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Larvicidal efficacy of *Hyptis capitata* Jacq. Against *Culex quinquefasciatus* mosquito (Culicidae)

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Abstract
Mosquitoes are the most important group of insects declared as major vectors for the transmission of life threatening diseases like malaria, filariasis, dengue fever, Japanese encephalitis, chikungunya fever etc. Now-a-days, the control and eradication of mosquitoes with synthetic insecticides raises a major problem, because of the development of resistance to chemicals by the mosquito as well as environmental pollution. Plant products may be used as an alternate source in control of mosquitoes from time immemorial. The present investigation focused on the evaluation of the larvicidal activity of methanolic extract of leaf of *H. capitata* against *Culex quinquefasciatus* mosquito followed by GC-MS analysis for the assessment of phytocompounds in the extract. Larvicidal bioassay exhibited 93.3 ± 11.54% of larval mortality at a concentration of 25 mg ml^{-1} ($P < 0.05$) and the LC_{50} was found to be 11.15 mg ml^{-1}. About nine chemically diversified volatile terpene compounds were detected and quantified. The major compounds present in the extract were Methyl Commate –D (55.50%) followed by phytol (16.51%), squalene (13.28%) and tocopherol (5.60%) The significant larvicidal activity may be attributed to the synergistic action of the detected phytochemicals especially phytol, squalene and tocopherol. The methanolic leaf extract of *H. capitata* may be used as an effective biolarvicide for the *Culex quinquefasciatus* mosquito.

Keywords: *Hyptis capitata* Jacq. Larvicidal bioassay, *Culex quinquefasciatus*, mortality

1. Introduction
Mosquitoes are a very large group of insects belonging to the order Diptera and the family Culicidae commonly known as Wing devil. They are declared as "public enemy number one'' [1]. Globally, every year 40,000, 000 people were infected by mosquito borne diseases [2]. About 3492 species of mosquitoes were recorded worldwide, of which about a hundred species are capable of transmitting various diseases in humans and other vertebrates [3]. They are the major vectors for the transmission of life threatening diseases like malaria, filariasis, dengue fever, Japanese encephalitis and chikungunya fever [4]. In many developing countries vector borne diseases are one of the causes of illness and death. World Health Organisation proposed a very effective approach as integrated vector management (IVM) for vector control [5]. Malaria, an important cause of mortality in infants, children and adults remains the most serious vector borne disease in India. More than 40% of the world's population lives in areas prone to malaria [6]. Recently, reports revealed that around 40 million of people of tropical and subtropical countries were infected with a chronic manifestation of lymphatic filariasis which is caused by *Culex quinquefasciatus* [7].
Generally, the mosquito control operation is adopted by the spraying of synthetic insecticides (organochlorines and organophosphates) and mosquito repellent DEET (N, N- diethyl -3-methyl toluamide) in their breeding sites. The use of synthetic mosquitocides or repellents causes harmful effects on the beneficially important non target organisms and also disturbs the ecological balance. Therefore, physical methods (mosquito traps), biological control (larvivorous fish), usage of phytochemicals (pyrethrin) and topically applied formulations have been used to control mosquitoes. The plant based insecticides are more advantageous over synthetic or chemical insecticides. They are the combination of multiple chemical compounds which are synergistically acted on the physiological and behavioural process of the mosquito [8]. Natural mosquito repellent such as odoriferous aromatic plants, leaves and barks can be used to control mosquito proliferation.

The essential oils obtained from the aromatic plants *viz*. lemon grass oil, eucalyptus oil, camphor, tulsi, Neem, peppermint, cedar essential oil are recognized as natural mosquito repellent [9, 10, 11]. Moreover, the plant derived control mechanism is a simple and sustainable method compared to the typical method of spraying chemicals.

The larvicidal potential of some medicinal plants of Lamiaceae like *Thymus vulgaris*, *Ocimum basilicum*, *Lavandula officinalis* and *Stachys byzantina* exhibited promising larvicidal activity against mosquitoes [12]. *Hyptis capitata* Jacq. of Lamiaceae is an ethnomedicinal herb widely used by the tribals of Bangladesh, Indonesia, Malaysia and Philippines for health care. The present research was carried out to assess the larvicidal activity of the methanolic leaf extract of *H. capitata* against *Culex quinquefasciatus* and to analyze the phytochemical composition with an aim to establish it as a safer alternative for mosquito control.

