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SUMMARY

Purpose

Conventional agriculture/crop production is based on the use of chemical pesticides in order to control plant diseases and pathogens. However, these applications may have adverse effects from the point of end-users, pollinators, inhibition of beneficial predator/parasitioids and helpful microbial communities.

Microbial biocontrol agents/biopesticides have emerged as an alternative to chemical pesticides to solve/prevent these problems. They are marketed as biopesticides, biofertilizers, plant growth stimulators and native immunity supplements. Among these *Trichoderma* based preparations are marketed worldwide for the crop protection against to several plant pathogens also to enhance the yield and growth of the plants.

Microorganisms particularly fungi, produce secondary metabolites which are not essential in primary metabolic processes but have industrial and economic importance. These metabolites are natural compounds which have low molecular weights [<3kDa], produced by microorganisms and plants and related to genus, species and race.

Results

Genus *Trichoderma*, from phylum *Ascomycota* order *Hypocreales* is a rhizo-competent fungus that has a worldwide distribution. *Trichoderma* species are well known to produce secondary metabolites including volatile and non-volatile compounds such as pyrones, terpenoids, steroids and polyketides and a group of antibiotics that are called peptaibiotics which have different antagonistic activities against to pathogens.

Since 1930's the members of this genus are known as biocontrol agents against to plant diseases and widely used in agriculture in both developed and developing countries since 1990's. The pathogens that against to are *Botrytris cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*,

Sclerotium spp., *Phythium ultimum*, *Phytophtora* spp, *Armillaria* spp., *Fusarium oxysporum*, *Verticillium* spp. and *Gauemannomyces graminis*. However, their action mechanisms and potential applications are not known fairly.

Discussion

In order to the compete in commercial market and also to have equivalent to/superior features than chemical formulations, there is a demand for the development of new formulations. Industrial production costs can be fairly reduced by using developed solid and submerged fermentation systems. Using recyclable materials from food production and processing industries or on the low cost substrates such as rice grains, fungal spores can be produced without using developed equipments or qualified workers. These processes can be helpful to the development for challenging to the pathogens and also to support to development of new pesticides and biofertilizers that base on bioactive metabolites.

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Essential Oil Composition of Endemic *Sideritis leptoclada* O. Schwarz& P. H. Davis (Lamiaceae) from Turkey by Using Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry (GCxGC-TOF/MS)

Gürkan SEMİZ[1,*] Mustafa Zafer ÖZEL[2]

[1]Pamukkale University, Faculty of Arts & Sciences, Department of Biology, 20070, Denizli, Turkey

[2]The University of York, Department of Chemistry, Heslington, YO10 5DD, York, U.K.

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Abstract: *Sideritis* genus is present by 46 species in Turkey with high endemism rate (ca. 82%). The chemical composition of essential oil obtained from the aerial parts of endemic *Sideritis leptoclada* O. Schwarz & P. H. Davis was investigated. The chemical composition of *S. leptoclada* from the Southern Turkey is reported for the first time by GCxGC-TOF/MS technique. Among the sixteen constituent representing 96.74% of the *S. leptoclada* oil, major components of *S. leptoclada* were found as α-pinene (24.84%), trans-β-caryophyllene (22.99%), β-pinene (15.14%) and caryophyllene oxide (6.65%). The results were discussed with the genus pattern in means of medicinal purpose and plant essential oils.

