Chemical Composition and Biological Activities of Essential Oil and Methanol Extract of *Teucrium scordium*

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ABSTRACT: *In this study, the chemical composition of the essential oil of Teucrium scordium was studied using capillary GC and GC/MS instruments. In addition, the antimicrobial and cytotoxic activities of the oil and methanol extract were evaluated by disc diffusion and MTT assays, respectively. Forty-three volatile components were identified from the oil of aerial parts, representing of 98.1% of total oil. The major constituents were trans-α-bergamotene (52.3%), (Z)-α-trans-bergamotol (18.1%), linalool (3.0%) and piperitenone oxide (2.9%). The best anti-bacterial activity was observed for the methanol extract against Staphylococcus epidermidis with ZI (19.0 ± 0.47) mm and also against Proteus mirabilis with MIC value of 1.25 µg/mL. Investigation of the samples on cell viability of HeLa cells showed good activity for the essential oil with an IC_{50} value of 5.2 µg/mL. Our results indicated that Teucrium scordium can be considered for further analyses as an effective and safe curing agent for cancer and pathogenic infection therapies.*

KEYWORDS: *Teucrium scordium; Essential oil; Antimicrobial activity; Cell viability*

INTRODUCTION

International Agency for Research on Cancer (IARC) reported that there are approximately 14.1 million new cases of cancer and 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2013 worldwide [1]. These facts inspired on increasing interest in plants as a source of novel therapeutic agents [2]. Plant-derived phytochemicals have been used as therapeutic agents for hundreds of years [3]. Medicinal plants are rich resources of different bioactive constituents that can be used for the treatment of several

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diseases and inhibition of various enzymes [4-6]. There have been studies on the use of plant products as disease control agents, with less toxicity and fewer environmental effects. Several compounds with anti-cancer activities have been identified which some of them (e.g. vinblastine, vincristine taxol and colehicine), either directly or after chemical modifications, have been used as anti-cancer drugs [7]. Some of these agents in compared to purely synthetic drugs have higher activity and lower toxicity [8].

The genus *Teucrium L.* (Lamiaceae) includes over 300 species commonly known as germanders and widespread all over the continents [9]. The species are mainly spread over central and southern America, Southeast Asia and Mediterranean region [10]. There are approximately 12 native *Teucrium* species in Iran [11]. The extract isolated from *Teucrium* species has been used for treatment of diabetes, obesity, inflammation, hyperlipidemia, and antioxidant, anticancer, and antibacterial activities [12]. Several studies have been performed on chemical compositions and antioxidant activities of the oils isolated from *Teucrium* species [13].

T. scordium is a wild-growing perennial herbaceous flowering plant with little branched stems up to 40 cm height. Leaves are without or with very short petiole, oval-elongated and slightly ineised. Flowers are white to light pink, densely clustered at the top of the branch. This study aims to report the chemical composition of the essential oil as well as the antibacterial and cytotoxicity activity of the oil and methanol extract of *T. scordium* collected from Yazd city, Iran.

EXPERIMENTAL SECTION

Plant material

The aerial parts of *T. scordium* were collected during the flowering stage in May 2014 from Herat of Yazd city, Iran. Their identities were confirmed and Voucher specimen (No. 3128335427131620) was deposited at the Herbarium of Yazd Agricultural and Natural Resources Research Center.

Essential oil isolation

The air-dried aerial parts of *T. scordium* (100 g) were subjected to a steam distillation for 3 h using a clevenger (DURAN, Germany) apparatus. The oil was dried in anhydrous sodium sulphate and stored at 4°C in tightly-closed dark container.

Extraction

Dried aerial parts of the plant (100 g) were powdered and extracted with methanol (2 × 1L) at room temperature for 48 h. The extracts were combined, filtrated through Whatman filter paper and then concentrated using a rotary evaporator (IKA, Germany) under reduced pressure at 40°C. The dried extract was weighed to give the yield of extraction which was expressed in terms of air dried weight of plant materials. Finally, the extract was stored at -20°C to be used for further analyses.

GC and GC/MS analyses

GC analysis of the oil was performed using a Shimadzu 15, a gas chromatograph equipped with a flame ionization detector (FID), a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm) and N_2 as carrier gas (1 mL/min). The column temperature programming was 60 ºC for 3 min, 60 to 220 ºC at the rate of 5 ºC/min and at 220 ºC for 5min. The injector and detector temperatures were 260 °C. The sample was injected in a split mode, using a split ratio of 1:50.

