Efficacy of *Hyptis suaveolens* against lepidopteran pests

Hyptis suaveolens (L.) Poit, a rigid sweetly aromatic herb belonging to the family Lamiaceae is a native of tropical America. It was introduced and naturalized in India[1]. The plant is used as green manure in certain parts of the west coast. The edible shoot tips are sometimes used for flavouring. In Java, the plant is used as cattle fodder. An infusion of the plant is used to treat catarrhal conditions, affections of the uterus and parasitical cutaneous diseases; the leaf juice is taken internally for colic and stomach-aches. The Mundas (group of tribals from Orissa and West Bengal) use the plant for headache; the powder of leaf is used as snuff to stop bleeding of the nose. In Philippines, the leaves are used for antispasmodic, antirheumatic and antisoporific baths. A decoction of the roots is used as appetizer and the root is chewed with betel nuts as a stomachic[2,3]. The leaves are used to treat cancer[4] and anti-fertility[5].

Some species of *Hyptis* have been shown to possess insecticidal properties. Insecticidal activity of volatile oils from *Hyptis martiusii* has been reported[6]. Also the chemical compositions of the essential oil from *H. martiusii*[6], *H. mutabilis*[7], *H. suaveolens*[8–10], *H. spicigera*[11], *H. verticillata*[12], *H. crenata*[13] and *H. pectinata*[14] have been reported.

The presence of ethereal oil, monoterpenes, diterpenes, suaveolic acid, suaveolol, triterpenoid, traces of hydrocyanic acid, sterol, campesterol, fucosterol, sesquiterpene alcohols and fatty acids[1,9,10] have been reported in this plant. 1,8-cineole and sabinene are the main constituents[10].

In India, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) is a serious pest feeding on more than 180 host plants belonging to 45 families[15]. It commonly destroys more than half the yield. The annual loss amounts to US$ 300–500 million in cotton and pulses[16]. *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) is another economically important insect pest of cosmopolitan distribution[17]. It has been reported to attack more than 112 different species of cultivated crop plants throughout the world[18]. Both the noctuids feed on tender leaves, flowers and immature pods and ultimately cause severe loss of production. Growing awareness on the negative impact of chemical pesticides on the environment has prompted a surge to look for alternatives. Plants have been identified to play a vital role in providing alternative source of biodegradable pesticides. The present study was undertaken to identify some new chemical compounds from *H. suaveolens* to control lepidopteran pests.

Fresh mature leaves of *H. suaveolens* were collected from Chennai, shade-dried and powdered using electric blender. One kg of plant powder was soaked in each solvent (hexane, diethyl ether, dichloromethane, ethyl acetate, methanol and water) for 24 h at room temperature (28 ± 2°C) sequentially and filtered. The solvent from the crude was evaporated using rotary vacuum evaporator, weighed and stored at 4°C for subsequent experiments. From the crude, 1000 ppm concentration was prepared and tested for antifeedant, oviposition deterrent, ovicidal and larvicidal activity against lepidopteran pests, *Helicoverpa armigera* and *Spodoptera litura*.

Antifeedant activity of the plant extracts was studied using leaf disc no choice method[19]. Fresh leaf discs (3-cm diameter) of castor and cotton were used for *S. litura* and *H. armigera* respectively. The leaf discs were treated with 1000 ppm concentration of plant extracts individually; one treatment with acetone alone was used as positive control and one treatment without solvent was considered as negative control. In each petri dish (1.5 cm × 9 cm) wet filter paper was placed to avoid early drying of the leaf disc and single fourth instar larva of *S. litura* and *H. armigera* was introduced individually. Five replicates were maintained for each concentration and the progressive consumption of leaf area by the larva after 24 h was recorded in control and treated discs using leaf area meter (Delta-T Devices, Serial No. 15736 F 96, UK).

