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RESEARCH ARTICLE

Chemical composition and antimicrobial activity of *Tanacetum tomentellum* (Boiss.) Grierson essential oil from Turkey

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Abstract

The chemical composition of essential oil obtained by hydrodistillation from the dried aerial parts of *Tanacetum tomentellum* (Boiss.) Grierson (Asteraceae) was analysed by GC-FID and GC-MS. Eighty-eight compounds, constituting about 84.1% of the total oil, were identified. The main constituents were camphor (9.4%), linalool (7.6%), *α*-terpineol (7.1%), *trans*-pinocarveol (5.3%) and *trans*-verbenol (4.5%). The oil was evaluated for antimicrobial and antimalarial activity. The oil showed antifungal activity against *Cryptococcus neoformans* with an IC_{50} value of 45 μg/mL, while it showed no antimicrobial activity against other tested microorganisms (*Candida albicans*, *Aspergillus fumigatus, Staphylococcus aureus* methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa* and *Mycobacterium intracellulare*) up to a concentration of 200 μg/mL. No antimalarial activity was observed against chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum* up to 15.9 μg/mL. We report for the first time the essential oil composition and biological activity of *T. tomentellum*.

Keywords: *Tanacetum tomentellum*, antimicrobial, antimalarial, GC-FID, GC-MS

Introduction

Emerging infectious diseases are substantially threat to global human health. Increasing population, poor sanitation, ecological changes, travel and threats could increase the spread of infections (Bueno, 2015; Coker et al., 2011; Haines et al., 2006; Daszak, Cunningham & Hyatt, 2000; Martinez, 2000). The emergence of multiple drug resistance in *Staphylococcus aureus,* methicillin resistant *S. aureus* (MRSA), *Streptococcus pneumoniae*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhi, Enterococcus faecalis*, *Klebsiella pneumoniae* and *Plasmodium falciparum* is of growing a major concern (Fauci & Morens, 2012; Lepelletier, Andremont & Grandbastien, 2011; Tängdén et al., 2010; McManus & Keylley, 2005; Byarugaba, 2004; White, 2004). The threat of emerging vector-borne diseases can also cause severe human morbidity and mortality (Benelli & Mehlhorn, 2016). The recent outbreak of Zika virus has issued alarms worldwide (Benelli & Mehlhorn, 2016; Samarasekera U & Triunfol, 2016). Discovery of potent, safe and new antimicrobial agents from plant extracts are urged to be encouraged (Ud-Daula et al., 2016; Tabanca et al., 2015; Demirci et al., 2015; Sadgrove, Greatrex & Jones, 2015; Krist et al., 2015; Stappen et al., 2015a; 2015b; 2015c; Kaczmarczyk et al., 2015; Stappen et al., 2014; Ghosh et al., 2014; Al-Rehaily et al., 2014; Iscan et al., 2012; Ozek et al., 2010; Kurkcuoglu et al., 2010). Furthermore, the effective and safe vector control tools and strategies are

also required by new research activities (Benelli&Mehlhorn, 2016; Samarasekera U. & Triunfol, 2016; Hemingway et al., 2006). To contribute to these studies, we investigated the chemical composition and antimicrobial and antimalarial activity of essential oil of *Tanacetum tomentellum* (Boiss.) Grierson (Asteraceae) from Turkey.

Materials and Methods

Plant Material

The aerial parts of *T. tomentellum* were collected during flowering from Sirnak: Senova-Hakkari, southeast of Turkey at an altitude of 1550 m in July. The voucher specimen has been deposited at the Herbarium in the Gazi University, Faculty of Science, Ankara, Turkey (Voucher specimen no: ZA8188).

Isolation of the Essential Oil

The air dried plant materials (flowers, leaves, and stems) were hydrodistilled for 3 hours using a Clevenger-type apparatus. The resulting oil was stored at 4 °C until the analysis. The oil yield was calculated as 0.38%, v/w on dry weight basis.

Gas Chromatography Analysis Conditions

Essential oil was analysed by GC using a Hewlett Packard 6890 system (SEM Ltd, Istanbul, Turkey) and an HP Innowax FSC column (60 m x 0.25 mm Ø, with 0.25 µm film thickness) was used with nitrogen at 1 mL/min. Initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220 °C, then kept constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage compositions of the individual components were obtained from electronic integration using flame ionization detection (FID, 250 °C). Relative percentages of the separated compounds were calculated from FID chromatograms as cited in Table 1.

