New enolic type bioactive constituents from *Hyptis suaveolens* (L.) Poit

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ABSTRACT

The objective of the present study is to isolate the lead phytochemical from the *Hyptis suaveolens* (L.) Poit. based on bioassay guided isolation from the promising crude extract. The crude extracts were prepared by using Hexane, petroleum ether, ethyl acetate, chloroform, methanol, acetone and water as solvents by cold percolation mechanical agitation method and hot extraction method, the dried crude extracts further subjected to bioactivity studies. Among the crude extracts, ethyl acetate extract is showed promising antifeedant, oviposition deterrent, ovicidal and insecticidal activity against *Helicoverpa armigera*, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae), it was further taken for flash column chromatography purification which yields twenty fractions. From the bioactivity studies, fraction II and IV contains active constituents which inhibit the feeding ratio of the *H.armigera* and *S. litura* and it is apparent from the table. As per the data, fraction II and IV showed statistically significant ovicidal activity and further purified by preparative liquid chromatography yields two bio active molecules with 99% purity (2E)-1-(2-hydroxy phenyl) pent-2-en-1-one (I) and 1-[(3-hydroxy-5, 5-dimethyl cyclohex-3-en-1yl) oxy] hexan-3-one (II). Presence of these two phyto chemicals is being reported for the first time from this plant. Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. But, at present role of natural products in the pharmaceutical industry has declined, due to lack of compatibility of traditional natural-product extract libraries with high-throughput screening. Attempts were made to enrich the bioactivity of phytochemical (I) & (II) through semi synthetic modification by employing boron reagents, which results crude mixture and it shows excellent bioactivity against *H.armigera*, *S.litura*.

Key words: *Hyptis suaveolens*, (2E)-1-(2-hydroxy phenyl) penta-2-en-1-one, 1-[(3-hydroxy-5, 5-dimethyl cyclohex-3-en-1yl) oxy] hexan-3-one, Antifeedant activity, Ovicidal activity.

INTRODUCTION

*Hyptis suaveolens* (L.) poit, a rigid sweetly aromatic herb belongs to the family (Lamiales: Lamiacae) is a native of tropical America. The plant is used as green manure in India [29], the edible shoot tips are used for flavoring the dishes. In Africa and 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 [6]. The leaves are used to treat cancer ailments [13] and anti-fertility causes [13], [20]. This plant is used for ethno botanical applications in rural communities in African countries and shows the promising results to control the *Sesamia calamistis* on Maize [2]. In India, *Helicoverpa armigera* is a serious pest feeding on more than 180 host plants belonging to 45 families [14]. It commonly destroys more than half of the yield. The annual loss amounts to US$ 300-500 million in cotton and pulses [12]. *Spodoptera litura* is another economically important pest of cosmopolitan distribution [18]. It has been reported to attack more than 112 different species of cultivated crop plants throughout the world [24]. Both the noctuids feed on tender leaves, flowers and immature pods and ultimately cause severe loss
of production. Also, recent research findings revealed that a combination of the aqueous extract of *H. suaveolens* (L.) Poit. with a lower dose of insecticides such as Thionex 350 EC (Endosulfan (350)) or Laser 480 EC (Spinosad (48)) helped to successfully control cotton bollworms [26].

The use of chemical pesticides against stem borers is limited not only because of their high costs (generally not affordable for small farmers) and scarce availability in rural areas, but also due to the health and environment concerns related to them. There is a need for cheaper, ecofriendly and safer alternative control practices. Over the years, farmers have learned to contain pest problems through the use of plant extracts. The potential advantages of botanical pesticides over synthetic pesticides have been highlighted by authors [22].

Many plants have been tested or identified as interesting botanical pesticides in sub-Saharan Africa [3],[9],[26],[27] and are potentially usable in pest control programs taking into account both the needs of increased food and preserving the health of a growing population. Plants have been identified to play a vital role in providing alternative source of biodegradable pesticides. *Hyptis suaveolens* is used for some ethno botanical applications in rural communities in African countries [2], [5], [21],[26] and the plant is readily available close to village, along roadsides, on farmsteads, etc. useful for human community.

