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MOSQUITO LARVICIDAL ACTIVITY OF EXTRACT'S PURIFIED FRACTION OF *HYPTIS SUAVEOLENS* (L) POIT.

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ABSTRACT: Bio-pesticides are playing a very important role in controlling mosquitoes and their larvae. In the present scenario, chemical pesticides are causing several side effects on non-target organisms, particularly in men. Moreover, mosquitoes and their larvae become resistant to chemical pesticides; therefore, chemical pesticides are not much more effective. The conventional use of temephos and pyrethrum creates several health hazards in men, especially in upper and lower respiratory disorders, including allergy, asthma, and rhinitis. The plant *Hyptis suaveolens* is well known for its aromatic properties. Therefore, it was proposed to apply its purified fraction on different strains of mosquitoes for the evaluation of mosquito larvicidal activities. In the present study, identified and authenticated shade-dried whole plant materials were pulverized to get powder, extracted in various solvents through Soxhletion, and percentage yields were noted down. The main phytoconstituents reported in the extract were alkaloids, flavonoids, terpenoids, and tannin in huge amounts. Due to the presence of the pungent smell of flavonoids, terpenoids, alkaloids, and tannin in *Hyptis suaveolens*, mosquito larvicidal activities were reported in the purified fraction.

INTRODUCTION: Nowadays, mosquitoes are causing enormous public health problems. Of these, few are very common in men, including malaria, filaria, dengue, Japanese encephalitis, yellow fever, zika virus, and chikungunya. *Aedes aegypti* mosquito is a vector of Flavivirus virus that spreads yellow fever, dengue fever, and chikungunya fever which are endemic in tropical and sub-tropical regions around the world.

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Similarly, various species of female Anopheles mosquito transmits various species of malaria parasites, including *Plasmodium vivex*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*, and among humans, approximately 91% of the 216 million malaria cases were reported in 2010, which were only due to Plasmodium falciparum transmitted through Anopheles stephensi [1].

The major problem associated with the use of chemicals for the control of mosquito is the development of resistance to the chemicals and their undesirable side effects, so the purified fraction of the extract isolated from plants *Hyptis suaveolens* could be served as an alternative source of chemical pesticides, which are biodegradable

into non-toxic products and are potentially suitable for use to control mosquitoes. Chemically, these are secondary metabolites found in plants for their defensive purposes only, and plant extracts, in general, have been an important natural resource of these bio-insecticides. These phytochemicals derived from plant sources can act as mosquito larvicidal, insect growth regulators, mosquito repellents, mosquito ovipositioning attractants. They can play an interrupted role in the transmission of mosquito-borne diseases at the individual and at the community level.

A number of extensive work has been reported on the effect of plant extracts against mosquito larvae. Bagavan and Rahuman [2] have reported toxicities of different plant extracts, including crude hexane extract of flower heads of *Spilanthes acmella*, *Spilanthes calva*, and *Spilanthes paniculata*, partially purified extracts of leaves of Vitex negundo, *Nerium oleander*, seeds of Syzygium, leaves of *Artemisia annua* and *Azadirachta indica*, the crude acetone extracts of *Fagonia indica*, *Arachis hypogaea*, *Nerium indicum* and *Thuja orientalis* on the larvae population of different mosquito species. Chore *et al.*, (2014) [3] isolated purified extract from 3 species of Aloe genus *viz. A. Ngongensis*, *A. Turkanensis*, and *A. Fibrosa* in hexane, chloroform, ethyl acetate, acetone and methanol solvents, respectively, and have applied all extract for larvicidal activity against mosquito Aedes aegypti.

Dengue haemorrhagic fever is also the most important public health problem in tropical and sub-tropical regions of the world, including India. Recent estimates of WHO indicate that about 50-100 million infections, including 0.5 million DHF cases and 24000 deaths (mostly among children) occur every year throughout the world due to Dengue as reported by Chore *et al.*, 3 Dengue fever have been affected 19 states in India and *Aedes aegypti* mosquito is the principal vector for transmitting dengue by carrying flavivirus. This mosquito breeds in storage clean water in containers *viz.* jars, drums, tanks, coolers, cement tanks, and clay pots available in and around houses. The transmission of flavivirus by *Aedes aegypti* vectors can be controlled effectively by targeting immature larvae of this mosquito. Moreover, mosquito larvae are relatively immobile and remain more accumulated than their adult stage. Development of resistance in *Aedes aegypti* against temephos has also been reported. In this regard, bio-insecticide development has received much attention as they are considered to be efficient, safe to the environment, and biodegradable compared to synthetic insecticides.

