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Research Article

Characterization of the Essential Oils from Commercial Chamomile Flowers and Chamomile Teabags by GC-MS Analysis

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ABSTRACT

Chemical composition of seven *Matricaria chamomilla* L. essential oils samples has been analyzed by GC-MS in order to evaluate the differences between trademarks. A total of 70 compounds accounting 92.16-95.76% of the total essential oil were identified. The existence of two types of chamomile essential oils, one rich in oxygenated sesquiterpenes (66.85-77.48%) and the other rich in sesquiterpene hydrocarbons (58.12%) was established. α-Bisabolol oxide B (6.57±1.20, 23.65±3.27%), α-bisabolone oxide A (7.89±0.36, 7.01±0.44%) and α-bisabolol oxide A (58.18±1.99%, 27.95±4.32%) were the main oxygenated compounds in the essential oils from both chamomile flowers and teabags at commercial food items, whereas large amount of the sesquiterpene hydrocarbon *trans*-β-farnesene (38.22%) followed of α-bisabolol oxide A (16.74%) were found in the chamomile essential oil at retail Pharmacies. Chamazulene was present (1.5-4.44%) in all analyzed essential oils. The results showed qualitative and quantitative differences that can affect not only to the organoleptic characteristics but also its pharmacological activity.

Keywords: *Matricaria chamomilla* L.; essential oil; α-bisabolol oxide A and B; *trans*-β-farnesene; chamazulene

INTRODUCTION

The sweet or German chamomile (*Matricaria chamomilla* L. or *Matricaria recutita* L.) belonging to the Asteraceae family is a well-known medicinal plant commonly used in herbal teas to relieve spasms or inflammatory disorders of the gastrointestinal tract[1]. The anti-inflammatory activity of chamomile essential oil has been attributed to the sesquiterpenes α-bisabolol or chamazulene. The oxygenated sesquiterpene α-bisabolol decreased leukocyte migration, protein extravasations and the amount of TNF-α in rat peritoneal cavity in response to carrageenan[2] showing also gastroprotective properties[3], whereas the sesquiterpene hydrocarbon chamazulene was able to suppress the formation of leukotiene B_4 in the neutrophilic granulocytes[4]. These compounds as well as the essential oil of *Matricaria chamomilla* have been tested against the L_3 larvae of *Anisakis* type I[5]. The essential oil at 125μg/ml produced the dead of all nematodes, which showed cuticles changes and intestinal wall rupture. *In vivo* assays the essential oil also has significant protection on gastric wall lesions. Chamazulene was ineffective, whereas α-bisabolol showed more activity *in vitro* test and less activity *in vivo* test than chamomile essential oil, suggesting that the larvicidal activity may be related with the presence of other compounds present in *M. chamomilla* essential oil[5]. Other studies indicated that both the essential oil and α-bisabolol show leishmanicidal activity[6,7]. At the highest concentrations tested (1000 and 500μg/ml) α-bisabolol and pentamidine (control), widely used anti-leishmaniasis drug, achieved 100% inhibition of *Leishmania infantum* promastigote, the main species responsible for human leishmaniasis in Spain. At the next highest concentrations (250 and 125μg/ml), α-bisabolol produced inhibition of 98% and 85%, respectively. The inhibitory activity of α-bisabolol was slightly higher than pentamidine at concentrations between 100-50 μg/ml and lightly lower than that of pentamidine between 15.6-6.25 μg/ml doses[7]. The essential oil of *M. chamomilla* has also been tested at topical level, in this sense chamomile essential oil not produce any irritation even applied undiluted to the highly sensitive choriallantoic membrane[8]. The antiviral activity against both acyclovir sensitive and acyclovir-resistant HSV-1, lack of irritating potential and high selectivity index of the chamomile essential oil in comparison with other tested essential oils leads the author to consider that chamomile essential oil might be a promising topical therapeutic agent in the treatment of recurrent herpes infection[8]. Finally, chamomile essential oil has been proved in comparison with other essential oils on mammalian DNA polymerase (pol) inhibitory activity, cancer cell (human colon carcinoma, HCT116) growth inhibitory activity, antiallergic activity and antioxidant activity[9]. Among all assayed essential oil, chamomile essential was the strongest inhibitor of pols α and λ and showed significant effects on both cancer cell growth and mast cell degranulation. The main compounds responsible of these pharmacological activities were the sesquiterpenes α-bisabolol oxide A (37.9%), (E)-β-farnesene (20.8%), α-bisabolol oxide B (6.47%), bisabolone oxide (5.78%),

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Table 1: Constituents of food supermarkets and pharmacy *Matricaria chamomilla* L. essential oil by GC-MS analysis.

