Middle-East Journal of Scientific Research 13 (5): 703-709, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.13.5.2939

Evaluation of Larvicidal Activity of *Hippophae rhamnoides* L. Leaves Extracts on *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae)

[1]Bashir Ahmad and [1,2]Javid Ali

[1]Center of Biotechnology and Microbiology, University of Peshawar, KPK Pakistan
[2]PCSIR Laboratories Complex Jamrude Road Peshawar, KPK Pakistan

Abstract: The crude leaves extract of *Hippophae rhamnoides* L. in different solvents were tested for larval mortality against fourth instars of *Anopheles stephensi* and *Aedes aegypti* viz. vector mosquito species. The ethanol and methanol extract was found highest percent mortality at 72 h was 84±0.82 and 81±1.71 at 2000 ppm against *Aedes aegypti*. Ethanol extract observed the lowest LC50 (ppm) at 24 h, 48 h and 72 h were 1424.45, 1104.53 and 1035.21 respectively against *Aedes aegypti.* The lowest LC90 (ppm) were 2841.20, 2632.08 and 2643.63 at 24 h, 48 h and 72 h calculated against *Aedes aegypti* of ethyl acetate, acetone and ethanol extract respectively. The highest mortality (%) of *Anopheles stephensi* at 72 h (2000 ppm) was 90±1.30 (ethanol) and 89.0±0.50 (methanol). The highest LC50 (ppm) of *Anopheles stephensi* at 24 h, 48 h and 72 h were 1725.80 for chloroform, 1508.97 for acetone and 1361.07 for chloroform. The lowest LC90 (ppm) were 2605.78 (ethanol) at 24 h, 2142.63 (ethanol) at 48 h and 2042.06 (methanol) at 72 h. These results suggest that *Hippophae rhamnoides* L. leaves exhibited noteworthy activity and could be measured as an effective natural larvicidal means against vector mosquitoes.

Key words: Seabuckthorn leaves · Extract larvicidal activity · Percent motatality · Mosquito control · LC50 · LC90

INTRODUCTION

Vector-borne diseases especially malaria and dengue are contribute to the main diseases load in Pakistan. One of the methods to control these diseases is to control the vectors for the disruption of disease propagation. Generally in budding countries the mosquitoes are very common due to less awareness shown toward hygiene. The sewage and especially the rain water without difficulty obtain sluggish present oftenly in the open places and on the roads. The stagnant water stagnant along with open sewage passages provides a very favorable condition for the growth of mosquitoes, which has made the civilians day to day life very miserable.

Vectors of pathogens of different ailments such as malaria, Japanese encephalitis, filariasis, chickungunya and dengue etc are mainly due to a number of mosquito species which belong to the genera Anopheles, Aedes and Culex [1]. Mosquito control programmes are established in various countries but still very little improvement in the management of malaria and infections can be seen, which decrease the ability of labor and can cause financial and human loses [2]. Due to increase pesticides resistance, unavailability of vaccines and medicines, it is very difficult to control mosquito born diseases in these days [1]. Mosquitoes in the larval stage are striking targets for pesticides because they rear in water and therefore very easy to handle them in this atmosphere [3].

Substances derived from plants have draw a greater concentration for researchers and round about 2000 plant species are formerly identified for its insecticidal activities [4]. Plant derived products has been used in many areas of the world against the species of insects and vectors by conventionally human communities. Many researchers have found that the phytochemicals constituents of plant origin can kill larvae, regulate the insect to grow, repel the insects and ovipositional attractants and have preventive actions [5]. Agents acting as repellents possess a very major role in saving humans from insect bites. The spreading risk of diseases and humans contact with vectors can only be reduced by effective repellents.

Corresponding Author: Bashir Ahmad, Center of Biotechnology and Microbiology, University of Peshawar, KPK Pakistan.

The compounds which are repellants should be non toxic, should not have any irritation and should have effects for longer time e.g. Amides, imides, esters and other multifunctional compounds [6].

