Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN 2320-3862
JMPS 2015; 3(4): 01-05

Received: 03-05-2015
Accepted: 07-06-2015

Murugesan Sakthivadivel
King Institute of Preventive Medicine and Research, Chennai-600 032, Tamil Nadu, India.

Palani Gunasekaran
King Institute of Preventive Medicine and Research, Chennai-600 032, Tamil Nadu, India.

Murugesan Sivakumar
Department of Zoology, Madras Christian College, Chennai-600 059, Tamil Nadu, India.

Subramanian Arivoli
Department of Zoology, Thiruvalluvar University, Vellore-632 115, Tamil Nadu, India.

Rajasingh Raveen
Department of Zoology, Madras Christian College, Chennai-600059, Tamil Nadu, India.

Samuel Tennyson
Department of Zoology, Madras Christian College, Chennai-600059, Tamil Nadu, India.

Correspondence:
Dr. Samuel Tennyson
Assistant Professor, Department of Zoology, Madras Christian College, Chennai-600059, Tamil Nadu, India.

Mosquito larvicidal activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae) aerial extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

Murugesan Sakthivadivel, Palani Gunasekaran, Murugesan Sivakumar, Subramanian Arivoli, Rajasingh Raveen and Samuel Tennyson

Abstract
Mosquito-borne diseases have intruded the globe since immemorial time. The present scenario for commanding the mosquitoes is aimed at application of target and stage-specific, cost-effective and biodegradable phytoproducts. Plant extracts are safer for non-target organisms including man. Plant based formulations would be more feasible environmental products with proven potential as insecticide. Therefore, in the present study the larvicidal activity of crude petroleum ether, chloroform and acetone aerial extracts of *Hyptis suaveolens* against the filarial vector, *Culex quinquefasciatus* was studied. Standard WHO protocols with minor modifications was adopted for the larvicidal bioassay. Larvicidal activity was evaluated at concentrations of 62.5, 125, 250, 500 and 1000 mg/L. Larval mortality was observed for 24 and 48 hours. Amongst the solvent extracts tested, acetone exhibited highest larvicidal activity and LC_{50} values was 485.61 followed by petroleum ether and chloroform extract which were 493.44 and 625.97 mg/L after 24 hours. In the case of 48 hours, petroleum ether extract (LC_{50} 298.76 mg/L) was found to exhibit highest larvicidal activity followed by acetone (LC_{50} 344.03 mg/L) and chloroform (LC_{50} 429.50 mg/L). Further investigations are needed to explore the larvicidal activity of acetone and petroleum ether aerial extract of this plant against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Keywords: *Hyptis suaveolens*, crude aerial extracts, *Culex quinquefasciatus*, larvicidal activity

1. Introduction

Mosquito-borne diseases have intruded the globe since immemorial time. Technical innovations during thirties [1] and forties including the introduction of DDT and other insecticidal chemicals were successful in apparent eradication of the disease during fifties and sixties, but last three decades suffered serious setback. Although effective, the repeated use of synthetic insecticides has disrupted natural biological control systems and sometimes resulted in widespread development of physiological resistance. These problems have warranted the need for developing alternative strategies using eco-friendly products [2]. These steadily growing problems demand an intensive search for new products that are environmentally safe, target specific and degradable. The present scenario for commanding the mosquitoes is aimed at application of target and stage-specific, cost-effective and biodegradable phytoproducts [3-5]. This has necessitated the continued effort for the search and development of environmentally safe, biodegradable and low cost larvicides for killing larva of mosquitoes from natural sources [6]. Plant extracts are safer for non-target organisms including man. Therefore, plant based formulations would be more feasible environmental products with proven potential as insecticide or repellent. It can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level [7]. Various reports on the natural plant products as larvicides against mosquito vectors have been published [8-20]. Therefore, in response to the urgent need for environment friendly mosquito control agents; a medicinal plant *Hyptis suaveolens* of the family Lamiaceae was tested for mosquito larvicidal activity against the filarial vector, *Culex quinquefasciatus*.

