



## Subcritical Water Extraction of Essential Oils from *Matricaria Chamomilla* L.

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### ABSTRACT

Subcritical water extraction (SWE) of essential oil from *Matricaria Chamomilla* L. (*M. Chamomilla* L.) was performed and compared with conventional method including hydro distillation. The percentage of main extracted components at different temperatures (100, 125, 150 and 175°C) and water flow rates (1, 2 and 4 ml/min) were investigated at 20 bar pressure and 0.50 mm mean particle size in SWE. The separation and identification of the components were carried out by gas chromatography (GC)-flame ionization detection and GC-mass spectrometry. The best operating conditions for the SWE of *M. Chamomilla* L. were determined to be at the temperature of 150°C and flow rate of 4 ml/min for 120 min extraction time. In compare to hydro distillation method, the SWE method resulted in more valuable essential oil with respect to the oxygenated components.

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## 1. INTRODUCTION

One of the important processes in essential oil industry for many years is isolation, concentration, and purification of essential oils, as a consequence of the extensive use of these compounds. Essential oils are currently being extracted from natural products either by conventional methods such as hydro distillation or solvent extraction or by more advanced methods such as supercritical fluid extraction and subcritical water extraction (SWE) [1]. SWE is a technique based on the use of water as an extractant, at temperatures between 100 and 374°C and pressure high enough to maintain the liquid state [2].

*M. chamomilla* L. is one of the most important and well-known plants that traditionally has been used for the treatment of various diseases [3]. The pharmacological and medicinal effects of *M. chamomilla* L. are mainly connected with its essential oil for its antimicrobial and antispasmodic properties [4]. Extracts of *M. Chamomilla* L. are used in antiseptic ointments, creams, and gels to treat cracked nipples, sore gums, inflammations, irritation of the skin and mucosa, respiratory tract inflammation, and for wound

healing [5]. The oils are used as fragrance components or active ingredients in soaps, detergents, creams, lotions, and perfumes. Recent researches support this uses [6-8].

Recently, supercritical carbon dioxide extraction of *M. Chamomilla* L. was performed [9] but SWE of it was not reported. The aims of this research work were to extract the essential oil from *M. Chamomilla* L. by SWE method and to investigate the effects of different parameters, such as temperature and water flow rate on this method for extracting of main components. The results were compared with those obtained by conventional techniques such as hydro distillation and Soxhlet extraction.

## 2. EXPERIMENTAL

**2. 1. Materials** The leaves of *M. Chamomilla* L. were collected from the Noor Abad farm (Shiraz, Iran) in May 2011. NaCl, Na<sub>2</sub>SO<sub>4</sub> and n-pentene (Merck, Darmstadt, Germany) were used as a demulsifier, drying agent and extractant, respectively, in the liquid-liquid extraction step of the aqueous extracts. High-performance liquid chromatography (HPLC) grade hexane (Aldrich Chemical Co., Milwaukee, WI) was used as the diluting solvent before gas chromatography

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(GC). The doubly distilled, degassed water purified through a Milli-Q deionizing unit (Millipore, Bedford, MA) was used as an extractant.

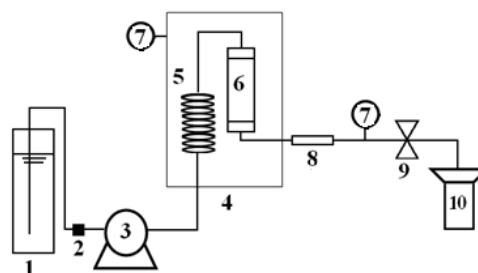
**2. 2. Sample Preparation** *M. Chamomilla* L. leaves were dried in shadow at room temperature for 2 days and then were stored in polyethylene bags in a refrigerator until the process of analysis. The moisture content of the leaves was 4 % (dry basis). The samples were ground by a grinder and screened by standard sieves immediately prior to extraction in order to avoid losses of volatiles. The mean ground leaf particles were 0.5 mm, equivalent to 35 meshes. The ground plant, without screening, was directly used in hydro distillation and Soxhlet extraction. Two replications of the extraction and analysis procedure were performed for each of the runs.

