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Original Research Article

Chemical composition and cytotoxicity of the essential oil of *Tanacetum abrotanifolium* (L.) Druce (Asteraceae) from Iran

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ABSTRACT

Chemical composition of the essential oil obtained from aerial parts of *Tanacetum abrotanifolium* (L.) Druce (Asteraceae) was investigated. Plant sample was collected at full flowering stage from West Azerbaijan Province of Iran. The hydrodistilled essential oil was analyzed by GC-FID and GC-MS. The major compounds were found to be (*E*)-sesquilavandulol (26.6%), camphor (16.2%) and 1,8-cineole (12.9%). Cytotoxicity activity of the oil was evaluated against four cancer cell lines, of which IC_{50} values estimated to be 312, 312, 625 and 1250 µg oil/mL for the monkey kidney Vero, human breast adenocarcinoma (MCF7), choriocarcinoma (JET 3) and human colon adenocarcinoma (SW480) cell lines, respectively.

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1. Introduction

The genus *Tanacetum* L. (Asteraceae, Anthemideae), commonly known as tansy, includes perennial herbs and subshrubs distributed in the Circum-Mediterranean region, Central, Southwestern, and Eastern Asia and some parts of North America (Sonboli et al., 2012; Shafaghat et al., 2017; Mohammadhosseini et al., 2021). The genus is represented in Iran by 36 species, 18 of which are endemic (Kazemi et al., 2014). *Tanacetum abrotanifloium* (L.) Druce is a perennial and sparsely pilose herb with leafy stems up to 100 cm tall growing in banks and rocky volcanic slopes (Mozaffarian 2008). The flowers of the plant have been traditionally used for the treatment of gastro-intestinal disorders by local people of some Iranian provinces. *Tanacetum* species generally produce essential oils with a high content of monoterpenoids (oxygenated monoterpenes) such as camphor, 1,8-cineole, chrysanthenyl alcohols and esters, thujone and borneol. However, some species exhibited different essential oil profiles. Some oxygenated monoterpenes, *e.g.*, artemisia alcohol (22.8%) and yomogi alcohol (19.4%) were identified as the major compounds in the essential oil of *T. hololeucum* (Bornm.) Podlech (Shamkhani et al., 2016). Moreover, some *Tanacetum* species such as *T. balsamita* L. subsp. *balsamita* and *T. balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson were found to be rich in carvone (Başer et al., 2001; Jaimand and Rezaee 2005).

Furthermore, essential oils of some other *Tanacetum* species have already been analyzed for their chemical composition and biological activities (Ghaderi and Sonboli, 2019). In this connection, the antimicrobial activity, cytotoxicity and composition of the essential oil of *T. balsamita* subsp. *balsamita* have been previously studied and carvone (51.0%) as well as β-thujone (20.8%) were the principal components of

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the essential oil (Yousefzadi et al., 2009). Firozy et al. (2012) investigated the essential oil composition and antioxidant activities of the various extracts of *T. sonbolii* Mozaff and reported α-cadinol (35.3%) and globulol (20.1%) as the major essential oil constituents. The ethyl acetate extract of the plant with high amount of total phenolics (85.0 ± 0.1 µg) exhibited higher antioxidant activity. In another study, antimicrobial property of the essential oil of *T. fisherae* Aitch. & Hemsl. from Iran has been reported. Accordingly, the potent antimicrobial activity of the essential oil has been attributed to the presence of 1,8-cineole (79.9%) as the main component (Rajaei et al., 2011). The essential oil composition and antimicrobial activity of *T. chiliophyllum* (Fisch. & Mey.) Sch.-Bip. var. *monocephalum* Grierson (Polatoğlu et al., 2012a) and two chemotypes of *T. chiliophyllum* var. *chiliophyllum* (Polatoğlu et al., 2012b) together with the compositions, antimicrobial and herbicidal effects of essential oils isolated from *T. aucherianum* Sch.Bip. and *T. chiliophyllum* var. *chiliophyllum* all from Turkey have been characterized, as well (Salamci et al. 2007). In a similar report, composition, insecticidal and other biological activities of the essential oil of flower and stem of *T. abrotanifloium* (L.) Druce from Turkey has been pointed out (Polatoğlu et al. 2015).

The main objectives of the present study were, 1) investigation of the chemical composition of the essential oil obtained from the aerial flowering parts of *T. abrotanifolium* from Iran and 2) evaluation of the cytotoxicity activity of the separated essential oil.