Gas Chromatography-Mass Spectrometry Analysis Conditions

GC-MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd, Istanbul, Turkey) and Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 425.

Identification of Components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes (Adams, 2011; Curves et al., 1985; Wang & Sun, 1987). The fragmentation patterns of the mass spectra were compared with the Wiley (McLafferty & Stauffer, 1989), MassFinder 3 (König, Joulain, & Hochmuth, 2004), in-house “Baser Library of Essential Oil Constituents” and as well as MS literature data (Jennings & Shibamoto, 1980; Joulain & König, 1998; ESO 2000, 1999).

Antimicrobial Activity

The modified Clinical and Laboratory Standards Institute (NCCLS) methods as described earlier (Tabanca et al., 2003; Tabanca et al., 2005) were followed for this study. Ciprofloxacin (ICN Biomedicals; ≥ 98%) for bacteria and amphotericin B (ICN Biomedicals; ≥ 98%) for fungi were used as positive controls. The tested organisms were from the American Type Culture Collection (ATCC): *Candida albicans* (ATCC 90028), *Aspergillus fumigatus* (ATCC 90906), *Cryptococcus neoformans* (ATCC 90113), *Staphylococcus aureus* (ATCC

29213), methicillin-resistant *S. aureus* (ATCC 33591), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068).

Antimalarial Activity

The antimalarial activity against two *Plasmodium falciparum* strains, D6 (chloroquine sensitive) and W2 (chloroquine resistant), was determined using parasitic LDH assay as described earlier (Tabanca et al., 2003; Tabanca et al., 2005). Chloroquine (Sigma-Aldrich; ≥ 98%) and artemisinin (Sigma-Aldrich; ≥ 98%) were used as positive controls.

Results and Discussion

Essential oil was obtained by hydrodistillation from air dried aerial parts of *T. tomentellum.* The oil was subsequently analyzed by GC and GC-MS and the individual identified components with their relative percentages are given in Table 1. Camphor (9.4%), linalool (7.6%), *α*-terpineol (7.1%), *trans*-pinocarveol (5.3%) and *trans*-verbenol (4.5%) were identified as the main components of *T. tomentellum*. The essential oil of this species was also found to be rich in oxygenated monoterpenes, and total 88 components were characterized with a sum of 84.1%.

Table 1. The Composition of *T. tomentellum* Essential Oil

RRI[a]	Compound	%[b]
1032	α-Pinene	0.1
1076	Camphene	<0.01
1118	β-Pinene	0.1
1132	Sabinene	0.1
1176	α-Phellandrene	0.2
1203	Limonene	1.0
1213	1,8-Cineole	2.1
1255	γ-Terpinene	0.1
1280	*p*-Cymene	0.6
1285	Isoamyl isovalerate	0.3
1286	2-Methyl butyl 2-methyl butyrate	0.1
1290	Terpinolene	<0.01
1300	Tridecane	0.2
1348	6-Methyl-5-hepten-2-one	0.1
1439	γ-Campholene aldehyde	0.2
1450	*trans*-Linalool oxide (Furanoid)	<0.01
1452	α,*p*-Dimethylstyrene	0.1
1452	1-Octen-3-ol	0.2
1467	6-Methyl-5-hepten-2-ol	0.4
1468	*trans*-1,2-Limonene epoxide	0.2
1474	*trans*-Sabinene hydrate	0.8
1480	Nerol oxide	0.2
1487	Isoneroloxide-I	0.5
1497	α-Copaene	0.2
1499	α-Campholene aldehyde	1.3
1532	Camphor	9.4
1535	β-Bourbonene	0.1
1553	Linalool	7.6
1556	*cis*-Sabinene hydrate	0.6
1562	Isopinocamphone	0.1
1571	*trans*-*p*-Menth-2-en-1-ol	0.2