GC-MS analysis was carried out on a Hewlett-Packard 6890/5973 using an HP-5MS column (30 m × 0.25 mm i. d., film thickness 0.25 µm). The GC conditions were performed the same as above. Mass range was scanned from 40 to 300 amu. Retention Indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C_6 to C_{25}) with linear interpolation. The oil components were identified by matching of their mass spectra with Wiley 275 GC-MS library, and also by comparing them with those of authentic compounds or with reported data in the literature [14, 15].

Assessment of antimicrobial activity

Biological materials

All tested microorganisms were obtained from the American Type Culture Collection (ATCC Rockville-MD-USA), Pasteur Institute of Iran and were as follows: *Staphylococcus epidermidis* (ATCC 12228), *Klebsiella pneumoniae* (ATCC 10719), *Staphylococcus saprophyticus* (ATCC 15805) and *Proteus mirabilis* (ATCC 43071).

Culture medium and inocula

The stock cultures of microorganisms used in this study were maintained on plate Nutrient Agar slants

at 4 ºC. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 mL of Nutrient Broth (MERK) and incubated at 37 ºC for 24 h. About 60 µl bacterial suspensions, adjusted to 10^6–10^7CFU/mL were taken and poured into Petri plates containing 6 mL sterilized Nutrient Agar medium (MERK). Bacterial suspensions were spread to get a uniform lawn culture.

Disc diffusion assay

The agar-well diffusion method was applied to detect anti-microbial activity [16]. The *T. scordium* extract was dissolved in DMSO to a final concentration of 40 mg/mL and filtered by 0.45 lm Millipore filters for sterilization using 100 µL of suspension containing 10^8 CFU/mL of bacteria and spread on the Nutrient Agar. The discs (6 mm in diameter) impregnated with 50 µL of the extract solution and essential oil. DMSO (as a negative control) was then placed on the inoculated agar. Gentamicin (30 µg/disc) and Ceftazidime (30 µg/disc) were used as positive controls for bacteria. The inoculated plates were incubated for 17 h at 37 ºC for bacterial strains. The diameter of the inhibition zone was measured in mm. All the assays were performed in triplicate

MIC Broth dilution assay

The lowest concentration of the compounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC). MIC value of the extract of *T. scordium* was evaluated against standard bacterial strains based on the Broth dilution method (MERK) [17]. Briefly, serial doubling dilutions of the extract and Essential oil were prepared in a 96 well microtiter plate ranged in 1000, 500, 250, 125, 62.5 and 31.25 µg/mL. To each well, 100 µL of indicator solution and 95 µL of Mueller Hinton broth were added. Finally, 5 µL of bacterial suspension (1.5×10^6 CFU/mL) was added to each well. The plates were prepared in triplicates.

These bacteria were tested in separate plates. Inoculated plates were incubated at 37°C, and the optical density (OD) of each well was measured at 0, 18h after initiation of incubation using an ELISA reader (BIOTEK) device set at 630 Nanometer. The mean of the OD of different concentrations of each bacterium and solvent was compared and analyzed using unilateral variance analysis (ANOVA). In our statistical analyses, $\alpha = 0.005$ was considered acceptable significant variation and the results were analyzed using SPSS Ver. 16.

Cytotoxicity assay

HeLa cell lines were purchased from National Cell Bank of Iran (NCBI, Pasteur institute, Tehran, Iran). The cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, Steinheim, Germany) supplemented with 10% heat-inactivated fetal calf serum (Biochrom, Berlin, Germany) and 100µg/mL streptomycin and 100u/mL penicillin in humidified 7% CO_2 atmosphere at 37 °C. The cytotoxic activities of the single agents were assessed in monolayer cultures by using MTT assay [18]. HeLa cells were seeded into 96 well plates (Betagen, Iran) at 5000 cells/well in 180 µL of medium and allowed to attach for overnight. The working concentrations of extract and essential oil of *T. scordium* were 40 µg/mL and 100% respectively. The final concentration of DMSO in the highest of applied concentration of extract was 0.2%. After 24 h, the cells were treated with different concentrations of *T. scordium* extracts and essential oil and then incubated for 48 hours at 37°C. Three wells containing tumor cells cultured in 180µl of complete medium were used as controls for cell viability. After incubation, the medium of cells was removed and 100 µL of RPMI 1640 containing 10 µg/µL MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) was added to each well and the plates were incubated for another 3h. The culture medium was then replaced with 50µl of DMSO and the absorbance of each well was measured by using a microplate reader (BioTek, USA) at 570nm. Each set of experiments was independently performed three times. Dose response curves were generated with respect to surviving fraction data and the relationship between dose and effect was analyzed for the 50% inhibitory concentrations (IC_{50}) by computer fitting the dose-response curves with linear and nonlinear regressions.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil of *T. scordium* was obtained from aerial parts of the plant by hydro-distillation. A yield of 0.36 % (w/w) was obtained for the essential oil. Fig. 1 shows the GC-MS chromatogram of the oil.

