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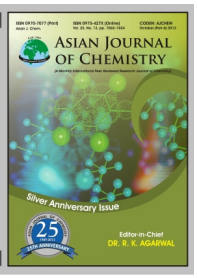

Extraction of Chamomile Essential Oil by Subcritical CO_2 and Its Analysis by UV-VIS Spectrophotometer

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Chamomile is one of the most known medicinal and aromatic plants in Albania. It is well known in applications of cosmetic and skin care products and in natural remedies as well. The essential oils of chamomile flowerheads were extracted by subcritical CO_2 and the extract fractions were analyzed by UV-VIS spectrophotometer. The extractions were carried out using high-pressure (65 bars) Soxhlet apparatus.

Key Words: Chamomile extraction, Subcritical CO_2, Spectrometer UV-VIS.

INTRODUCTION

Chamomile is an old herbal medicine, which is widely used in medical practice. The plant is a native to and cultivated in Southern and Eastern Europe. Chamomile is a well known medicinal and aromatic plant in Albania as well. The water and ethanol extracts of chamomile flowers are mainly used for their anti-inflammatory and antiseptic properties. Chamomile (*Matricaria recutita L.*), once it has been called as *Marticaria chamomilla, Chamomilla recutita* and *Chamomilum nobile* family *Asteraceae*[1]. The main constituents of this plant include the terpenoids α-bisabolol and its oxides (75 %)[2], dicycloethers[2] (13 %) and matricine as chamazulene[2]. Also in Albania, there are studies carried out on essential oils of chamomile by Delibashi[3]. Essential oil extractions of chamomile are commonly done by hydrodistillation (steam distillation)[2] using a Clevenger apparatus. However, in the recent years, the use of supercritical fluid for extraction has been considered one of the most promising novel methods of sample preparation. The miscibility of essential oil with carbon dioxide leads to several applications of supercritical fluid extraction on the study of essential oil, as well as of flavour and fragrance compounds in the food industry. There are studies of chamomile extraction using supercritical CO_2, for example, by Kaiser[4] and by Reverchon and Senatore[2].

In the present work in comparison to other studies instead of supercritical CO_2, we have used subcritical CO_2 to extract chamomile essential oil. This is a new method developed in our laboratory, namely Soxhlet extraction. It occurs in vapour-liquid equilibrium at 33 ºC and 61 bars. The objective was to obtain chamomile essential oil by assessing near critical CO_2 conditions (*i.e.* extraction at different temperatures) that optimize the process performance and attempting afterwards to characterize near critical fluid chamomile extracts by UV-visible spectrophotometer.

EXPERIMENTAL

The origin of the plant used (flowerheads) is not specifically known but it is a local Albanian plant. The plant is initially dried until constant weight and subjected afterwards to grinding process and then used such as for all the extractions. All the reagents and chemicals were of analytical reagent grade. Instrumental grade carbon dioxide was supplied in a cylinder. The extractions with subcritical CO_2 were carried out in an autoclave, as shown in the Fig. 1. Different thermodynamic parameters were used, *i.e.* different temperatures, 0, 20 and 40 ºC and respective pressures 25 bars, 35 bars and 65 bars. The extractions were continued up to 51 cycles (16-51 cycles depending on the temperature used during the extraction). A cycle has ended when liquid CO_2 reaches the maximum level at Soxhlet recipient (Fig. 1) and starts to spill over, down to the collection beaker. The amount of CO_2 used was 230 g. The crude extracts were taken after the evaporation of the solvent (CO_2) and were dissolved in dichloromethane and further analyzed by UV-visible spectrophotometer (Shimadzu UV 2400 PC).