1574	Menthyl acetate	0.4
1586	Pinocarvone	2.8
1591	Bornyl acetate	0.1
1600	β-Elemene	0.3
1611	Terpinen-4-ol	1.0
1612	β-Caryophyllene	0.2
1639	*trans*-*p*-Mentha-2,8-dien-1-ol	3.3
1648	Myrtenal	0.5
1663	*cis*-Verbenol	0.5
1670	*trans*-Pinocarveol	5.3
1678	*cis*-*p*-Mentha-2,8-dien-1-ol	1.2
1682	γ-Terpineol	0.1
1683	*trans*-Verbenol	4.5
1700	*p*-Mentha-1,8-dien-4-ol (=Limonen-4-ol)	0.3
1706	α-Terpineol	7.1
1719	Borneol	0.8
1725	Verbenone	0.6
1726	Germacrene D	1.2
1738	*p*-mentha-1,5-dien-8-ol	0.3
1742	β-Selinene	0.4
1744	α-Selinene	0.3
1751	Carvone	1.2
1755	Bicyclogermacrene	0.4
1758	*cis*-Piperitol	0.4
1773	δ-Cadinene	0.8
1797	*p*-Methyl acetophenone	0.2
1802	Cumin aldehyde	0.3
1804	Myrtenol	0.9
1811	*trans*-*p*-Mentha-1(7),8-dien-2-ol	3.0
1823	*p*-Mentha-1(7),5-dien-2-ol	0.3
1845	*trans*-Carveol	2.2
1864	*p*-Cymen-8-ol	0.6
1871	Neryl isovalerate	0.3
1882	*cis*-Carveol	0.4
1896	*cis*-*p*-Mentha-1(7),8-diene-2-ol	3.1
1900	*epi*-Cubebol	0.1
2007	*p*-Mentha-1,8-dien-10-ol	0.2
2008	Caryophyllene oxide	0.3
2029	Perilla alcohol	0.3
2030	Methyl eugenol	0.1
2069	Germacrene D-4β-ol	0.4
2092	β-Oplopenone	0.2
2100	Heneicosane	0.3
2144	Spathulenol	3.0
2187	T-Cadinol	0.5
2209	T-Muurolol	0.6
2219	δ-Cadinol	0.2
2239	Carvacrol	0.4
2245	Elemicine	0.2
2247	*trans*-α-Bergamotol	0.2
2255	α-Cadinol	1.1
2300	Tricosane	0.7
2384	Hexadecanol	0.6

2500	Pentacosane	1.0
2607	1-Octadecanol	0.7
2622	Phytol	0.1
2700	Heptacosane	0.5
	Total	**84.1**

[a]RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data

T. tomentellum essential oil was evaluated for antimicrobial activity against human pathogenic bacteria, filamentous fungi, and yeasts in addition to antimalarial activity. No antimicrobial activity was observed at the highest test concentration of 200 μg/mL against *Candida albicans*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRS), *Mycobacterium intracellulare*, *Pseudomonas aeruginosa* and *Aspergillus fumigatus*. The oil only demonstrated mild activity against *Cryptococcus neoformans* with an IC_{50} value of 45 μg/mL where the positive control, amphotericin B, exhibited an IC_{50} of 0.70 μg/mL (Table 2). *T. tomentellum* essential oil showed no antimalarial activity against *P. falciparum* D6 or W2 clone up to 15.9 μg/mL.

Table 2. Antimicrobial activity of *T. tomentellum* essential oil

	IC_{50} (μg/mL)[1]						
Sample	***C. albicans***	***C. neoformans***	***S. aureus***	**MRSA**	***P. aeruginosa***	***M. intracellulare***	***A. fumigatus***
T. tomentellum oil	-	45	-	-	-		-
Ciprofloxacin	NT	NT	0.06	0.06	0.04	0.15	NT
Amphotericin B	0.40	0.70	NT	NT	NT		NT

"-"= inactive at the highest dose of 200 μg/mL; [1]IC_{50}= The concentration (μg/mL) that affords 50% inhibition of growth; NT = Not tested; Ciprofloxacin and Amphotericin B= positive controls

In our study, *T. tomentellum* essential oil showed very poor antimicrobial activity and no antimalarial activity. It appears that the major compounds may not be responsible for the activity or the quantity of these compounds may not be enough to generate the biological activity. In a previous study, (+)-camphor and (-)-camphor were reported to demonstrate insignificant antimicrobial activity on *C. albicans*, however, combination of 1,8-cineole and (-)-camphor produced greater inhibitory effect against *C. albicans* (Viljoen et al., 2003). To the best of our knowledge, this is the first published report describing the chemical composition and biological activity of *T. tomentellum* essential oil. In conclusion, since medicinal and aromatic plants are gaining much interest as antimicrobial agents, the research into the chemistry of plant extracts and responsible active compounds should be encouraged.

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