The objective of the present study was undertaken to identify some new chemical compounds from *H. suavelons* (L.) to control lepidopteran pests and their semi synthetic modification.

**Previously isolated constituents**
The presence of ethereal oil, Monoterpenes, Diterpenes, Suaveolic acid, Suaveolol, Triterpenoid, Campesterol, Fucosterol, Sesquiterpene alcohols [10] and essential oils have been reported in this plant [20]. Insecticidal activities of volatile oils from *Hyptis martiusii* [2], *Hyptis mutabilis* [7], *H. spicigera* [5] and *H. pectinata* [17] have been reported.

**MATERIALS AND METHODS**
The plant materials of *Hyptis suaveolens* (L.) were collected from Ambattur-Padi village area in Chennai district and identified with the help of flora of Madras Presidency (Gamble,1928). The authentication of the plant was confirmed by Dr. Narasimhan, Taxonomist, Department of Botany, Madras Christian College, Chennai. The voucher specimen was deposited at the Entomology Research Institute (ERI) herbarium collections, (LC/ERI/Herb.206) Loyola College.

**Plant preparation**
The fresh leaf and stem of plant was rinsed with water to remove sand and it was dried in an incubator at room temperature for one month. It was pulverized to reduce the surface area using pulverizer machine, the leaf powder (10 kg) and stem powder (12 kg) was kept in air-tight cellophane bags until it’s used [32], [33].

(a) Cold percolation method:
The leaf coarse powder is further taken for sequential solvent extraction in different solvents (Hexane, petroleum ether, ethyl acetate, chloroform, methanol, acetone and water). 2kg of leaf powder is taken into 10 litre three necked round bottom flak and about 7L of solvent was added to it, the mixture was agitated with the help of mechanical overhead agitator at room temperature, 50 RPM for 48 hours. The extract was separated using fine muslin cloth and then filtered under vacuum, filtrate was taken in 10 litre single neck round bottom flask and solvent is removed in room temperature by rotary evaporator, it was further dried under high vacuum condition to remove trace amount of solvent. The crude extract was kept in cold room (5°C) under nitrogen atmosphere.

(b) Hot extraction method:
The leaf coarse powder (2kg) is taken into 10 litre three necked round bottom flak and about 7L of solvent was added to it, the mixture was agitated with the help of mechanical overhead agitator at 45°C by water bath for 32hours. The extract was separated using fine muslin cloth and then filtered under vacuum, filtrate was taken in 10 litre single neck round bottom flask and solvent is removed in room temperature by rotary evaporator, it was further dried under high vacuum condition to remove trace amount of solvent. The crude extract was kept in cold room (5°C) under nitrogen atmosphere.
In comparison with two methods, better yields were obtained with cold percolation method over hot extraction method.

**Experimental Procedure**

All solvents and reagents were obtained from Aldrich, Fluka and were used without purification unless stated. Liquid substrates were distilled prior to use. $^1$H NMR and $^{13}$C spectra were measured on a Bruker AVANCE (400 MHz) spectrometer using TMS as the internal standard and specified deuterated solvents. Chemical shifts were expressed in ppm and Infrared (IR) spectra were recorded as KBr pellets on a Perkin-Elmer FTIR spectrometer.

**Bio assay guided isolation of lead active molecules [32], [33]**

Shade dried, powdered leaf material (2 Kg) was subjected to sequential solvent extraction and the respective crude extract was then tested for bio-activity studies. The crude extract, which shows promising bio activity, is taken for further column chromatographic isolation. Ethyl acetate crude extract (50g) showed promising activity was fractionated through flash column chromatography, column size (15cm X 100cm), using silica gel (230-400 mesh AR) and gradient of solvent Hexane / Ethyl acetate (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50, 100). Totally 20 fractions were obtained. Each fraction was tested for its bioactivity at various concentrations of 100ppm, 250ppm, 500ppm, 1000ppm and 2000ppm. Among these fractions, fraction II and IV shows maximum antifeedant and ovicidal activities, it was further taken for preparative liquid chromatography which yields two compounds with purity 99% by HPLC. The molecular structure of bioactive compound II and IV was identified by using FTIR, $^1$H-NMR and $^{13}$C-NMR technique.