Hyptis suaveolens (L.) Poit (Figure 1) is a potent medicinal herb commonly called as 'American mint' in English is found in West Africa especially Northern Nigeria, Philippines and Tropical America. The plant is also found in Deccan Peninsula, North East India and Andaman and Nicobar islands [21]. It is called as 'wilaiti' in Hindi, 'bhustrena' in Sanskrit and 'wilayati tulsi' and 'kattu thumbai' in Tamil [22, 23]. The herb is used as a stimulant, carminative and as a wound healing agent [24]. The leaves are used to treat skin diseases and bronchial disorders [25]. The plant is used for treating wounds, uterine infection, parasitic skin diseases [21], headache and stomachache [26]. Pharmacological properties of this plant includes antioxidant [27], antimicrobial [28], antibacterial [29], anticancer [30], antiinflamatory, antiseptic [31] and antimalarial [32]. Phytochemical constituents of *Hyptis suaveolens* include alkaloids, terpenes, phenols, tannins and steroids [33]. The plant gained increasing attention when it exhibited insecticidal properties [34]. The plant showed feeding deterrent [35] and insect repellent activity [36]. *Hyptis* extracts were used effectively to control cow pea borer, *Maruca testulalis* [37] and *Trogoderma granarium* [38]. The plant is reported to possess repellent activity against insect pests of stored grains [39, 40] and mosquitoes [30, 41]. Raja *et al*. [42] reported the ethyl acetate leaf extract of *Hyptis suaveolens* to possess insecticidal activity against *Spodoptera litura* and *Helicoverpa armigera*. The essential oil of *Hyptis suaveolens* exhibited repellent activity against *Sitophilus granarius* [43]. Olotuah [44] pointed out that methanolic extract of *Hyptis suaveolens* possessed insecticidal activity against *Sitophilus oryzae*, *Sitophilus zeamais* and *Callosobruchus maculatus*. In East and West Africa, plants of the *Hyptis* genus are commonly utilized against mosquitoes. In Guinea Bissau (West Africa), it was demonstrated that smoldering leaves of *Hyptis suaveolens* showed repellent activity beyond 80%, whereas for fresh leaves the repellent activity was greater than 70% [45]. The plant also exhibits larvicidal activity against *Aedes aegypti* [46] and *Culex quinquefasciatus* [47]. Further, the plant was also screened for larvicidal, adult emergence inhibition and ovicidal activity against vector mosquitoes *viz*., *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* [48]. Therefore, in the present investigation, the crude aerial extracts of *Hyptis suaveolens* has been tested for its larvicidal activity against *Culex quinquefasciatus*.

Fig 1: *Hyptis suaveolens* (L.) Poit

2. Materials and methods

2.1. *Plant collection and extraction*

Mature aerial parts of *Hyptis suaveolens* collected from places in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered aerial parts (1 kg) was macerated sequentially with 3 L of petroleum ether, chloroform and acetone for a period of 96 hours and filtered. The filtrate was then concentrated at reduced temperature on a rotary vacuum evaporator. The crude petroleum ether, chloroform and acetone aerial extracts thus obtained were lyophilized and a stock solution of 1,00,000 mg/L prepared by adding adequate volume of Tween 80 was refrigerated at 4°C until testing for bioassay.

2.2. *Larvicidal bioassay*

Bioassay was carried out against laboratory reared vector mosquitoes free of exposure to insecticides. Standard WHO [49] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. Mosquito immature particularly third instar larvae were obtained from laboratory colonized mosquitoes of F_1 generation. From the stock solution, concentrations of 62.5, 125, 250, 500 and 1000 mg/L was prepared. Twenty healthy larvae were released into each 250 ml glass beaker containing 200 ml of water and test concentration. Larval mortality was observed for 24 and 48 hours after treatment. Larvae were considered dead when they showed no signs of movement when provoked on their respiratory siphon by a needle. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of Tween 80 to water. Distilled water served as control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula [50]. SPSS 11.5 version package was used for determination of LC_{50} and LC_{90} values [51]. Two way ANOVA followed by Duncan Multiple Range Test (DMRT) was performed to determine the difference in larval mortality between concentrations.