2. Experimental

2.1. Plant material

The aerial parts of *Tanacetum abrotanifolium* (L.) Druce, were collected at the full flowering stage from West Azerbaijan Province, Khoy, Kaput village (35° 50′, 48° 56′ and 2100 m) of Iran and dried at ambient temperature. The plant material was identified by a plant taxonomist (Dr. Ali Sonboli) from Shahid Beheshti University and a voucher specimen (MPH-1250) was deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University of Tehran, Iran.

2.2. Essential oil isolation

The powdered aerial flowering parts (100 g) of the collected plant were subjected to hydrodistillation using a Clevenger type apparatus for 3 h. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until analyzed and tested.

The comparison of the main components of the essential oil of *T. abrotanifolium* from Iran and Turkey has been presented in Table 2. While in the flower essential oil of *T. abrotanifolium* originated from Turkey camphor (35.2%), (*E*)-sesquilavandulol (19.0%) and 1,8-cineole (13.5%) were reported as the principal constituents, hexadecanoic acid (41.8%) and (*E*)-sesquilavandulol (16.2%) characterized as the major components from the stem essential oil (Polatoğlu et al. 2015). As can be seen in Table 2, about 50% of the stem essential oil was composed of saturated fatty acids *i.e.*, hexadecanoic acid (41.8%) and tetradecanoic acid (6.6%). However, no traces of these compounds were detected in the essential oil obtained from the aerial flowering parts of *T. abrotanifolium* originated from Iran. Silphiperfol-6-ene with 4.5% in the essential oil of the Iranian sample of *T. abrotanifolium* has not been detected in Turkish one (Table 2). The outputs of the present study and that of Polatoğlu et al. (2015) indicated the observed differences between essential oil profiles that could be attributed to different plant organs used and the geographical origin of samples.

(*E*)-Sesquilavandulol as the main component of the essential oil of *T. abrotanifolium* has already been characterized in the Turkish *T. argenteum* subsp. *flabellifolium* (Tabanca et al., 2007) and *T. chiliophyllum* var. *chiliophyllum* profiles (Polatoğlu et al., 2012a) along with a wild population of *T. parthenium* (Mirjalili et al., 2007) from Iran (Table 2). Although camphor, 1,8-cineole, thujone and borneol have almost been identified as the major components of the essential oils of different *Tanacetum* species, some deviating species *e.g.*, *T. hololeucum* (Shamkhani et al. 2016) and *T. kotschyi* (Polatoğlu et al., 2011) comprised irregular monoterpenes such as artemisia alcohol, artemisia ketone and yomogi alcohol as the main compounds which are not common in the genus *Tanacetum*. Nevertheless, yomogi alcohol, as a monoterpene alcohol has been reported in *Artemisia feddei* Lev. et Van. essential oil (Hayashi et al., 1968). Also, some rare compounds *i.e.*, fragranol (26.7%) in *T. dumosum* (Jassbi et al., 2013), myroxide (19.8%) in *T. khorassanicum* (Majed-Jabari et al. 2002), β-cadinol (35.3%) in *T. sonbolii* (Firozy et al. 2012), myrcene (39.4%) in *T. vulgare* (Keskitalo et al., 2001), menthyl isovalerate (20.0%) in *T. elbursense* (Habibi et al., 2007) and *epi*-bicyclosesquiphellandrene (31.4%) in *T. densum* subsp. *laxum* (Bagci, 2009) have been found as the major components.

2.3. Essential oil analysis

GC-FID analyses of the obtained volatile oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 µm). Nitrogen was used as the carrier gas at a constant flow-rate of 1.1 mL/min. The split ratio was adjusted at 1/50. The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min. The injector and detector (FID) temperatures were kept at 250 °C and 280 °C, respectively. GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with a quadrupole analyzer on the same column and temperature programming as mentioned for GC-FID analysis. The transfer line temperature was set at 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min, with a split ratio equal to 1/50. The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6-C_{24}) and the oil on a DB-5 column under the same

conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectral library (Adams, 2017) or with authentic compounds.

2.4. Cell line and culture

Four cancer cell lines were used in the present study *i.e.*, human colon adenocarcinoma (SW480), human breast adenocarcinoma (MCF7), choriocarcinoma (JET 3) and monkey kidney (Vero) and all were obtained from Pasteur Institute of Iran. Cells were cultured in RPMI-1640 supplemented with fetal bovine serum (10%, Gibco) and penicillin-streptomycin (1.0%), at 37 ºC, in humidified air containing CO_2 (5%).