Hippophae rhamnoides L. commonly known as seabuckthorn is a shrub belongs to the plant family Elaeagnaceae. The leaves of seabuckthorn are normally green on upper side while green silver ash color on lower side with a solid and coarse bark [7]. Seabuckthorn is found in Kurram Agency, Gilgit Baltistan and Khyber Pakhtunkhwa (Chitral, upper Swat, Utror-Gabral) of Pakistan [8]. The seabuckthorn leaves and berry are rich in vitamins (E, C, A and K) and other major vital compounds (flavonoids, carotenoids and sterols), which acquire top medicinal and dietetic characteristics [9, 10]. Seabuckthorn has been used conventionally as a good source of medication, nutrition, firewood and as boundary marker. The plant has been broadly used for cure of stomach, cancer and liver disorder, thrombosis, ulcer, tendon and ligament injuries [11]. Numerous minerals such as Na, K, Fe Mg, Ca, Zn, P and Ag are found in Sea buckthorn spp Turkestanica seed [12]. Similarly seed and leaf crude extract of Seabuckthorn (*Hippophae salicifolia* D. Don) have potent antifungal and antibacterial activities [13]. The *Hippophae rhamnoides* stem and root extracts contains a variety of constituents contributing to antioxidant, antibacterial and antifungal activities [14]. The leaves extracts of *Hippophae rhamnoides* [15] has a major role against dengue virus.

MATERIALS AND METHODS

Plant Leaves Collection: The *Hippophae rhamnoides* L. (*H. rhamnoides*) leaves were collected from PCSIR Demonstration Cum- Training Center Skardu, Gilgit Baltistan Pakistan. The washing of leaves was done by distilled H_2O, dried under shade at 20-25°C, grinded into powder form and kept in an air-tight plastic bag until used.

Plant Leaves Extracts: From *H. rhamnoides* leaves powder 50 g were taken and macerated with 250 ml of ethyl acetate, acetone, aqueous, methanol, ethanol, chloroform and n-hexane for a period 48 h and filtered (repeated five times for each solvent). The extracts were concentrated in rotary evaporator and stored at 4 degree centigrade till used.

Test Mosquitoes: Mosquitoes *Aedes aegypti* (*A. aegypti*) and *Anopheles stephensi* (*A. stephensi*) were reared in the Food Technology Center, PCSIR Laboratories Complex Peshawar KPK Pakistan. The mosquito's vectors cyclic generations were under specified conditions of relative humidity i.e. 80-90% and temperature i.e. 25-29°C in insectarium. The food used for the growth of larvae consisted of yeast and powdered dog biscuits (1:3).

Larvicidal Activity Test: The larvicidal activity test was carried out by means of World Health Organization method [16] with minor modifications. Twenty-five instar (IV) larvae of *A. stephensi* and *A. aegypti* were transfer to plastic cups (500 ml) having 249 ml (distilled H_2O) and *H. rhamnoides* L. leaves extract of one milliliter in concentration (each extract). Four replicates for each concentration were set up. Distilled H_2O having 25 larvae were used as a control. Abbott's formula [17] was applied to correct the control mortality and probit analyses [18] were used to calculate regression equation, LC90 and LC50.

$$\% \text{ mortality} = \frac{\% \text{ Mortality (sample)} - \% \text{ Mortality (control)}}{100 - \% \text{ Mortality (control}} \times 100$$

Statistical Study: Statistical analysis of the study records were carried out with the help of SPPS to find the mean, standard deviation (±SD), LC50, LC90, regression equations and Chi-square value (X^2) values.

RESULTS

The analysis of larvicidal activity of *Hippophae rhamnoides* L. leaves extracts against vector mosquitoes (*Aedes aegypti*) are shown in Table 1 and was found dose and time dependent. The percent mortality increased by increasing the dose and time of exposure of larvae for each extract. The aqueous extract of 1000 ppm showed 12±0.82%, 19±0.96% and 39±2.5% mortality after 24 h, 48 h and 72 h hours respectively. When the dose of the same extract were increased to 1500 ppm with respect to its time, the percent mortality was found high as compared to 1000 ppm i.e. 42±1.29%, 45±0.96% and 47±1.7%. High mortality rate 77% was detected in a concentration of 2000 ppm of the same extract. The Table 1 showed that at a concentration of 1000 ppm high percent mortality 25±1.7, 46±0.53 and 50±1.30% were noted in chloroform extract. The least larvicidal activity 29±0.96%, 38±1.3% and 42±1.30% were shown by ethanol extract at 1500 ppm concentration at 24 h, 48 h and 72 h respectively. The high percent mortality 84±0.82% was recorded against 2000 ppm of ethanolic extract at 72 h, while the lowest value 55±0.96% mortality was determined against the same

concentration at 24 h of ethyl acetate. The second highest % mortality 81±1.71 observed in a methanol extract (2000 ppm) at 72 h (Table 1).