2. Materials and Methods

2.1 Plant Collection and Extraction

The study material *Hyptis capitata* Jacq. was collected from Thiruvananthapuram district. The plant was identified and authenticated by Department of Botany, University of Kerala and the voucher specimen (KUBH-6166) has been deposited at the same department herbarium. Fresh leaves were collected, washed thoroughly and shade dried without any contamination. The dried leaf samples were powdered. About 25 gm dried leaf powder was extracted in Soxhlet extractor with methanol for 48 hrs. to72 hrs. The extract was filtered through Whatman No.1 filter paper and evaporated to dryness at 55^0-60 ^{0}C [13]. The solidified extract was stored in an airtight container in a refrigerator for bioassay.

2.2 GC-MS Analysis

Methanolic leaf extract of *H. capitata* was subjected to GCMS analysis to determine the phytocompounds. A Shimadzu, QP 2010 S model was used for the GC-MS analysis. Gas chromatograph was equipped with a capillary column in 30 m x 0.25mm ID x 0.25 µm thickness in size. The instrument was set to an initial temperature of 80 ^{0}C and maintained for 4 minutes. The oven temperature was rose up to 280 ^{0}C after of 2 minutes and maintained at this temperature at the rate of an increase of 50 ^{0}C for 6 minutes. The temperature of injection port was ensured as 260 ^{0}C and a flow rate of 1ml min^{-1}. The ionization voltage was 1.06 KV + 0.001KV. The samples were injected in split mode. The spectral scan range was set at 50-500 m/z. The spectrum of the components was compared with the spectrum of known components stored in the NIST 11 & WILEY 8 library. The name of the compound, nature and molecular weight of the components of the test samples was confirmed [14].

2.3 Larvicidal Bioassay

The mosquito species selected for the study was *Culex quinquefasciatus*. They are cosmopolitan in distribution prevalent in urban and rural regions of India. The habitat of *Culex quinquefasciatus* is swamps, marshes, rice fields, bogs, pastures. It is commonly known as 'Southern house mosquito' is a potential vector of bancroftian filariasis [15]. They are also called 'wrigglers' due to the peculiar style of swimming of larvae. It is medium sized, brown coloured, blood feeder and active only at night. Mostly, they feed during the evening and morning. The entire life cycle of *Culex quinquefasciatus* is completed in 10-14 days, through egg, larva, pupa and adult. The lifespan of male and female *Culex* mosquitoes is about one month and 1 to 2 weeks [16].

The larvicidal bioassay was carried out following standard guidelines with minor modification [17]. Different concentrations such as 5 mg ml^{-1}, 10 mg ml^{-1}, 15 mg ml^{-1}, 20 mg ml^{-1} and 25 mg ml^{-1} were prepared from the 5% stock solution. About 10 mosquito larvae were introduced into the beaker containing 20 ml of control and test sample solutions. The complete set up was exposed for 24 hours at room temperature. The percentage of larval mortality was calculated after 24 hours of treatment. Larvae were considered dead if they settled and remained motionless at the bottom of the beaker with no response to light stimulus.

$$\% \text{ of Mortality} = \frac{\% \text{ of Mortality in treated} - \% \text{ of Mortality in control}}{100 - \% \text{ of Mortality in control}} \times 100$$

2.4 Statistical Analysis

The experiments were done in triplicate the mean and standard deviation were computed by Graph Pad Instat DTCG. Analysis of Variance was done by Tukey-Kramer Multiple Comparison Test. The larvicidal effectiveness of the plant extract was expressed as LC_{50}. Results with $P < 0.05$ were considered statistically significant.

3. Results

3.1 Mosquito Larvicidal Bioassay

The larvicidal activity of methanolic leaf extract of *H. capitata* showed a concentration dependent mortality against *Culex quinquefasciatus* mosquito larvae after 24 hours of exposure (Table1 & Fig. 1) while no mortality was observed in control.

Table 1: Larvicidal Effect of Methanolic Leaf Extract of *Hyptis capitata* Jacq. Against *Culex quinquefasciatus* Mosquito

Concentration of Extract (mg ml^{-1})	% of Mortality	LC $_{50}$
Control	0	11.15 mg ml^{-1}
5	33.33 ± 11.55*	
10	46.6 ± 10.54*	
15	66.6 ± 11.25*	
20	80 ± 12.01*	
25	93.3 ± 11.54*	

Each value represents the mean ± SD of triplicate measurements and the superscript represents the level of significance compared to control value (control-no activity) *significant at $P < 0.05$ (according to Tukey-Kramer Multiple Comparison test)

The percentage of mortality was moderate (33.33 ± 11.55%) at the concentration of 5 mg ml^{-1}. Meanwhile, the highest percentage of mortality 93.3 ± 11.54% was found at the concentration of 25 mg ml^{-1} ($P < 0.05$). The LC $_{50}$ value of 11.15 mg ml^{-1} was recorded in the larvicidal bioassay of *Culex quinquefasciatus* with methanolic leaf extract of *H. capitata*.