2.5. Cytotoxicity assay

Cytotoxicity was assessed by the tetrazolium-based colorimetric assay (MTT), which measures the reduction of the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Roche) into a blue formazan product, mainly by the activity of the mitochondrial enzymes, cytochrome oxidase and succinate dehydrogenase. The experiment was performed according to our published procedure (Sonboli et al., 2014). Cytotoxicity is expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50}). All tests and analyses were run in triplicate and mean values recorded. Cisplatin was used as a reference anticancer drug.

3. Results and Discussion

3.1. Essential oil analysis

The hydrodistillation of the aerial parts of *T. abrotanifolium* gave a yellow oil with a mean yield of 0.3 (w/w%), based on the dry weight of the plant. Thirty-five components were identified representing 97.6% of the total oil. The qualitative and quantitative essential oil compositions are presented in Table 1, where compounds are listed in order of their elution on the DB-5 column. The major compounds were found to be (*E*)-sesquilavandulol (26.6%), camphor (16.2%), 1,8-cineole (12.9%), α-bisabolol (5.5%) and camphene (4.7%). The classification of the identified constituents based on functional groups is summarized at the end of Table 1. The oxygenated monoterpenes were found to be the principal group of natural compounds constituting 38.5% of the total oil. Oxygenated sesquiterpenes, monoterpene and sesquiterpene hydrocarbons represented 38.3, 16.7 and 4.1% of the oil composition, respectively.

The comparison of the main components of the essential oil of *T. abrotanifolium* from Iran and Turkey has been presented in Table 2. While in the flower essential oil of *T. abrotanifolium* originated from Turkey camphor (35.2%), (*E*)-sesquilavandulol (19.0%) and 1,8-cineole (13.5%) were reported as the principal constituents, hexadecanoic acid (41.8%) and (*E*)-sesquilavandulol (16.2%) characterized as the major components from the stem essential oil (Polatoğlu et al. 2015). As can be seen in Table 2, about 50% of the stem essential oil was composed of saturated fatty acids *i.e.*, hexadecanoic acid (41.8%) and tetradecanoic acid (6.6%). However, no traces of these compounds were detected in the essential oil obtained from the aerial flowering parts of *T. abrotanifolium* originated from Iran. Silphiperfol-6-ene with 4.5% in the essential oil of the Iranian sample of *T. abrotanifolium* has not been detected in Turkish one (Table 2). The outputs of the present study and that of Polatoğlu et al. (2015) indicated the observed differences between essential oil profiles that could be attributed to different plant organs used and the geographical origin of samples.

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3.2. Cytotoxicity activity

The growth of all four studied cell lines (human and monkey), *i.e.*, human colon adenocarcinoma (SW480), breast adenocarcinoma (MCF7), and choriocarcinoma (JET 3), as well as monkey kidney (Vero) cells, was inhibited in a dose-related manner after 24 h of exposure to the essential oil of *T. abrotanifolium* (Table 3). IC_{50} values estimated to be 312, 312, 625 and 1250 µg oil/mL, respectively, for Vero, SW480, MCF7, and JET 3 cell lines. According to the obtained results, lower cytotoxicity activity of the essential oil compared to the reference anticancer drug, cisplatin was expected. As can be seen in Table 3, cisplatin remarkably inhibited the growth of the studied cancer cell lines with lower IC_{50} values compared to the essential oil. The *in vitro* study of the essential oil of *T. balsamita* subsp. *balsamita* based on the MTT cytotoxicity assay on human fetal skin fibroblast (HFSF) and monkey kidney

Table 1
Percentage composition of the essential oil of *Tanacetum abrotanifolium*.