As can be seen from Table 2, 3 and 4 the chloroform extract showed highest LC50 value 1860.16 ppm followed by n-hexane extract 1564.11 ppm and 1513.22 at 24 h, 48 h and 72 h respectively. The lowest LC90 value 2783.35 was recorded for aqueous extract at 24 h and 4266.78 LC90 value was found high for ethanolic extract at 48 h. The aqueous extract showed maximum LC90 value 4374.21 ppm and the lowest LC90 value 2643.83 ppm was recorded for ethanolic extract at 72 h. The regression equation values of *Hippophae rhamnoides* L. leaves aqueous, methanolic, ethanolic, chloroform and n-hexane extract for larvae were Y=-12.75+5.53x, -8.85+4.30x, -8.01+4.13x, -7.39+3.79x and -9.76+4.52x at 24 h respectively. The regression equation value of ethyl acetate extract at 48 h was Y=-6.39+3.58x, while for acetone extract the regression value was -4.85+3.18x at 72 h (Table 2, 3 and 4).

The percent larval mortality of the *Hippophae rhamnoides* L. leaves extracts against vector mosquitoes (*Anopheles stephensi*) are presented in Table 5. The maximum mortality 17±0.96% of the 1000 ppm ethanolic extract of *Hippophae rhamnoides* L. leaves was recorded against *Anopheles stephensi* at 24 h and the lowest larvicidal activity 30±1.30% was noted in the 1500 ppm chloroform extract at 48 h. The mortality rate was found high 90±1.30% in the 2000 ppm ethanolic extract, followed by methanol and aqueous extracts were showing 89±0.50% and 88±0.82% mortality respectively at the same concentration and incubation time, while the less mortality rate 79±0.96% was recorded in the n-hexane extracts at 72 h (2000 ppm).

It is evident that LC50 (24 h) value 1725.80 ppm was found high for chloroform extract and regression equation was Y=-13.45+5.70x and the lowest LC50 (24 h) value was 1494.30 ppm was found in ethanolic extract with regression equation, Y= -12.19+5.41x (Table 6).

Table 1: Larval mortality (%) of *Hippophae rhamnoides L.* leaves extract against vector mosquitoes (*Aedes aegypti).*

	Concentration (ppm)								
	1000			1500			2000		
Solvents	24h	48h	72h	24h	48h	72h	24h	48h	72h
Aqueous	12±0.82	19±0.96	39±2.5	42±1.29	45±0.96	47±1.7	56±1.3	70±1.30	77±0.96
Methanol	20±0.82	28±0.82	36±0.81	33±1.26	47±1.26	59±1.7	68±0.82	71±1.30	81±1.71
Ethanol	18±1.30	26±1.30	32±1.80	29±0.96	38±1.3	42±1.30	59±1.7	76±2.94	84±0.82
Ethyl Acetate	13±0.50	34±1.91	37±1.26	43±1.26	53±2.63	53±2.63	55±0.96	64±1.63	73±0.96
Acetone	15±0.96	24±1.63	36±2.6	51±0.96	59±1.50	62±0.56	65±0.96	75±0.96	79±0.96
Chloroform	25±1.7	46±0.53	50±1.30	57±0.96	62±1.30	65±1.50	68±0.82	76±2.94	74±1.30
n-Hexane	13±0.50	16±0.82	25±1.7	31±0.96	41±0.96	52±0.82	60±2.16	65±1.50	76±2.94
*Control (distilled water)	-	-	-	-	-	-	-	-	-

*Control= - (no mortality), Values are mean (%) of the four replicates of two trials ±standard deviation.