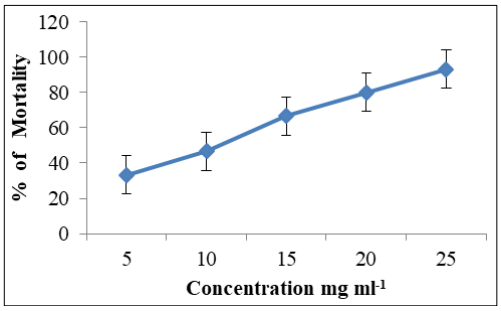

Fig 1: Larvicidal Activity of Methanolic Leaf Extract of *Hyptis capitata* Jacq. Against *Culex quinquefasciatus* Mosquito

3.2 GCMS analysis and identification of compounds

The Gas Chromatogram and Mass Spectrum of methanolic leaf extract of *H. capitata* showed nine peaks which indicated the presence of nine active phytochemicals (Fig.2) such as various terpenes like monoterpenes, diterpenes, triterpene, glycosides and sesquiterpenoids with significant biological activities. The identified phytochemical constituents of the leaf extract with the retention time (RT), peak area in terms of percentage are presented in Table 2. The major phytocompound was Methyl Commate–D (55.50%) followed by Phytol (16.51%) squalene (13.28%) and Alpha tocopheryl acetate (5.60%).

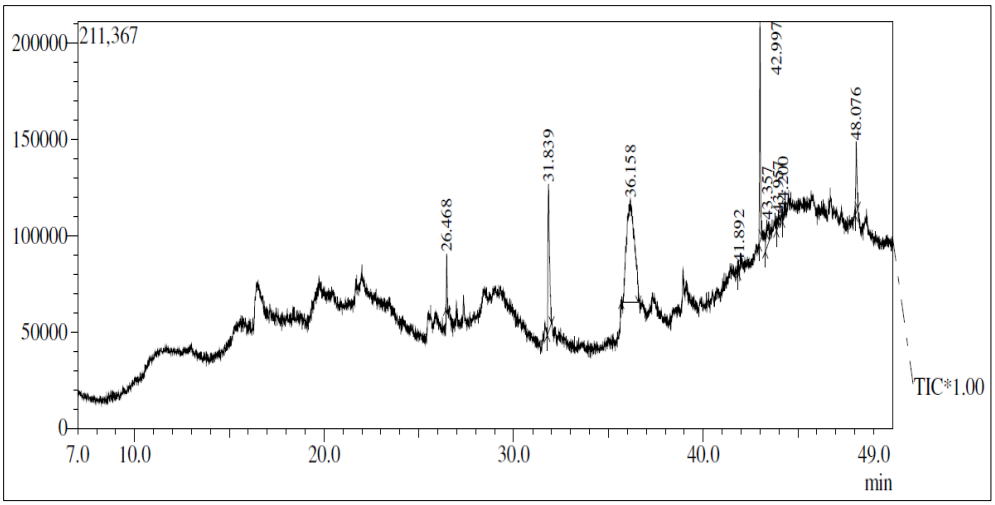

Fig 2: GC-MS Chromatogram of Methanolic Leaf Extract of *Hyptis capitata* Jacq.

Table 2: Phytocompounds Identified from the Methanolic Leaf extract of *Hyptis capitata* Jacq. by GC- MS Analysis.