**Spectral data of Compound (1):**

The chemical name of the compound (1) is 5-Ethyl-8-Allyl-9-sec Benzopyrone, the IUPAC name of the compound is (2E)-1-(2-hydroxy phenyl) pent-2-en-1-one.

$^{1}$H-NMR (400 MHz CDCl$_3$): $\delta$ 0.756-2.07 (14 H, s, -CH$_3$ and –CH$_2$ protons); 3.21 (2H, dd); 5.24 and 5.28 (2H dd); 7.48 (C1H, m, aromatic proton); 7.83 (1H, m, aromatic proton).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta$ 16.0(C-1), 36.2 (C-2), 117.8 (C-3), 121.6 (C-4), 178.6 (C-5), 120.4 (C-6), 140.2 (C-7), 123.4 (C-8), 120.2 (C-9), 120.0 (C-10), 118.3 (C-11).

**Spectral data of Compound (2):**

The chemical name of the compound (2) is 5-Pentyl-Methylene Oxy-4, 4-Dimethyl-Cyclohexenol, the IUPAC name of the compound is 1-[(3 hydroxy-5, 5-dimethyl-cyclohex-3-en-1-yl) oxy] hexan-3-one.

$^{1}$H-NMR (400 MHz CDCl$_3$): $\delta$ 0.937 (3H, s); 1.09 (6H, s); 1.261 (3H, s); 1.51-1.76 (10H m); 1.910-1.941 (2H dd); 3.20-3.24 (2H, q).

$^{13}$C-NMR (400 MHz, CDCl$_3$): $\delta$ 14.08 (C1), 17.6 (C2), 56.2 (C3), 178.0 (C4), 58.2 (C5), 62.2 (C6), 116.4 (C7), 60.5 (C8), 42.2 (C9), 124.0 (C10), 142.3 (C11), 60.4 (C12), 15.2 (C13, C14).
RESULTS AND DISCUSSION

Antifeedant activity
Antifeedant activity of the compound I and II was studied using leaf disc no choice method [10], [5], [4]. 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 1000ppm concentration of compound I and II individually. One treatment with acetone alone was considered as positive control and one treatment without solvent was considered as negative control. In each petri dish (1.5 cm X 9 cm) wet filter paper was placed to avoid early drying of leaf disc and single fourth instar larva of S. litura and H. armigera was introduced. Five replicates were maintained for each concentration and the progressive consumption of leaf area by the larva, every 24 h was recorded in control and treated discs using leaf area meter (Delta- T Devices, Serial No. 15736 F 96, UK).

Ovicidal activity
For evaluation of ovicidal activity, scales from the egg masses of S.litura were carefully removed using fine camel brush. 500 eggs from both the lepidopteran 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 [1].

Oviposition deterrent activity
For oviposition deterrent activity, 1000ppm concentration of compound I and II was sprayed on fresh castor and cotton leaves and single fourth instar larva of Spodoptera litura and Helicoverpa armigera was introduced individually. Similarly 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 (60cm X 45cm X 45cm). Ten pairs of S. litura and H. armigera moths were introduced on castor and cotton leaves respectively. 10% (w/v) sucrose solution with multivitamin tablets drops was provided for adult feeding to increase fecundity. Five replicates were maintained for control and treatments. After 48h, the number of egg masses (S. litura) and eggs (H. armigera) lay on treated and control leaves was recorded and the percentage of oviposition deterrence was calculated. The ovicidal activity and larvicidal activity were studied, experimental measurements carried out according to [15], [11], [23], [16], [8] and also the values expressed in table 1.