Natural insecticide comprises of number of secondary metabolites which act on both behavioural and physiological processes and on the target species of mosquitoes have been reported by Subramaniam *et al.*, [4] Hence, the present study was proposed to isolate a purified fraction of extracts of *Hyptis suaveolens* of family Lamiaceae for controlling mosquitoes and its larvae. *Hyptis suaveolens* is an herbaceous aromatic plant of height approximately 30-150 cm. Leaves are hispid, having a strong scented smell, broadly ovate 2-8 cm long, 2-6 cm wide with finely toothed edges which are grafted an oval limb and an inflorescence in axillaries cymes.

MATERIALS AND METHODS: The fresh plant materials of the whole plant *Hyptis suaveolens* (L.) **Fig. 1** was collected from Barkatullah University Campus, Bhopal (M.P.) after identification and authentication from BSI, Allahabad (No.97880), which was shade dried at room temperature and pulverized to get powder of 50-70 mesh size and loss in weight of the material was measured, then extracted in various solvents *viz.* dichloromethane (DCM), chloroform ($CHCl_3$), methanol (MeOH) and finally in distilled water in increasing order of polarity by Soxhlet apparatus and has reported maximum percentage yield in distilled water as shown in **Table 1**.

FIG. 1: SHOWING PLANT *HYPTIS SUAVEOLENS*

TABLE 1: ISOLATION OF EXTRACTS FROM PLANT MATERIALS OF *HYPTIS SUAVEOLENS* BY SOXHLETION

S. no.	Plant materials	Weight of powdered materials	Solvent used for extraction	Obtained extract (In gm)	% Yield
1	Whole plants	100 gm	Distilled water	19.74	19.74

The obtained crude extract was separated and further purified by thin layer and column chromatography as reported by Tswett (1906) [5], Dubey *et al.*, [6] by applying different solvent systems to get purified fractions. The spots developed on TLC Lux plates (Merck) were visualized in UV chamber at 356 nm, in iodine chamber, and in visible light and R_f values as shown in **Table 2** and **Fig. 2A**, **B**, **C** of all spots which were calculated by applying the formula of Brimley and Barrett (1953) [7] as described below:

R_f = (Distance travelled by solutes) / (Distance travelled by solvent)

TABLE 2: THIN LAYER CHROMATOGRAPHY OF *HYPTIS SUAVEOLENS* WATER EXTRACT

S. no.	Extracts used	Solvent system used	Spots obtained	RF Value (cm)	Colour characterization		
					Visible light	Iodine chamber	UV light
1	Whole plant distilled water extract	Toluene: ethyl acetate (70:30)	HS-1	0.26	Brown	Violet	Dark brown
			HS-2	0.52	Yellow	Pink	Light brown
			HS-3	0.67	Green	Brown	Brown
			HS-4	0.74	Light green	Light red	Green
			HS-5	0.82	Light yellow	Yellow	Pink

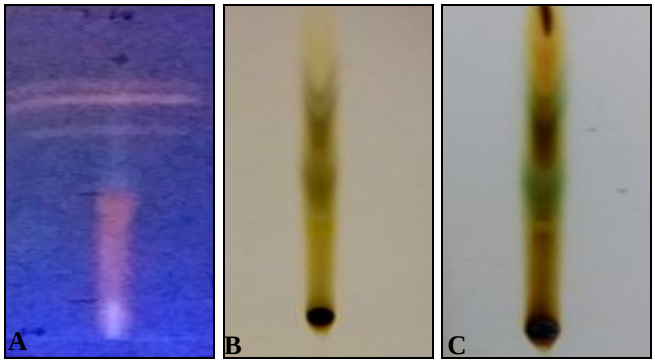


FIG. 2: SHOWING THIN LAYER CHROMATOGRAPHY OF *HYPTIS SUAVEOLENS* (L.) IN (A) UV CHAMBER (B) VISUAL LIGHT (C) IODINE CHAMBER

Then, column chromatography (Stock and Rice, 1974)8 of the water extract was performed in solvent system *viz.* petroleum ether: toluene: ethyl acetate (5:3:2) and two purified fractions were obtained (HS^{-1} and HS^{-2}) as shown in **Table 3**. Both purified fractions of *Hyptis suaveolens* (L.) plant extracts were tested on the larvae of *Aedes aegypti* and *Anopheles stephensi* for this purpose, adult larvae of *Aedes aegypti* and Anopheles stephensi of order Diptera and family Culicidae were collected from Saket Nagar huts, Bhopal by the door to door visit.

