COMPARATIVE STUDIES OF DIFFERENT EXTRACTION METHODS OF ESSENTIAL OIL FROM *MATRICARIA RECUTITA* L. IN IRAN

SEYED SAIED HOMAMI[1]; KAMKAR JAIMAND[2] ; MOHAMMAD BAGHER REZAEE[2] AND REZA AFZALZADEH[3]*

[1]*Islamic Azad University, South Tehran branch, Tehran, Iran.*
[2]*Phytochemistry Group, Department of Medicinal Plants & By-products, Research Institute of Forest and Rangelands, P.O.Box 1318, Tehran, Iran.*
[3]*Faculty of physic , Industry Khajeh Naseer Toosy University of Technology, Tehran, Iran.*

ABSTRACT

Chamomile, *Matricaria recutita* L. from family of Asteracae, is a well-known medicinal plant in folk medicine cultivated all over the world. Chamomile essential oil is widely used in pharmaceutical, cosmetic, and food industries. This paper focuses on isolation methods and chemical constituents of essential oils from *Matricaria recutita* L. in Iran as to optimize these methods and achieving the proper economic procedures. The volatile constituents of *Matricaria recutita* L. cultivated in Iran were extracted by different methods of extraction by microwave usage, pilot plants and laboratory scale. The oil yields obtained from microwave was 0.08%, laboratory scale 0.06%, pilot 0.06% and pilot with heating element 0.07% . Essential oils were identified by GC and GC/MS. A total of 34 components were identified and significant qualitative and quantitative differences were observed amongst the studied. The main constituents of the essential oils of the studied three different methods were as follows: Microwave were α-bisabolol oxide A (42.3%), chamazulene (15.1%), α-bisabolol oxide B (9.6%), (Z,Z)-farnesol (8.1%) and in laboratory scale were cis-pinocamphone (73.5%), α-bisabolol oxide A (7.9%), in pilot were α-bisabolol oxide A (62.1%), chamazulene (10.2%), (Z,Z)-farnesol (8.3%) and in pilot with heating element were α-bisabolol oxide A (50.5%), E- β - farnesene (12.8%), chamazulene (12.3%) . The best method of extraction was pilot method ,because α-bisabolol oxide A (62.1%), chamazulene (10.2%), were major components, which are showing different pharmacological activities like anti-inflammatory, anti-cancer, anti-allergic, a treatment for stress and depression.

Key words: *Matricaria recutita* L. (*M. chamomilla* L.), chamomile, volatile oil SDE extract, GC/FID, GC/MS.

INTRODUCTION

Matricaria chamomilla L. (syn: M. recutita L.; German chamomile) resides in the Asteraceae (Compositae) family and is one of the most widely used medicinal plants in the world. Chamomile essential oil is widely used in pharmaceutical, cosmetic, and food industries. The pharmacological effect of chamomile is mainly connected with its essential oil for its spasmolytic, antimicrobial, and dis-infective properties. The biologically active substances in chamomile essential oil are α-bisabolol, bisabolol oxides, chamazulene, and en-yne- dicycloethers [1–5].

The plant is an annual herb with erect branching and finely divided leaves growing between 50–90 cm tall. The flowers are daisy-like, with hollow conical yellowish centre surrounded by silver-white to cream colored florets. *M. chamomilla* L. is a safe plant and is used in different commercially available forms such as tea, infusion, liquid and capsules in human nutrition. It has a stable natural monocyclic sesquiterpene alcohol named α-bisabolol as the main component, so the plant essential oil has a long shelf life of 6 to 24 months. In addition to the high stability and safety points, the plant has no proven potentially toxic compound and therefore, no acute toxicity for human and animals [15]. Consequently, it has been listed as GRAS by the FDA [14,15].

Different essential oil isolation techniques (hydro-distillation [2,3,5,7], supercritical fluid extraction [6,8,9,10,11], headspace analysis [4]) have been applied for studying the volatile constituents of chamomile by several investigators. Different analytical techniques were in the literature for the qualitative and quantitative assessment of the oil. These include elemental micro-analysis, liquid sampling mass spectrometry (LS/MS), TLC, GC/MS. In addition, an enantioselective HPLC method was developed for the separation of the four stereoisomers of α-bisabolol and a RP-HPLC method was reported to separate the isomeric en-yne-dicycloethers and chamazulene. (E)-β-Farnesene, α-bisabolol, bisabolol oxide A and B, chamazulene, and en-yne-dicycloethers were found to be the main constituents in chamomile oil [2–11].

Comparison of different methods in extraction of essential oils from aromatic plants, *Matricaria recutita* L. in Iran, as to optimize extraction methods and examine the compositions of the oil during hydro-distillation were the main goals of this research. Qualitative and quantitative achievements for their values in the economical methods were of concerned.

