International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 22-01-2022, Accepted: 07-02-2022, Published: 22-02-2022
Volume 7, Issue 2, 2022, Page No. 141-146

GC-MS and larvicidal activity of seed aqueous and ethanol extract of *Carica papaya* against *Aedes aegypti* (Dengue vector)

P Malathi*, S Sujitha
PG and Research, Department of Zoology, Periyar E.V.R College, Thiruchirappalli, Tamil Nadu, India

Abstract
The present study was designed to evaluate the larvicidal activity of aqueous and ethanol extract of *Carica papaya* against the dengue vector *Aedes aegypti.* The 4th instar larvae of *Aedes aegypti* were exposed to various concentrations (1%, 2%,3%,4%, and 5%) for 12 and 24 hrs. Seed ethanol extract of *Carica papaya* against 4th instar *Aedes aegypti* after 12 hrs and 24 hrs exposure reveals the most effective larvicidal activity than aqueous extract. The lowest LC_{50} and LC_{90} values of seed ethanol at 12 hrs exposures were 1.779 g/100ml and 5.855 g/100ml and 24hrs were 1.298 g /100ml 3.527 g/100ml, respectively. The composition of seed aqueous and ethanol extract of *Carica papaya* was qualitatively analysed by using GC-MS. The major phytocomponents identified were alcohol (34.68%), ester (39.18%) and Benenepropanoic acid alpha (26.09%),undeconoic acid ethyl ester (8.86%). Results of the present study revealed that the phytocompounds which is present in aqueous and ethanol extract of *Carica papaya* considered as a potent source for the production of predictable larvicides.

Keywords: *Carica papaya*, phytochemicals, GC-MS, 4th instar, *Aedes aegypti*, dengue vector, larvicide

Introduction

Mosquitoes cause more human suffering than any other organism. Nearly 700 million people get a mosquito borne illness each year resulting in greater than one million dealth [3]. Dengue ranks as the most important mosquito borne viral disease in the world and outbreak exert a huge burden on populations, health systems and economies in most tropical countries of the world. Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue [18].

Aedes aegypti is the main vector that transmits the viruses that cause dengue. It belongs to a family Culicidae, is an insect closely associated with human and their dwellings where anthropogenic activities contribute to the increase of larva in standing freshwater environments [24, 25]. Dengue also known as breakbone fever caused by genus flavivirus. The virus has five different types of serotypes [11]. Infection with one type usually gives life long immunity to that type, but only short term immunity to the others. Subsequent infection with a different type increases the risk of severe complications. As there is no commercially available vaccine, prevention is sought by reducing the habitat and the number of mosquitoes and limiting exposure to bites.

Chemical pesticides like DDT, Malathion, Pyrethrois are used to control vectors in rural and urban areas. Along with their useful effects, they also bring out severe destruction to human health as well. Furthermore, these chemicals are expensive and are often toxic to both human and other animals and natural enemies [4]. In order to reduce these problems, it is an urge to search an alternate and safer control measures from plant based molecules which are eco- friendly, biodegradable and target specific [12].

Carica papaya commonly called pawpaw (English), belongs to a family Caricaceae. It is a mono sexual plant of Central American origin. Due to their pharmacological properties, it is quite common in Asia. Papaya has potential medicinal value and has been widely used in traditional Asian medicines remedy against variety of diseases [10]. Various Pharmacological actions and medicinal uses of different parts of papaya are well reported in the ancient literature [16, 7, 2]. Papaya leaves made into decoction as a treatment for malarial and antiplasmodial activity has been noted in some preparations of the plant [14]. Dry seeds of papaya used for skin irritant to lower fever [1]. The seed of papaya has antimicrobial activity against several enteropathogens [13]. The papaya seed is also used in the ethno veterinary practices [9].

Objective of the study

- To evaluate the larvicidal activity of *Carica papaya* seed extracts against 4th instar *Aedes aegypti.*
- To explore the phytochemical constituents of *Carica papaya* seed extracts by using GC-MS.

Materials and Methods

Collection of seeds and Preparation of extract

Fresh seed were collected, washed thoroughly and were shade dried, pulverized in to a moderately coarse powder. 20 grams of the powdered seed were extracted with ethanol (70%) and distilled water for aqueous separately by using Soxhlet apparatus for 48 h [15]. After extraction the aqueous and ethanol seed extract was condensed under reduced pressure. The extract was stored in refrigerator until used.