RESULTS AND DISCUSSION

Extractions with liquid CO_2 were carried out at 0, 20 and 40 ºC with the respective pressures 25, 35 and 65 bars. The overall results for chamomile extracted with liquid CO_2 for

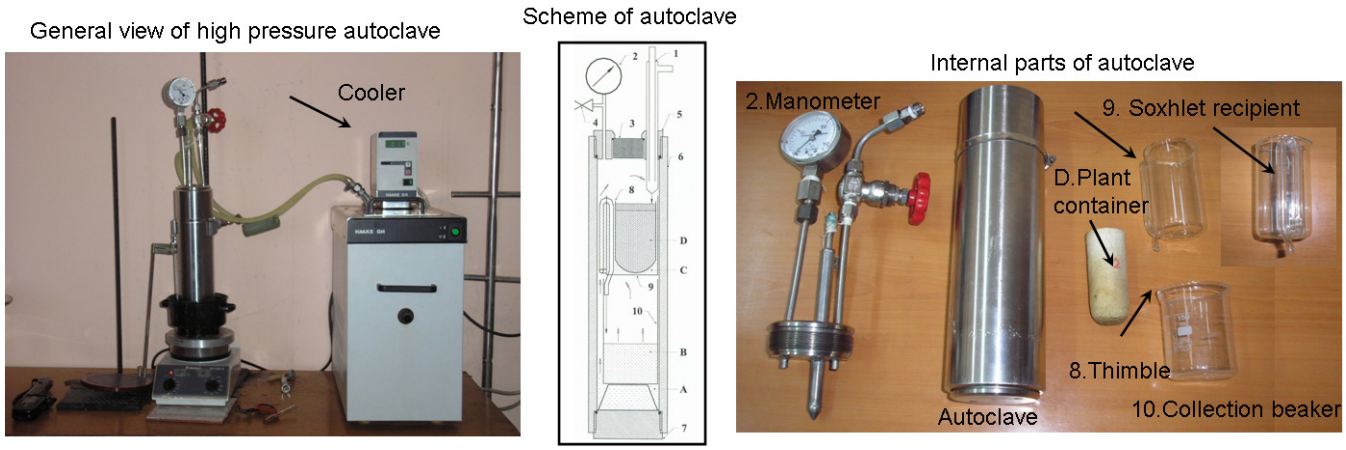

Fig. 1. Left: Photo of autoclave for CO_2 extraction; Middle: Schematic representation of the autoclave: 1. Cooler, 2. Manometer, 3. Window, 4. Pressure releaser, 5. Joint, 6. Cylinder, 7. Foundation, 8. Thimble, 9. Soxhlet recipient, 10. Collection beaker, A.C. Subcritical CO_2, B. Extract, D. Plant to be extracted (Plant container); Right: Photo of internal parts of the high pressure autoclave

TABLE-1
EXTRACTIONS CARRIED OUT WITH LIQUID CO_2 AT 0, 20 AND 40 ºC FOR CHAMOMILE

Amount of plant (g)	Amount of extract (g)	Bath temperature (ºC)	Number of cycles	Time of extraction for one cycle (min.)	Time of the overall extraction (min.)	Yield (%)
15	0.533	40	51	~14	~684 (~12h)	3.55
15	0.488	20	34	~21	~705 (~12h)	3.25
15	0.362	0	16	~50	~779 (~13 h)	2.41

