GC-MS analysis of constituents of essential oil from *Stachys pubescens* in *in-vitro* conditions

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**Abstract**

The genus *Stachys* distributed in the Mediterranean regions and south-west Asia. Tissue culture is the practical solution to produce these metabolites. *Stachys pubescens* seeds were sterilized using detergents and were grown on 0.8% agar for seedling production, then the upper part of the seedling were moved in sterile conditions on autoclaved MS medium containing plant hormones. After growing a sufficient quantity of green callus, callus DCM extracts were prepared and analyzed using the GC system. Based on analysis of the essential oil from the plant shoot, seven compounds identified, which was in total 96.2% of the essential oil including 90% of the oxygenated monoterpenes, 5.5% of aliphatic compounds and 7% other ingredients.

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Introduction

The genus *Stachys* which belongs to the Lamiaceae family is found in mild regions of the Mediterranean and in south-west Asia. This genus consists of 300 species widespread throughout the world (Luteyn and Churchill, 1999). 34 of these are found in Iran, of which 13 are endemic. Several *Stachys* species are used in Iranian folk medicine as medicinal plants (Rezazadeh et al., 2006). In addition, pharmacological studies confirmed that extracts or components of plants belonging to the genus *Stachys* exert significant antibacterial, anti-inflammatory and antitoxic effects (Usubillaga et al., 1999). Biosystematic and chemotaxonomic studies have been carried out on *Stachys* species, in which flavonoids and flavonoid glycosides, quinones, iridoids, phenolic acids, diterpenoids and essential oils were reported (Rezazadeh et al., 2006).

The compositions of the oils from some *stachys* species, e.g., *S. acerosa*, *S. athorekalyx* and *S. recta*, have been reported. *Stachys* species also have several folkloric uses. For example, the leaves of *S. officinalis* L.Trev. are used as a carminative and to relieve headache. *S. botenica* L. is used as a tonic, astringent and to relieve headache, while *S. byzantina* is used as an antiseptic, to relieve gout and to stop hemorrhage. *S. byzantia* is used to treat ulcers and as an antiseptic. Two previous studies of oil from *S. pubescens* showed rather different results, prompting the present study (Akhlaghi et al., 2011).

Salimi et al., (2011) conducted an experiment on the essential oil of *Stachys pubescens*. Growing wild in north-west of Iran was examined by GC and GC-MS methods. The yield of total volatiles was 0.06% (v/w). A total of 21 compounds were characterized in the essential oil. The main components of the oil were thymol (87.4%), trans-4-octene (4.8%) and linalool (1.6%). Other compounds present in appreciable amounts were nerol (0.7%), docosane (0.7%), a-terpineol (0.5%), and linalyl acetate (0.5%). There are limited studies on constituents of essential oil of stachys and there is no report in the study of essential oil of this plant in in-vitro condition. This study conducted to determine the differences between components of essence in this condition and other studies on collected materials.

Material and methods

Herbal material

Plant materials collected from Sabalan Mountain in 2010. Sabalan is in the north-west of Iran. Seeds had sterilized 30 minutes in distilled water, 2 minutes in ethanol 70% and 8 minutes in sodium hypochlorite.

In-vitro culture

Seeds washed several time again and transferred to a Medium with 0.8% agar. The dishes left in a dark place and 25°C for 12 days. To callus culture seedlings sliced and cultured in MS medium consist of 100 mgr.L⁻¹ myo inositol, 2 mgr.L⁻¹ Glycine, 0.5 mgr.L⁻¹ nicotinic acid, 0.5 mgr.L⁻¹ pyridoxine, 0.1 mgr.L⁻¹ thymine, 30 gr.L⁻¹ sucrose, 15% coconut milk, 0.8% agar, 1 mgr.L⁻¹ IAA, 1 mgr.L⁻¹ 2,4-D and 0.2 mgr.L⁻¹ Kinetin. The pH of all media was adjusted to 5.8. Cultures were incubated at 25°C under darkness.

Essential oil extraction

To extraction of essential oil, first of all 5 mL dichloromethane added to 10 gr plant material. After completely digestion and 3min vortex essential oil was isolated. After centrifuge, dichloromethane phase isolated and condensed to 100 µL. 1 µL of essential oil used for GC and GS/MS. The analysis and identification of essence of plants was performed through Spectrometer gas/ chromatography coupled with volume. Identification of spectrums performed trough their prevention indices in references books, papers and digital library information.

Result and discussion

Generating the seedlings was very successful (Fig.1). Calluses were Light green and very fragile. Considering results of GC/MS 7 mixtures with 96.2 percent was identified from the essential oil. Based on the analysis of the essential oil, the components were classified into three groups. Oxygenated monoterpenes (87.8%), aliphatic mixture (4%) and
other mixture (0.7%). The components identified and their percentages are given in Table 1.

**Table 1. Chemical composition of essential oils from *Stachys pubescens***.

<table>
<thead>
<tr>
<th>Components</th>
<th>RI</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>1 Docosane</td>
<td>2200</td>
<td>0.6</td>
</tr>
<tr>
<td>2 Thymol</td>
<td>1289</td>
<td>85.4</td>
</tr>
<tr>
<td>3 Linalyl acetate</td>
<td>1255</td>
<td>0.7</td>
</tr>
<tr>
<td>4 Nerol</td>
<td>1231</td>
<td>0.5</td>
</tr>
<tr>
<td>5 α-Terpionel</td>
<td>1189</td>
<td>0.7</td>
</tr>
<tr>
<td>6 Linalool</td>
<td>1089</td>
<td>1.2</td>
</tr>
<tr>
<td>7 Trans-4-octene</td>
<td>802</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>92.5</strong></td>
</tr>
</tbody>
</table>

Previous investigations on the oils of the *Stachys* genus showed varying compositions. The dominant compound in the oil of *S. balansae* and *S. recta* were betacaryophyllene (24.3%) and 1-octen-3-ol (33.8%) respectively (Cakir et al., 1997). The dominant compound in the oil of *S. aegyptiaca* was α-pinene (54.5%) (Halim et al., 1991).

**References**