Keywords: Essential oil, GCxGC-TOF/MS, monoterpenes, sesquiterpenes, *Sideritis leptoclada*,

1. Introduction

The genus *Sideritis* L. (Lamiaceae) with its nearly 150 species distributed in Northern hemisphere, occuring generally in the Mediterranean area [1-3]. The *Sideritis* name derives from the Greek word 'sideros' (iron) in reference to these vulnerary plants that heal the wounds [4]. Species of this genus, like *Sideritis leptoclada* O. Schwarz & P. H. Davis possess significant pharmacologic as well as economic values. Local people use this plant as herbal tea. *Sideritis* species are mainly named as mountain tea (*dağ çayı* in Turkish) in Turkey and comprises one of the most frequently traded herbs in bazaars. The genus *Sideritis* representing by 46 species and is an important species among the other Lamiaceae genera because the ratio of endemism (*ca*. 80%) in Turkey [5]. *Sideritis* species are frequently used in folk medicine due to their anti-inflammatory, antimicrobial, anti-spasmodic, anti-rheumatic, digestive and diuretic activities [6]. Recently, several studies have been reported on the chemical composition of *Sideritis* oils of different origins [7-12]. However, there are no studies on the *Sideritis* oil were conducted by GCxGC-TOF/MS. GCxGC with TOF/MS is highly desirable for identification and increases

*Corresponding Author E-mail: gsemiz@pau.edu.tr

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sensitivity of volatile compounds. Therefore, the purpose of the this study was to investigate the content and composition of essential oil in the leaves of *Sideritis leptoclada* O. Schwarz & P. H. Davis (Lamiaceae), a local endemic species in Turkey.

2. Material and Methods

2.1. Plant Material

The samples of *S. leptoclada* were collected from different locations from Sandras Mountain-Turkey. Voucher specimens were deposited in the Herbarium of the University of Pamukkale (Denizli, Turkey). Air-dried aerial parts were cut in small pieces (ca.10 mgr) and subjected to GCxGC-TOF/MS.

2.2. Direct Thermal Desorption (DTD) and GCxGC-TOF/MS analysis

The volatile compounds in *S. leptoclada* were analysed using DTD followed by GCXGC-TOF/MS. A GCXGC-TOF/MS system was used with a dual stage commercial thermal desorption injector. This incorporated a thermal desorption unit (TDU) which was connected, using a heated transfer line, to a programmable-temperature vaporisation (PTV) injector, CIS-4 plus (Gerstel, Mulheim an der Ruhr, Germany). The injector was equipped with a Gerstel MPS autosampler. Empty glass thermodesorption tubes were conditioned for 2h before use at a temperature of 400 °C [13]. Approximately 10 mg of *S. leptoclada* was placed into the quartz microfiber filter (QM-A sheets, Whatman, VWR) and loaded into the thermodesorption tubes. To keep the sample in position, glass wool was employed. Initial desorption of the sample was effected by heating the TDU from 40 °C (initial time 0.2 min) to 150 °C at a rate of 120 °C min^{-1} with a final hold time of 5 minutes under 1.5 mL min^{-1} helium flow in splitless mode. Volatile analytes emanating from this were cryo-focused at -40 °C in the CIS which had been cooled by liquid nitrogen prior to injection. The CIS was then heated at a rate of 10 °C s^{-1} to a final temperature of 150 °C. During this CIS temperature ramp, analytes were transferred to the GC column [13].

2.3. Chromatographic Analysis

The GCxGC–TOF/MS system comprised an HP 6890 (Agilent Technologies, Palo Alto, CA, USA) GC and a Pegasus III TOF/MS (Leco, St Joseph, MI, USA). The first column was a non-polar DB5 (30 m x 0.32 mm i.d. x 0.25 μm) and the second column a DB17 (1.9 m x 0.10 mm i.d. x 0.10 μm). Both columns were purchased from J&W Scientific (Folsom, CA, USA). The columns were connected using a press-fit connector [14]. The first dimensional separation is based on separation by volatility, whilst the second dimensional separation is based on separation by polarity [15]. The modulator secondary oven was operated at +15 °C higher than the GC oven temperature. The modulation time was 5 s and helium was employed as a carrier gas. The initial temperature of the first-dimension column was 60 ºC for 1 min; the temperature was then increased at 5 °C min^{-1} to 250 ºC, which was held for a 1 min. The initial temperature of the second-dimension column was 75 ºC for 1 min; the temperature was then raised at 5 °C min^{-1} to 265 ºC and held for a 1 min [16]. TOF/MS with electron-impact ionisation was used to identify peaks. Analytes were identified by employing GC-MS software; according to the NIST mass spectral library, and also by comparing their Kovats retention indices.