**2. 3. SWE** The SWEs were carried out in a laboratory-built apparatus (Semnan University, Semnan, Iran) shown in Figure 1. The main parts of SWE apparatus were the following: one N<sub>2</sub> cylinder for purging and removing dissolved O<sub>2</sub>, one burette, three glassy tanks, one pump, one extraction vessel, one oven for heating of the extraction vessel, one heat exchanger for cooling of extract, one back pressure regulator for maintaining the appropriate pressure in the equipment and one sample collection system.

An extraction vessel (height = 120 mm, inner diameter = 12 mm) was preloaded with 1 g of ground *M. Chamomilla* L. leaves. To prevent moving of the particles from the fixed bed, two pulpy and cloth-filters sandwiched in between two stainless steel filter was inserted in both sides of the fixed bed. The extractor was assembled in the oven and pressurized. After that, the pump (BFRL Co., SY-8100 series, Germany) was turned off, the by-pass valve was closed and the oven was brought up to the required temperature. At that time, the degassed and deionized water was then delivered at the adjusted flow rate with the HPLC pump to a 3-m stainless steel capillaries preheating section installed in the oven to heat it to the required temperature before passing through the extraction vessel. The pressure of the system was adjusted to the desired condition using the back-pressure regulator valve at the outlet coil.

Regarding to the selected flow rate and a system void volume between the extractor and collection vessel (~40 ml), in all runs, around 30 ml of the water coming out of the system was discarded. Using GC analysis, it was observed that this amount of the extracts was clean and no peak was detected. After that, the collection of the extract in a separating funnel was started. The extraction process was supposed to be started at that time (extraction time = 0).

Using GC analysis, it was proved that it was free from any components.



**Figure 1.** Schematic diagram of SWE extraction system; 1: water reservoir; 2: burette; 3: pump; 4: oven; 5: preheater; 6: extraction cell; 7: pressure indicator; 8: heat exchanger; 9: pressure regulator; 10: cooling extract out.

After collecting the required volume of extract, a liquid-liquid extraction step using n-pentene (95%) was carried out. The volumetric ratio of n-pentene to extract was 1:2 in all experiments and extractions were completed by two equal volume of solvent in two steps. Around 8 g NaCl was added to facilitate the breaking of the emulsion. Essential oil was stored in 1 ml HPLC grade hexane before GC analysis. For the kinetic experiments, the collection vial was replaced at appropriate time intervals.

**2. 4. Hydro Distillation** 100 g ground *M. Chamomilla* L. was extracted with 1000 ml of water steam for 3 h using a Clevenger-type hydro distillation extractor. In this case, around 0.6 ml of essential oil was obtained after hydro-distillation. The extracted essential oil was transferred into a volumetric flask, using three rinses of hexane. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> prior to the GC.

**2. 5. Analysis** The GC-flame ionization detection analyses were performed using a Varian Model CP-3800 gas chromatograph (Varian Co., Palo Alto, CA) equipped with a 60-m CP Sil 8 CB fused silica column (0.32-mm i.d., 0.25- mm film thickness). An injection volume of 1.0 ml of the hexane extracts was injected using an autosampler. The oven temperature program was a 3 °C/min temperature ramp from 50 to 230 °C. The carrier gas was nitrogen (99.999%, Roham Gas Co., Tehran, Iran). The column head pressure was 0.70 bar. The detector and injector temperatures were 250 and 230 °C, respectively.