COMPOUND	RI	Peak area (%) Chamomile flowers	Peak area (%) Chamomile teabags	Peak area (%) Pharmacy EO
Monoterpene hydrocarbons		0.36	0.11	1.15
α-Pinene	939	-	-	0.03
Camphene	953	-	-	0.02
Sabinene	978	-	-	0.03
β-Pinene	980	-	-	0.01
δ-3-Carene	1013	-	-	0.03
p-Cymene	1027	0.24±0.23	-	0.10
Limonene	1031	0.04±0.02	0.11±0.05	0.04
cis-Ocimene	1044	-	-	0.09
trans-Ocimene	1051	0.05±0.03	-	0.61
γ-Terpinene	1061	0.03±0.02	-	0.19
Oxygenated monoterpene		0.34	0.90	0.30
1,8-Cineole	1035	-	-	0.02
Artemisia ketone	1064	0.28±0.07	-	0.22
Menthone	1154	-	0.41±0.21	-
iso-Menthone	1163	-	0.14±0.09	-
Menthol	1172	-	0.35±0.31	-
Neral	1240	-	-	0.02
Geranial	1270	-	-	0.04
α-Terpinyl acetate	1343	0.06±0.07	-	-
Sesquiterpene hydrocarbons		5.57	12.56	58.12
α-Copaene	1378	-	-	0.07
Modhephene	1381	-	-	0.11
α-Isocomene	1388	-	0.09± 0.03	0.43
β-Elemene	1394	-	-	0.16
α-Gurjunene	1411	-	-	0.03
β-Caryophyllene	1421	-	0.08±0.03	0.27
trans-β-Farnesene	1458	2.98±0.65	7.74±1.55	38.22
Germacrene D	1487	0.17±0.03	0.22±0.03	4.32
Bicyclogermacrene	1500	0.19±0.09	-	4.11
(E, E)-α-Farnesene	1515	-	-	8.61
δ-Cadinene	1528	-	-	0.26
Chamazulene	1732	2.221±0.481	4.44±0.05	1.53
Oxygenated sesquiterpene		77.48	66.85	24.89
Dehydrosesquicineole	1462	0.12±0.10	0.69±0.12	-
Sesquirosefuran	1556	-	-	0.19
Nerolidol	1559	0.20±0.00	0.30±0.08	-
Dendrolasin	1570	-	0.22±0.03	0.19
Spathulenol	1579	1.50±0.02	1.58±0.08	0.62
Caryophyllene oxide	1581	0.14±0.00	-	-
Salvial-4(14)-en-1-one	1592	0.19±0.07	0.17±0.04	-
cis-Cadin-4-en-7-ol	1637	-	0.54±0.15	-
epi-α-Cadinol	1646	0.72±0.02	-	-
β-Eudesmol	1655	-	0.56±0.10	-
α-Bisabolol oxide B	1660	6.57±1.20	23.65±3.27	3.58
α-Bisabolone oxide A	1699	7.88±0.36	7.01±0.438	3.56
α-Bisabolol	1700	1.98*	4.18±0.19	-
α-Bisabolol oxide A	1747	58.18±1.99	27.94±4.32	16.74
Phenylpropanoids		0.33	1.16	0.00
trans-Anethole	1284	0.22±0.03	1.16±0.57	-
Metyl Eugenol	1397	0.11±0.11	-	-
Others		13.81	10.76	10.60
2-Methyl-ethylbutanoate	854	0.20±0.07	-	0.03