3. Results and Discussion

The chemical composition of essential oil from *S. leptoclada*, an endemic species from the Southwestern Anatolia region of Turkey, was studied for the first time using GCXGC-TOF/MS. Table 1 represents the chemical composition of the essential oil from *S. leptoclada*. As can be seen from this table, 16 compounds, representing about 99.99% of the essential oil, were characterized. The major components are as follows: α-pinene (24.84%), trans-β-

caryophyllene (22.99%), β-pinene (15.14%) and caryophyllene oxide (6.65%). Some of the *Sideritis* species of Turkey have been collected and their oils have been analysed by GC-MS techniques [9, 10, 12, 17, 18]. Current literatures showed that α-pinene and β-pinene were already proposed as the main constituents of essential oils from certain other *Sideritis* species such as *S. bilgerena* P. H. Davis (51.2% and 30.2%, respectively), *S. congesta* P. H. Davis et Hub.-Mor. (19.5% and 28.8%, respectively), *S. argyrea* P. H. Davis (16.5% and 23.9%, respectively) and *S. lycia* Boiss. et Heldr. (21.6% and 32.2%, respectively) [8, 11, 18-20]. *Sideritis* species classified as 6 groups; monoterpene, oxygenated monoterpene, sesquiterpene, oxygenated sesquiterpene, diterpene and others [21]. 57% of the *Sideritis* species existing in Turkey belong to the "monoterpene hydrocarbon-rich" group as shown in Tabanca et al. [22]. For our results, *Sideritis leptoclada* is also included in this group. But Başer [23,24] and Kırımer [20] classified *Sideritis* essential oils based on their main components, and *S. leptoclada* was included in the sesquiterpene-rich group, however, its whole essential composition was not presented previously; only the major component was given β-caryophyllene. In fact, *Sideritis* species are not rich in essential oil, but their smell and aroma are pleasant [25]. The percentage of trans-β-caryophyllene was found as 22.99% in the *S. leptoclada* studied. The result is different from the other *Sideritis* species. These differences might have been derived both from sampling time and, climatic/seasonal factors particularly genetical features (different chemotype).

Table 1. Percentage composition of components identified in the leaves of *S. leptoclada.*

No	Compound	RI	Percentage (%)
1	Pentanal	675	0,05
2	(E)-2-Hexenal	827	0,35
3	(E,E)-2,4-Hexadienal	858	0,12
4	Heptanal	879	0,16
5	α-Thujene	924	1,18
6	α-Pinene	933	15,14
7	β-Pinene	972	24,84
8	Limonene	1023	4,25
9	trans-β-Ocimene	1032	1,82
10	cis-Geraniol	1237	3,61
11	α-Terpinyl acetate	1333	2,29
12	D-Longifolene	1400	4,19
13	Trans-β-Caryophyllene	1405	22,99
14	Aromadendrene oxide	1440	4,40
15	α-Bisabolene	1496	4,69
16	Caryophyllene oxide	1573	6,65
	Unknown		3.26
TOTAL			100

4. Conclusions

The results showed that the species was rich by monoterpene constituents than sesquiterpenes. Various factors such as genetic, environmental, physiological and edaphic factors may affect the composition of the essential oil of *S. leptoclada*. *Sideritis* species are of great commercial interest for local people, becuase they collect this species from natural populations and use them in their life and they also sell in local bazaars for healty purposes. However, certain wild species from different environments (under different edaphic, climate and polluted sites) have not yet been studied. The essential oils were described as natural products preventing the growth of pathogens or other organisms in the test systems. Due to increasing demand on this species, further works are necessary to find the efficacy and suitable concentrations of these essential oils in folk use. The results of our work can be provided also useful data for the chemotaxonomy of *Sideritis* species.

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