EXPERIMENTATIONS

Plant material

Matricaria chamomilla L. was collected during June –July 2011 from Esfahan province, central of Iran, plant specimen determined by Iranian Botanical Garden(IBG) staff. voucher number MPH. 531. Plant materials (flowers) were extracted by three different apparatus .

MADH apparatus and procedure

The home-made microwave extraction apparatus is depicted in Fig.1. Microwave-assisted hydro-distillation (MAHD) was purchased from Feller Germany microwave apparatus Model MW 420 GS. The multimode microwave reactor has a rated voltage 220-240V ~ 50-60 Hz, rated input power Microwave 1550 W, rated output power Microwave 1100 W, rated input power(Grill) 1200-1400 W, oven capacity 42 L, turntable diameter 345 mm, external dimensions (LxWxH) 553 x 465 x 326 mm. Temperature was controlled by feedback to the microwave power regulator.

Fig. 1 – Microwave-assisted hydro-distillation apparatus used in this study.

Isolation of essential oil

Dried aerial part biomass (80g per sample) of chamomile was extracted

e-mail : saeedhomami@yahoo.com

by different methods and apparatus like hydro-distillation extraction on microwave, pilot plants and laboratory scale. The distillation time was 3h at a rate of 3–4 mL/min. The oils were separated from the water by decantation and were dried by filtration over anhydrous sodium sulfate. The oils were stored prior to analysis in ampoules in a refrigerator at 4°C, and were analyzed within a week. The averaged oil yields obtained from microwave system was 0.08%, laboratory scale was 0.06%, pilot was 0.06% and pilot with heating element was 0.07%, then the essential oils were identified by GC and GC/MS.

Table.1 : Results of three different methods of extracting Oils from *Matricaria chamomilla.*

Compounds	R.I[a]	Method of Extraction			
		Microwave	Lab	Pilot	Pilot -element
Sabinene	969	---	2.14	---	---
β -Pinene	985	---	---	0.54	---
(E)-β- Ocimene	1058	---	---	0.34	---
γ- Terpinee	1068	---	---	1.21	---
Cis-Pinocamphone	1176	---	**73.54**	---	---
p-Cymen-9-ol	1205	---	3.50	---	---
Cis-Carvone oxide	1262	---	0.72	---	---
Cis-Dihydr-α- terpinyl acetate	1315	---	3.46	---	---
E-Caryophyllene	1419	---	---	---	0.41
E-β-Farnesene	1463	4.67	1.24	2.17	**12.87**
(Z)-γ-Bisabolene	1512	---	---	---	1.00
(E)-γ-Bisabolene	1547	---	0.55	---	---
Germacrene B	1553	---	0.82	---	0.44
n-Tridecanol	1570	1.62	---	0.27	0.81
Spathulenol	1576	0.75	---	---	---
Dihydro(10,11)-ar-α-bisabolol	1607	0.75	---	0.31	---
α-Bisabolol oxide B	1650	9.58	1.27	5.67	5.24
E-Bisabol-11-ol	1664	1.05	---	---	---
β-Bisabolol	1671	1.86	---	0.41	0.68
α- Bisabolone oxide A	1679	0.99	---	0.48	---
α-Bisabolol	1693	0.61	---	0.21	0.37
(Z,E)-Farnesyl acetate	1701	---	---	---	0.28
(Z,Z)- Farnesol	1718	8.14	1.14	8.30	5.82
(E,E)-Farnesol	1725	2.57	---	1.22	2.87
(E,E)-Farnesyl acetate	1726	0.98	0.45	1.82	1.52
Chamazulene	1732	15.08	1.67	10.25	12.33
(E,Z)-Farnesol	1746	2.27	---	1.03	1.92
α-Bisabolol oxide A	1755	42.27	7.97	62.16	50.50
2,7(14),10-Bisabolatrien-1-ol-4-one	1850	3.19	---	0.98	2.11
n-Hexadecanol	1878	---	---	---	0.76
n-Nonadecane	1893	---	1.46	0.38	---
Not identified	1961	3.55	---	1.09	---
1-Eicosene	1995	---	---	0.56	---
n-Octadecanol	2088	---	---	0.50	---

[a]R.I. = retention indices on DB-5 column

Gas Chromatography :

GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/ non-split injector and a flame ionization detector (FID) at 250°C. N_2 was used as a carrier gas (1 ml min-1) and a DB-5 type was utilized as the capillary (50m x 0.2 mm, film thickness 0.32 μm). Temperature within the column for 3 min was retained at 60°C, after that the column was heated at a rate of 5°C min-1 until it reached at 220°C and maintained in this condition for 5 min. The split ratio was 1:100.