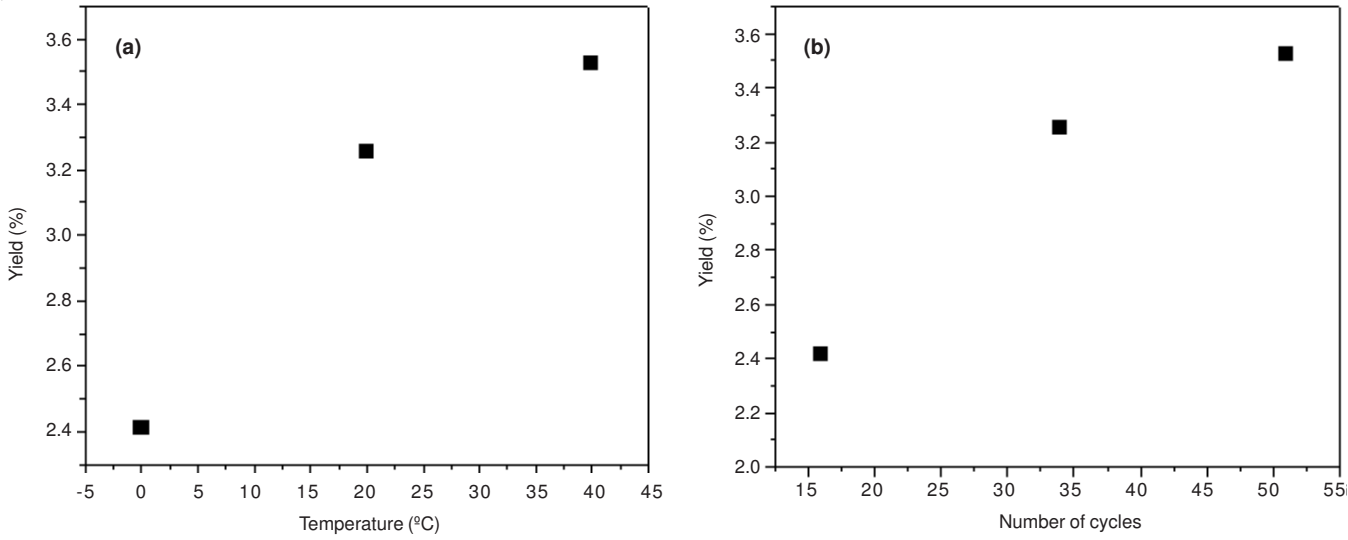

Fig. 2. Graphs of yield as a function of temperature a) and number of cycles b) for chamomile extracted with liquid CO_2. Data are taken from Table-1

different temperatures and pressures (Table-1). It is apparent from Table-1 that the optimal yield is reached at 20 ºC (3.25 %) whereas the maximum yield is achieved at 40 ºC (3.55 %). Therefore, the best result is attained at 40 ºC with the corresponding pressure 65 bars. The dependence of the yield as function of the temperature is plotted in the graph a) of Fig. 2. It is also evident from Table-1 that time of extraction for one cycle decreases with increased temperature as expected. A cycle ends when liquid CO_2 pours in the Soxhlet recipients as noted in the experimental part. However, it is important to mention that the first cycle takes always longer than the successive ones. In the first cycle the system requires additional time to reach the thermodynamic equilibrium. After the first cycle the following ones run faster.

Therefore, we have included average values (in min) in the column entitled; time of extraction for one cycle. Consequently, in the column entitled; time of the overall extraction, approximate values have been inserted. Additionally, number of cycles for the overall extractions decreases with decreased temperature as expected. At a lower temperature the thermodynamic system necessitates additional time to reach the equilibrium as mentioned. Graph b) of Fig. 2 displays yields obtained for each temperature as function of total cycles for each temperature (augmented values). As the temperature rises up, the numbers of cycles also enhance causing the yield of extraction to increase too.

As the maximum yield was achieved at 40 ºC (pressure 65 bars) we carried out additional experiments by varying the

time of extractions. 15 g of chamomile was extracted by liquid CO_2 in order to assess maximal yield. Table-2 shows the results of the obtained extracts (g) and corresponding yields as extraction time increases. The data shown in Table-2 are plotted in the graph of Fig. 3. Yield values are plotted as function of time including the number of cycles for each extraction. The yield of extraction reaches the optimal value after 8 h (2.4) and after 10 h remains almost constant.

TABLE-2
RESULTS OF EXTRACTIONS CARRIED OUT WITH LIQUID CO_2 at 40 ºC AS FUNCTION OF TIME FOR CHAMOMILE

Amount of plant (g)	Amount of extract (g)	Bath temp. (0 ºC)	Number of cycles	Time of the overall extraction (min.)	Yield (%)
15	0.027	40	1	17	0.18
15	0.105	40	3	40	0.7
15	0.167	40	6	80 (1.3 h)	1.1
15	0.28	40	20	300 (5 h)	1.9
15	0.367	40	30	480 (8 h)	2.4
15	0.378	40	40	600 (~10 h)	2.5