The essential oil composition was analyzed by GC-FID and GC–MS. The qualitative and quantitative

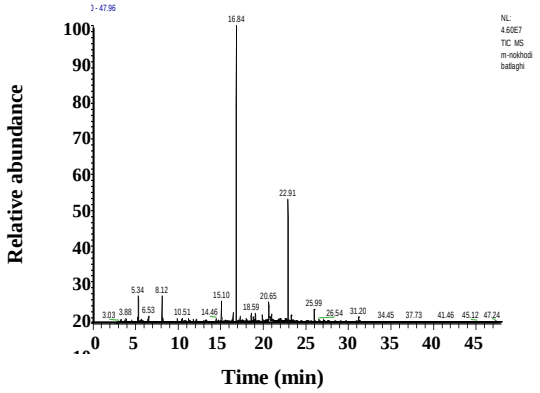

Fig. 1: Gas chromatogram of the essential oil of T. scordium on DB-5 capillary column

essential oil constituents are presented in Table 1, where compounds are listed in order of their elution on the DB-5 column. 43 components were identified from this oil, representing of 97.5 % of the total oil. The most abundant compounds were trans-α-bergamotene (52.3%), (Z)-α-trans-bergamotol (18.1%), linalool (3.0%) and piperitenone oxide (2.9%). The oil of comprised two monoterpene hydrocarbons (0.6%), nine oxygenated monoterpenes (8.7%), seven sesquiterpene hydrocarbons (57.2%), thirteen oxygenated sesquiterpenes (24.9%) and two diterpenoid derivatives (2.2%) as well as nine alkane derivatives (4.2%).

A summary of the previous published data on the main compounds of essential oils in some species of *Teucrium* has been presented in Table 2. The several investigations show that *Teucrium* essential oils were characterized by mono and sesquiterpenes (oxygenated and hydrocarbon) compounds [19, 20]. Sabinene, β-pinene, caryophyllene oxide, germacrene-D, pulegone and β-caryophyllene have almost been reported as the major compounds in the *Teucrium* species. Of which, two compounds pulegone and β-caryophyllene were found as the main constituents of the essential oil of *T. scordium* species.

Antimicrobial activity

In this study, the antimicrobial activity of the methanol extract and essential oil of *T. scordium* was investigated by an *in vitro* assay against both Gram-positive and Gram-negative bacteria. As shown in Table 3, the oil revealed a weak antimicrobial activity against the bacteria strains but the extract exhibited moderate to high antimicrobial effect against all strains tested, with minimum inhibitory concentration (MIC) ranging from 1.25 to 10 µg.mL^{-1} and zone of inhibition (ZI) ranging from 20 to 30 mm. Based on the MIC value, the best antibacterial activity of extract was obtained against *P. mirabilis* with a MIC value of 1.25 µg.mL^{-1}. The lowest ZI (20 mm) was recorded for extract when tested against *S. saprophyticus*. The inhibitory effect of *T. scordium* has been previously reported in the literature, indicating the moderate antibacterial activity on different bacteria strains [10, 42, 43].

Cytotoxicity activity

During this study, the effect of essential oil and methanol extract of the plant on cell viability of HeLa cells was investigated with the MTT assay. Both samples reduced the cell viability of cell line in a dose-dependent manner and the essential oil was more active. The IC_{50} values of oil and extract were 5.2 and 18 µg/mL on HeLa cells, respectively. These samples exhibited moderate cytotoxicity in comparison with the IC_{50} value of paclitaxel (4 nM for HeLa). The cytotoxic activity of the extracts of *T. scordium* has previously been evaluated. Kundakovi et. al studied the cytotoxicity of the cyclohexane and dichloromethane extract of this species on estrogen-dependent breast cancer cell lines. The IC_{50} values of 130 and 189 mg/mL were obtained for the extracts, respectively.[10] Our results suggest that the essential oil as well as the methanol extract of *T. scordium* could be considered for further investigations to evaluate their cytotoxic activity.