3. Results

The results of *Culex quinquefasciatus* larval mortality tested against *Hyptis suaveolens* crude petroleum ether, chloroform and acetone aerial extracts are presented in Table 1 and 2; Figure 2 and 3. The results of the present study revealed that among the solvent extracts tested, acetone exhibited highest larvicidal activity and LC_{50} value was 485.61 followed by petroleum ether and chloroform extract which were 493.44 and 625.97 mg/L after 24 hours. In the case of 48 hours, it was petroleum ether extract (LC_{50} 298.76 mg/L) which showed maximum larvicidal activity followed by acetone (LC_{50} 344.03 mg/L) and chloroform (LC_{50} 429.50 mg/L) (Table 3). Further, one hundred per cent larval mortality was observed at 1000 mg/L in petroleum ether and acetone extracts after 48 hours exposure.

Table 1: Larval mortality of *Culex quinquefasciatus* against crude aerial extracts of *Hyptis suaveolens* at 24 hours

Solvents	Concentration (mg/L)						
	Untreated control	Treated control	62.5	125	250	500	1000
Petroleum ether	0.00 ± 0.00^a (0.0)	0.00 ± 0.00^a (0.0)	0.66 ± 1.15^a (3.3)	3.33 ± 1.15^b (16.6)	6.00 ± 2.00^c (30.0)	12.00 ± 2.00^d (60.0)	18.00 ± 2.00^e (90.0)
Chloroform	0.00 ± 0.00^a (0.0)	0.00 ± 0.00^a (0.0)	0.00 ± 0.00^a (0.0)	0.66 ± 0.00^a (3.3)	4.00 ± 2.00^b (20.0)	10.00 ± 2.00^c (50.0)	16.00 ± 2.00^d (80.0)
Acetone	0.00 ± 0.00^a (0.0)	0.00 ± 0.00^a (0.0)	0.66 ± 1.15^a (3.3)	2.00 ± 2.00^a (10.0)	7.33 ± 1.15^b (36.6)	10.66 ± 3.05^c (53.3)	18.66 ± 1.15^d (93.3)

Values are mean of the three replicates of three trials ±standard deviation. Figures in parenthesis denote per cent larval mortality.
Different superscript alphabets indicate statistical significant difference at P <0.05 level by two way ANOVA followed by DMRT.

Table 2: Larval mortality of *Culex quinquefasciatus* against crude aerial extracts of *Hyptis suaveolens* at 48 hours

Solvents	Concentration (mg/L)						
	Untreated control	Treated control	62.5	125	250	500	1000
Petroleum ether	0.00 ±0.00[a] (0.0)	0.00 ±0.00[a] (0.0)	1.33 ±1.15[a] (6.6)	4.66 ±1.15[b] (23.3)	9.33 ±1.52[c] (46.6)	16.66 ±2.30[d] (83.3)	20.00 ±0.00[e] (100.0)
Chloroform	0.00 ±0.00[a] (0.0)	0.00 ±0.00[a] (0.0)	0.66 ±1.15[a] (3.3)	2.00 ±0.00[a] (10.0)	7.33 ±3.05[b] (36.6)	12.66 ±1.15[c] (63.3)	19.33 ±1.15[d] (96.6)
Acetone	0.00 ±0.00[a] (0.0)	0.00 ±0.00[a] (0.0)	0.66 ±1.15[a] (3.3)	6.00 ±2.00[b] (30.0)	10.66 ±1.15[c] (53.3)	12.66 ±3.05[c] (63.3)	20.00 ±0.00[d] (100.0)

Values are mean of the three replicates of three trials ±standard deviation. Figures in parenthesis denote per cent larval mortality.
Different superscript alphabets indicate statistical significant difference at $P < 0.05$ level by two way ANOVA followed by DMRT.

