**Contact Toxicity and Chemical Composition of Essential Oil of Acantholimon scorpius**

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**Abstract**

Background: Plumbaginaceae plants family is a valuable natural insecticidal compound. This research focused on contact toxicity and chemical composition of essential oil obtained from *Acantholimon scorpius*.

Methods: The essential oil from the aerial parts of *A. scorpius* was extracted by hydrodistillation method and tested for their toxicity against *Oryzaephilus mercator* (Coleoptera: Silvanidae) and *Tribolium castaneum* (Coleoptera: Tenerbrionidae). Chemical compounds of the essential oil was analyzed by the gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID).

Results: The essential oil showed toxic effect on tested insects. This oil showed 90.0% mortality of *O. mercator* and 85.2% mortality of *T. castaneum* at a dose of 12 μl/l air after 48h of exposure. The constituents of this oil were identified, representing more than 82.9% of the total essential oil composition. Hexadecanoic acid, tetrahydrogeranyl acetone and oleic acid were the main compounds of the essential oil.

Conclusion: According to the result the essential oil of *A. scorpius* showed a noticeable insecticidal activity in contact toxicity model.

**Introduction**

Plumbaginaceae plants family is a valuable natural insecticide in the several nations such as Chinese, Mediterranean and Iranian traditional medicines. Plants of this family have been used as effective treatment of many diseases.1-6 Recent studies have indicated that various biological activities such as antiplasmodial and insecticidal properties.7-12 In different researches, various active constituents were reported from the parts of this genus like anthocyanins, flavonoids, tannins and quinines.13-19 Some of these secondary metabolites such as quinines act as a part of plants defense mechanism against insects.20-22 Because of insect's resistance against to many synthetic insecticide, development of the safe natural insecticides or repellents is an important need for insect borne transmitted diseases control and reduction. Insecticide properties of plant and secondary metabolites lead researchers to find novel new insecticidal compounds form plants.23-27 Among plants metabolites, the essential oils have appropriate insecticidal potencies for using against stored product insects.28 *Acantholimon* genus belongs to Plumbaginaceae family, consists of 83 species of woody sub shrub flowering plants commonly have spikes with pink to purple flowers and needle leaves, widely distributed in many different climatic regions of Iran and known as ‘Prickly thrift’ in Europe.29 *A. scorpius* is an endemic plant of northwest and Zagros region of Iran. Although this genus shows high biodiversity among Plumbaginaceae family, there are no reports on chemical profile and insecticidal activity of this genus till now. Hence, this is the first report on the essential oil compositions and insecticidal activities of *Acantholimon* genus.

**Materials and Methods**

**Plant Material**

Aerial parts of *A. scorpius* were collected at the flowering period from wild population growing in East Azerbaijan province, Iran. Voucher specimens were authenticated by the Pharmacognosy Department and voucher specimens, 2558, deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.

**Extraction of the Essential Oil**

The air-dried ground aerial parts of *A. scorpius* (500 gr) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus, yielding 1.5% v/w of dry

**Keywords:** Essential oils, Insecticidal activities, Contact toxicity, Mass spectroscopy, Gas chromatography

**References:**

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The essential oil sample was dried over anhydrous sodium sulphate (Na₂SO₄) and stored at 4 °C in the dark until tested and analysis by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

**Analysis of the Essential Oil**
The essential oil was analyzed by Shimadzu GC-MS-QP5050A fitted with a fused methyl silicon DB-5 column (60 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1.3 mL/min. The column temperature was kept at 50 °C for 3 min, increased to 300°C at a rate of 5°C/min, and finally kept at 300°C for 5 min. The injector temperature was 270°C and split ratio was adjusted at 1:33. The injection volume was 1 µL. The mass spectral (MS) data were obtained at the following conditions: ionization potential 70 eV; ion source temperature 200°C; quadrupole temperature 100°C; solvent delay 2 min; resolution 2000 amu/s and scan range 30-600 amu; EM voltage 3000 volts. Identification of compounds was based on direct comparison of the Kovats indices and MS data with those for standard compounds, and computer matching with the NIST NBS54K Library, by comparison with references. For quantitation (area %), the GC analyses were also performed on an Agilent 6890 series apparatus fitted with a FID detector. The FID detector temperature was 300 °C. To obtain the same elution order as with GC-MS, simultaneous auto-injection was performed on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

