0.163x + 16.00	0.94
4th	24	63.76	620.49	0.15x + 3.866	0.99
	48	51.16	515.37	0.166x + 4.462	0.99
	72	39.13	279.80	0.18x + 5.066	0.99

x = concentration of ethyl acetate extractives (in ppm)

Table 4
Completely randomized three way ANOVA analyses of the larvicidal activity using concentration (C), hour (H) and instars (I) as three independent parameters

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean of squares (MS)	F value	p-level
Instars (I)	1079.91	3	359.97	285.44	0.00
Hours (H)	670.08	2	335.04	265.67	0.00
Conc. (C)	1223.02	4	305.76	242.45	0.00
I × H	50.99	6	8.50	6.74	0.00
I × C	31.44	12	2.62	2.05	0.02
H × C	15.31	8	1.91	1.52	0.10
I × H × C	20.51	24	0.85	0.68	0.86
Within groups	151.33	120	1.26	---	---
Total	3242.57	179	18.12	----	---

Table 5
Result of qualitative analyses of phytochemicals from crude leaf extract of tested plant

Name of the Plant	Plant Part	Tannin	Saponin	Steroid	Flavonoid	Terpenoid
Nicotiana plumbaginifolia	Leaves	+ +	_ _	+ +	+ +	_ _

+ + …… Present
_ _ ….. Absent

DISCUSSION

Mosquito control is always challenging. It will be highly effective if the mosquito managements are practiced at the wriggler stage. Much attention to be paid in the plant derived pesticides which are cost effective and biodegradable. As several plant products are reported with promising mosquitocidal properties, they may be suitable alternatives to chemical ones as herbal products are compatible with the animal and human systems [27]. Eco-safety measures are also a large foundation of choosing the newer alternatives. Plant derived commodities were found to exert diverse mosquitocidal properties as larvicides [28], [29, 30] adulticides, pupicides, repellents, smoke toxicants [31, 32]

etc. against different mosquito species. The present study well presented the larvicidal capability of *N. plumbaginifolia* leaves as a novel source against *An. stephensi*, the most crucial vector of malaria, under laboratory conditions for the first time. Shahi *et al.* 2010 [33] reported that the alcoholic extract of leaves of *Calotropis procera* showed a remarkable mosquito larvicidal effect against *An. stephensi*. LC_{50} and LC_{90} values were 109.71 and 234.61 ppm respectively after 24 h of exposure for the 3rd instars. In our experiment, LC_{50} and LC_{90} concentrations were found 17.07 and 99.33 ppm respectively after 24 h of exposure with ethyl acetate extractives for the 3rd instars. Methanolic extracts of three plants viz, *Andrographis paniculata*, *Eclipta alba* and *Cardiospermum halicacabum* showed good result against *An. stephensi* larvae with 79.68, 112.56 and 133.01 ppm values of median lethal concentrations respectively [34]. Singha Ray *et al.* (2014) [35] established that *Nelumbo nucifera* seed coat act as mosquito larvicide promisingly against 3rd instar larvae of *An. stephensi*. Kamaraj *et al*. (2010) [36] reported that ethyl acetate extract of *Annona squamosa* bark had a good larvicidal effect where LC_{50} and LC_{90} values were 25.18 and 94.04 ppm respectively after 24 h of exposure against 4th instar larvae of *An. stephensi.* Methanol extracts of *Chrysanthemum indicum* and *Tridax procumbenns* leaves found effective against 4th instar larvae of *An. subpictus* [37]. We found that whole population of 1st and 2nd instar larvae were diminished with 0.1% concentration of crude extract when observation period was set as 48 h and 72 h respectively. Non-target organisms were found safe after exposure; therefore, the bioactive fractions are selectively toxic only against target species.

CONCLUSION

Concisely, the present work revealed strong mosquito larvicidal ability of *N. plumbaginifolia* leaves. The result is very promising but at its preliminary stage. To find out the pure compound out of the active portions of the extract, further concentrated research is necessary.

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CONFLICT OF INTEREST

We have no conflict of interest.