TABLE 3: COLUMN CHROMATOGRAPHY OF *HYPTIS SUAVEOLENS* EXTRACT

S. no.	Extracts used	Solvent system used	Fractions obtained	Colour characterization	Biologically active fractions
1	Whole plant distilled water extract	Petroleum ether: Toluene: Ethyl acetate (5:3:2)	HS-1	Yellow	Highly active
			HS-2	Green	Less active

Total 130 healthy larvae of *Aedes aegypti* and *Anopheles stephensi* both the species were reared in the glass vials. Total 10 larvae per vial were released in 500 ml normal water in glass vials capped with muslin cloth for ventilation. Cultures were maintained in the laboratory under controlled conditions *viz.* temperature (27 ± 1 ºC), relative humidity (75 ± 5% RH). Toxicity assays of the purified fraction of crude water extracts of *Hyptis suaveolens* (L.) were conducted by using larvae of *Aedes aegypti* and *Anopheles stephensi*, respectively. A stock solution of temephos was prepared by applying the standard formula of NVBDCP, and herbal extracts concentration were prepared by dissolving purified fraction of extracts from 50 mg to500 mg in 10% to 100% volume of solvents, and 10 IV instars larvae were released in it and mortality was scored after 24 h of treatments. Beakers were kept at 28 ºC ± 2 ºC room temperature, and the larvae were exposed to 500 ml water and kept as controlled. Experiments as shown in **Fig. 3** were performed in triplicates, and in each experiment, early aged larvae were used. In the bio-assay protocol, groups I was served as a control in

which larvae were reared in maintained laboratory conditions in a beaker of standard size, and no treatment was given to the larvae of this group.

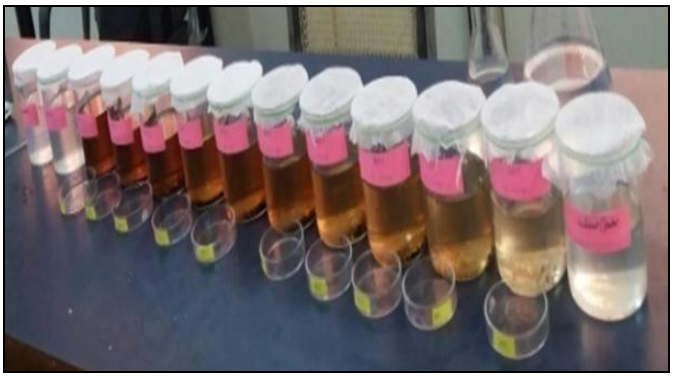


FIG. 3: MOSQUITO LARVICIDAL EFFICACY OF 10%-100% CONCENTRATION OF *HYPTIS SUAVEOLENS* WATER EXTRACTS PURIFIED FRACTION ON MOSQUITO LARVAE

Group II was served as vehicle control, in which larvae were reared in a beaker with vehicle solvent (distilled water), which was used finally for extraction of plant extract to observe the consequences of vehicle solvent on animals. Group III was served as treated, in which larvae were reared by treating it with different concentration of plant extract in increasing order from 10% (50 mg extracts in 500 ml water) to 100% (500 mg extracts in 500 ml water) to monitor the larvicidal efficacy of different extracts of the plant. Group IV was served as the standard in which larvae were reared by treating it with the standard.

Reference drug temephos was used by dissolving it 0.75 ml in 500 ml water, and concentration was maintained 100% to observe the efficacy of the standard drug for comparing it with herbal extracts. For observing the efficacy, a purified fraction of the plant extract of *Hyptis suaveolens* was tested on the larvae for larvicidal activity, and three replicates were placed. Mortality in *Aedes aegypti* and *Anopheles stephensi* was recorded after 24 h of the treatment. Finally, LC_{50} values were calculated according to the method of Finney (1971) [9]. The results were compared with the help of PROBIT analysis, standard deviation (SD); standard error (SE), and significant value (P-value) calculated as shown in **Tables 4** and **Graphs 1** and **2**.

