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# Influence of the Processes Extraction on Essential Oil of *Origanum glandulosum* Desf

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**Abstract:** Essential oils obtained from *Origanum glandulosum* Desf. using supercritical carbon dioxide, micro wavedistillation, hydrodistillation and solvent ethanol were analyzed with GC/MS. The extraction with pressurized $CO_2$ was performed at 15°C and 67 bar. The major valuable component extracted was thymol (63.8, 75.3, 55.6 and 82.4%), respectively, wile The p-cymene and γ-tepinene were revealed only in $CO_2$ extract, microwavedistillation and hydrodistillation (13.7, 6.0 and 12.5%) and (6.8, 8.4 and 11.2%), respectively.

**Key words:** *Origanum glandulosum*, essential oil, supercritical fluid extraction, hydrodistillation, microwave distillation, ethanol extraction

## INTRODUCTION

Much scientific research is currently focused on industrial development coupled with environmental preservation. Recently, the use of the extraction techniques with pressurized fluids and microwave have increased due to their advantages compared to conventional extraction methods (hydro-distillation and solvent extraction). The advantages of this methods are:

- By microwave distillation, we have reduction in extraction time with free solvent (Zlotorzynski, 1995; Lucchesi *et al*., 2004a,b).
- That extracts with Supercritical fluid (SFE) are free of residues, it is possible to work at lower temperatures, reducing the decomposition of thermolabile compounds present in the extract and the preservation of organoleptic characteristics of the oil (Pellerin, 1991).

Extraction processes that employ fluids under high pressure as solvents to obtain Essential Oils (EO) and microwaves energy are generally regarded as clean processes and are increasingly replacing traditional extraction methods. Such methods are known as SFE which typically utilize $CO_2$ as a solvent (because it is a relatively non toxic and inert gas) and MD for microwave distillation.

Utilization of this two processes have shown excellent results, characterized by the introduction of high selectivity and excellent extraction capacity when compared to conventional extraction processes. Therefore, these techniques are finding wider application in natural product separation (Calinescu *et al*., 2002; Gaspare and Leeke, 2004).

The genus *Origanum*, which is concentrated in the Mediterranean area, has many species and several varieties. *Origanum* species are important sources of antimicrobial and antioxidant agents. *O. glandulosum* Desf. is a member of the genus that grows in Algeria. Two chemotypes characterizes the EO composition of Algerian *O. glandulosum* Desf.: (I) thymol; (ii) carvacrol (Ruberto *et al*., 2002). According to Ietswaart (1980), *O. glandulosum* is endemic to the Algerian and Tunisian areas. In Algeria, the species it is called *zaâtar* and used against whooping cough, fever and bronchitis. *O. glandulosum* was the most widespread plant of the western region of Algeria (Quezel and Santa, 1962).

The objective of this study is to examine the extraction processes of the essential oil from the specie named above with pressurized $CO_2$, microwave distillation and to compare the results with conventional processes (hydrodistillation and ethanol extraction).

## MATERIALS AND METHODS

**Characterization and preparation of the samples:** The natural samples were collected in the region of Tlemcen (Algeria) [1190 m, 34°49 latitude north et 1°19 longitude west ] in June 2004. They were dried naturally in the shade

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for one week. Voucher specimens have been deposited in the Herbarium of the Laboratory of Ecology Vegetable, University Aboubekr Belkaid-Tlemcen.

After harvesting, the samples were cleaned, packed in plastic bags under vacuum, then shipped to Brazil where they were stored in a domestic freezer at -20°C. The water humidity of the raw material was determined using the Jacobs (1973) method, utilizing xylene for the distillation, as recommended for odoriferous plants. The moisture content was 10.6% (m/m). The plant materials were finely chopped using a domestic food processor (ARNO, model PRO, Brazil), for 15 sec. The chopped solids were separated according to size using sieves from the Tyler series for 15 sec in a shaker (Prod test, n°. 3614, rheostat 10). The chopped plant materials in these experiments were composed of the following particle sizes: of 28, 35 and 48 mesh, with a percentage of 42, 42 and 16%, in weight, respectively. The average diameter and apparent density of particles were 0.40 mm and 0.5346 g $cm^{-3}$, respectively.

