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Chemical composition and antimicrobial activity of essential oil from *Pyrethrum pulchrum* Ledeb.

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ABSTRACT

The chemical composition and antimicrobial activity of the essential oil from the aerial parts of *Pyrethrum pulchrum* Ledeb. were investigated. Dried plant material was hydro-distillated yielding 0.1% of essential oil. The oil was analyzed by GC-MS techniques. Fifty-five compounds were identified representing 99.7% of the total oil composition. Camphor was the predominant compound (33.9%) followed by linalool (21.1%) and α-pinene (9.0%). The antimicrobial activity of the oil was determined using the disk diffusion method against Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Enterococcus faecalis*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), *Mycobacterium vaccae* and fungi (*Candida albicans, Sporidiobolus salmonicolor* and *Penicillum notatum*). The essential oil of *P. pulchrum* displays an intermediate activity against selected bacteria.

Keywords: Asteraceae, *Pyrethrum pulchrum*, *Tanacetum*, essential oil, camphor

INTRODUCTION

The Asteraceae also known as Compositae or sunflower family, comprises of the largest family of flowering plants with over 1900 genera and ca. 40000 species [1]. The genus *Tanacetum* Ledeb., formerly *Pyrethrum Zinn*, is a large, poorly defined classification group in the Asteraceae containing 150-200 species distributed over West Asia and Europe [2, 3]. Many species of this genera have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically active compounds [4]. Moreover, *Tanacetum* has been used as medicinal plants for over 2000 years [5] and it is a known remedy for the treatment of many diseases, including women's ailments, psoriasis, toothache, insect bites, rheumatism, asthma, vertigo and migraine prophylaxis [6, 7], while *Pyrethrum* is used for treating various inflammatory disorders, wound, anthrax, bone fractures as well as reducing fever [8, 9].

The strong and aromatic odor of the *Tanacetum* species is mainly due to high concentrations of volatile terpene constituents in their essential oil, especially in their leaves and flowers [10]. Camphor, 1,8-cineole, α-thujone, carvone, thymol, *trans*-sabinyl acetate, borneol, caryophyllene oxide, (E)-myroxide, sabinene, bornyl acetate, isopulegone, artemisia ketone, limonene, and camphene were identified as the main constituents of *Tanacetum* species essential oils [3,11]. It is well known, this genus is also found to contain sesquiterpene lactones, a large group of molecules with several biological activity [11]. Several studies have shown that essential oils and extracts of the genus *Tanacetum* exhibit anti-inflammatory, anticancer, antibacterial, antifungal, anthelmintic, insecticidal and antiprotozoal effects [12-14].

According to literature, six species present in Mongolia that belong to two different genera of *Tanacetum* and *Pyrethrum*, namely *Tanacetum tanacetoides, T. vulgare, Pyrethrum pulchrum, P. lanuginosum, P. alatavicum and P. changaicum* [15]. *P. pulchrum* is

an impressive flowering plant with about 15-45 cm in height, growing on large-stoned screes at the snow line, usually on glacier moraines in the mountain regions. To our knowledge, no previous studies have been reported neither on the phytochemical analysis of *P. pulchrum* nor on its biological activity. This is the first report on the chemical composition and antimicrobial activity of the essential oil from *P. pulchrum* growing in Mongolia.

EXPERIMENTAL

Plant material: Aerial parts of *P. pulchrum* were collected in the Mongolian Altai mountain region of Govi-Altai province, in July 2012. Plants were dried in shade at ambient temperature. Plant material was authenticated by Prof., ScD, Ch.Sanchir, Institute of General and Experimental Biology of the Mongolian Academy of Sciences.