Propyl-2-methylbutanoate	950	0.08±0.03	-	-
6-Methyl-5-hepten-2-ona	990	-	-	0.02
2-Pentylfuran	994	-	-	0.06
Octanal	1005	-	-	0.02
Nonanal	1107	-	-	0.03
2-Methyl buthyl-2-methylbutyrate	1109	-	-	0.01
Decanal	1208	-	-	0.01
cis-3-Hexenyl isovalerate	1241	-	-	0.05
Hexyl isovalerate	1247	-	-	0.01
cis-2-hexenyl isovalerate	1250	-	-	0.02
Phenylmethylpentanoate	1396	-	-	0.03
6,10,14-trimethyl-2-pentadecanone	1847	0.10±0.01	0.89±0.08	0.23
Z-Spiroether	1888	9.73±3.92	4.19±0.30	8.83
E-Spiroether	1897	1.75±2.20	1.04±0.73	0.70
Methyl hexadecanoate	1936	0.05±0.03	0.22±0.10	-
Methyl linoleate	2127	0.07±0.02	-	-
Linoleic acid	2192	0.28±0.02	0.86±0.05	-
Tricosane	2333	0.35±0.03	0.68±0.08	0.20
Tetracosane	2424	0.08±0.04	-	0.04
Pentacosane	2528	0.86±0.08	2.21±0.05	0.31
Hexacosane	2631	0.03±0.01	0.09±0.00	-
Heptacosane	2704	0.20±0.02	0.45±0.05	-
Nonacosane	2901	0.06±0.01	0.13±0.01	-
TOTAL		95.76±3.11	92.16±0.56	95.05

Compounds listed in order of elution in the HP-5MS UI column. RI: retention index relative to C_8-C_{32} *n*-alkanes on the HP-5MS UI column. *: only one sample

chamazulene (2.70%), α-bisabolol (1.93%) and germacrene D (1.58%). α-Bisabolol oxide A (followed of α-bisabolol) showed the strongest pol λ inhibition, but not influence cell growth of HCT116 human colon carcinoma cells, suggesting that it exerted no inhibition of pol α, a DNA replicative pol. Although not antioxidant activity was observed, this compound inhibited mast cell degranulation. The antiallergic and anti-inflammatory activities of α-bisabolol oxide A, could have been produced by specifically pol λ inhibition, indicating that the antiallergy and anti-inflammatory effects of chamomile essential oil should be largely generated by this compound[9] and not only by α-bisabolol or chamazulene as previous works. Taking into account the interesting results obtained in the recently different assays carried out with chamomile essential oil, the aim of the present work was a comparative study by gas chromatography-mass spectrometry between the essential oil obtained by hydrodistillation from commercial dried chamomile flowers or chamomile teabags at food supermarkets used in tea preparation and the essential oil of *M. chamomilla* purchased in Pharmacy and used for therapeutic purposes.

MATERIAL AND METHODS

Plant material

Dried chamomile flowers (3x100g) or chamomile teabags (3x100g) at food supermarket were subjected to hydrodistillation for 3 hr in a Clevenger-type apparatus yielding 0.63±0.05% and 0.36±0.05% respectively of blue-green essential oils. The essential oils were dried over anhydrous sodium sulphate and stored at 4ºC until gas chromatographic-mass spectrometry analysis. A commercial sample of *Matricaria chamomilla* L. essential oil from Plantis Laboratory and available in Pharmacy was also analyzed.

GC-MS analysis

Gas Chromatography-Mass Spectrometry analysis was carried out with a 5973N Agilent apparatus, equipped with a capillary column (95 dimethylpolysiloxane-5% diphenyl), Agilent HP-5MS UI (30 m long and 0.25 mm i.d. with 0.25 µm film thickness). The column temperature program was 60°C during 5 min, with 3°C/min increases to 180°C, then 20°C/min increases to 280°C, which was maintained for 10 min. The carrier gas was helium at a flow-rate of 1 mL/min. Split mode injection (ratio 1:30) was employed. Mass spectra were taken over the *m/z* 30–500 range with an ionizing voltage of 70 eV. Kovat's retention index was calculated using co-chromatographed standard hydrocarbons. The individual compounds were identified by MS and their identity was confirmed by comparison of their RIs, relative to C_8-C_{32} *n*-alkanes, and mass spectra with those of authentic samples or with data already available in the NIST 2005 Mass Spectral Library and in the literature[10].