For oviposition deterrent activity 1000 ppm concentration of plant extracts was sprayed on fresh castor and cotton leaves for *S. litura* and *H. armigera* respectively; similar controls as mentioned above were also used here. The petioles of the treated leaves were tied with wet cotton plug to avoid early drying and placed inside the cage (60 cm × 45 cm × 45 cm). Ten pairs of *S. litura* and *H. armigera* moths were introduced on castor and cotton leaves respectively. 10% (w/v) sucrose solution with multivitamin drops was provided for adult feeding to increase fecundity. Five replicates were maintained for control and treatments. After 48 h, the numbers of egg masses (*S. litura*) and eggs (*H. armigera*) laid on treated and control leaves were recorded and the percentage of oviposition deterrence was calculated[20].

For ovicidal activity, scales from the egg masses of *S. litura* were carefully removed using fine camel brush. 500 eggs from both the lepidopterans were separated into 5 lots each having 100 eggs and dipped in 1000 ppm concentration of plant extracts and controls as mentioned above. Number of eggs hatched in control and treatments were recorded and the percentage of ovicidal activity was calculated using Abbott's formula[21].

For evaluation of larvicidal activity against *S. litura*, fresh castor leaves were treated with 1000 ppm concentration of plant extracts and controls as mentioned above. Petioles of the leaves were tied with wet cotton plug to avoid early drying and placed in plastic trough (29 cm × 8 cm); 20 prestarved (2 h) IV instar larvae of *S. litura* were introduced individually and covered with muslin cloth. For *H. armigera* 1000 ppm concentration of plant extracts was mixed with artificial diet[22]. Small pieces of artificial diet were separated and placed in plastic containers. Single IV instar larva was introduced in each container. Five individual containers were considered as one replication. Five replicates were maintained and the number of larvae dead after 48 h was recorded and the percentage of larval mortality was calculated using Abbott's formula[21]. All the data collected were subjected to Analysis of Variance (ANOVA) and the significant difference within the mean was separated using Least Significant Difference test (LSD; $P < 0.05$).

Crude ethyl acetate extract (20 g) was dissolved in 10 ml of ethyl acetate and 5 g of silica gel and macerated well using mortar and pestle to make fine powder. The powdered material was fractionated through a silica gel (100–200 mesh LR) column chromatography (4 cm × 60 cm) using the combination of hexane/ethyl acetate (95 : 5; 90 : 10; 85 : 15; 80 : 20). Totally 11 fractions were obtained; each fraction was confirmed using Thin Layer Chromatography (on Aluchrosep Silica gel 60 UV_{254} gel coated sheets); each fraction was tested for its bioactivity at 500 ppm concentration. Promising fractions were further studied for their bioactivity at 100, 250, 500, 1000 and 2000 ppm. Purified promising fractions were subjected

Table 1. Bioactivity of ethyl acetate extract of *Hyptis suaveolens* at 1000 ppm concentration

Bioactivity	Tested insects	
	Spodoptera litura	*Helicoverpa armigera*
Antifeedant (%)	65.3 ± 3.37	71.0 ± 1.90
Oviposition deterrent (%)	39.0 ± 3.48	24.0 ± 4.21
Ovicidal (%)	69.4 ± 2.99	65.7 ± 2.7
Insecticidal (%)	19.4 ± 2.55	11.5 ± 2.28

Values are expressed as percentage mean ± SD (n = 5).

Table 2. Antifeedant and ovicidal activity of fraction (II) against selected pests

Concentration (ppm)	*Spodoptera litura*		*Helicoverpa armigera*	
	Antifeedant (%)	Ovicidal (%)	Antifeedant (%)	Ovicidal (%)
100	26.6 ± 2.70a	28.0 ± 1.58a	32.4 ± 4.15a	25.4 ± 5.31a
250	29.6 ± 4.82ab	29.0 ± 3.97a	39.2 ± 4.43a	31.4 ± 6.10ab
500	34.0 ± 4.84b	31.6 ± 4.27a	37.8 ± 5.40a	44.8 ± 8.67b
1000	63.6 ± 3.84c	65.2 ± 3.03b	67.6 ± 6.38b	69.2 ± 7.62c
2000	74.6 ± 4.97d	72.8 ± 4.08c	78.4 ± 3.36c	73.4 ± 6.98c

Values are expressed as percentage mean ± SD (n = 5). Within the column different alphabets are statistically significant ($P < 0.05$; LSD).