Isolation of the essential oil: For essential oil extraction, 25 grams of milled plant material were extracted for 2 h in a Clevenger apparatus using hydro-distillation process.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: The oil was analyzed using a ThermoQuest Trace gas chromatography mass spectrometry system (TRACE 2000 series, Thermo Finnigan, Bremen, Germany) equipped with a ZB-5 capillary column (15 m×0.25 mm, film thickness 0.25 μm, Pheno menex, Torrance, USA). The essential oil was diluted in *n*-hexane at a ratio of 1 to 10. The samples were then injected and separated under programmed conditions to achieve complete separation of the essential oil. The oven temperature was kept at 40 ºC for 2 min and then ramped up to 280 ºC at 10 ºC min^{-1} followed by a heating to 320 ºC with a rate of 30 ºC min^{-1}. The injector temperature was set to 220 ºC and helium served as carrier gas with a flow rate of 1.5 mL min^{-1} and a split ratio of 1:10. Mass spectra were recorded from 33-401 Da (m/z) in full scan mode representing the total ion current chromatogram (TIC, Figure 1).

The relative composition of the essential oil was calculated based on GC peak area. Series of C_8-C_{40} of *n*-alkanes were injected for the calculation of retention indices (RI) of individual components [16]. Proposals for the identity of the separated compounds were obtained by matching their recorded mass spectra and RIs with library mass spectra and RIs from NIST, Adams, and Massfinder databases [17-19].

Antimicrobial activity assay: *P. pulchrum*, essential oil antimicrobial activity was investigated against Gram-negative bacteria, Gram-positive bacteria and fungi in accordance with reports by Krieg *et al*. [20].

In essence: *Bacillus subtilis* (6633), *Staphylococcus aureus* (134/94 MRSA; 511), *Escherichia coli* (458), *Enterococcus faecalis* (1528, VRSA), *Pseudomonas aeruginosa* (SG137, K799/6) and *Mycobacterium vaccae* (10670) were cultivated on standard I nutrient agar (NA I) in Petri dishes at 37 ºC. Antifungal bioassays were conducted on yeast morphology agar (YMA) at 37 ºC for *C. albicans* and on malt agar (MA) at 30 ºC against *Sporobolomyces salmonicolor* (549) and *Penicillium notatum*. After inoculation, a disc (9 mm in diameter) was removed from the center of the Petri dish and 50 μL of the test solution was added to the cavity. After 18 h of incubation at the respective temperatures the inhibiting areola were measured. Ciprofloxacin (5 μg·ml^{-1} in deionized water) and amphotericin B (10 μg·ml^{-1} in DMSO/MeOH 1:1) were used as reference compounds against bacterial and fungal strains, respectively.