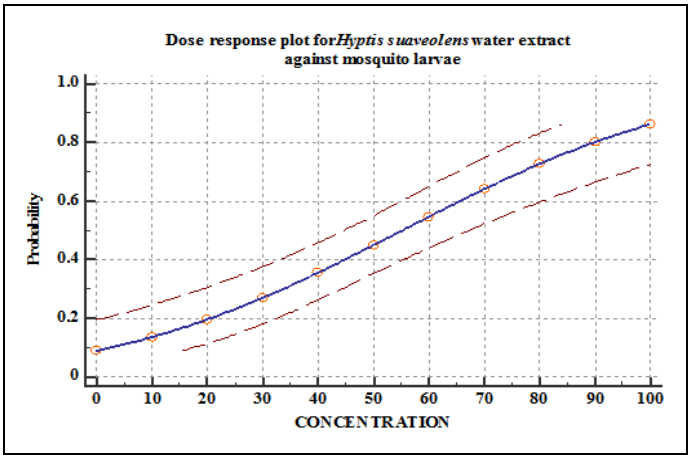
TABLE 4: PROBIT ANALYSIS OF *HYPTIS SUAVEOLENS* (L) WATER EXTRACT

Experimental Groups	Concentration (per ml)	Total number of larvae per beaker	Percentage mortality	Lethal dose (95% Confidence limit)		DF	Chi-Square	Cox& Snell R^2	Nagelkerke R^2	Wald statistics
				LC_{50} (Upper-Lower)	LC_{90} (Upper-Lower)					
Controlled	-	10	0.00±00.00	55.20	100.86	1	38.69	0.27	0.36	32.41
Vehicle controlled	-	10	0.00±00.00	(44.71-67.28)	(90.39-140.78)		(P<0.001)			(P<0.0001)
Purified fraction of water extract of *Hyptis suaveolens* in different concentrations	10%	10	23.33±23.00							Coefficient=0.02
	20%	10	26.66±05.77							
	30%	10	40.00±05.77							Std. Error =0.25
	40%	10	46.66±10.00							
	50%	10	53.33±34.10							
	60%	10	60.00±20.00							
	70%	10	66.66±25.45							
	80%	10	73.33±10.00							
	90%	10	80.00±10.00							
	100%	10	83.33±16.77							
Standard Temephos 2%	0.25 ml/litre	10	92.66±10.00							

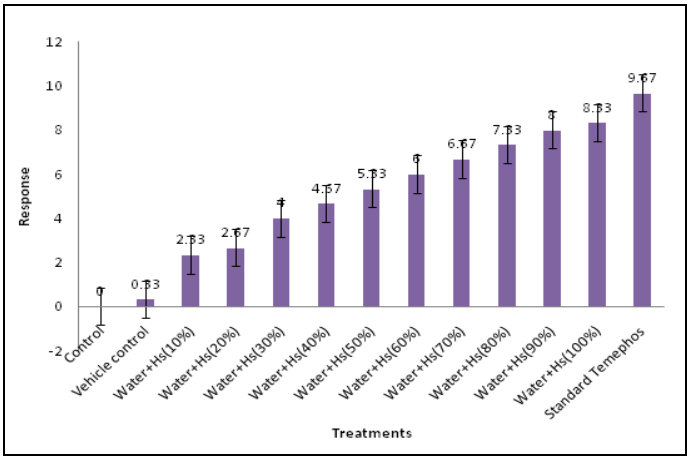
(n=120; Positive cases = 56.67 %, Negative cases = 43.33%),*DF: Degree freedom; LC: Lethal Concentration

RESULTS AND DISCUSSION: In the present study, the percentage yield of the extract of *Hyptis suaveolens* whole plant was reported to be 19.74% in distilled water **Table 1**. Similarly, Edeoga *et al.*, (2006) [10] reported the chemical composition of *Hyptis suaveolens* and found that *Hyptis suaveolens* had the highest percentage yield of alkaloids (14.32%) followed by flavonoids (12.54%) in distilled water extracts after de-fating with 100ml of diethyl ether in Soxhlet apparatus for 2 h, then boiled with 50ml of petroleum for the extraction of the bio-active compound *i.e.* alkaloids, flavonoids, tannins, and saponins. They have also noticed that alkaloids are well known to play some metabolic role and developmental control in living beings.