CONCLUSIONS

This study was carried out to evaluate the biological properties and chemical composition of *Teucrium scordium*. Our findings showed that *T. scordium* is a rich source of bioactive metabolites specially both type of oxygenated and hydrocarbon sesquiterpens. Also, *T. scordium* exhibited considerable antibacterial and cytotoxic activities, which can be used as natural antimicrobial and anticancer agents for human and infectious diseases.

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Table 1: Chemical composition of the essential oil of aerial parts of T. scordium.

No	Compounds	RI[a]	Concentration (%)
1	2-Hexenal	#	0.3
2	*o*-Xylene	#	0.2
3	*α*-Pinene	935	0.1
4	1-Octen-3-ol	976	2.5
5	*3*-Octanol	994	0.2
6	*n*-Decane	1000	0.1
7	Limonene	1029	0.2
8	1,8-Cineole	1032	0.6
9	Linalool	1100	3.0
10	*n*-Nonanal	1103	0.4
11	Borneol	1171	0.4
12	*α*-Terpineol	1196	0.4
13	*n*-Decanal	1207	0.1
14	Pulegone	1243	0.3
15	Geraniol	1256	0.3
16	Thymol	1296	0.3
17	Piperitenone	1345	0.5
18	Piperitenone oxide	1370	2.9
19	*trans-β*-Damascenone	1387	0.2
20	*β*- Caryophellene	1424	1.3
21	*trans-α*-Bergamotene	1438	52.3
22	(*E*)-β-Farnesene	1455	0.9
23	Undecanoic acid	1465	0.2
24	Germacrene D	1483	0.5
25	*trans-β*-Ionone	1486	0.4
26	*β*-Bisabolene	1507	1.1
27	Cuparene	1518	0.6
28	*δ*-Cadinene	1525	0.5
29	*α*-Copaen-11-ol	1528	1.2
30	Nerolidol	1563	1.0
31	Spathulenol	1580	0.3
32	Caryophyllene oxide	1587	0.5
33	Isoaromadendrene epoxide	1612	1.2
34	α-Cadinol	1647	0.2

Table 1: Chemical composition of the essential oil of aerial parts of T. scordium. (Continued)

35	Valerianol	1656	0.3
36	*β*- Acorenol	1661	0.2
37	*β*-Sinensal	1683	0.4
38	(*Z*)-*α*-*trans*-Bergamotol	1697	18.1
39	(*Z*)-β-Curcumen-12-ol	1715	0.9
40	Hexahydrofarnesyl acetone	1844	1.6
41	Nonadecane	1900	0.2
42	Phytol	2120	0.6
	Monoterpene hydrocarbons		0.3
	Oxygenated monoterpenes		8.7
	Sesquiterpene hydrocarbons		57.2
	Oxygenated sesquiterpenes		24.9
	Diterpenes		2.2
	Alkane derivatives		4.2
	Total identified		97.5

Notes: [a]RI, Retention indices relative to C_8 –C_{28} n-alkanes on DB-5 column. The components are listed in order of elution from the DB-5 column.

Table 2: Comparison of the percentage of main essential oil components from the previous reports of Teucrium species.