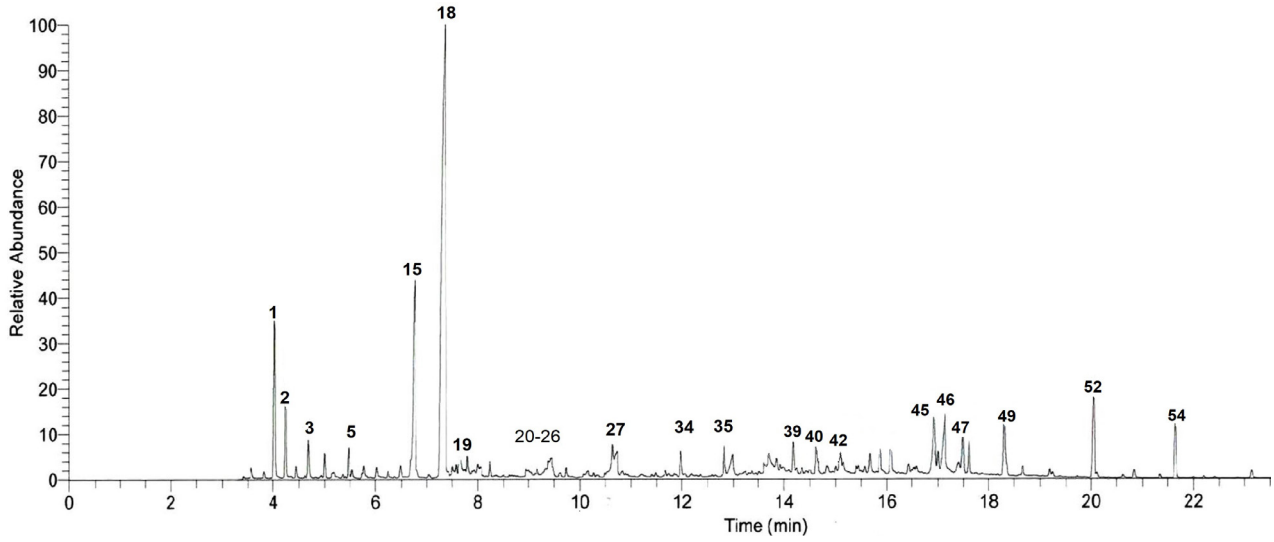

Fig. 1. Typical TIC-GC/MS chromatogram of *P. pulchrum* oil separated on a semi - polar ZB - 5 capillary column. Compounds are listed by their peak number in Table 1.

RESULTS AND DISCUSSION

The yield of essential oil obtained by hydro-distillation from aerial parts of *P. pulchrum* was 0.1%. The literature data indicate that the essential oil content in the genus *Tanacetum* ranges between 0.1% to 6.98% [5, 12, 21]. Our result is close to those reported for *T. audibertii* (0.1%) [11] and *T. chilliophyllum* (0.06-0.16%) [22]. The general chemical profiling of the essential oils, the identity and percentage content of the individual components are summarized in Table 1. The GC-MS analyses of *P. pulchrum* essential oil provided the separation of 55 components, representing 99.7% of the oil. In particular, camphor (33.9%), linalool (21.1%), α-pinene (9.0%), (Z)-γ-curcumyl 2-methylbutyrate (4.8%), pentylcurcume (3.2%), camphene (2.9%), tricosane (2.8%) and nerolidol (2.6%) were present as the main constituents.

Generally, essential oils are comprised of two or three major components in relatively high concentrations (20-95%) and other components present in trace levels. On the other hand, quantity and quality of essential oil composition depended considerably on the type of variety, growth stage, time of collection and climatic conditions of the habitat. Table 2 shows a comparison between the major components of *P. pulchrum* essential oil and some species of *Tanacetum*. A comparison of the data presented in this paper with those in the literature for other species of *Tanacetum* shows that there are qualitative and quantitative differences in the levels of some of the compounds present. Our results indicate that the camphor, linalool and α-pinene are three major compounds of *P. pulchrum* essential oil.