Mosquito Larvicidal assay

Aedes aegypti Mosquito colonies were collected from ICMR (Centre for Medical and Entomology Research Institute) Madurai. It was maintained at 26 ± 2°C, 70 ± 10% relative humidity and a photoperiod of 12:12, Light: Dark at the PG and Research Department of Zoology, Periyar E.V.R College, Tiruchirappalli, Tamil Nadu, India.

One gram of Papaya seed aqueous and seed ethanol extract dissolved in 100 ml of respective solvent and kept as stock. From the stock five different concentrations (1%, 2%, 3%,4% and 5%) were prepared to test the larval mortality [19]. The larvicidal bioassay was done using standard WHO Protocols [17]. Twenty numbers of 4th stage larvae were introduced in each test. For each concentration three replicates were performed. Mortality of larvae of the treated and control was observed over a period of 12 and 24 hours. LC_{50}, LC_{90} were calculated from toxicity data by using probit analysis [5].

Statistical analysis

Statistical analysis of the experimental data was executed using the computer software SPSS 17.0 to find the LC_{50}, and LC_{90}.

Gas Chromatograph-Mass Spectrometer (GC- MS) analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of *Carica papaya* was passed out by Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software.

Identification of Compounds

The identification of compounds was founded on the associations of their mass spectra with NIST Library 2008. Each component was calculated by comparing its average peak area to the total areas.

Result and Discussion

Percentage mortality and lethal concentrations (LC_{50} and LC_{90}) values of aqueous and ethanol extract of *Carica papaya* against *Aedes aegypti* for 12 and 24 hrs are summarized in (Table 1). In the present analysis 4th instar larvae of *Aedes aegypti* were exposed to 1% to 5% of seed aqueous and ethanol extract of *Carica papaya*. The larval mortalities were noticed after 12 and 24 hours and larval mortality was concentration dependent. Seed ethanol extract of *Carica papaya* against 4th stage *Aedes aegypti* after 12 hrs exposure reveals the lowest LC_{50} and LC_{90} values of 1.779 g/100ml and 5.855 g/100ml, respectively. It shows that *Carica papaya* seed ethanol extract is the most active in terms of mosquito larvicidal activity compared to aqueous extract. This result was closely like the previous work conducted by [19]. On the other side aqueous extract shows the minimum effective when compared to ethanol extract of *Carica papaya*. It has highest LC_{50} and LC_{90} values of 3.334 g/100ml and 12.486 g/100m, respectively. Seed ethanol extract against 4th stage *Aedes aegypti* after 24 hrs exposure shows the LC_{50} and LC_{90} values of 1.298 g /100ml and 3.527 g/100ml, respectively. Aqueous extract reveals the LC_{50} and LC_{90} values of 1.733 g/100ml and 8.212 g/100ml, respectively.

The findings of the present investigation revealed that the seed extract of *Carica papaya* possess significant larvicidal, activity against *Aedes aegypti* mosquito. The result of the present study is similar to that of the study conducted by [8]. Insecticidal activity of seed powder of the Hawaiian, Mamey, Maradol, and Yellow varieties of *Carica papaya* caused high levels of larval mortality against *S. frugiperda* was reported by [6]. Similar reports have also been observed in the study conducted by [23]. The effect of the seed extract was comparable to various seed extracts, which are reported to be effective against *Aedes aegypti* mosquito larvae.

The chemical constituents of aqueous extract of *Carica papaya* were analyzed by Gas Chromatography Mass Spectrum (GC-MS). Chemical components are listed in the (Table. 2 and Figure 1). In addition 12 components were identified with the retention time were methional, 2,3-butanediol, butanoic acid, butane, 2-isothiocyanato, 1, 3-propanediol, phosphonic acid, hexaethylene glycol monododecyl ether, n-hexadecanoic acid, glycerine, heptanediamide, N, N'- di-benzoyloxy, 3-oxo-4-phenylbutyronitrile, niacin.