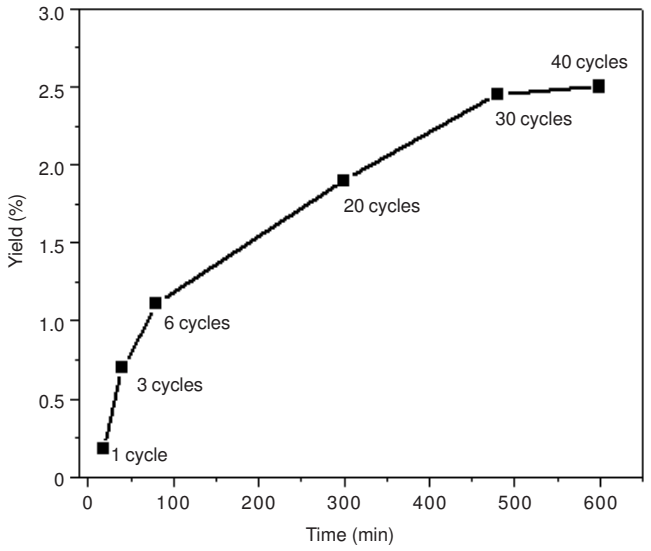

Fig. 3. Graph of yield as a function of time for chamomile (number of cycles in the inserts). Extractions were carried out at 40 ºC

However a slight discrepancy is observed when comparing the yield after 10 h (Table-2) with that obtained after 12 h (Table-1). The acquired yield after 10 h was 2.5 %, while the yield after 12 h was 3.5 %. This difference, although not very significant, can be attributed to the fact that the extraction carried out for 12 h was a non-stop experiment. Whereas the extractions reported in Table-2 (Fig. 3) were carried out by interrupting regularly the experiments after a certain number of cycles.

After the first cycle the extraction was stopped by emptying the autoclave (releasing the valve) and weighing afterwards the obtained extract (after 1 cycle). The collection beaker was cleaned while the extract was preserved in a dark vial diluted in CH_2Cl_2. The same sample/plant was re-inserted in the plant container (inside the autoclave). The autoclave was re-filled with CO_2 and the second extraction was allowed to run for 2 additional cycles (1 + 2 = 3 cycles in total). After the third cycle the extraction was stopped and the extract was weighed. The amount of the extract obtained after 2 cycles was added to the amount obtained after 1 cycle. The sum gives the amount of extract for 3 cycles. The experiments were carried likewise up to 40 cycles. During the continuous discharge of the autoclave volatile compounds also escape. The latter gives rise to weigh loss of the obtained extract and the yield will be as a result diminished.

Lastly, we attempted to characterize the extract obtained at 40 ºC by UV-visible spectrophotometer. UV-visible analyses of chamomile extracts are reported in the literature[4,5]. It is important to mention that all the obtained extracts were yellow in colour. In this respect, it is reported that due to thermal degradation matricine converts to chamazulene giving the essential oil a blue colour[1,5,6]. This is especially common for the traditional methods *i.e.* steam distillation[2]. This suggests that the extracts of this work did not undergo thermal degradation. Also the extracts obtained at 0 and 20 ºC were yellowish. UV-visible spectra of the extract obtained at 40 ºC are shown in Fig. 4. The spectrum of concentrated extract (5 % in CH_2Cl_2) features two peaks at *ca.* 620 nm and *ca.* 670 nm (Fig. 4, left panel). Upon dilution in dichloromethane the UV-visible spectrum appears slightly different. The peaks at *ca.* 620 nm and *ca.* 670 nm attenuate, whereas a wide band in the region *ca.* 240-350 nm becomes apparent (Fig. 4, left panel). Additionally, three peaks-shoulders appear in the region *ca.* 400-500 nm. Upon further dilution of the extract the broad band at *ca.* 240-350 nm appears perturbed and a minor peak at *ca.* 245 nm becomes visible. It is reported that matricine and dicycloethers absorbs at 244 nm[4]. Furthermore, liquid CO_2