Species	Main Components (%)				Ref.
Teucrium africanum Thunb.	*α*-Cubebene (29.3%)	β-Cubebene (20.5%)	Calamanene (4.0%)	Pathoulene (3.7%)	[21]
T. algarbiense (Cout.) Cout.	Limonene (11.8%)	β-Pinene (10.2%)	α-Pinene (8.3%)	Sabinene (7.2%)	[22]
T. arduini L.	β-Caryophyllene (28.8%)	Caryophyllene oxide (14.2%)	Germacrene D (12.5%)	Linalool (3.4%)	[23]
T. carolipaui C. Vicioso ex Pau	α-Cadinol (12.32%)	*t*-Muurolol (12.3%)	β-Eudesmol (10.08%)	-	[24]
T. chamaedrys L.	Germacrene-D (16.5%)	(Z)- β-farnesene (12.2%)	β-Caryophyllene (10.5%)	α-Pinene (9.1%)	[25]
T. flavum L.	β-Caryophyllene (30.7%)	Germacrene-D (21.3%)	α-Humulene (8.4%)	-	[26]
T. flavum subsp. *flavum*	β-Caryophyllene (13.5%)	Caryophyllene oxide (8.5%)	4-Vinyl guaiacol (6.0%)	α-Humulene (5.0%)	[27]
T. fruticans L.	β-Pinene (21.0%)	Germacrene-D (18.0%)	β-Myrcene (13.0%)	β-Caryophyllene (12.0%)	[28]
T. lepicephalum Pau	Sabinene (57.4%)	a-Pinene (19.2%)	Limonene (7.4%)	-	[24]
T. leucocladum Boiss.	Patchouli alcohol (31.2%)	β-Pinene (12.7%)	α-Pinene (11.0%)	t-Cadinol (5.48%)	[29]
T. lusitanicum Schreb.	Elemol (12.0%)	β-Pinene (2.5–11.9%)	Limonene (11.5%)	Sabinene (9.6%)	[22]
T. marum Boiss. & Hausskn.	Isocaryophyllene (20.2%)	β-Bisabolene (14.7%)	β-Sesquiphellandrene (11.3%)	α-Santalene (11.0%)	[30]
T. orientale L. subsp. *orientale*	Caryophyllene oxide (33.5%)	Linalool (17.0%)	β-Caryophyllene (9.3%)	-	[17]
T. orientale L. subsp. *taylorii* (Boiss.) Rech. f.	Linalool (28.6%)	Caryophyllene oxide (15.6%)	3-Octanol (9.6%)	β-Pinene (8.8%)	[31]
T. persicum Boiss.	Caryophyllene oxide (10.6%)	α-Pinene (9.4%)	Geranyl Linalool (7.8%)	γ-Cadinene (7.4%)	[32]
T. persicum Boiss.	α-Cadinene (9.7%)	1,4-Cadinadiene (9.2%),	α-Terpinyl acetate (7.9%)	Linalyl acetate (7.7%)	[12]

Table 2: Comparison of the percentage of main essential oil components from the previous reports of Teucrium species. (Continued)

T. polium subsp. aurasiacum (Maire) Greuter & Burdet	α-Cadinol (46.8%)	3β-Hydroxy-a-muurolene (22.5%)	α-Pinene (9.5%)	β-Pinene (8.3%)	[33]
T. polium subsp. *capitatum* Arcang.	α-Cadinol (46.2%)	Caryophyllene oxide (25.9%)	α-Muurolol epi (8.1%)	Cadalene (3.7%)	[34]
T. polium subsp. *capitatum* Arcang.	α-Pinene (28.8%)	p-Cymene (17.0%)	β-Pinene (7.2%)	-	[35]
T. scordium L.	β-Caryophyllene (22.8%)	(E)-β-Farnesene (10.4%)	Caryophyllene oxide (8.6%)	1,8-Cineole (6.1%)	[36]
T. scordium L.	Pulegone (39.1%)	β-Cryophyllene (20.1%)	β-Farnesene (5.7%)	Menthofuran (4.2%)	[37]
T. scorodonia L. subsp. *scorodonia*	Germacrene B (26.2%)	β-Caryophyllene (25.2%)	α-Cubebene (8.0%)	β-Cubebene (6.5%)	[38]
T. scorodonia L. subsp. *scorodonia*	*(E)*-β-Caryophyllene (25.2%)	a-Cubebene (11.3%)	Germacrene-D (10.1%)	Germacrene B (8.8%)	[39]
T. stocksianum Boiss.	δ-Cadinene (12.9%)	α-Pinene (10.3%)	Myrcene (8.6%)	β-Caryophyllene (8.2%)	[40]
T. stocksianum subsp. *stocksianum*	Camphene (20.6%)	α-Cadinol (19.7%)	β-Myrcene (10.2%)	Carvacrol (9.9%)	[41]
T. trifidum Retz.	β-Cubebene (31.0%)	α-Cubebene (11.4%)	β-Caryophyllene (7.7%)	δ-Cadinene (5.2%)	[21]

Table 3. Antimicrobial activity of the extract of Teucrium scordium.

Microorganism	MIC (µg/mL)			Zone of Inhibition (mm)[b]	
	oil	extract	reference[a]	extract	reference
Klebsiella pneumoniae	>100	10	16	30±0.70	11±0.40
Proteus mirabilis	>100	1.25	16	25±0.47	12±0.23
Staphylococcus epidermidis	>100	2.5	8	23±0.47	19±0.49
Staphylococcus saprophyticus	>100	2.5	8	20±0.47	11±0.94

a) Ampicillin, Tetracycline and Fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively.
b) The values represent the mean of four experiments ± SD. Ampicillin, gentamicin and ketoconazole (10 µg/disc) were used as references

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