The major constituents recognized in ethanol extract of *Carica papaya* were listed in (Table 3 and Figure 2). There are 12 components were identified with retention time. The suggested compound includes: Furfural, Benzaldehyde, 2,4,6 –Cycloheptatrien, Cyclohexanone, Benzenepropanoic acid, alpha-(hydroxyimino), 1 Cyclohexyl-N-(2,5-dichlorophenyl)-2-oxo-pyrolidine-4-carboxamide, Thiocyanic acid, phenyl methyl ester, Undecanoic acid,ethyl ester, Diglycolic acid, Benzeheacetic acid, Benzeneacetamide at, Formamidine, N,N'-dibenzyl. The presence of fatty acid esters and phenolic compounds would be the probable reason for mosquito larvicidal activity. These phytochemicals would be the very ideal preliminary role on mosquito larvicide which was reported on the aqueous and ethanolic extracts of *Carica papaya* seeds. Our study report similar to that of [21, 22, 23].

Table 1: Toxicity of aqueous and ethanol effect of *Carica papaya* against 4th instar *Aedes aegypti*

Extracts	concentration	12hrs			24hrs		
		Mortality (%)	LC_{50} (g/100ml)	LC_{90} (g/100ml)	Mortality (%)	LC_{50} (g/100ml)	LC_{90} (g/100ml)
Aqueous	1%	20	3.334	12.486	40	1.733	8.212
	2%	20			50		
	3%	40			50		
	4%	50			80		
	5%	80			90		
	Control	0			0		
Ethanol	1%	30	1.779	5.855	1%	1.298	3.527
	2%	50			2%		
	3%	70			3%		
	4%	80			4%		
	5%	90			5%		
	Control	0			Control		

Table 2: Major phytocompounds identified in aqueous extract of *Carica papaya*

	Name of the compound	Molecular formula	MW	Peak Area (%)
10.871	Methanol	C4H8OS	104	0.077
12.374	2,3 –Butanediol	C4H10O2	90	1.084
12.920	Butanoic acid	C4H8O2	88	1.844
13.328	Butane,2-Isothiocyanato	C5H9NS	115	0.486
14.839	1,3-Propanediol	C3H8O2	76	3.033
17.162	Phosphonic acid (p-hydroxyphenyl)	C6H7O4P	174	1.717
18.973	Hexaethylene glycol monododecyl ether	C24H50O7	450	1.971
19.942	n-Hexadecanoic acid	C16H32O2	256	2.248
20.411	Glycerin	C3H8O3	92	34.682
21.050	Heptanediamide,N,N'-di- benzoyloxy	C21H22N2O6	398	2.293
22.275	3-Oxo-4-phenylbutyronitrile	C10H9NO	159	3.548
24.310	Niacin	C6H5NO2	123	1.818

Table 3: Major Phytocompounds identified in ethanol extract of *Carica papaya*

RT	Name of the compound	Molecular Formula	MW	Peak Area (%)
10.725	Furfural	C5H4O2	96	1.133
11.627	Benzaldehyde	C7H6O	106	0.345
13.186	2,4,6 –cYCycloheptatrien	C8H8O	120	0.978
14.667	Cyclohexanone	C6H10O	98	0.239
16.388	Benzenepropanoic acid,alpha-(hydroxyimino)-	C9H9NO3	179	26.097
17.029	1 Cyclohexyl-N-(2,5- dichlorophenyl)-2-oxo-pyrolidine-4-carboxamide	C17H20C12N2O2	354	0.166
18.084	Thiocyanic acid,phenyl methyl ester	C8H7NS	149	1.774
19.375	Undecanoic acid,ethyl ester	C13H26O2	214	8.862
20.631	Diglycolic acid	C4H6O5	134	39.186
22.287	Benzeheacetic acid	C8H8O2	136	2.337
24.581	Benzeneacetamide	C8H9NO	135	1.272
25.628	Formamidine, N,N'-dibenzyl	C15H16N2	224	0.099