Table 1. Constituents of the essential oil from *Pyrethrum pulchrum*

No	$RI_{exp.}$	$RI_{db.}$	Compound	RPA, %
1	928	932	α-Pinenez	9.0
2	942	946	Camphene	2.85
3	971	974	β-Pinene	1.40
4	990	981	2-Pentylfuran	0.40
5	1001	1002	α-Phellandrene	1.03
6	1003	1004	2-methyl-, 2-methylpropyl ester	0.14
7	1013	1014	α-Terpinene	0.18
8	1022	1020	*p*-Cymene	0.64
9	1025	1025	Limonene	0.31
10	1038	1032	(*E*)-β-Ocimene	0.27
11	1044	1036	Benzene acetaldehyde	0.14
12	1057	1054	γ-Terpinene	0.22
13	1072	1067	*cis*-Linalool oxide	0.15
14	1087	1084	*trans*-Linalool oxide	0.52
15	1100	1095	Linalool	21.12
16	1103	1100	Methyl butyl-2-methyl butyrate-2	0.61
17	1108	1103	Methyl butyl isovalerate-2	0.19
18	1139	1141	Camphor	33.94
19	1162	1165	Borneol	0.67
20	1175	1174	Terpinen-4-ol	0.43
21	1190	1186	α-Terpineol	0.18
22	1235	1232	Thymol, methyl ether	0.05
23	1243	1244	Geranial	0.06
24	1295	1289	Thymol	0.16
25	1317	1315	(2E,4E)-Decadienal	0.08
26	1358	1356	Eugenol	0.01
27	1375	1373	*n*-Decanoic acid	1.06
28	1382	1379	Geranyl acetate	0.38
29	1415	1421	(*E*)-β-Caryophyllene	0.08
30	1455	1454	(*E*)-β-Farnesene	0.24
31	1467	1466	Dehydrosesquicineol	0.17
32	1472	1474	β-Chamigrene	0.01
33	1477	1484	Germacrene D	0.07
34	1492	1494	α-Selinene	1.80
35	1563	1561	(*E*)-Nerolidol	2.63
36	1579	1582	Caryophyllene oxide	0.25
37	1623	1626	Benzophenone	0.06
38	1634	1639	allo-Aromadendrene epoxide	0.10
39	1683	1673	α-Bisabolol	0.70
40	1726	1730	Chamazulene	0.52
41	1738	1742	(2*E*,6*E*)-Farnesol	0.07
42	1747	1741	α-Cyperone	0.54
43	1845	1844	2-Pentadecanone, 6,10,14-trimethyl	0.86
44	1926	1821	Methyl hexadecanoate	0.17
45	1952	1951	Pentylcurcumene	3.21
46	2010	2011	(*Z*)-γ-Curcumyl 2-methylbutyrate	4.82
47	2024	1701	Arglabin	0.57
48	2091	2095	Methyl linoleate	0.02
49	2098	2100	Heneicosane	1.24
50	2139	2132	Linoleic acid	0.78
51	2199	2200	Docosane	0.08
52	2299	2300	Tricosane	2.78
53	2400	2400	Tetracosane	0.15
54	2499	2500	Pentacosane	1.36
55	2699	2700	Heptacosane	0.20
			SUM	**99.67**

Retention indices (RI) measured on a ZB-5 column; exp: experimental values, db: values from databases; RPA: relative peak area.

Table 2. Major components of essential oils from *P. pulchrum* and some species of *Tanacetum*

Species	Major constituents	References
P. pulchrum	camphor - 33.9%, linalool - 21.1%, α-pinene - 9.0%	our result
T. parthenium	camphor - 45.1%, chrysanthenyl acetate - 21.5%, camphene - 9.6%	[23]
T. parthenium	camphor - 53.8%, *trans*-β-farnesene - 8.3%	[24]
T. punctatum	camphor - 45.5%, *trans*-β-farnesene - 7.4%	[24]
T. chiliophyllum	camphor - 32.5%, 1,8-cineol - 16.1%	[14]
T. armenum	camphor - 27%, 1,8-cineol - 11%	[25]
T. vulgare	camphor - 22.3-41.4%, 1,8-cineol - 10.5-26.4%	[26]
T. pinnatum	camphor - 23.2%, α-penine - 8.5%, camphene - 7.7%	[27]
T. angulatum	1,8-cineol - 75.3%, camphor - 8.1%	[22]
T. praeteritum	borneol - 28%, 1,8-cineol - 12%	[3]
T. audibertii	artemisia keton - 39.8%, *trans*-linalool oxide - 32%	[11]
T. messicyticu	thujone - 51%	[3]
T. argyrophyllum	*cis*-thujone - 69.9%	[13]
T. balsamita	carvone - 52%	[25]
T. longifolium	eudesmol - 22.5%, 1,4-dimethyl azulene - 13.5%, germacrone - 8.2%	[28]

Table 3. Antimicrobial activity of essential oil from *P. pulchrum*

Microorganism	Inhibition zone (mm)		
	Essential oil	Ciprofloxacin	Amphotericin B
Bacillus subtilis (6633)	11	29	-
Staphylococcus aureus (511)	11	19	-
Staphylococcus aureus (134/94, MRSA)	-	-	-
Enterococcus faecalis (1528, VRSA)	14	16	-
Escherichia coli (458)	0	24/32	-
Pseudomonas aeruginosa (K977/61)	17	28/35	-
Pseudomonas aeruginosa (SG 137)	-	25	-
Mycobacterium vaccae (10670)	19	22	-
Sporidiobolus salmonicolor	-	-	19
Candida albicans	12	-	20
Penicillium notatum	-	-	19

One of the major constituents of the *P. pulchrum* is camphor. There are many pharmaceutical applications for camphor such as topical analgesic, antiseptic, antispasmodic, antipruritic, anti-inflammatory, anti-infective and cough suppressant [29]. Linalool, identified as the other major constituent in the essential oil of *P.pulchrum*, is a well-known sedative [30].