Gas Chromatography - Mass Spectrometry :

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph which was coupled to a Saturn II ion trap detector was used . The column was the same as GC , and the GC conditions were as above. Mass spectrometer conditions were : ionization potential 70 eV; electron multiplier potential is 2000 V.

The identity of the oil components were established from their GC retention indices, relative to C_7- C_{25} n-alkanes, by comparison of their MS spectra with those reported in the literature (16), (17), (18), and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

RESULTS AND DISCUSSION

For both hydro-distillation laboratory scale and microwave-assisted hydro-distillation, the extraction temperature was equal to the boiling point of water under the conditions of the study (~100°C). The oil yields obtained from 80 grams of samples plant by microwave-assisted hydro-distillation was 0.08%, hydro-distillation laboratory scale was 0.06%, pilot was 0.06% and pilot with element was 0.07% . To reach such temperature level, where the actual distillation started, it was necessary to heat the samples for only 15.0 minutes with microwave-assisted hydro-distillation, while 38 minutes was necessary in the case of hydro-distillation laboratory scale. This was due to the more efficient microwave heat flow. According to the Table.1, components in hydro- distillation laboratory scale are completely different with microwave-assisted hydro-distillation , pilot and pilot with heating element.

For example in hydro-distillation laboratory scale main compound was cis-pinocamphone (73.5%), but in other methods this was not observed.

Main component in microwave-assisted hydro-distillation, pilot and pilot with element was α-bisabolol oxide A 42.27%, 62.16% and 50.50%, respectively, and the same compound in hydro-distillation laboratory scale was 7.97%.

Another compound was chamazulene which was 15.08%, 10.25% and 12.33%, respectively, but in hydro-distillation laboratory scale this was 1.67%. Also for (Z,Z)-farnesol compound in microwave-assisted hydro-distillation , pilot and pilot with heating element was 8.14%, 8.30% and 5.82%, respectively, but in hydro-distillation laboratory scale is 1.14%.

When our results were compared with other reports, the extraction, behaviors depending on the different conditions used. Golmakani and Rezaei (2008a, b) also reported similar results for the extraction yield of essential oils from *Thymus vulgaris* L. and *Zataria multiflora* Boiss. obtained by hydro-distillation and microwave-assisted hydro-distillation [19,20]. Final extraction yield of hydro-distillation after 240 minutes (4.18%, w/w) was statistically similar to those obtained by microwave at 180, 360, and 540 W after 210, 150, and 120 minutes, respectively. As final extraction yield results indicated, microwave-assisted hydro-distillation could decrease the time required for obtaining the same amount of essential oil by about 50% compared to hydro-distillation, i.e. 120 minutes instead of 240 minutes.

These results are in good agreement with the results of Stashenko *et al.* (2004) for Colombian *Xylopia aromatica*. They found that for the same extraction yield, the time required for microwave-assisted hydro-distillation was one-fourth of that for hydro-distillation [21]. Rezvanpanah *et al.* (2008), extracted essential oils from *Satureja hortensis* and *Satureja montana* using microwave-assisted hydro-distillation, where the extraction time was reduced significantly as the microwave power was changed from 220 to 660 W. The difference in the extraction yield at 180 and 540 W seemed to be more pronounced during the first 2 hours of extraction. The required time to reach the boiling point of water at 540 W was nearly one-fourth of that at 180 W. After 20 minutes of operation, the extraction yield at 540 W (1.53%, w/w) was about five times more than that at 180 W (0.31%, w/w). Meanwhile, the extraction yield for 540 W after 45 minutes of operation was the same as that obtained in 60 minutes at 360 W [22]. In another study, Özek *et al.* (2005) extracted essential oils of three endemic Turkish *Heracleum* species by different techniques. Their results indicated some quantitative differences amongst some of the extracted components. The microwave-assisted hydro-distillation extracted oils showed slightly lower amounts of octyl acetate which was 59% compared to those extracted by hydro-distillation and other extraction techniques which was 93.7–94.9% [23]. Instead, some other compounds were extracted at higher levels when using microwave-assisted hydro-distillation .

Compared to hydro-distillation, no new compound was found in the essential oils extracted by microwave-assisted hydro-distillation in the current study. These results indicated that using microwave did not influence the quality of the extracted essential oil, but the extraction time was shorter. Similar findings were reported by Golmakani and Rezaei (2008a) for the compositions of essential oils extracted with hydro-distillation [thymol (37.20%), p-cymene (16.85%), γ-terpinene (9.06%)] and microwave-assisted hydro-distillation [thymol (40.20%), p-cymene (17.57%), γ-terpinene (8.54%)] from *Thymus vulgaris* L [19].

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