GC-mass spectrometry (MS) analysis was conducted on the Varian Saturn Model 3400. The GC-MS system (Varian Co.) was equipped with a DB-5 fused silica column (30 m × 0.25 mm; film thickness 0.25 mm) and interfaced with a Varian ion trap detector. The GC conditions were oven temperature from 70 to 230 °C at 4 °C/min; injector 250 °C; carrier gas, helium at a flow rate of 40 ml/min; and splitting ratio, 1:100. The detector temperature was maintained at 250 °C. The MS conditions were ionization energy, 70 eV; mass range,

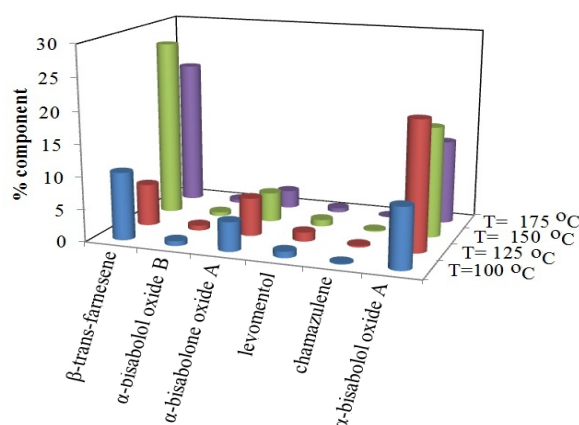
40–400 amu and scan mode electron impact (EI). The components were identified by comparing their retention times and mass spectra with those of pure reference components. The mass spectra were also compared with those in the National Institute of Standards and Technology, WILEY5 and TERPENOIDES mass spectra libraries. The concentration of each component as a percentage was determined from normalizing method.

### 3. RESULTS AND DISCUSSION

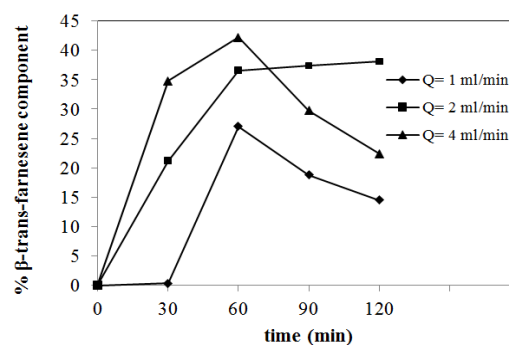
In using the SWE, hydro distillation and Soxhlet methods to isolate the essential oils from *M. Chamomilla* L. oil, six components were identified;  $\beta$ -trans-farnesene,  $\alpha$ -bisabolol oxide B,  $\alpha$ -bisabolone oxide A, levomentol, chamazulene and  $\alpha$ -bisabolol oxide were the main and important components. They were chosen as the key components to find the best SWE operating conditions. Among the operating conditions that may affect the extraction, the pressure is of minor importance [10]. For all SWEs, the extraction pressure was selected to be at 20 bar to maintain the water as a liquid at the extraction temperatures. The mean particle size was 0.5 mm in SWEs. The other operating conditions to be optimized were chosen as the extraction temperature and water flow rate in the range of 100–175 °C and 1–4 ml/min, respectively.

The univariate method was used in all experiments. The time for SWE was chosen to be 120 min to make sure that all the essential oil had been extracted. The kinetic studies were then carried out under these chosen conditions of SWE. The results are the means of the two experiments. Relative SD percent (%RSD) was calculated on the basis of the obtained peak areas. The %RSD values were ranged from 3 to 14%.

**3. 1. Effect of Temperature** Temperature was one of the most important parameters affecting SWE. Increasing the extraction temperature above a certain value gives rise to the degradation of the essential oil components. The maximum permitted extraction temperature must be obtained experimentally for different plant materials [11]. Regarding the extraction of essential oils, it has been shown that temperatures between 100 and 175 °C will be the best condition [12]. The extraction temperature for *M. Chamomilla* L. was optimized in order to maximize the percentage of main components. Its influence was studied between 100 and 175 °C, and the mean particle size, flow rate, extraction time and pressure were selected to be 0.5 mm, 2 ml/min, 60 min and 20 bar pressure, respectively. As can be seen in Figure 2, the percentage of main components increased generally with increase in temperature up to 150 °C. At 175 °C, it decreased, and an extract with a burning smell was produced.