RESULTS AND DISCUSSION

Hydrodistillation during 3 hours of the six samples (6x100g) of two chamomile trademark available at food supermarket gave a blue-green essential oils (0.63±0.05 and 0.36±0.05 respectively), with a specific density lower than water and aromatic odor. Greater variability in yield and color was found among trademark and not between samples. The higher yields (0.6-0.7 ml/100g) of essential oil together deep green color was obtained from dried

commercial chamomile flowers. Lower oil content (0.3-0.4/100g) and dark blue color was found in powdered commercial chamomile teabags. The dark blue color is due to the higher content in this trademark (4.44±0.05) of the sesquiterpene chamazulene formed by decomposition of the sesquiterpene lactone matricin, during the hydrodistillation of the raw material. Seventy compounds accounting for 92.16-95.76% of the essential oils from 6 samples at food supermarket and one Pharmacy essential oil were identified by capillary GC/MS. Components are listed (Table1) as homologous series of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, phenylpropanoids and others and listed according to Kovats retention index. The qualitative and quantitative differences found among the homologous series can establish two groups of chamomile essential oil. The first one includes essential oils obtained from dried chamomile flowers and chamomile teabags from both trademarks for oral consumption with oxygenated sesquiterpenes (66.85-77.48%) as the main fraction and the second group corresponds to *Matricaria chamomilla* L. essential oil purchased in Pharmacy for medicinal use, rich in sesquiterpene hydrocarbons (58.12%) followed by oxygenated sesquiterpenes (24.89%). Although oxygenated sesquiterpenes is the main fraction for samples available at food supermarket high variability in the biologically active compounds was observed. In the essential oils obtained from samples of dried chamomile flowers, α-bisabolol oxide A reached 58.18±1.99%, followed by α-bisabolone oxide A (7.88±0.36%) and α-bisabolol oxide B (6.57±1.20%), whereas in the essential oils from chamomile teabags more similar quantity between α-bisabolol oxides A and B (27.94±4.32% and 23.65±3.27%, respectively) were observed. In the commercial *Maticaria chamomilla* essential oil purchase in Pharmacy large amount of *trans*-β-farnesene (38.22%) followed by α-bisabolol oxide A (16.74%), Z-Spiroether (8.83%), (E, E)-α-farnesene (8.61%), germacrene D (4.32%), bicyclogermacrene (4.11%), α-bisabolol oxide B (3.58%) and α-bisabolone oxide A (3.56%) were found. It is interesting to note the qualitative differences obtained in the minority fraction of the oxygenated monoterpenes. 1,8-Cineole (0.02%), neral (0.02%) and geranial (0.04%) were only detected in chamomile essential oil purchase in Pharmacy, whereas menthone (0.41±0.21%), *iso*-menthone (0.14±0.10%) and menthol (0.35±0.31%) are only present in the essential oils from samples of chamomile teabags. Artemisia ketone was found in both chamomile flowers essential oil from food supermarket (0.28±0.07) and *M. chamomilla* essential oil from Pharmacy (0.22%) and α-terpinyl acetate was only detected in dried chamomile flowers. The maximum content of chamazulene (4.44±0.05%) was found in the essential oil from chamomile teabags, followed from samples also at food supermarket (2.22±0.48%) and the essential oil purchase at Pharmacy (1.53%). The qualitative and quantitative differences in the biological active compounds between trademarks obtained in this work could affect not only the odor and taste of tea infusion but also the intensity of its pharmacological activity[5,8], mainly of the anti-inflammatory nature[9].

CONCLUSION

German chamomile is commonly consumed in herbal teas to relieve spasms and inflammatory disorders mainly of the gastrointestinal tract. It is a natural source of food flavouring, safe and although allergic reactions known. The main biologically active compounds in chamomile essential oil are bisabolol oxides, bisabolone oxide, α-bisabolol, spirooethers, *trans*-β-farnesene and chamazulene. The results obtained in this study confirm the presence in the essential oils of large amounts of the active compounds in both chamomile flowers and chamomile teabags available at food supermarkets and in Pharmacy being able to be used for their pharmacological properties.

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