Table 3. Antifeedant and ovicidal activity of fraction (IV) against selected pests

Concentration tested (ppm)	*Spodoptera litura*		*Helicoverpa armigera*	
	Antifeedant	Ovicidal	Antifeedant	Ovicidal
100	23.4 ± 3.20a	31.0 ± 5.78a	31.2 ± 5.63a	22.0 ± 4.63a
250	30.8 ± 4.76b	42.4 ± 6.50b	40.2 ± 7.39ab	28.0 ± 5.00ab
500	29.2 ± 4.14ab	43.2 ± 8.46b	45.2 ± 8.49b	30.0 ± 6.59b
1000	60.2 ± 5.44c	61.8 ± 3.70c	63.4 ± 7.09c	69.0 ± 2.82c
2000	69.4 ± 3.64d	68.8 ± 2.58d	77.0 ± 3.60d	67.6 ± 7.36d

Values are expressed as percentage mean ± SD (n = 5). Within the column different alphabets are statistically significant ($P < 0.05$; LSD).

to FTIR, ^{1}H NMR and ^{13}C NMR for identification of bioactive compounds.

Maximum antifeedant and ovicidal activity were recorded in ethyl acetate extract of *H. suaveolens* and the results are presented in Table 1. No antifeedant and ovicidal activity was recorded in positive and negative control. Among the 11 fractions tested, fraction II and IV showed maximum antifeedant and ovicidal activity. Statistically significant antifeedant and ovicidal activity were recorded at 1000 ppm and 2000 ppm concentrations (Tables 2 and 3). The bioactivity of fraction II seems to be due to the presence of long aliphatic chain group containing α, β-unsaturated keto-moiety, attached to phenolic nucleus. The presence of α, β-unsaturated ketone group seems to impart synergistic activity of phenolic compound. Also, the presence of methyl residue seems to enhance the hydrophobic nature of the molecule, thereby indirectly enriching the bioactivity of the parent phenolic compound. Earlier bioactivity of polyphenolic rich fractions from the stem bark of *Streblus asper* against *Dysdercus cingulatus* has been reported[23] and several polyphenolic compounds have been reported to have insecticidal activity[24–26].

The molecular structure of bioactive fraction (II) was identified as 5-keto-pent-3,4-enyl-2′-phenol using FTIR, ^{1}H-NMR and ^{13}C-NMR and the IUPAC name was found to be (2E)-1-(2-hydroxyphenyl)pent-2-en-1-one (Figure 1).

The bioactive nature of fraction IV seems to be due to the presence of enolic –OH group and ether linkage. Gem-Diallyl group and the ethyl residue, which impart the hydrophobicity of the molecule, seem to be responsible for inhibiting fatty acid metabolism. The molecular structure of

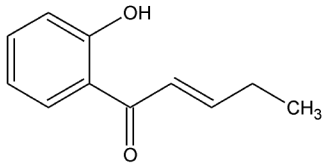

Figure 1. (2E)-1-(2-hydroxyphenyl)pent-2-en-1-one.

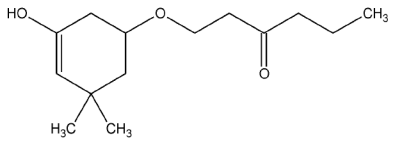

Figure 2. 1-[(3-hydroxy-5,5-dimethylcyclohex-3-en-1-yl)oxy]hexane-3-one.

fraction IV was identified as 5-pentyl-methylene oxy-4,4-dimethyl–Cyclohexenol using FTIR, ^{1}H-NMR and ^{13}C-NMR and the IUPAC name was found to be 1-[(3-hydroxy-5,5-dimethylcyclohex-3-en-1-yl)oxy]hexane-3-one (Figure 2).

The new compounds isolated from *H. suaveolens* showed promising results against lepidopteran pests. This is the first report on bioactivity of these newly isolated compounds. Earlier essential oil extracted from the leaves of this plant showed antibacterial activity[27] and ovicidal activity against eggs of *Callosobruchus maculatus*[28]. Further work is in progress to use them in bio-pesticide formulations.

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