**Figure 2.** Effect of temperature on the main essential oil components SWE of *M. Chamomilla* L., Operating conditions: flow rate = 2 ml/min, particle size = 0.50 mm, pressure = 20 bar, extraction time = 60 min



**Figure 3.** Effect of flow rate on percentage of  $\beta$ -trans-farnesene in SWE of *M. Chamomilla* L. Operating conditions: Particle size = 0.50 mm, Temperature = 150°C, Pressure = 20 bar.

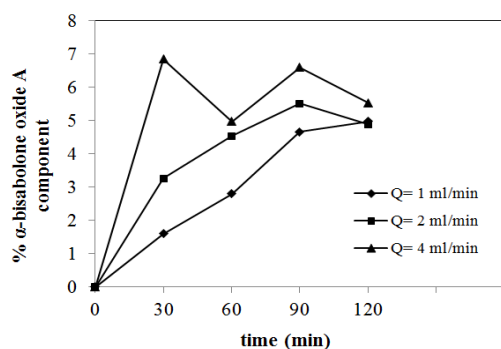
It may be the result of degradation of some of the constituents at higher temperatures. Because of the highest percentage of main components of essential oil at 150 °C and the disagreeable odor of the extract at higher temperatures, further experiments were carried out at this temperature.

**3. 2. Effect of Flow Rate** The effect of water flow rate on the percentage of main components ( $\beta$ -trans-farnesene,  $\alpha$ -bisabolone oxide A,  $\alpha$ -bisabolol oxide A) at 150 °C temperature, 0.5 mm particle size, 20 bar pressure and 120 min extraction time is shown in Figures 3, 4 and 5. The water flow rate was studied at 1, 2 and 4 ml/min. As can be seen in Figure 3, the rate of  $\beta$ -trans-farnesene was faster at the higher flow rate up to 60 min. It is in accordance with previous works [13–15]. It means that the mass transfer of the  $\beta$ -trans-farnesene from the surface of the solid phase into the water phase regulated most of the extraction process. Increase in flow rate resulted in increase in superficial

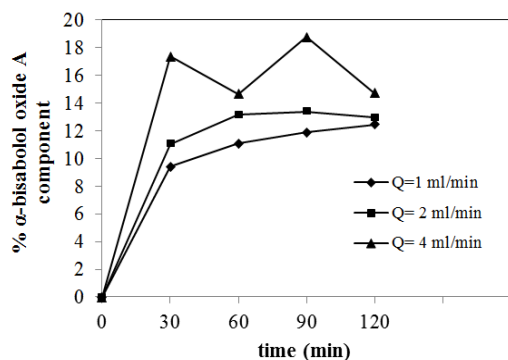
velocity, and thus, quicker mass transfer [16, 17]. After 60 min, the percentage of  $\beta$ -trans-farnesene at flow rate 4 ml/min was lower than the percentage of  $\beta$ -trans-farnesene at flow rate 2 ml/min. It may be because of other extracted components. It causes that the percentage of  $\beta$ -trans-farnesene decreases. The maximum percentage of  $\beta$ -trans-farnesene was extracted in 4 ml/min after 60 min.

The percentage of extracted  $\alpha$ -bisabolone oxide A in SWE is shown in Figure 4. It is clear that by increasing the flow rate, the percentage of  $\alpha$ -bisabolone oxide A in volatile oils increases. The maximum percentage of  $\alpha$ -bisabolone oxide A was extracted in 4 ml/min.

In Figure 5, the percentage of extracted  $\alpha$ -bisabolol oxide A in SWE is shown. As can be seen in Figure 5, similar to Figures 3 and 4, the most rate of  $\alpha$ -bisabolol oxide A was at the flow rate 4 ml/min. Increasing of percentage may be due to increase of non-volatile compounds that decrease the percentage of extracted  $\alpha$ -bisabolol oxide A in SWE extract.