Table 2: Probit analysis of larvicidal activity of *Hippophae rhamnoides* L. leaves extract against *Aedes aegypti.*

Extracts	*LC50 24 hr	**LC90 24 hr	Regression equation	Chi-square value ($\times^2$)
Aqueous	1631.646	2783.35	Y= -12.75+5.53x	0.00
Methanol	1659.193	3294.58	Y= -8.85 +4.30x	2.08
Ethanol	1424.45	4395.42	Y= -8.01+4.13x	0.315
Ethyl Acetate	1627.14	2841.20	Y=-12.00 +5.29x	0.01
Acetone	1588.05	2963.47	Y= -10.14+4.73x	1.12
Chloroform	1860.16	4053.22	Y= -7.39+3.79x	2.20
n-Hexane	1832.87	3518.84	Y= -9.76+4.52x	0.62

*LC50: Lethal concentration needed to kill 50% of the exposed population and **LC90: Lethal concentration needed to kill 90% of the exposed population.

Table 3: Probit analysis of larvicidal activity of *Hippophae rhamnoides* L. leaves extract against *Aedes aegypti.*

Extracts	*LC50 48 hr	**LC90 48hr	Regression equation	Chi-square value (X^2)
Aqueous	1563.188	2903.35	Y= -9.84+4.65x	0.095
Methanol	1506.914	3268.89	Y= -5.98+3.45x	0.228
Ethanol	1104.53	4266.78	Y= -1.65+2.18x	0.008
Ethyl Acetate	1525.43	3480.17	Y= -6.39+3.58x	0.512
Acetone	1393.10	2632.08	Y= -9.58+4.64x	0.264
Chloroform	1389.74	2718.32	Y= -8.82+4.39x	0.040
n-Hexane	1564.11	2671.16	Y= -12.61+5.51x	0.881

*LC50: Lethal concentration needed to kill 50% of the exposed population and **LC90: Lethal concentration needed to kill 90% of the exposed population.

Table 4: Probit analysis of larvicidal activity of *Hippophae rhamnoides* L. leaves extract against *Aedes aegypti.*

Extracts	*LC50 72 hr	**LC90 72 hr	Regression equation	Chi-square value (X^2)
Aqueous	1375.185	4374.21	Y= -3.00+2.55x	1.310
Methanol	1268.71	2871.44	Y= -6.21+3.61x	0.047
Ethanol	1035.21	2643.63	Y= -4.49+3.14x	0.422
Ethyl Acetate	1322.13	3461.05	Y= -4.57+3.07x	0.363
Acetone	1250.74	3163.00	Y= -4.85+3.18x	0.110
Chloroform	1467.78	3068.31	Y= -7.67+4.00x	0.608
n-Hexane	1513.22	3061.26	Y= 4.18+-8.32x	1.271

*LC50: Lethal concentration needed to kill 50% of the exposed population and**LC90: Lethal concentration needed to kill 90% of the exposed population.

Table 5: Per cent larval mortality of *Hippophae rhamnoides* L. leaves extract against vector mosquitoes (*Anopheles stephensi).*

	Concentration (ppm)								
	1000			1500			2000		
Extracts	24h	48h	72h	24h	48h	72h	24h	48h	72h
Aqueous	12±1.2	16±0.82	27±6.75	41±0.96	61±0.96	67±0.50	71±1.30	84±0.82	88±0.82
Methanol	13±0.50	19±0.50	21±0.96	49±1.71	54±1.73	60±0.82	75±0.96	85±0.96	89± 0.50
Ethanol	17±0.96	22±1.30	40±0.82	51±0.96	66±1.30	75±0.96	77±0.96	86±0.56	90±1.30
Ethyl Acetate	8±0.82	23±0.96	41±0.96	56± 0.82	64±1.63	63±0.96	67±0.50	73±0.96	79±0.96
Acetone	11±0.96	18±0.58	35±1.70	45±0.96	50±1.30	62±1.30	68±0.82	77±0.96	81±1.71
Chloroform	10±0.58	14±0.55	21±1.70	30±1.30	56±0.82	63±0.96	72±0.96	76±2.94	82±1.30
n-Hexane	15±0.96	22±1.70	30±1.30	35±1.70	54±1.30	61±0.96	70±1.30	74±1.30	79±0.96
*Control (distilled water)	-	-	-	-	-	-	-	-	-

*Control= - (no mortality), Values are mean (%) of the four replicates of two trials ±standard deviation.