Table 3: Probit analysis of larvicidal efficacy of crude aerial extracts of *Hyptis suaveolens* against *Culex quinquefasciatus*

Solvents	LC_{50} (mg/L)		LC_{90} (mg/L)	
	24h	48h	24h	48h
Petroleum ether	493.44	298.76	875.75	511.70
Chloroform	625.97	429.50	1032.88	729.92
Acetone	485.61	344.03	840.79	623.17

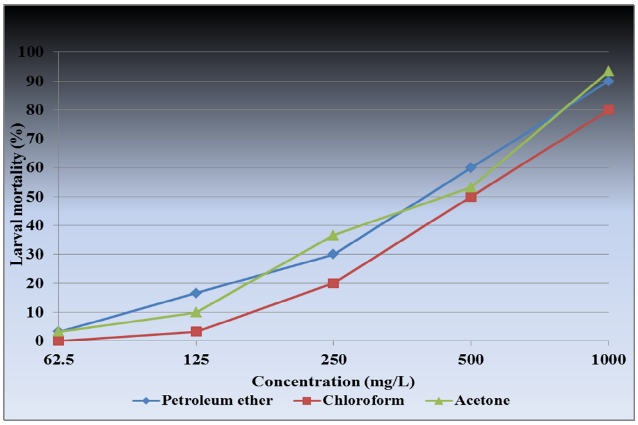

Fig 2: Per cent larval mortality of *Culex quinquefasciatus* against *Hyptis suaveolens* crude aerial extracts at 24 hours

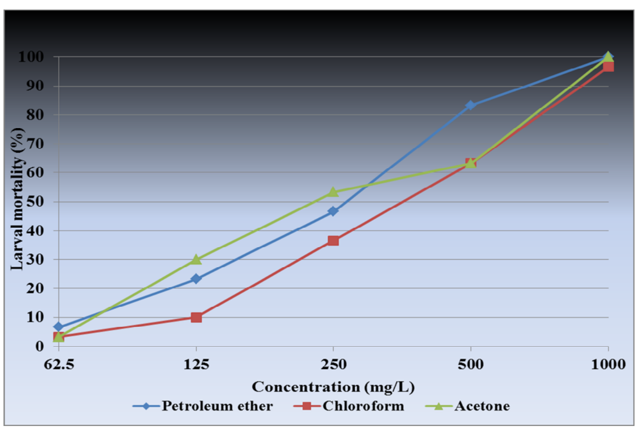

Fig 3: Per cent larval mortality of *Culex quinquefasciatus* against *Hyptis suaveolens* crude aerial extracts at 48 hours

4. Discussion

Plants are a rich source of biologically active chemicals and can be considered a potential source of mosquito control agents [52]. There are reports of medicinal plants with insecticidal activity including ovicidal, larvicidal, antifeedant and repellence belonging to the plant families Annonaceae, Papilionaceae, Meliaceae, Mimosaceae and Lamiaceae [53]. Mosquito larvae control using larvicidal agents is a major component in the control of vector-borne diseases. Larvicidal, repellent or adulticidal activities against different species of mosquitoes have been reported from members of the plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae [10]. Thus, investigation into plants as potential larvicides is considered as a viable and preferred alternative in the control of the mosquito species at the community level.

Plants belonging to the family Lamiaceae have been screened/studied for their larvicidal activity against mosquitoes. Plants that showed promising larvicidal activity were ethanolic aerial extracts of *Teucrium divaricatum* (LC_{50} 18.6ppm), *Mentha longifolia* (LC_{50} 26.8ppm), *Melissa officinalis* (LC_{50} 39.1ppm), *Salvia sclarea* (LC_{50} 62.7ppm) and *Mentha pulegium* (LC_{50} 81.0ppm) [54]. Sakthivadivel and Daniel [12] revealed that the petroleum ether leaf extracts of *Leucas aspera* and *Ocimum sanctum* also possessed LC_{50} ranging between 100 and 200ppm against the larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Plants reported with LC_{50} values less than 500ppm were methanolic stem extracts of *Origanum vulgare* (LC_{50} 256.0ppm), *Stachys cretica* (LC_{50} 292.0ppm), *Salvia verbenaca* (LC_{50} 311.0ppm), *Teucrium hircanicum* (LC_{50} 316.0ppm) and *Salvia verticillata* (LC_{50} 410.0ppm) [55, 56]. The results of the above mentioned reports were comparable with the LC_{50} value of the present study thereby exhibiting larvicidal activity.