In addition, in the essential oil of *P. pulchrum*, compounds with an interesting biological activity, such as arglabin and chamazulene were detected with a relative amount of 0.57% and 0.52%, respectively.

Arglabin belongs to the guaianolide, the class of sesquiterpene lactones, previously isolated from Artemisia species [31]. Arglabin shows a promising antitumor activity against different tumor cell lines [32].

Chamazulene is an aromatic sesquiterpene found in a variety of plants including chamomile (*Matricaria chamomilla*), wormwood (*Artemisia absinthium*) and yarrow (*Achillea millefolium*) [33]. Chamazulene has anti-inflammatory properties in vivo and inhibits the CYP1A2 enzyme involved in leukotriene biosynthesis [34].

During the last decades, antimicrobial plant products have gained special interest because of the resistance to antibiotics that some microorganisms have acquired, the increasing popular concern about the safety of food and the potential impact of synthetic additives on health. One of the novel ways to reduce the proliferation of microorganisms is the use of essential oils [35]. Thus, the antimicrobial activities of the essential oil of *P.pulchrum* were studied against eight bacterial and three fungi strains (Table 3).

Antimicrobial activity was detectable in six out of 11 cases (*Bacillus subtilis, Staphylococcus aureus,*

Enterococcus faecalis, Pseudomonas aeruginosa, Mycobacterium vaccae and *Candida albicans*). The results of the antimicrobial tests of the essential oil from the aerial parts displayed an intermediate effect [20, 36] on the Gram-negative bacteria *P. aeruginosa* (K799/61) and *M. vaccae* (10670). On the other hand, the essential oil exhibited a weak activity against Gram-positive bacteria, *B. subtilis* (6633), *S. aureus* (511) and *E. faecalis* (VRSA), and against the fungus *C. albicans*. No inhibition was observed against *S. aureus* 134/94 (MRSA), *E. coli* (458), *P. aeruginosa* (SG137) and the fungi *S. salmonicolor* and *P. notatum*.

Several studies have also reported that *T. parthenium* essential oils which are rich in camphor, prevent the growth of some bacteria such as *S. aureus, S. epidermides, S. flexneri, Klebsialla pneumonia, E. coli* (25923 and 157), methicillin resistant *S. aureus, B. subtilis, S. saprophyticus and P. aeruginosa* [23, 24, 37]. However, other publications reported that the essential oils of *T. parthenium* (from various regions) were resistant to *E. coli, B. subtilis* and *S. aureus* [5, 38].

As already mentioned, natural habitat of plant species was having a bigger impact on the chemical composition and even the biological activity of the essential oils.

CONCLUSION

In the current study, the chemical composition and antimicrobial activity of the essential oil of *P. pulchrum* from Mongolia was investigated for the first time. The yield of essential oil obtained by hydro-distillation from *P. pulchrum* was 0.1 percent of the dried weight. In total, 55 components were identified representing 99.7% of the oil. Camphor (33.9%), linalool (21.1%), α-pinene (9.0%), (Z)-γ-curcumyl-2-methylbutyrate (4.8%), pentylcurcumene (3.2%), camphene (2.9%), tricosane (2.8%), nerolidol (2.6%), α-selinene (1.8%) and β-pinene (1.4%) were the main components. The essential oil from *P. pulchrum* exhibited an intermediate antimicrobial effect on bacteria *P. aeruginosa* (K799/61) and *M. vaccae* (10670).

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