Table 6: Probit analysis of larvicidal activity of *Hippophae rhamnoides* L. leaves extract against (*Anopheles stephensi).*

Extracts	*LC50 24 hr	**LC90 24 hr	Regression equation	Chi-square value (X^2)
Aqueous	1601.29	2630.36	Y= -14.05+5.94x	0.202
Methanol	1566.65	2621.11	Y= -13.32+5.73x	0.392
Ethanol	1494.30	2605.78	Y= -12.19+5.41x	0.013
Ethyl Acetate	1509.58	2292.18	Y= -17.46+7.06x	1.614
Acetone	1631.69	2786.68	Y= -12.71+5.51x	0.346
Chloroform	1725.80	2895.69	Y= -13.45+5.70x	1.885
n-Hexane	1636.23	2834.38	Y= -12.26+5.37x	1.873

*LC50: Lethal concentration needed to kill 50% of the exposed population and **LC90: Lethal concentration needed to kill 90% of the exposed population.

Table 7: Probit analysis of larvicidal activity of *Hippophae rhamnoides* L. leaves extract against (*Anopheles stephensi).*

Extracts	LC50 48 hr	LC90 48 hr	Regression equation	Chi-square value (X^2)
Aqueous	1399.08	2158.66	Y= -16.40 +6.80x	0.232
Methanol	1408.33	2274.36	Y= -14.38+6.16x	0.210
Ethanol	1320.38	2142.63	Y= -14.02+6.10x	0.18
Ethyl Acetate	1330.39	2165.49	Y= -13.92+6.05x	0.067
Acetone	1508.97	2697.33	Y= -11.15+5.08x	0.010
Chloroform	1505.49	2452.96	Y= -14.21+6.04x	0.010
n-Hexane	1427.86	2571.96	Y= -10.82+5.01x	0.001

LC50: Lethal concentration needed to kill 50% of the exposed population and LC90: Lethal concentration needed to kill 90% of the exposed population.

Table 8: Probit analysis of larvicidal activity of *Hippophae rhamnoides L.* leaves extract against (*Anopheles stephensi).*

Extracts	*LC50 72 hr	**LC90 72 hr	Regression equation	Chi-square value (X^2)
Aqueous	1266.99	2081.43	Y= -13.44+5.94x	0.001
Methanol	1310.64	2042.06	Y= -15.75+6.65x	0.084
Ethanol	1117.36	2057.01	Y= -9.74+4.84x	0.070
Ethyl Acetate	1170.87	2176.37	Y= -9.60+4.76x	0.964
Acetone	1252.71	2641.97	Y= -7.25+3.95x	0.001
Chloroform	1361.07	2289.54	Y= -12.78+5.67x	0.335
n-Hexane	1295.49	2409.09	Y= -9.80+4.76x	0.020

*LC50: Lethal concentration needed to kill 50% of the exposed population and **LC90: Lethal concentration needed to kill 90% of the exposed population.

The lowest LC50 value 1320.38 ppm (Regression equation, Y=-14.02+6.10x) for ethanolic extract at 48 h (Table 7). The maximum LC50 values 1361.07 ppm was recorded for chloroform extract followed by 1310.64 ppm, 1295.49 ppm, 1266.99 ppm, 1252.71 ppm, 1170.87 ppm and 1117.36 ppm at 72 h for methanolic, n-hexane, aqueous, acetone, ethyl acetate and ethanolic extracts respectively (Table 8). The highest LC90 value 2895.69 ppm was noted for chloroform extract at 24 h, with regression equation Y= -13.45+5.70x (Table 6), while the least LC90 value 2142.63 ppm was determined for ethanolic extract at 48 h and regression equation was Y= -14.02+6.10 (Table 7). The LC90 (72 h) value 2641.97 ppm was found highest for acetone extract, with regression equation Y=-7.25+3.95x and the LC90 values were detected nearly equal 2042.06 ppm (Regression equation, Y=-15.75+6.65x) and 2057.01 ppm (Regression equation, Y=-9.74+4.85x) for methanolic and ethanolic extracts respectively (Table 8).