Moreover, alkaloids also possess a protective role in animals as reported by Edeoga and Eriata (2001) [11]. Therefore, in the present study, distilled water extracts of *Hyptis suaveolens* were isolated and purified by thin-layer chromatography, respectively. For this purpose, TLC of the extract was done by applying solvent system *viz.* toluene: ethyl acetate (70:30) and 5 spots (HS-1 to HS5) were visualized with RF value 0.26, 0.52, 0.67, 0.74, and 0.82, respectively, as shown in **Table 2** and **Fig. 2A, B, C**. Obtained distilled water extract was again purified by applying solvents system petroleum ether: toluene: ethyl acetate (5:3:2) in column chromatography and the purified fraction was used for mosquito larvicidal activities. Grassi *et al.,* [12] have separated and isolated two main compounds suaveolol and methyl suaveolate, from leaves of *Hyptis suaveolens* Poit. (also known as Chichinguaste) of family Lamiaceae by means of repeated preparative thin layer and column chromatography by using five-step petroleum ether and ethyl acetate elution system (5% < 10% < 20% < 40% and finally 100% ethyl acetate in petroleum ether) and detected TLC plates by anisaldehyde reagent. Recently, Baranitharan *et al.,* (2019) [13] have also reported the phytochemical analysis of *Erythrina variegata* and observed the efficacy of *Erythrina variegata* methanol extract purified fraction against malarial and filarial vector and their results were found to be very similar to the results obtained in the present study as shown in **Table 4**.



GRAPH 1: DOSE RESPONSE CURVE OF *HYPTIS SUAVEOLENS* (L.) DISTILLED WATER EXTRACT



GRAPH 2: MEAN STANDARD GRAPH OF *HYPTIS SUAVEOLENS* (L.) DISTILLED WATER EXTRACT

In the present study, mosquito larvicidal activity of whole plant *Hyptis suaveolens* water extract purified fraction was tested against larvae of *Aedes aegypti* and *Anopheles stephensi*, and distilled water was used as vehicle solvent alone for testing its efficacy, then water extract of this plant, when applied against larvae of both the species, it was noticed that the maximum efficacy was observed with 83.33 ± 16.77% mortality of the extract reported in triplicates at 100% concentration as shown in **Table 4**, **Graphs 1** and **2** and **Fig. 3**. Kamraj *et al.,* (2011) [14] have reported larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. They have also assessed the role for larvicidal activities of hexane, chloroform, ethyl acetate, acetone, and methanol dried leaves and barks extracts of *Annona squamosa* L., *Chrysanthemum indicum* L., *Tridax procumbens* L., respectively against the 4th instars larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* mosquitoes. They have noticed that all plant extracts showed moderate effects after 24 h of exposure. In the present study, PROBIT analysis of the bioassay results of the purified fraction of plant extracts was done with the help of Latha (1999) [15] for the testing of the goodness of fit, which was assessed by Chi-square test, Cox and Snell R^2 regression analysis **Table 4**. Bioassay result of purified fraction HS^{-1} of water extract of *Hyptis suaveolens* whole plants showed significant effect 99% (P<0.0001) on mortality of larvae of *Aedes aegypti* and *Anopheles stephensi* mosquitoes. Chi-square value was reported 38.69 (P<0.001) at 100% concentration of the purified fraction of the extract of *Hyptis suaveolens* on larvae of *Aedes aegypti* and *Anopheles stephensi*, respectively. The findings of the present investigation revealed that the *Hyptis*

suaveolens (L.) water extract purified fraction possess remarkable mosquito larvicidal activities against two important mosquito species *Aedes aegypti* and *Anopheles stephensi*, respectively. Recently, Scalvenzi *et al.,* (2019) [16] have noticed larvicidal activity of *Ocimum campechianum*, *Ocotea quixos* and *Piper adimicum* essential oils against *Aedes aegypti*, and a moment ago, Goyal *et al.*, (2019) [17] have also assessed the chemical composition and larvicidal efficacy of secondary metabolites from aromatic plant extracts against dengue fever vector *Aedes aegypti* of order Diptera and of family Culicidae.

Awosolu *et al.,* [18] have noticed larvicidal effects of plant *Codiaeum variegatum* (Croton) and plant *Azadirachta indica* (Neem) aqueous extract against *Culex quinquefasciatus* mosquito. Desiyamani *et al.,* [19] have tested the toxicity of plant Sargassum polycystum extract for mosquito larvicidal, mosquito repellent and mosquito adulticidal activities against dengue filarial vectors. Rahuman *et al.,* [20] have isolated and identified a compound from *Abutilon indicum* (L.) for mosquito larvicidal activities.

CONCLUSION: In the present study, the efficacy of water extract purified fraction (HS^{-1}) of the *Hyptis suaveolens* whole plants was reported against the larvae *Aedes aegypti* and Anopheles stephensi for mosquito larvicidal activity that is an eco-friendly and economical means for the management of mosquito strain by controlling their larvae of without side effects of the biopesticides on non-target organism including men.

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