REFERENCES

1. Fradin MS and Day JF, Comparative efficacy of insect repellents against mosquito bites. N Engl J Med, 347: 13 – 18, (2002).
2. Caraballo H and King K, Emergency Department Management of Mosquito-Borne Illness: Malaria, Dengue, And West Nile Virus. Emergency Medicine Practice, 16 (5), (2014).
3. World Health Organization. World Malaria Report 2012 (Report). accessed on August 14, 2014. http://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_no_profiles.pdf
4. WHO. “Malaria fact sheet”. accessed on September3, 2014. http:// www.who. int/mediacentre/ factsheets/ fs094/en/
5. Ojha K and Pattabhiramaiah M, Evaluation of Phytochemicals, Larvicidal Activity of *Jatropha Curcas* Seed Oil against *Aedes Aegypti.* International Journal of Applied Research and Studies, 2 (12): 1 – 12, (2013).
6. Brown AWA, Insecticide resistance in mosquitoes: pragmatic review. J Am Mosq Control Assoc, 2: 123 – 140, (1986).
7. Tabashnik BE, Evolution of resistance to Bacillus thuringiensis. Annu Rev Entomol, 39: 47 – 79, (1994).
8. Bhattacharya K and Chandra G, Phagodeterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata* L. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus* (Diptera:

Culicidae). Asian Pac J of Trop Dis, 4 (Suppl 1): S226 - S232, (2014).
9. Bhattacharya K, Burman S, Nandi S, Roy P, Chatterjee D and Chandra G, Phytochemical extractions from the leaves of *Ravenala madagascariensis* from Sundarban area and its effect on southern house mosquito (*Culex quinquefasciatus* Say 1823) larvae. Journal of Mosquito Research, 4 (12): 1-6, (2014).
10. Rawani A, Mallick Haldar K, Ghosh A and Chandra G, Larvicidal activities of three plants against filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae*).* Parasitol Res**,** 105: 1411 – 1417, (2009).
11. Chowdhury N, Chatterjee SK, Laskar S and Chandra G, Larvicidal activity of *Solanum villosum* Mill (Solanaceae: Solanales) leaves to *Anopheles subpictus* Grassi (Diptera: Culicidae) with effect on non-target *Chironomus circumdatus* Kieffer (Diptera: Chironomidae). J Pest Sci**,** 82: 13 – 18, (2009).
12. Ghosh A, Chowdhury N and Chandra G, Plant extracts as potential mosquito larvicides. India J Med Res, 135(5): 581 - 598, (2012).
13. Trinh TH, Mante S, Pua EC and Chua NH, Rapid Production of Transgenic Flowering Shoots and F1 Progeny from *Nicotiana plumbaginifolia* Epidermal Peels. Nature Biotechnol*,* 5, 1081 – 1084, (1987).
14. Foyer CH, Lescure JC, Lefebvre C, Morot-Gaudry JF, Vincentz M and Vaucheret H, Adaptations of Photosynthetic Electron Transport, Carbon Assimilation, and Carbon Partitioning in Transgenic Nicotiana plumbaginifolia Plants to Changes in Nitrate Reductase Activity. Plant Physiol, 104 (1): 171 – 178, (1994).
15. Magnien E, Dalschaert X and Devreux M, Different radiosensitivities of *Nicotiana plumbaginifolia* leaves and regenerating protoplasts. Plant Science Letters, 19 (3): 231 – 241, (1980).
16. World Health Organization, Guidelines for laboratory and field testing of mosquito larvicides. WHO, Geneva WHO/CDS/WHOPES/GCDPP/13pp, (2005).
17. Macedo M, Consoli RAGB, Grandi TSM, Des Anjos AMG, De Olivira AB, Mendes MM, Queiroz RO and Zani CL, Screening of Asteraceae (Compositae) plant extract for larvicidal activity against *Aedes fluviatilis* (Diptera : Culicidae). Mem Inst Osw Cruz, 92: 565 - 570, (1997).
18. G.E. Trease and W.C. Evans, Pharmacognosy Brailliar Tiridel can, 13th Edn, Macmillian Publishers, (1989).
19. A. Sofowara, Medicinal plants and Traditional medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria: 289, (19930).
20. J.B. Harborne, Phytochemical methods, Chapman and Hall, Ltd, London: 49 -188, (1973).
21. A.A. Siddiqui and M. Ali, Practical Pharmaceutical chemistry, Ist Edn, CBS publishers and distributors, New Delhi, India: 126-131, (1997).
22. Dutta S and Ray S, Evaluation of antioxidant potentials of leaf aqueous and methanolic extracts of calophyllum inophyllum in relation to total phenol and flavonoid contents. International Journal of Pharma & Bio Sciences, 5 (3), (2014).
23. Kantamreddi VSSN, Lakshmi YN and Kasapu VVVS, Preliminary phytochemical analysis of some important indian plant species. International Journal of Pharma and Bio Sciences, 1 (4): 351 - 357, (2010).
24. Bhattacharya K and Chandra G, Bioactivity of *Acyranthes aspera* (Amaranthaceae) Foliage against the Japanese Encephalitis Vector *Culex vishnui* Group. J Mosq Res, 3 (13): 89 - 96, (2013).
25. Bhattacharya K, Chandra I, Kundu P, Ray S, Halder D and Chandra G, Larval control of *Culex vishnui* group through bio-active fraction of traveller's tree, *Ravenala madagascariensis Sonn*. (Strelitziaceae). Journal of Mosquito Research, 4 (15): 1 – 6, (2014).
26. Abott WS, A method of computing the effectiveness of an insecticide. J Econ Enotomol, 18 (2): 265 - 267, (1925).
27. Chaithong U, Choochote W, Kamsuk K, Jitpakdi A, Tippawangkoso P and Chaiyasit D, Larvicidal effect of pepper plants on *Aedes aegypti* (L.) (Diptera:

Culicidae). Journal of Vector Ecology, 31: 138 - 44, (2006).
28. Singha S, Adhikari U, Ghosh A and Chandra G, Mosquito Larvicidal Potentiality of *Holoptelea integrifolia* Leaf Extract against Japanese Encephalitis Vector*, Culex vishuni* Group. J Mosq Res, 2 (4): 25 – 31, (2012).
29. Hossain E, RawaniA, ChandraG, Mandal S C and Gupta J K, Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*. Asian Pac J Trop Med, 4: 436-441, (2011).
30. Chakraborty S, Singha S, Bhattacharya K and Chandra G, Control of human filarial vector, Culex quinquefasciatus Say 1823 (Diptera:Culicidae) through bioactive fraction of Cayratia trifolia leaf. Asian Pac J Trop Biomed, 3(12), 980-84, (2013).
31. Singha S, Adhikari U and Chandra G, Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pac J Trop Biomed, 1 (1): S119 - S123, (2011).
32. Chowdhury N, Bhattacharjee I, Laskar S and Chandra G, Efficacy of *Solanum villosum* Mill. (Solanaceae: Solanales) as a biocontrol agent against fourth instar larvae of *Culex quinquefasciatus* Say. Turk J Zool, 31 (4): 365 - 370, (2007).
33. Shahi M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H and Hanafi-Bojd MY, Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales*:* Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). *J Vector Borne Dis,* 47, 185 – 188, (2010).
34. Govindarajan M, Evaluation of indigenous plant extracts against the malarial vector, Anopheles stephensi (Liston) (Diptera: Culicidae). Parasitol Res, 109 (1): 93 – 103, (2011).
35. Singha Ray A, Bhattacharya K, Singh A and Chandra G, Larvicidal Activity of *Nelumbo nucifera* Gaertn. (Nymphaeaceae) against *Anopheles stephensi* (Liston 1901) and its Effect on Non-target Organisms. Journal of Mosquito Research, 4 (10): 1-7, (2014).
36. Kamaraj C, Rahuman AA, Bagavan A, Zahir AA, Elango G, Kandan P, Rajakumar G, Marimuthu S and Santhoshkumar T, Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). Tropical Biomedicine, 27 (2): 211 – 219, (2010).
37. Kamaraj C, Bagavan A, Elango G, Abduz Zahir A, Rajakumar G, Marimuthu S, Santhoshkumar T and Abdul Rahuman A, Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus.* Indian J Med Res, 134: 101-106, (2011).