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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 monoterpene, 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). Biosysternatic and chernotaxonomic 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* speies also have several flolkloric uses. For example, the leaves of *S. officinalis* L.Trev. are used as a carminative and to revileve headache. *S. botenica* L. is used as a tonic, astringent and to relieve headach, 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 30minutes in distilled water, 2minutes 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 condensated 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 Table1.

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

	Components	RI	Percentage
1	Docosane	2200	0.6
2	Thymol	1289	85.4
3	Linalyl acetate	1255	0.7
4	Nerol	1231	0.5
5	α-Terpionel	1189	0.7
6	Linalool	1089	1.2
7	Trans-4-octene	802	3.4
Total			92.5

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)

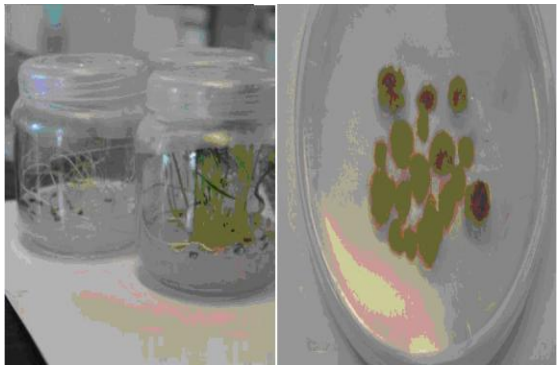

Fig. 1. *In-vitro* culture of a) Seedling b) callus.

Ramezani *et al.*, (2002) reported spathulenol and caryophyllene oxide as the main constituents of *S. lavandulifolia*. Comparison of the results with the different conditions showed significant differences for the oils, which can be attributed to their different locality, weather and some other climatic conditions of the plants. Nik and Mirza (2006) investigated on essential oil of *Stachys pubescens,* reported that the oil characterized by a high content of germacrene D (37.7%), (Z)-β-ocimene (20.3%) and bicyclogermacrene (11.6%), which were the major constituents of the oil. Other components present in appreciable amounts were β-pirene (7.5%), (E)-β-ocimene (5.5%), β-bourbonene (11.6%), α-pinene (2.5%), octen-1-olacetate (1.3%), α-cadinol (1.1%) and spathulenol (1%). Mirza and Baher (2003)reported that the oil of *S. lanata*Jacq. collected from the National Botanical Garden in Tehran, Iran, was rich in α-thujone (25.9%), α-humulene (24.9%), β -caryophyllene (12.6%) and viridiflorol (10.5%). Khanavi *et al.,* (2004)reported that both hydrodistilled and steam-distilled essential oils of the aerial parts of *S. byzantina*growing in Iran were rich in sesquiterpenes, such as α-copaene (16.6% and 10.4%), spathulenol (16.1% and 18.5%) and β -caryophyllene (14.3% and13.5%), respectively.

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