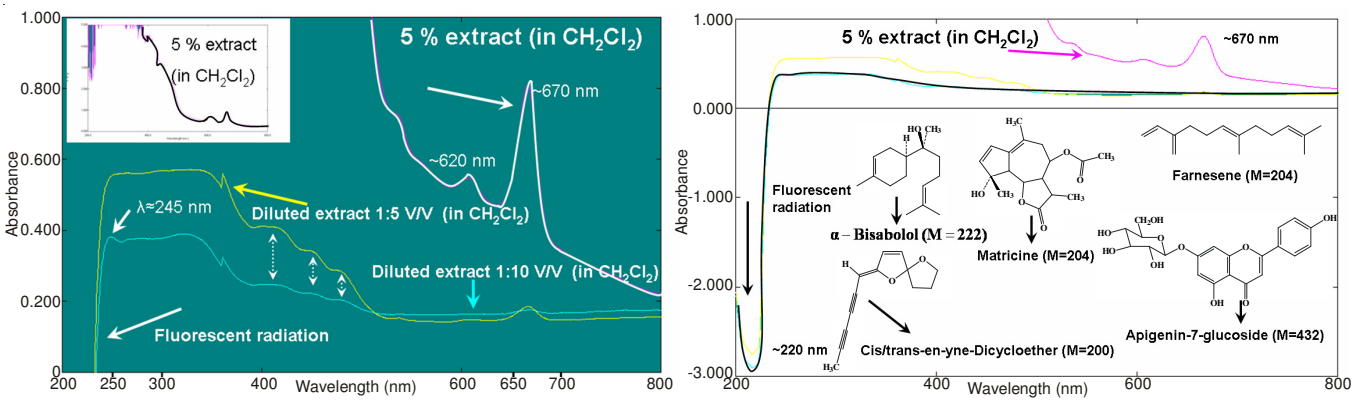

Fig. 4. UV-visible spectra of the chamomile extract obtained at 40 ºC. The extract was diluted in dichloromethane. In the inserts are shown chemical structures of the main constituents of chamomile essential oil as reported in the literature[1,2,4-6]

dissolves well compounds with molecular weight < 250 g/mol[7]. In this context, matricine and *cis/trans*-en-yne-dicycloether (see chemical structures in the inserts of the right panel of Fig. 4) have molecular weights 204 g/mol and 200 g/mol respectively. It is well known that liquid CO_2 extracts also sesquiterpenes[7]. In this respect, α-bisabolol the major constituent of the chamomile essential oil is a sesquiterpene[5] with a molecular weight 222 g/mol (chemical structure in the inserts of the right panel of Fig. 4). We cautiously attribute the broad band at 400-500 nm to α-bisabolol and its oxides[8,9]. Farnesene is another compound present in the chamomile essential oil. It has a molecular weight of 204 g/mol. Farnesene contains conjugated double bond. When a double bond is part of a conjugated chain the energies of the molecular orbitals lie closer together and the (π-π^*) -transitions shift into the visible region of the spectrum[9]. Therefore, it is logical to assume that the features in the region *ca*. 620-670 nm may originate from the conjugated double bond of farnesene.

Additionally, an unidentified fluorescent peak appears at *ca*. 220 nm. Finally, extracts obtained at 0 ºC and 20 ºC were also analyzed by UV-visible spectrophotometer. The UV-visible spectra of the extracts acquired at 0 ºC and 20 ºC featured great similarities with UV-visible spectrum of the extract obtained at 40 ºC. The latter suggests the presence of the same constituents/compounds in the extracts obtained at 0, 20 and 40 ºC. Lastly, apigenin-7-glucoside with molecular weight of 432 g/mol can be extracted under supercritical conditions (pressures above the critical point, 70 bars)[4]. Under critical conditions (this work) apigenin-7-glucoside can also be extracted at temperatures 32-33 ºC and pressures 55-65 bars. However, bearing in mind that UV-visible spectra of the extracts obtained at 0 ºC and 20 ºC featured great similarities with UV-visible spectrum of the extract obtained at 40 ºC, we exclude the possible presence of apigenin-7-glucoside in the essential oil extract of chamomile.

Conclusion

The essential oils of chamomile were extracted by subcritical CO_2 using high-pressure (65 bars) Soxhlet apparatus. Different thermodynamic parameters were applied assessing the best near critical conditions. The extraction carried out at 40 ºC provided the highest yield whereas, the extraction carried out at 0 ºC gave rise to the lowest yield. Analysis of UV-visible spectra revealed presence of *cis/trans*-en-yne-dicycloether and/or matricine. We cautiously attributed the appearance of the additional peaks in the UV-visible spectrum (400-500 nm) to the α-bisabolol and its oxides known as the major constituents of chamomile essential oil. We also revealed the probable presence of farnesene in the essential oil extract of chamomile.

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