DISCUSSION

Plants are the main reservoir of bioactive compound which are less toxic, minimum chance to cause resistance and are easily biodegradable [6]. Phytochemicals can be used as suitable alternative over synthetic insecticides which are comparatively safe, less expensive and easily available through the world. Neem is a rich source of phytochemicals mainly containing high amounts of steroids, tannins saponins and alkaloids which is responsible for the high antimosqutocidal activity [19]. The phytochemicals present in twigs of *H. rhamnoides* extracts i.e. cold chloroform/ methanol (1:1) and methanol showed glycosides, terpenoids, steroids, flavonoids, reducing sugars and tannins [20]. The phytochemicals including alkaloids, terpenoids and Phenols may act independently or jointly ovicidal activity and are responsible as skin repellent against *A. Stephensi* [6]. It has been reported that the phenolic constituents showed high activity against larvae (mosquito) and the extract especially tannin from *Eclipta prostrate*, *Hemidesmus indices* and *Gymnema sylvestre* which is responsible for the mortality in *C. quinquefasciatus* larvae [3]. Total phenolic content (mg GAE/g extract) of *Hippophae rhamnoides* L. extracts of leaves methanol, leaves aqueous, seed methanol, seed aqueous, pomace methanol and pomace aqueous were found 278.80, 184.89, 162.56, 109.57, 107.01 and 87.35 respectively [21]. It is reported that mosquito larvicidal activity are mainly due tannins, phytosterols, flavonoids, phenols, saponins and carbohydrates [1]. The phenolic and flavonoids contents in methanolic extract of seabuckthorn leaves were 34.6 (mg quercetin equivalent/100 g dry leaf) and 18.1 respectively [22]. In fact the leaves and twigs of the plant species are most commonly used for malarial therapy which is very useful to sustain the harvesting of plants for long time. The bark and roots harvesting is the main threat for plants population harvesting roots and bark can easily threaten the local populations of plants if a sustainable harvesting plan has been made [2]. According to Mallikarjun *et al*. 2010 [1] chloroform extract was found more effective against larvae, while the ethanolic and petroleum ether extracts showed less larvicidal activity. The study of Nassima *et al*. 2011 [23] concluded that less mortality were observed in the larvae and adult stages as compared to pupae stage. In the study of Kalu *et al.* 2010 [24] was concluded that the ethanol extract from garlic bulb exhibited effective larvicidal properties. Bioassays of leaf and fruit showed [25] that the extract of petroleum ether has been found more effective than methanol and chloroform extract against larvae. Lokesh *et al*. 2010 [26] studied the Aedes mortality was the highest followed by Culex with moderate mortality rate, while the mortality of the Anopheles was found very low as compared to the former two genera. It has been documented that [24] biological activity and chemical composition of the plant depend upon the geographical origin of plant, the plant tissues, plant age, the age and species of the tested pest organism. Arivoli and Samuel 2011 [27] reported that all four crude (Hexane, Ethyl acetate, Dichloromethane and Diethyl ether) extracts of *Abutilon indicum* were found highly effective against larvae while the hexane extract showed maximum larvicidal activity against *Aedes aegypti*.

The study of Arivoli and Samuel 2011 [27] observed the plant extracts can injured both the eggs and egg shells, which may be by endosmosis. The eggs become desiccated after the initial phase of bump and shrink followed by the death of larvae. Larval toxicity effect of *Euphorbia hirta* leaf extract [28] against malarial vector *A. stephensi* (IV instar) at 250 ppm observe percent larval mortality of was 66.2, while LC50 (197.40) and LC90 (371.34). Beside it was observe that mortality was increased as the concentration increased. Chandran and Madanagopal 2012 [29] study revealed that larvicidal activity of *P. tithymaloides* aqueous stem extract against the *A. aegypti* (instar IV) at 1.0% concentration calculated the percent mortality was 16 with LC50 (2.210).