It has been demonstrated that the extraction of active biochemical from plants confides upon the polarity of the solvents utilized. Polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules. This was gained by using solvent systems ranging from hexane/petroleum ether, the most non polar (polarity index of 0.1 that mainly extracts essential oil) to that of water, the most polar (polarity index of 10.2) that extracts biochemical with higher molecular weights such as proteins, glycans, *etc.* Chloroform or ethyl acetate are moderately polar (polarity index of 4.1) that mainly extracts steroids, alkaloids, *etc.* It has been obtained that in most of the studies solvent with minimum polarity have been used such as hexane or petroleum ether or that with maximum polarity such as aqueous/ steam distillation. However, those biochemical that were extracted using moderately polar solvents were also seen to give good results as reported by a few bioassay. Thus, different solvent types can significantly affect the potency of extracted plant compounds and there is difference in the chemo-profile of the plant species [57].

Use of plant extracts in insect/mosquito control is an alternative pest control method for minimizing the noxious effects of some pesticidal compounds on the environment [58]. The findings of the present investigation revealed that the acetone and petroleum ether extracts of *Hyptis suaveolens* possesses larvicidal activity against *Culex quinquefasciatus*. Therefore, the results reported in the present study open the possibility of further investigations on evaluation, identification and isolation of the bioactive component(s) of

Hyptis suaveolens extracts and its systemic effects on target mosquitoes, which may enable the application of the extract as larvicidal, in small-volume aquatic habitats or breeding sites of limited size in and around human dwellings for the effective control of vector mosquitoes. However, more concerted efforts would be needed to make these environment friendly plant extracts viable for field use and for small/large scale vector control operations.

5. Acknowledgements

Authors thank the Director, King Institute of Preventive Medicine and Research, Chennai 600032, Tamil Nadu, India for the facilities provided.