**Extraction with pressurized $CO_2$:** The SC experimental device used for these experimental tests is illustrated in Fig. 1. The experimental apparatus consists basically of a cylinder of $CO_2$, jacketed extractor column, lung tank to stabilize the operational conditions (temperature and pressure), thermostatic bath, separator vase, manometers, thermocouples and a flux measurer.

For each test, the sample was weighed and placed inside the extractor cell with the help of a funnel and compressed with a stem in order to obtain a completely uniform layer. After packing the extractor, the system was initially stabilized by regulating the temperature and pressure. Once these conditions were realized, the extraction process was started by opening the valve placed before the extractor after having opened the downstream valves and the micrometric valve regulated. The chronometer started with the appearance of the first drops of the oil. For pre-determined times intervals the collector flasks were closed, weighed and stored in the freezer for later analysis.

Solubility was determined using the dynamic method Rodrigues *et al.* (2002) and Elisa *et al.* (2002). Extraction curves were generated for each experiment (oil mass *vs.* time). Using this method, it is possible to determine the extraction rate constant as well as the particular extraction rate. All the experiments were repeated for twice.

**Microwave distillation:** An Arthur Martin multimode microwave oven operating at 2450 MHZ and 850W equipped with an external cooling system as described by Lucchesi *et al.* (2004a, b), was used for the extraction of the essential oil of *O. glandulosum* at atmospheric pressure. Twenty five gram of dry plant material was moistened prior to extraction by soaking in 60 mL of water for 1 h and then draining off excess water.

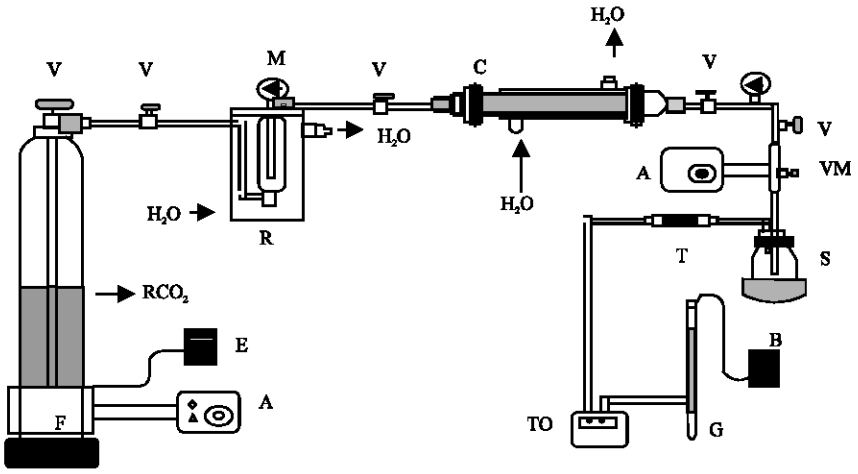


Fig. 1: Experimental Device: $RCO_2$ = Siphoned Cylinder of stainless steel with a fishing (capacity of 25 kg), conditioned with liquid $CO_2$; F = Heating tape (FISATON-Brazil); M = Manometer type Bourdon, with capacity for 100±1 kgf $cm^{-2}$ (RECORD- Brazil); $H_2O$ = Water bath (Tecnal, model TE 184); R = Jacketed cylinder to keep the solvent as a sub cooled liquid with capacity of $0.5\times10^{-3}$ $m^3$; T = capillary glass tube filled with porapak-Q (Supelco, 80/100 mesh, 75CC, lot 113, USA); S = glass flask, capacity for 5 mL; B = glass recipient with ice-cubes and water; A = Voltage regulator (VARIAC, STP- Paulista Technical Society); E = digital thermometer (Lutron, model TM-905); F = heating tape (Fisaton, model 5, Brazil); V = needle type valves (HOKE); C = Extractor column of double tube, in stainless steel, (0.6 m× 0.0216 m× 0.028 m), connected to the thermostatic bath. VM = Micrometric valve (HOKE, model 1335G2Y, USA); B = Glass Bublemeter; TO = flow totalizer (LAO, model G1, Brazil)

After, the moistened material was placed in a reactor and heated by microwave irradiation for 30 min without adding any solvent. A refrigerating system outside the microwave cavity condensed the distillate continuously. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used. The method yielded 3.3% of a deep yellow oil.