CONCLUSIONS

In budding countries mosquito borne diseases are the alarming issue in the public health communities. These diseases can be prohibited to prevent the mosquito bite by the use of plant extract having larvicidal activity and showed good repellent and killing properties against mosquito. The rich sources of novel bioactive compounds are from higher plants and can be used to control insect by developing environmentally safe methods. It can be concluded from the present study that the larvicidal activity is due to the phytoconstituents present in the extracts of Seabuckthorn leaves. The extracts of this plant can be used to control vector born diseases like dengue and malaria etc. Moreover, further studies are needed for the isolation and identification of the principal constituents responsible for antimosquito activity and their mode of action by different trails to recommend its use as a useful antimosquito agent.

REFERENCES

1. Mallikarjun, N., C.M. Sunita, K.T.R. Prashith, S.J. Sudharshan and K.S. Vinayaka, 2010. Antimosquito (Insecticidal) activity of extracts of Hemidesmus indicus and Swertia chirata against Aedes aegypti mosquito larvae-A Comparative Study. Drug Invention Today, 2(2): 106-108.
2. Alex, A., A.O.Y. Alfred, T.O. George and S.J.S. Monique, 2005. Ethnobotanical study of some Ghanaian anti-malarial plants. Journal of Ethnopharmacology, 99: 273-279.
3. Nandita, C., G. Anupam and C. Goutam, 2008. Mosquito larvicidal activities of Solanum villosum berry extract against the dengue vector *Stegomyia aegypti*. BMC Complementary and Alternative Medicine, 8: 10-17.
4. Arivoli, S. and T. Samuel, 2011. Studies on the Mosquitocidal Activity of *Murraya koenigii* (L.) Spreng (Rutaceae) Leaf Extracts Against *Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus* (Diptera: Culicidae). Asian J. Exp. Biol. Sci., 2(4): 721-730.
5. Babu, R. and K. Murugan, 1998. Interactive effect of neem seed kernel and neem gum extracts on the control of Culex quinquefasciatus say. Neem Newslett, 15(2): 9-11.
6. Prabhu, K., K. Murugan, A. Nareshkumar, N. Ramasubramanian and S. Bragadeeswaran, 2011. Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). Asian Pacific J. Trop. Biomed, pp: 24-129.
7. Raj, K., K.G. Phani, O.P. Chaurasia and B.S. Shashi, 2011. Photochemical and pharmacological profile of seabuckthorn Oil: a Review. Research Journal of Medicinal Plant, 5: 491-499.
8. Shah, A.H., D. Ahmad, M. Sabir, S. Arif, I. Khaliq and F. Batool, 2007. Biochemical and nutritional evaluations of sea buckthorn (*Hyppophae rhamnoides L. Spp. Turkestanica)* from different locations of Pakistan. Pak. J. Bot., 39: 2059-2065.
9. Chen, T.G., MK. Ni, R. Li, F. Ji and T. Chen, 1991. Investigation of the biological properties of Central Asian Seabuckthorn growing in the province of Kansu, China. Chem. Nat. Compd, 27: 119-121.
10. Yang, B. and H.P. Kallio, 2001. Fatty acid composition of lipids in seabuckthorn (Hippophae rhamnoides L.) berries of different origins. Journal of Agricultural and Food Chemistry, 49: 1939-1947.
11. Tsering, S., O.P. Chaurasia, K. Girish, M. Sunil, A. Zulfikar, Y. Ashish and B.S. Shashi, 2010. Attributes of Seabuckthorn (Hippophae rhamnoides L.) to meet nutritional requirements in high altitude. Defense Science Journal, 60: 226-230.
12. Alam, Z. and M. Ijaz, 2009. Biochemical characterization of sea buckthorn (*Hippophae rhamnoides* L. spp. turkestanica) seed. African Journal of Biotechnology, 8(8): 1625-1629.
13. Sanjay, M.G., K.G. Atul, A. Zakwan and K. Anil, 2011. Antibacterial and antifungal activity in leaf, seed extract and seed oil of Seabuckthorn (*Hippophae salicifolia D. Don*) Plant. J. Plant Pathol. Microbiol, 2(2): 1-4.
14. Jong, H.J., W.L. Ji, S.K. Kyoung, K. Ju-Sung, N.H. Sang, Y.Y. Chang, K.L. Ju, S.K. Yong and J.K. Myong, 2010. Antioxidant and Antimicrobial Activities of Extracts from a Medicinal Plant, Sea Buckthorn. J. Korean Soc. Appl. Biol. Chem., 53(1): 33-38.
15. Jain, M., L. Ganju, A. Katiyal, Y. Padwad, K.P. Mishra, S. Chanda, D. Karan, K.M. Yogendra and R.C. Sawhney, 2008. Effect of *Hippophae rhamnoides* leaf extract against dengue virus infection in human blood-derived macrophages. Phytomed, 15(10): 793-9.