6. References

1. Park RGA. Insecticide as a major measure in the control of malaria, being an account of the methods and organizations put into force in Natal and Zululand during the past six years. Quarterly Bulletin of the Health Organization of the League of Nations 1936; 5:114-133.
2. Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. Asian Pacific Journal of Tropical Medicine 2010; 3(9):691-695.
3. Rajkumar S, Jebanesan A. Mosquitocida1 activities of octacosane from *Moschosma polystachyum* Linn. (Lamiaceae). Journal of Ethnophannacology 2004; 90:87-89.
4. Sharma P, Mohan L, Srivastava CN. Larval susceptibility of *Ajuga remota* against Anopheline and Culicine mosquitoes. Southeast Asian Journal of Tropical Medicine and Public Health 2004; 35:608-610.
5. Mohan L, Sharma P, Srivastava CN. Evaluation of *Solanum xanthocarpum* extracts as mosquito larvicides. Journal of Environmental Biology 2005; 26:399-401.
6. Chakkaravarthy VM, Ambrose T, Vincent S, Arunachalam R, Paulraj MG, Ignacimuthu S, Annadurai G. Bioefficacy of *Azadirachta indica* (A. Juss) and *Datura metal* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Dipteral: Culicidae). Journal of Entomology 2011; 8(2):191-197.
7. Rana IS, Rana AS. Efficacy of essential oils of aromatic plants as larvicide for the management of filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae) with special reference to *Foeniculum vulgare*. Asian Pacific Journal of Tropical Disease 2012; 2:184-189.
8. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. Journal of the American Mosquito Control Association 1991; 7(2):210-237.
9. Macedo MF, Consoli RAGB, Grandi TSM, dos Anjos AAMG, de Oliveria AB, Mendes NM *et al.* Screening of Asteraceae (Compositae) plant extracts for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). Memórias do Instituto Oswaldo Cruz 1997; 92(4):565-570.
10. Shaalan EAS, Canyonb D, Younesc MWF, Abdel-Wahaba H, Mansoura AH. A review of botanical phytochemicals with mosquitocidal potential. Environment International 2005; 3:1149-1166.
11. Sakthivadivel M, Daniel T. Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* L. seed. Bioresource Technology 2003; 89:213-216.
12. Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitoes, *viz*., *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Applied Entomology and Zoology 2008; 43(1):57-63.
13. Arivoli S, Ravindran KJ, Samuel T. Larvicidal efficacy of plant extracts against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). World Journal of Medical Sciences 2012; 7(2):77-80.
14. Arivoli S, Ravindran KJ, Raveen R, Samuel T. Larvicidal activity of botanicals against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). International Journal of Research in Zoology 2012; 2(1):13-17.
15. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential larvicides. Indian Journal of Medical Research 2012; 135:581-598.
16. Samuel T, Ravindran KJ, Arivoli S. Bioefficacy of botanical insecticides against the dengue and chikungunya vector *Aedes aegypti* (L.) (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine 2012; 2:S1842-S1844.
17. Samuel T, Ravindran KJ, Arivoli S. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine 2012; 2:S1130-S1134.
18. Sakthivadivel M, Gunasekaran P, Therasa JA, Samraj DA, Arivoli S, Samuel T. Larvicidal activity of *Wrightia tinctoria* R. BR. (Apocynaceae) fruit and leaf extracts against the filarial vector *Culex quinauefasciatus* Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Disease* 2014; 4(1):S373-S377.
19. Sakthivadivel M, Gunasekaran P, Jothis M, Samraj A, Arivoli, S, Samuel T. Evaluation of larvicidal efficacy of *Cleome viscosa* L. (Capparaceae) aerial extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease 2014; 4(2):S795-S798.
20. Samuel T, William SJ. Potentiality of botanicals in sustainable control of mosquitoes (Diptera: Culicidae). In: Achieving Sustainable Development: Our Vision and Mission, Ed. William, S.J. Loyola College, Chennai, Tamil Nadu, India, 2014, 204-227,
21. Anonymous. The Wealth of India. CSIR, New Delhi, ISBN 2001; 81:230-237.
22. Malar RJJ, Sushna SL, Johnson M, Janakiraman N, Ethal RJJ. Bio-efficacy of the leaves extracts of *Hyptis suaveolens* (L.) Poit against the fish pathogens. International Journal of Life Science and Pharma Research 2012; 2(1):128-133.
23. Udayakumar M, Bharathidasan E, Sekar T. Invasive alien flora of Thiruvallur District, Tamil Nadu, India. Scholars Academic Journal of Biosciences 2014; 2(4):295-306.
24. Chitra S, Patil MB, Ravi K. Wound healing activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae). International Journal of Pharm Tech Research 2009; 1(3):737-744.
25. Koche D, Shirsat R, Imran S, Bhadange DG. Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (MS) India. International Journal of Pharma and Bio Sciences 2010; 1(4):253-256.
26. Nadkarni KM. Indian Materia Medica, Mumbai, India, 1976.
27. Rajiv S, Santosh S, Sugandha S. Antioxidants accelerate cellular health. International Journal of Green Pharmacy 2010; 4(3):212.
28. Satish V, Padmaa M, Usha G, Ravichandran V. Antimicrobial on the extract of *Cocculus hirsutus* Linn. and *Hyptis suaveolens* Poit. Indian Journal of Natural Products and Resources 2010; 1:49-52.
29. Kavitha H, Satish S. Antibacterial potential of crude

extract of some medicinal plants on plant and human pathogenic bacteria. Journal of Pharmacy Research 2010; 3:2964-2967.