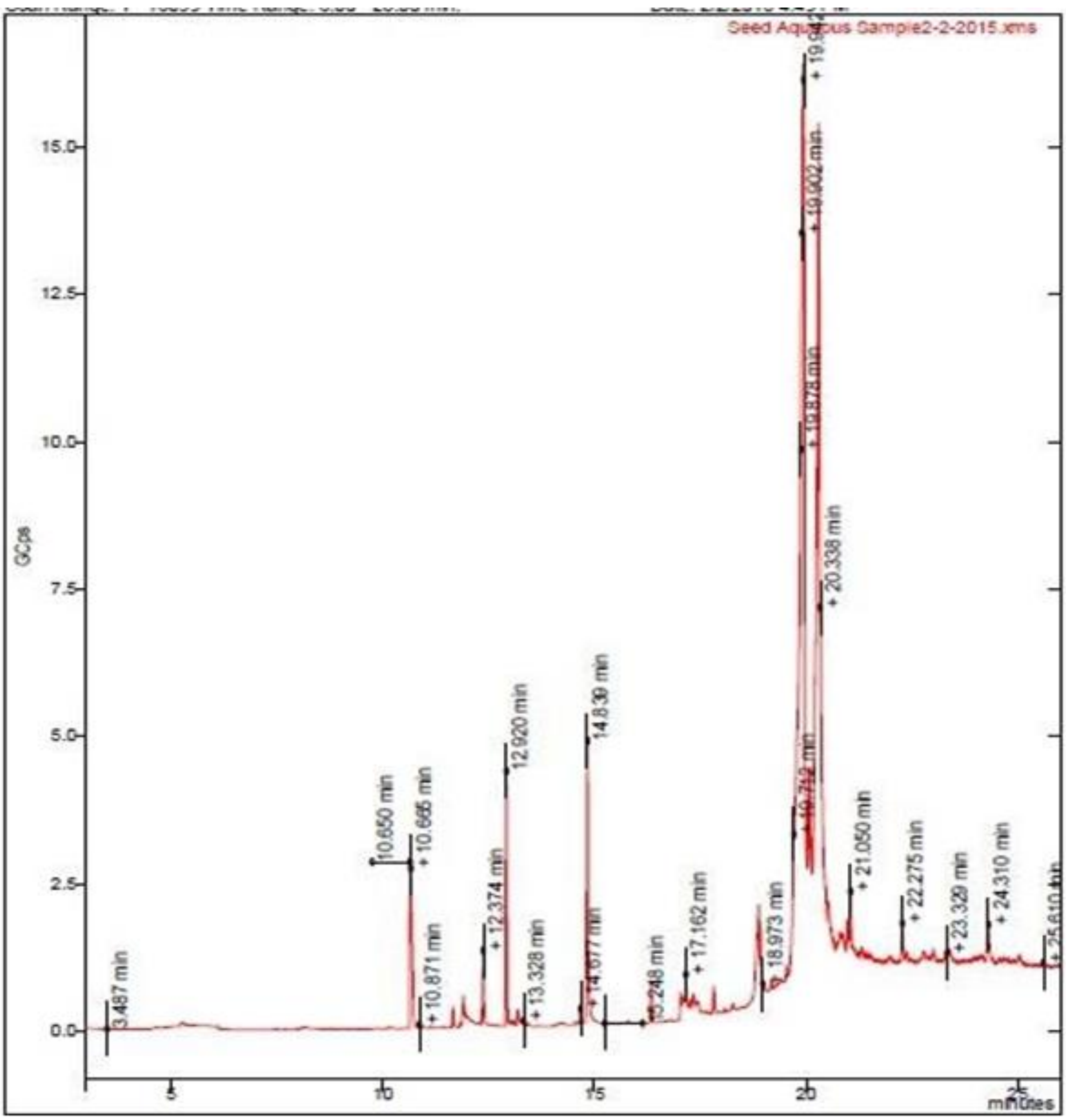

Fig 1: GC-MS Chromotogram of Carica Papaya Seed Aqueous

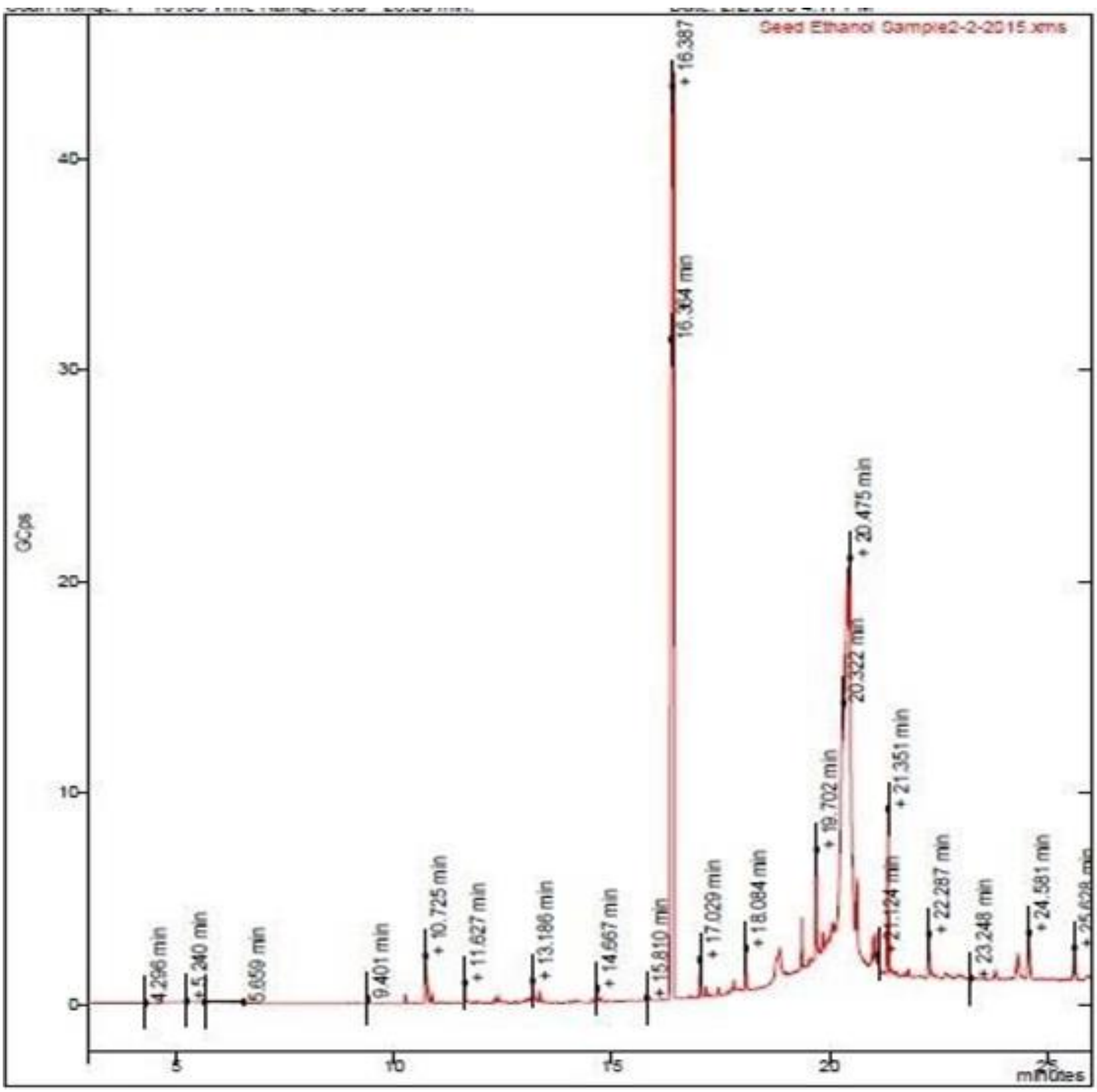

Fig 2: GC-MS Chromotogram of *Carica papaya* seed ethanol

Conclusion

The current study revealed that, the extracts of *Carica papaya* seed contains lots of phytocompounds and substances. Further isolation and purification of these extracts are in progress and assessment of these active phytocompounds involved, and their mode of action and field trials are needed, to endorse *Carica papaya* as an mosquitocidal product against *Aedes aegypti* (dengue) vector.

Acknowledgement

My Sincere thanks to my Research Mentor Dr.S.R. Vasugi for her valuable suggestion towards this research.

Conflicts of Interest

The authors declare no conflict of interest

References

1. Aravind G, Debjit Bhowmik, Duraivel S, Harish G. Traditional and Medicinal Uses of *Carica papaya*. Journal of Medicinal Plants Studies,2013:1:7-15.
2. Arya Vaidya Sala. Carica papaya, In: Indian Medicinal Plants and Compendium of 500 species, 1st Edn. Orient Longman Pvt. Ltd, Hyderabad,2005:1:383-384.
3. Caraballo, Hector. "Emergency Department Management of Mosquito-Borne Illness: Malaria, Dengue, and West Nile Virus". Emergency Medicine Practice, 2014, 16(5).
4. Cartilla P, De la Cruz J. Termiticidal Potential of *Stachytarpheta Jamaicensis (L.)* Vahl,2012:1:1-5.
5. Finney DJ. Probit analysis, Statistical methods in biological assay, 3rdedn. Griffin press, London,1971:508: 68-72.