Sr. No.	Component	RI	%	Identification methods
1	santolina triene	905	0.2	RI, MS
2	α-thujene	929	0.8	RI, MS
3	α-pinene	939	2.5	RI, MS
4	camphene	956	4.7	RI, MS
5	sabinene	979	0.7	RI, MS
6	β-pinene	985	1.3	RI, MS
7	dehydro-1,8-cineole	996	0.1	RI, MS
8	p-cymene	1027	2.6	RI, MS
9	limonene	1033	3.7	RI, MS
10	1,8-cineole	1036	12.9	RI, MS
11	γ-terpinen	1061	0.2	RI, MS
12	*cis*-sabinene hydrate	1070	0.2	RI, MS
13	linalool	1098	0.7	RI, MS
14	*trans-p*-mentha-2.8-dien-1-ol	1125	0.3	RI, MS
15	*cis-p*-mentha-2.8-dien-1-ol	1139	0.1	RI, MS
16	camphor	1154	16.2	RI, MS
17	terpinen-4-ol	1183	1.8	RI, MS
18	α-terpineol	1195	1.2	RI, MS
19	silphiperfol-5-ene	1340	0.5	RI, MS
20	silphiperfol-6-ene	1360	4.5	RI, MS
21	α-copaene	1390	0.4	RI, MS
22	α-patchoulene	1400	0.5	RI, MS
23	(*E*)-caryophylene	1438	0.9	RI, MS
24	α-humulene	1472	0.1	RI, MS
25	α-selinene	1490	0.6	RI, MS
26	germacrene D	1498	1.3	RI, MS
27	bicyclogermacrene	1514	0.2	RI, MS
28	δ-cadinene	1536	0.1	RI, MS
29	spathulenol	1597	1.6	RI, MS
30	caryophyllene oxide	1605	1.3	RI, MS
31	(*E*)-sesquilavandulol	1638	26.6	RI, MS
32	α-bisabolol	1694	5.5	RI, MS
33	(*E*)-sesquilavandulyl acetate	1738	0.7	RI, MS
34	(*Z*)-sesquilavandulyl acetate	1829	0.4	RI, MS
35	(*Z*)-nerolidol	1870	2.2	RI, MS
Monoterpene hydrocarbons			**16.7**	
Oxygenated monoterpenes			**38.5**	
Sesquiterpene hydrocarbons			**4.1**	
Oxygenated Sesquiterpenes			**38.3**	
Total			**97.6**	

RI, Retention index; MS, mass spectrum

Table 2
Comparison of the main components (%) of the essential oil of *T. abrotanifolium* from Iran and Turkey.

No.	Component	Turkey		Iran
		Flower	Stem	Aerial part
1	Camphene	3.1	0.4	4.7
2	Limonene	0.4	-	3.7
3	1,8-Cineole	13.5	4.5	12.9
4	Camphor	35.2	2.2	16.2
5	Silphiperfol-6-ene	-	-	4.5
6	(*E*)-Sesquilavandulol	19	16.2	26.6
7	α-Bisabolol	0.7	0.8	5.5
8	Phytol	tr	4.9	-
9	Tetradecanoic acid	tr	6.6	-
10	Hexadecanoic acid	0.6	41.8	-

Table 3
Cytotoxicity activity of *T. abrotanifolium* essential oil from Iran.

Cell lines	IC_{50} (µg/mL)[a]	
	essential oil	Cisplatin[b]
Human breast cancer (MCF7)	312 ± 5.8	6.001
Monkey kidney (Vero)	312 ± 7.1	36
Choriocarcinoma (JET 3)	625 ± 7.4	-
Human colon adenocarcinoma (SW480)	1250 ± 9.4	4.8

[a] Values are expressed as the mean ± S.D.
[b] Cisplatin was used as a reference drug.

(Vero) cell lines showed IC_{50} values of 2500 and 1250 µg/mL, respectively (Yousefzadi et al., 2009). In another study, cytotoxicity of the essential oil of *Sclerorhachis leptoclada* Rech. f. was evaluated and the best inhibitory result with IC_{50} value of 312 µg oil/mL reported against the Vero cell line.

4. Concluding remarks

Nowadays, there is an increased demand for finding valuable natural compounds more specifically plant-derived essential oils and extracts that exhibit promising and therapeutic anti-cancer properties. It is always anticipated that fewer side effects compared to chemotherapy synthetic drugs are the advantage of these herbal medicines. To realize the potential of medicinal plants, therefore, the first step is to identify the constituents of essential oils and extracts, and the next plan is to investigate their potential biological effects. In this research, the composition and cytotoxicity activity of the essential oil of *Tanacetum abrotanifolium* from Iran were determined. However, further phytochemical analysis, identification of active compounds, and their applications in the health, pharmaceutical and pharmacological fields could be suggested for future investigations.

Conflict of interest

The authors declare that there is no conflict of interest.

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