30. Mudgal V, Khanna KK, Hazra PK. Flora of Madhya Pradesh II. Botanical Survey of India, 1997, 403-404.
31. Shenoy C, Patil MB, Kumar R. Wound healing activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae). International Journal of Pharm Tech Research 2009; 1(3):737-744.
32. Ziegler HL, Jensen TH, Christensen J, Staerk D, Hagerstr H. Possible artifacts in the *in vitro* determination of antimalarial activity of natural products that incorporate into lipid bilayer. Apparent antiplasmodial activity of dehyroabientinol, a constituent of *Hyptis suaveolens.* Journal of Planta Medica 2002; 68:547-549.
33. Gills LS. Ethnomedicinal uses of plants in Nigeria, 1st Edition, University of Benin Press, Benin City, Nigeria, 1992, 276.
34. Peerzada N. Chemical composition of the essential oil of *Hyptis suaveolens*. Molecules 1997; 2:165-168.
35. Simmond MSJ, Blaney, WM. Labiate-insect interaction: Effect of Labiate-derived compounds on insect behavior. In: Harley, R.M. and Reynold, T. (Ed.). Advances in Labiate Science. Royal Botanical Garden, Kew, 1992, 375-392.
36. Oparaeke AM, Dike MC, Onu L. Control of *Callosobruchus maculates* (F.) on stored cowpea with African curry (*Ocimum gratissimum* L.) and African bush tea (*Hyptis suaveolens* Poit) leaf powders. Nigerian Journal of Entomology 2002; 19:99-108.
37. Gbehounou G. *Hyptis suaveolens*: A good weed? In: International Weed Science Society. Newsletter, 2007, 3-4.
38. Musa AK, Dike MC, Onu I. Evaluation of Nitta (*Hyptis suaveolens* Poit.) seed and leaf extracts and seed powder for the control of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) in stored groundnut. American-Eurasian Journal of Agronomy 2009; 2(3):176-179.
39. Ijeh II, Edeoga HO, Jimoh MA, Ejeke C. Preliminary phytochemical nutritional and toxicological studies of leaves and stems of *Hyptis suaveolens*. Research Journal of Pharmacology 2007; 1(2):34-36.
40. Alok S, Sanjay J, Monika S, Alok M, Padmini S, Prabodh S. *In-vitro* evaluation of antioxidant activity of leaves of *Hyptis suaveolens* (L.) Poit. International Journal of Pharmacy Research 2010; 1(2):86-93.
41. ICMR. Prospects of using herbal products in the control of mosquito vectors. ICMR Bulletin 2003; 33:1-10.
42. Raja N, Jeyasankar N, Venkatesan SJ, Ignacimuthu S. Efficacy of *Hyptis suaveolens* against lepidopteran pests. Current Science, 2005; 88(2):220-222.
43. Benelli G, Flamini G, Canale A, Molfetta I, Cioni PL, Conti B. Repellence of *Hyptis suaveolens* whole essential oil and major constituents against adults of the granary weevil *Sitophilus granarius*. Bulletin of Insectology 2012; 65(2):177-183.
44. Olotuah OF. Laboratory evaluation of pesticidal activities of *Hyptis suaveolens* in pest management. International Journal of Agricultural Research 2013; 8(2):101-106.
45. Pålsson K, Jaenson TGT. Plant products used as mosquito repellents in Guinea Bissau. Acta Tropica 1999; 72:39-52.
46. Cavalcanti ESB, de Morais SM, Ashley ALM, William PSE. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. Memorias do Instituto Oswaldo Cruz 2004; 99:541-544.
47. Okigbo RN, Okeke JJ, Madu NC. Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum and Hyptis suaveolens* against mosquito larvae. Journal of Agricultural Technology 2010; 6(4):703-719.
48. Arivoli S, Samuel T. Mosquitocidal activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae) extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Recent Scientific Research 2011; 2(5):143-149.
49. W.H.O. Guidelines for laboratory and field testing of mosquito larvicides, Geneva, 2005.
50. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18:265-267.
51. SPSS. SPSS for windows, Version 11.5. SPSS, Chicago, IL, 2007.
52. Wink M. Production and application of phytochemicals from an agricultural perspective. In: Van Beek TA, Breteler H, editors. Phytochemistry and Agriculture. Oxford, UK: Clarendon Press. 1953; 171-213.
53. Kalyanasundaram M, Das PK. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian Journal of Medical Research 1985; 82:19-23.
54. Cetin H, Cinbilgel I, Yanikoglu A, Gokceoglu M. Larvicidal activity of some Labiatae (Lamiaceae) plant extracts from Turkey. Phytotherapy Research 2006; 20(12):1088-90.
55. Pavela R. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitology Research 2008; 102:555-559.
56. Pavela R, Vrchotova N, Triska J. Mosquitocidal activities of thyme oils (*Thymus vulgaris* L.) against *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research 2009; 105:1365-1370.
57. Chowdhury N, Bhattacharjee I, Laskar S, Chandra G. Efficacy of *Solanum villosum* Mill. (Solanaceae: Solanales) as a biocontrol agent against fourth instar larvae of *Culex quinquefasciatus* Say. Turkish Journal of Zoology 2007; 31:365-370.
58. Fatope MO, Ibrahim H, Takeda Y. Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. International Journal of Pharmacognosy 1993; 31(2):250-254.