**Solvent Extraction (SE):** Ethanol was used for solvent extraction of each plant sample as allowed by the Brazilian Legislation for the Use of Food, following the procedure described by Povh *et al.* (2001): Thorty gram of chopped plant material was mixed with 180 ml ethanol and the mixture was agitated (190 tr $min^{-1}$) under refrigeration for 8 h, in order to minimize extractions of fats, fatty acids and pigments, as well as to minimize solvent evaporation. After extraction, the mixture was filtered under vacuum and 20 mL of the filtrate was evaporated in Petri dishes at 18°C over 48 h in order to minimize evaporation of the essential oil. The Petri dishes were then weighed and reweighed after 6 h to check for variation in mass.

The yield of crude ethanol extract (essential oil, fats of cuticles and pigments) was calculated by dividing the plant sample mass by the extracted oil mass. In order to determine the mass of essential oil, the Petri plates were put in a hothouse with air rotation at a temperature of 165°C and the samples were weighed at 30 min intervals until a constant weight was obtained. At this temperature the essential oils are evaporated, leaving behind only non-volatile greases and pigments. The quantity of the essential oil was calculated as the difference between the mass of crude extract and the mass after hothouse treatment.

**Hydro-Distillation (HD):** The essential oils were obtained by Hydro-Distillation (HD) using a Clevenger apparatus (European Pharmacopoeia, 1975). Plant material (100 g) and water were placed inside a flask and connected to the condenser. The essential oil and the water mixture were finally separated by decantation. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used for analysis.

**Chemical analysis:** The chemical analysis of the substances was determined using a gas chromatograph coupled to a mass spectrometer system (GC/MS): SHIMADZU, model QP-5000, with capillary column DB-5 (30 m×0.25 mm×0.25 μm J and W Scientific). The carrier gas (helium); Flow: 1.0 mL $min^{-1}$; Split: 1/20; Detector temperature = 230°C, injector temperature = 240°C. Injection volume: 1 μL of solution (ethyl acetate). The temperature programming was from 60-240°C, 3°C $min^{-1}$. The identification of the substances was made through the comparison of its spectra of masses with the database of the system GC-EM (Nist.62 library) (McLaferty and Stauffer, 1989) and retention index (Adams, 1995). The quantitative analysis was conducted using gas chromatography (GC-FID), SHIMADZU, model GC-17, operating at the same conditions of the GC-MS.

## RESULTS AND DISCUSSION

Figure 2 show typical curve of the kinetics of the essential oil extraction processes in $CO_2$ as solvent under high pressure, microwave-distillation and hydro-distillation at different times. The curves SFE and HD are characterized by three distinct regions: (I) the constant extraction step, where the solute is extracted essentially by convection; (ii) period of decreased rate, where convection and diffusion actuate; (iii) diffusional period, where the oil diffusion of the mixture solute/solvent prevail in the solid. While with microwaves, we observe a rapid diffusion oil. According to Gaspar and Leek (2004) and Benjilali (2003) the increases in SFE and HD yields of oil were related to the disruption of the oil gland and the experimental extractions. In MD process, the rapidly oil yield obtained is related to the energy transfered by dielectric loss but not by conduction or convection (Fini and Brecia, 1999; Walter and Chalk, 1999).