**Figure 4.** Effect of flow rate on percentage of  $\alpha$ -bisabolone oxide A in SWE of *M. Chamomilla* L. Operating conditions: Particle size = 0.50 mm, Temperature = 150°C, Pressure = 20 bar.



**Figure 5.** Effect of flow rate on percentage of  $\alpha$ -bisabolol oxide A in SWE of *M. Chamomilla* L. Operating conditions: Particle size = 0.50 mm, Temperature = 150°C, Pressure = 20 bar

**TABLE 1.** The percentage composition of essential oil of *M. Chamomilla* L. extracted by SWE, hydro distillation and Soxhlet extraction.

Components	SWE <sup>a</sup>	Hydro distillation <sup>b</sup>
$\beta$ -trans-farnesene	22.40	14.82
$\alpha$ -bisabolol oxide B	1.92	1.23
$\alpha$ -bisabolone oxide A	4.89	3.16
levomentol	1.46	1.11
chamazulene	0.55	0.23
$\alpha$ -bisabolol oxide A	12.97	9.89

<sup>a</sup> Particle size = 0.5 mm, flow rate = 4 ml/min, temperature = 150°C, pressure = 20 bar, extraction time = 120 min.

<sup>b</sup> Extraction time = 180 min.

As can be seen from Figures 3-5, flow rate 4 ml/min have maximum percentage of main extracted components. The main disadvantage of applying higher water flow rates is increasing the extract volume and consequently, lower concentration of the final extracts. In practice, the best flow rate must be selected considering two important factors, including the extraction time and the final extract concentration. It is clear that a shorter extraction time and more concentrated extracts are desirable. To prevent a slower extraction rate and longer extraction times, despite the larger amount of the final extracts, a flow rate of 4 ml/min was selected as the optimum value.

#### 4. COMPARISON WITH HYDRO DISTILLATION TECHNIQUE

The comparison among the amount of main components by SWE and hydro distillation extraction is shown in Table 1. The amounts of valuable oxygenated components in the SWE method are significantly higher than hydro distillation extraction. As mentioned in our previous work, in general, non-oxygenated components present lower vapor pressures compared to oxygenated components, and in this sense, its content in hydro distilled extracts are increased. Because of the significant presence of the oxygenated components, the final extract using the SWE method was relatively better and more valuable.

#### 5. CONCLUSION

In this study, the subcritical water extraction of essential oil from *M. Chamomilla* L. and the effect of temperature and flow rate of extraction were investigated on the main components. The best operating conditions for the SWE of *M. Chamomilla* L. were concluded to be at 150°C temperature and 4

ml/min flow rate for 120 min extraction time. In compare to with hydro distillation method, the SWE method resulted in more valuable essential oil with respect to the oxygenated components.

## 6. ACKNOWLEDGEMENT

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استخراج اسانس برگ‌های بابونه با آب فوق داغ انجام و با شیوه مرسوم شامل تقطیر آبی مقایسه شد. درصد ترکیبات استخراج شده عمده‌ی دماها (۱۰۰، ۱۲۵، ۱۵۰ و ۱۷۵°C) و شدت جریان‌های مختلف (۱، ۲، ۴ و ۸ ml/min) در فشار ۲۰ bar و متوسط اندازه ذرات ۰/۵ mm تحقیق شدند. جداسازی و شناسایی ترکیبات با استفاده از کروماتوگرافی گازی و آشکارساز یونش شعله‌ای GC-FID و طیف سنجی جرمی GC-MS انجام شدند. بهترین شرایط عملیاتی برای استخراج اسانس بابونه دمای ۱۵۰°C و دبی آب ۴ ml/min به مدت ۱۲۰ min تعیین شد. در مقایسه با شیوه تقطیر آبی، شیوه استخراج با آب فوق داغ اسانس با ارزشی را از نظر ترکیبات اکسیژن‌دار می‌دهد.

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