Sl. No.	Retention Time	Name of the compound	Molecular Formula	Nature of the compound	Peak Area (%)
1	26.468	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Terpene alcohol	2.90
2	31.839	Phytol	$C_{20}H_{40}O$	Di terpene	16.51
3	36.158	Methyl Commate -D	$C_{31}H_{50}O_4$	Triterpene glycosides	55.50
4	41.892	12 – Chlorobicyclo (8.2.0) dodecan-11-one	$C_{12}H_{18}Cl_2O$	-	0.55
5	42.997	Squalene	$C_{30}H_{50}$	Triterpene	13.28
6	43.357	1- Methyl verbenol	$C_{10}H_{16}O$	Monoterpene	3.88
7	43.957	Di- methylamino pyrimidine	$C_6H_9N_3$	-	0.92
8	44.200	Beta Santalol	$C_{15}H_{24}O$	Sesquiterpenoids	0.86
9	48.076	Alpha tocopheryl acetate	$C_{31}H_{52}O_3$	Vitamin -E	5.60

4. Discussion

The larval treatment is found to be very effective in controlling the growth of mosquitoes because the larvae were restricted to small spaces due to low mobility in this stage [18]. Alkofahi *et al*., 1989 [19] recommended the application of botanicals as an alternative for the control of mosquitoes due to the emergence of resistance of mosquitoes to synthetic insecticides. Large number of plant extracts have mosquitocidal or repellent activities against disease causing mosquito vectors [20]. In the present study, the methanolic leaf extract of *H. capitata* demonstrated that the larval mortality increased with an increase in the concentration of extract and its chemical constituents. Maximum larval mortality (>90%) was found to be at the higher concentration against *Culex quinquefasciatus* mosquito. It was chiefly attributed to the increased levels of active ingredients present in higher doses [21]. The methanolic leaf extract of *Hyptis suaveolens* and *Ocimum sanctum* induced larval mortality with LC_{50} values 73.25 ppm and 125 ppm respectively activity against *Anopheles gambiae* mosquito [22]. The petroleum ether leaf extracts of *Leucas aspera* showed LC_{50} ranging from 100- 200 ppm against the larvae of *Culex quinquefasciatus*, *Anopheles stephanie* and *Aedes aegypti* mosquito [23].

Generally, medicinal plants have various bioactivity due to the presence of primary and secondary metabolites. In addition to the curative properties, these bioactive chemicals may act as insecticides, antifeedants, molting hormone, repellents, juvenile hormone mimics as well as growth inhibitors [24]. The significant percentage of larval mortality or toxicity observed in the present study might be due to the presence of a diverse group of terpenoids (triterpene alcohol, triterpene, triterpene glycosides, monoterpene, diterpene and sesquiterpenoids) detected in the methanolic leaf extract of *H. capitata*. Terpenoids are one of the chief phytochemicals that are neurotoxic with mosquito larvicidal properties. The mechanism of action of terpenoids on larvicidal activity might be due to the interference of acetylcholine esterase activity and octopaminergic system of larval mosquito which are

responsible for neurotransmission and neuromodulation [25]. Similarly, the identified major compounds like phytol, squalene and tocopherol have been reported to possess mosquito larvicidal properties [26-28].

The potential and bioactive phytochemicals such as alkaloids, coumarins, flavonoids, glycosides, phenols, steroids, tannins and terpenoids were also prevalent in *Hyptis capitata* [29]. USTA *et al*., 2002 [30] opined that the phytochemicals interfered with the functioning of mitochondria. Tannins are toxic by inhibiting the food digestion process and causing growth interruption and water absorption disorder in the larvae, thus affects the death of the larvae [31]. The flavonoids act on the respiratory system and disrupt the electron transport system in the body of larva and thus decreasing ATP production [32]. Alkaloids inhibit the acetylcholine esterase activity, constricting the blood vessels and depressing the autonomous nervous system thereby contributing the insecticidal property in killing the larvae of mosquitoes [33-35].

Plant derived chemical compounds acts toxicants against larval and adult stages of mosquitoes while some other phytochemicals act as a repellent, they interfere with the growth, development, reproduction and produce olfactory stimulus [36]. Mayura *et al*., (2007) [37] opined that the crude plant extract may be more effective than individual compounds, due to the synergism effect that discourages the development of resistance which resulted in mortality. The larvicidal efficacy of the methanolic leaf extract of *H. capitata* against *Culex quinquefasciatus* mosquito may be due to the synergic or antagonistic action of phytochemicals including the diverse types of terpenoids.

5. Conclusion

The present investigation highlighted that an ethnomedicinal plant *H. capitata* as a larvicidal agent against *Culex quinquefasciatus* mosquito. The methanolic extract may act as a source of phytochemicals chiefly terpenoids with mosquito larvicidal properties. These results could encourage the search for a novel phytocompound that may serve as biolarvicide offering an alternative to chemically synthesized insecticides. Future perspectives include the isolation of bioactive compound (s) and their mode of action and a systematic study on non-target organisms as well as field trials before commercialization.

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