The results of extraction yields using SFE, MD, SE and HD are presented in Table 1. The oils obtained by the $CO_2$ and microwavedistillation methods were clear, slightly viscous and showed a deep yellow coloration. The solubility value for SFE oil was $0.69.10^{-2}$ g/g $CO_2$. Those obtained by ethanol extraction showed higher

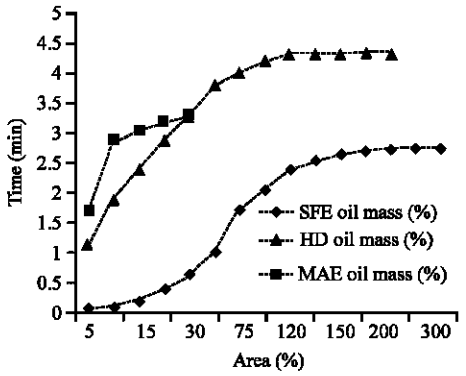


Fig. 2: Extraction kinetics curves of the essential oils obtained by SFE, MD and HD, SFE: Supercritical fluid $CO_2$ extraction (T= 15°C, P = 66.7 bar rate, Flow of $CO_2$ = 1.23 g $CO_2$ $min^{-1}$), MD: Microwavedistillation, HD : Hydrodistillation

viscosity, due to the presence of waxes and pigments while the essential oil obtained by hydrodistillation was mobile and showed a pale yellow coloration. We observe that extraction using ethanol as a solvent shows superior yield (3.89±1.11%) compared to the SFE (2.74±0.34%) and MD (3.30±0.31%) methods. However, in this total extract the pigments and the waxes that are in the raw material are also included. Hence, considering only the essential oil, the conventional technique HD shown superior results compared to those of the supercritical, microwave distillation and ethanol (1.20±0.4%) extractions. Nevertheless, the yield of EO obtained by MD was higher than the yields of EO obtained by SFE and ethanol.

**Chemical analysis:** Table 2, present the results of the chemical analyses of oils, obtained by the different extraction methods.

As shown in Table 2, the analysis allowed 21 compounds identified consisting of more than 95.4% (SFE), 98.1% (MD), 89.9% (ethanol) and 93.9% (HD). A variation is observed in the composition of the oils. The extracts obtained with all four methods contain a considerable amount of thymol, which varies according to the technique: 63.8% by SFE, 75.3% by MD, 82.4% by ES and 55.6% by hydrodistillation. The oxygenated monoterpenes were higher in al oils (60.2-88%) compared to the monoterpenes hydrocarbons (00.0-31.8%). The essential oil obtained by hydrodistillation contain more constituents, while the EO obtained by Ethanol had fewer components. The concentration of γ-terpinene and p-cymene were higher in HD oil (11.2%) and SFE oil (6.8%), respectively.

Table 1: Extraction yield

| Specie | | *Origanum glandulosum* (%) |
|---|---|---|
| SFE (15°C 67 bar) | | 2.74 |
| | MD (30 min) | 3.30 |
| SE | Total extract | 3.89 |
| | Essential oil | 1.20 |
| | HD (3 h) | 4.50 |

SFE: Supercritical $CO_2$ extraction, MD: Micowavedistillation, SE: Solvent Extraction, HD : Hydrodistillation

Table 2: Composition of the extracts of *O. glandulosum* (area %)