**Insecticidal toxicity assay**
Insect toxicity of the essential oil was determined according to Kordali et al. method. The insects were collected from a laboratory culture and reared on a mixture of whole wheat and maize flour at the ratio 1:1 (0.5 kg). All insect species were reared at 27±2°C, 12% moisture content and continuous darkness for about 3 weeks without exposing to insecticides. Adults used in the experiments were 1-3 week old and of mixed sex. The essential oil was applied with an automatic pipette on a paper strip (6cm x 3cm). The amounts of essential oil applied were 12, 24, 36 and 48 µl, corresponding to 3, 6, 9 and 12 µl/l air. Each dose was applied with automatic pipette as 100 µl acetone solution also acetone used as control. After evaporation of the acetone, the strip was attached to the underside of the desiccators to avoid direct contact of test material. Twenty adults of *O. mercator* and *T. castaneum* were placed in petri dishes (9cm), maintained at 27±2°C, 12% moisture content and 12 h photoperiod. The experimental design was completely randomized, with three replicates. Insect mortality was evaluated after 12, 24, and 48 h of exposure. Control treatments without the essential oil were treated in the same way.

Responses to treated sample versus control were converted to "percentage of mortality".31

**Data analysis**
Statistical analysis of the data was done using SPSS 10.0 software package. The results were showed significant difference at *p* <0.05 levels.

**Results**
The composition of the essential oil and insecticidal activity of *A. scorpius* investigated for the first time. Hydrodistillation of air dried aerial parts of *A. scorpius* yielded yellowish oils (1.5% v/w on dry weight basis). The major components of the essential oil are hexadecanoic acid (34.3%), tetrahydrogeranyl acetone (18.1%), 9, 12-Octadecadienoic acid, methyl ester (6.2%) and oleic acid (4.7%). The major volatiles in our study showed in Table 1 where the compounds are listed in order of their elution on DB-5 column.

**Table 1. Major constituents of the essential oils of *A. scorpius*.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>K.I* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonanal (Pelargonaldehyde)</td>
<td>1098 0.5</td>
</tr>
<tr>
<td>Tetradecane</td>
<td>1399 3.7</td>
</tr>
<tr>
<td>Geranyl acetone</td>
<td>1436 0.4</td>
</tr>
<tr>
<td>Tetrahydrogeranyl acetone</td>
<td>1469 18.1</td>
</tr>
<tr>
<td>Tetradecanoic acid (Myristic acid)</td>
<td>1720 0.9</td>
</tr>
<tr>
<td>Nonadecane</td>
<td>1900 2.2</td>
</tr>
<tr>
<td>2-methyl-Nonadecane</td>
<td>1966 1.1</td>
</tr>
<tr>
<td>n-Hexadecanoic acid(Palmitic acid)</td>
<td>1984 34.3</td>
</tr>
<tr>
<td>Eicosane</td>
<td>2000 2.1</td>
</tr>
<tr>
<td>9,12,15-Octadecatrien-1-ol</td>
<td>2034 2.6</td>
</tr>
<tr>
<td>Oleic acid(1-Octadecenoic acid 9)</td>
<td>2085 4.7</td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid, methyl ester</td>
<td>2092 6.2</td>
</tr>
<tr>
<td>Docosane</td>
<td>2200 4.4</td>
</tr>
<tr>
<td>Palmitaldehyde diallyl acetone</td>
<td>2237 1.7</td>
</tr>
</tbody>
</table>

**Grouped components**
- Long chain hydrocarbons (%) 13.5
- Alcoholic hydrocarbons & derivatives (%) 2.6
- Aldehydes and ketones hydrocarbons (%) 20.7
- Fatty acid and derivatives (%) 46.1
- Others (%) 82.9

* Kovats Indice (K.I) was calculated according to the method described by Kovats.

The toxicity of the essential oil on *O. mercator* and *T. castaneum* were determined in 3, 6, 9 and 12 µl/l air doses of the essential oil. The mortality increased with increase in the exposure times and dose. The oil caused 90.0% mortality of *O. mercator* and 85.2% mortality of *T. castaneum*, respectively (in 12 µl/l air). In the present study the essential oil of *A. scorpius* that containing hexadecanoic acid (34.3%), tetrahydrogeranyl acetone (18.1%) and 9, 12-Octadecadienoic acid, methyl ester (6.2%) as major compounds showed potent insecticidal activity (Table 2).
Discussion
In this research the chemical composition and insecticidal activity of the essential oil of Acantholimon genus reported for first time. Based on this report and according to the previous studies toxicity of the essential oil is attributed to their main components such as 9. 12-Octadecadienoic acid, methyl ester, docosane and farnesyl derivatives. The essential oil caused significant mortality (80-90% for two pests) at 12 μl/l air after 48h of exposure (Table 2).

Conclusion
The results showed that the essential oil of A. scorpious has varying degree of insecticidal activity against O. mercator and T. castaneum. However, more investigation needs to investigate potential insecticidal activity of Acantholimon genus.

Conflict of interests
The authors claim that there is no conflict of interest.

References
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