Table1. Bioactivity of ethyl acetate extract of Hyptissuaveolens at 1000 ppm concentration

<table>
<thead>
<tr>
<th>Bioactivity</th>
<th>Spodoptera litura</th>
<th>Helicoverpa armigera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antifeedent (%)</td>
<td>65.3 ± 3.57</td>
<td>71.0 ± 1.90</td>
</tr>
<tr>
<td>Oviposition deterrent</td>
<td>39.0 ± 3.48</td>
<td>24.0 ± 4.21</td>
</tr>
<tr>
<td>Ovicidal (%)</td>
<td>69.4 ± 2.99</td>
<td>65.7 ± 2.7</td>
</tr>
<tr>
<td>Insecticidal (%)</td>
<td>19.4 ± 2.55</td>
<td>11.5 ± 2.28</td>
</tr>
</tbody>
</table>

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

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 20 fractions tested, fraction II and IV showed maximum antifeedant and ovicidal activity. Statistically, significant antifeedant and ovicidal activity were recorded at 1000 ppm concentrations. The bioactivity of fraction II seems to be excellent response due to the presence of long aliphatic chain group containing α, β-unsaturated ketone moiety which is 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 that the several poly phenolic compounds have insecticidal activity [28], [30], [31].

Table 2 Antifeedant and ovicidal activity of compound I against selected pests

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th><em>Spodoptera litura</em></th>
<th><em>Helicoverpa armigera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antifeedant (%)</td>
<td>Ovicidal (%)</td>
</tr>
<tr>
<td>1000</td>
<td>63.6 ±3.84c</td>
<td>65.2 ±3.03b</td>
</tr>
<tr>
<td>2000</td>
<td>74.6±4.97d</td>
<td>72.8±4.08c</td>
</tr>
</tbody>
</table>

Table 3 Antifeedant and ovicidal activity of compound II against selected pests

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th><em>Spodoptera litura</em></th>
<th><em>Helicoverpa armigera</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antifeedant (%)</td>
<td>Ovicidal (%)</td>
</tr>
<tr>
<td>1000</td>
<td>60.2±5.44c</td>
<td>61.8±3.70c</td>
</tr>
<tr>
<td>2000</td>
<td>69.4±3.64d</td>
<td>68.8±2.58d</td>
</tr>
</tbody>
</table>

Semi synthetic modification of compound (I) & (II):
Small bioactive molecules produced in Nature possess exquisite chemical diversity and continue to be an inspiration for the development of new therapeutic agents through semi synthetic concept. The semi synthetic modification of (2E)-1-(2-hydroxyphenyl) pent-2-en-1-one (I) and 1-[(3-hydroxy-5, 5-dimethylcyclohex-3-en-1-yl) oxy] hexan-3-one (II) was performed with following reagents: 1) NaBH₄-I₂ 2) [n-Bu]₄N.BH₄ 3) N,N-DMAN.BH₃ leads to a mixture of compounds. Chromatography isolation and bioactivity studies is in progress, the results will be published within due course.

![Chemical structures](image)

**CONCLUSION**

An increasing prevalence of plant damage and infections caused by newer emerging pests, fungal pathogens which severely affect the economy and plant distribution & population, at present the need of IPM (Integrated Pest Management) is new effective, safe botanical pesticides. The two bioactive molecules (2E)-1-(2-hydroxy phenyl) pent-2-en-1-one (I) and 1-[(3-hydroxy-5, 5-dimethyl cyclohex-3-en-1-yl) oxy] hexan-3-one (II) shows promising activity against *H.armigera* and *S.litura*, was reported first time from *H.suaveolens* (L.) Poit. Semi-synthetic modifications of natural products aimed at enhancing their biological properties serve as great opening for drug discovery. Since year 2000, 12 of 15 natural product derived drugs are semi synthetic products. Based on this, the attempted semi synthetic modification on (I) and (II) phytochemicals yields crude mixture. Studies on the isolation, structural elucidation and bioactivity of purified compound will be reported in the due course.

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