| Compound | RI[a] | SFE (area %) | MD (area %) | SE (area %) | HD (area %) |
|---|---|---|---|---|---|
| α-Thujene | 923 | 0.8 | - | - | 0.7 |
| a-pinene | 931 | - | - | - | 0.6 |
| 1-Octen-3-ol | 959 | - | - | - | 0.3 |
| 3-Octanone | 963 | - | - | - | 0.1 |
| b-Pinene | 971 | - | - | - | 1.4 |
| β-Myrcene | 980 | 0.5 | 1.0 | - | 1.4 |
| Δ-3-Carene | 1005 | - | Tr | - | 0.5 |
| α-Terpinene | 1010 | 0.7 | - | - | 0.6 |
| p-Cymene | 1014 | 13.7 | 6.0 | - | 12.5 |
| Limonene | 1022 | 0.3 | 0.3 | - | 2.5 |
| γ-Terpinène | 1050 | 6.8 | 8.4 | | 11.2 |
| Tr-Sabinene hydrate | 1055 | - | 0.2 | - | 0.3 |
| Linalool | 1086 | 1.6 | 0.9 | - | 1.2 |
| Terpinene-4-ol | 1166 | 0.6 | - | - | 0.4 |
| α-Terpineol | | 0.4 | - | - | Tr |
| Thymol | 1277 | 63.8 | 75.3 | 82.4 | 55.6 |
| Carvacrol | 1283 | 3.6 | 4.7 | 5.6 | 2.7 |
| trans-Caryophyllene | 1420 | 1.2 | 0.9 | - | 0.9 |
| β-Sesquiphellandrene | 1516 | 0.9 | 0.4 | - | 0.8 |
| Caryophyllene oxide | 1572 | 0.5 | Tr | 1.9 | 0.2 |
| Total | | 95.4 | 98.1 | 89.9 | 93.9 |
| Monoterpenes hydrocarbons | | 22.8 | 15.7 | - | 31.8 |
| Oxygenated monoterpenes | | 70.0 | 81.1 | 88.0 | 60.2 |
| Sesquiterpenes | | 2.6 | 1.3 | 1.9 | 1.9 |

SFE: Supercritical fluid extraction, MD: Microwavedistillation, SE: Solvent extraction, HD: Hidrodistillation, [a]RI: Retention index on DB-5, tr: trace ($p<0.05\%$)

Table 3: Comparative results of the literature

| | | Setif (Sari *et al.*, 2006; Ruberto *et al.*, 2002) | Alger (Houmani *et al.*, 2002) | | Tlemcen | | |
|---|---|---|---|---|---|---|---|
| Origin (Algeria) | | HD | HD | HD | SFE | MD | SE |
| Yields (%) | | 2.56 | 1.56 | 4.50 | 2.74 | 3.30 | 1.20 |
| | Thymol | 18.5-73.1 | 21-31 | 55.60 | 63.80 | 75.30 | 82.40 |
| Major | Carvacrol | 7.6-72.6 | 2.4-7.4 | 2.70 | 3.60 | 4.70 | 5.60 |
| components (%) | p-Cymene | 1.7-18.5 | 6.7-10.3 | 12.50 | 13.70 | 6.00 | - |
| | γ-Terpinene | 1.1-18.7 | 8.7-18.4 | 11.20 | 6.80 | 8.40 | - |

SFE: Supercritical fluid extraction, MD: Microwavedistillation, SE: Solvent Extraction, HD: Hidrodistillation

Ruberto *et al.* (2002) and Sari *et al.* (2006) have reported the chemical composition of EO of *O. glandulosum* collected from different region. The main components were thymol and/or carvacrol, p-cymene and γ-terpinene with account 88% for all samples. The same percentage was obtained with ethanol and SFE extracts. But regarding the concentration of thymol, our specie was more richer from 55.6 to 82.4% (Table 3).

The differences in yield and composition of extracts were caused by the extraction conditions (Gaspare and Leek, 2004). In SFE and Ethanol extracts, the oil was obtained without heating and amount used of water. While in MD, the polar substances were rapidly heating for a short time without solvent. As such, the principal compounds were conserved. But in HD, the temperature and the water accelerated oxidation/hydrolysis reactions.

## CONCLUSIONS

The results showed that the mode of the extraction highly influences the yield and composition of EO. The chemical composition of the extracts varied with operational conditions. The chemical profiles of the extracts also showed differences. The ethanolic extracts were less rich in components compared to their respective extracts using the other methods. However, the alcoholic extract contains thymol as the major compound. Finally, because the extracts with the MD and SFE process were richer in components, in particular oxygenated monoterpenes, we can safely conclude than the SFE extraction is a good alternative method to give natural oils than the essential oils obtained by hydrodistillation.

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