16. WHO. Report of the WHO informal consultation on the evaluation and testing of insecticides CTD/WHO PES/IC/96.1. Geneva: WHO; 1996, pp: 69.
17. Abbott, W.S., 1987. A method of computing the effectiveness of an insecticide. J. Amr. Mosq. Control. Assoc., 3(2): 302-303.
18. Finney, D.J., 1971. Probit analysis. 3rd ed. London: Cambridge, Great Britain: Cambridge University Press, pp: 333.
19. Mgbemena, I.C., 2010. Comparative Evaluation of Larvicidal Potentials of Three Plant Extracts on *Aedes aegypti.* J. Amer. Sci., 6(10): 435-440.
20. Keshab, B., M.S. Tirtha, B. Ramita, S.C. Jain and L. Janardan, 2010. Biological activities of three different medicinal plants from Himalayan Region of Nepal. Nepal J. Sci. and Technol., 11: 139-146.
21. Richa, A., M. Sunil, Y. Ashish, B.S. Ravi and S. Tsering, 2012. Antimicrobial activity of seed, pomace and leaf extracts of sea buckthorn (*Hippophae rhamnoides* L.) against food borne and food spoilage pathogens. Afr. J. Biotechnol, 11(45): 10424-10430.
22. Gill, N.S., R. Sharma, R. Arora and M. Bali, 2012. Antioxidant and antibacterial activity of *Hippophae rhamnoides* methanolic leaf extracts from dry temperate agro-climate region of Himachal Pradesh. Journal of Plant Sciences, DOI: 10.3923.
23. Nassima, R., A. Abdelouaheb and S. Noureddine, 2011. Efficacy of Azadirachtin against Mosquito Larvae *Culex Pipiens (Diptera: Culicidae)* under Laboratory Conditions. Eur. J. Sci. Res., 57(2): 223-229.
24. Kalu, I.G., U. Ofoegbu, J. Eroegbusi, C.U. Nwachukwu and B. Ibeh, 2010. Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus.* J. Medi. Plants Res., 4(6): 496-498.
25. Anamika, S., B. Roli, T. Shobhita, S.S. Srivastava and K.K. Maharaj, 2008. Larvicidal activity of an indigenous plant, Centratherum anthelminticum. J. Environ. Biol., 29(5): 669-672.
26. Lokesh, R., B.E. Leonard, P. Madhuri, K. Saurav and K. Sundar, 2010. Larvicidal Activity of *Trigonella foenum* and *Nerium oleander* Leaves against Mosquito Larvae Found in Vellore City, India. Current Res. J. Biol. Sci., 2(3): 154-160.
27. Arivoli, S. and T. Samuel, 2011. Larvicidal and adult emergence inhibition activity of Abutilon indicum (Linn.) (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). J. Biopesticides, 4(1): 27-35.
28. Karthikeyan, A.P., M. Kadarkarai, P. Chellasamy, P. Sekar, H. Jiang-Shiou and N. Marcello, 2012. Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using Euphorbia hirta against Anopheles stephensi Liston (Diptera: Culicidae). Parasitol. Res., 111: 997-1006.
29. Chandran, S. and N. Madanagopal, 2012. Biolarvicidal effect of phyto-synthesized silver nanoparticles using Pedilanthus tithymaloides (L.) poit stem extract against the dengue vector Aeded aegypti L. (Diptera: Culicidae). Asian Pacific J. Trop. Biomed, 1: 1-8.