6. Franco-Archundia SL, Jiménez-Pérez A, Luna-León C, Figueroa-Brito R. Efecto toxico desemillas de cuatro variedades de *Carica papaya* (Caricaceae) en *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Folia Entomol. Mex,2006:45:171-177.
7. Kirtikar KR, Basu BD. Indian medicinal Plants Reprint, 2nd Edn,International Book Distributors, Dehra Dun,1998:2:1097-1099.
8. Kovendan K, Murugan K, Kumar AN, Vincent S, Hwang JS. Bioefficacy of larvicdial and pupicidal properties of *Carica papaya* (Caricaceae) leaf extract and bacterial insecticide, spinosad, against chikungunya vector, *Aedes aegypti* (Diptera: Culicidae), Parasitol. Res,2011:110(2):669-678.
9. Lans C, Harper T, Georges K, Bridgewater E. Medicinal plants used for dogs in Trinidad and Tobago. Prev Vet Med,2000:45(3-4):201-220.
10. Mello VJ, Gomes MT, Lemos FO, Delfino JL, Andrade SP, Lopes MT. The gastric ulcer protective and healing role of cysteine proteinase from *Carica candamarcensis*. Phytomedicine,2008:15:237-244.
11. Normile D. "Surprising new dengue virus throws a spanner in disease control efforts". Science,2013:342:6157:415. doi:10.1126/science.342.6157.415.PMID 24159024
12. Nathan SS, Kalaivani K. Efficacy of nucleopolyhydro virus (NPV) and azadirachtin on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Biol Control,2011:34:93-98.
13. Osato JA, Santiago LA, Remo GM, Cuadra MS, Mori A. Antimicrobial and Antioxidant activities of unripe papaya. Life Sci,1993:53(17):1383-1389.
14. Udoh P, Essien I, Udoh F. Effect of *Carica papaya* (paw paw) seeds extract on morphology of pituitary – gonadal axis of amle Wistar rats. Phytotherapy Res,2005:19:1065-8.
15. Vogal AL. In: Text book of practical organic chemistry. The English Language Society and Longman, London, 1978, 1368-1372.
16. Wealth of India. A Dictionary Indian Raw Materials and Industrial Products: Raw Materials Series, Revised edn, Ca Ci, Publications and Information Directorate, CSIR, New Delhi,1992:3:276-293.
17. WHO. Guild lines for laboratory and field testing of mosquito larvicides. WHO/CDS/WHOPES/GCDPP/2005, 2005, 13.
18. WHO/WPRO. Dengue fever and dengue haemorrhagic fever prevention and control. Regional Committee resolution WPR/RC59.R6, adopted by the WHO Regional Committee for the Western Pacific, 2006.
19. Malathi P, Vasugi SR. Evaluation of mosquito larvicidal effect of *Carica papaya* against *Aedes aegypti*. International Journal of Mosquito Research,2015:2(3):21-24.
20. Junwe Z, Xiaopeng Z, Yanma Ting L, Kuen Q, Yuhua H, Suquin X *et al*. Adult repellency and or resistance of larvicidal activity of five plant essential oils against mosquitoes. J. Am. Mosq Contr. Assoc,2006:3:515-522.
21. Service MW. Management of vectors.In: Pest and vetors management in Tropics, (Eds): A. Youdeowei and M.W. Service, 1983, 265-280.
22. Mackenzie JS, Gubler DJ, Peterson LR. Emerging flaviviruses the spread and resurgence of Japanese encephalitis, West Nile and Dengue viruses. Nat. med,2004:10:S98-S109.
23. Sushama R, Katade, Pushpa V, Pawar, Radhika D, Wakharkar, Nirmala. Deshpande *Sterculia guttata* seeds extractives-An effective mosquito larvicide. Indian journal of experimental biology,2006:44:2662-665.