# Comparison of Microwave-Assisted Hydrodistillation and Traditional Hydrodistillation Methods for the *Rosmarinus eriocalyx* essential oils from Eastern Morocco

**O. Fadel [1], Z. Ghazi [1], L. Mouni [1], N. Benchat [1], M. Ramdani [1*], H. Amhamdi [2], J.P. Wathelet [3], A. Asehraou [4], R. Charof [5]**

[1] *LCAE-URAC18, Faculté des Sciences, Université Mohammed Premier, Oujda, Morocco*
[2]*Laboratoire de Chimie Physique des Ressources Naturelles et Environnement, Ecole Nationale des Sciences Appliquées d'Al Hoceima, Université Mohammed Premier BP: 3, Ajdir, 32 003 Al Hoceima, Morocco*
[3] *Gembloux Agro-Bio Tech, Département de Chimie et Bio-industries, Unité de Chimie générale et organique, Université de Liège. Gembloux, Belgique*
[4] *Laboratoire LBPM, Faculté des Sciences, Université Mohammed Premier, Oujda, Morocco*
[5] *Institut National d'Hygiène, Rabat, Morocco*

Received in 06 Sept 2008, Revised 11 Oct, Accepted 13 Oct 2010.
Corresponding Author: E-mail: moharamdani2000@yahoo.fr, Fax : +212536500603, Tel : +212676889949

## Abstract

Volatile compounds of *Rosmarinus eriocalyx* samples were extracted by steam-hydrodistillation (HD) and microwave-assisted hydrodistillation (MAH). GC-MS and GC-FID analysis of the oils revealed the presence of 22 and 26 compounds in the essential oils obtained through HD and MAH, respectively. The total yield of the volatile fractions obtained through HD and MAH was 1.21% and 1.47%, respectively.
The two oils contained the same dominant components: camphor (35.92% HD; 35.33% MAH), camphene (19.74%; 17.07%), α-pinene (14.53%; 12.87%), and 1,8-cineol (6.52%; 6.73%). Higher amounts of oxygenated monoterpenes such as borneol, 1,8-cineol, isobornyl acetate, α-terpeneol, caryophyllene oxide (13.59%) were present in the oil of MAH in comparison with HD (12.36%). However, HD oil contained more monoterpene hydrocarbons such as tricyclene, α-pinene, camphene, o-cymene and limonene (39.04%) than MAH extracted oil (34.45%).

*Keywords: Rosmarinus eriocalyx,* Essential oil, Hydrodistillation, Microwave-assisted hydrodistillation, Oxygenated monoterpenes, Monoterpene hydrocarbons.

## 1. Introduction

There exist three rosemary species of the Lamiaceae family that naturally grow in the Mediterranean region: *Rosmarinus officinalis* L., *R. eriocalyx* Jordan & Four and *R. tomentosus* Hub.-Mor & Maire. *R. officinalis* is wildly growing and mainly cultivated in Mediterranean countries, such as Morocco, Tunisia, Spain, France and Italy [1-3].

While, *R. eriocalyx* grows in a small region of Andalucia (Southern Spain) and in the northwest of Africa. This specie differs from *R. officinalis* in its smaller leaves, only 5-15 mm long and less than 2 mm broad, and densely hairy flower stems. It also tends to be lower-growing, often under 25 cm tall and prostrate, and never exceeding 1 m tall (*R. officinalis* can reach 1.5 m, exceptionally 2 m, tall).

Rosemary essential oils have been used for thousands of years for food preservation [4], pharmaceuticals, alternative medicine and natural therapies [5-7]. The plant is also known for its powerful antioxidant activity [8]. Recently, in our laboratory, Hammouti et al. [9] reported that *Rosmarinus officinalis* oil can be used as a green inhibitor for the corrosion of steel in 0.5 M $H_2SO_4$.

Essential oils are isolated using a number of methods [10] such as steam distillation (SD), hydrodistillation (HD), organic solvent extraction, microwave assisted hydrodistillation (MAH), microwave hydrodiffusion and gravity (MHG) [11,12], high pressure solvent extraction (HPSE), supercritical $CO_2$ extraction (SCE) [13], ultrasonic extraction (UE) and solvent free microwave extraction (SFME) [14].

The essential oil volatile composition of the *R. officinalis* has been the subject of considerable research in recent years [15]. Rosemary essential oil contains mainly monoterpenes and monoterpene derivatives (95–98%), the remainder (2–5%) being sesquiterpenes [16,17]. The principal volatile compounds in rosemary are camphor and 1,8-cineole, followed by borneol, verbenone, α-pinene and camphene [18,19]. The chemical analysis of *R. officinalis* oil harvested by Hammouti et al. [9] in October 2006 from Morocco on Jerada locality shows 1,8-cineol (54.1%) as the major component followed by camphor (14%), α-pinene (9.6%), α-terpineol (4.8%), β-pinene (3.4%) and borneol (2.9%).

In Greece [20], Myrcene was the major component present in the essential oils with concentrations always exceeding 20 %, 1,8-Cineole (8.0-12.2 %), α-pinene (8.8-16.5 %) and camphor (4.4-14.1 %).

The main compounds of the Brazilian oils [21] were: α-pinene (40.55-45.10%), 1,8-cineole (17.40-19.35%), camphene (4.73-6.06%), verbenone (2.32-3.86%), borneol (2.24-3.10%) and camphor (1.63-2.42%). Compared with other rosemary oils, Brazilian oils were more similar to those of French origin due to their 1,8-cineole and camphor contents [22-24]. There was also similarity between Brazilian oils and oils of North American cultivars [25].

However, *R. Eriocalyx* was rarely investigated by the researchers. The essential oils of Rosmarinus eriocalyx which were produced from the leaves of six populations of plants collected in Algeria [26] under different ecological conditions were rich on camphor (32.3-37.0%), followed by camphene (17.0-20.0%), α-pinene (15.2-18.2%) and 1,8-cineole (7.6-11.4%).

To the best of our knowledge, there is no previous report on the chemical composition of the essential oil of *R. Eriocalyx* collected from Tafoughalt (Beni-Snassen Mountains) in the north-eastern Morocco. In this paper we report the chemical composition of the oils isolated by hydrodistillation (HD) and Microwave-assisted hydrodistillation (MAH) from the leaves of *R. Eriocalyx*. A comparative study of the chemical composition of the essential oil extracted by the two methods was investigated. Our results were then compared with these previously published in the literature.

## 2. Materials and methods

### *2.1. Plant material*

Fresh leaves of R. *E*riocalyx were collected from Tafoughalt (Beni-Snassen Mountains) in the north-eastern Morocco on March 2008. Leaves were dried in the air protected against direct sunlight.

### *2.2. Hydrodistillation (HD)*

400 g of dried leaves were hydrodistilled with 900 ml of water for 220 min using a Clevenger apparatus (until no more essential oil was recovered). The essential oil was collected and dried over anhydrous sodium sulphate yielding 1.21 %.

### *2.3.Microwave-assisted hydrodistillation (MAH)*

400 g of dried leaves were hydrodistilled with 500 ml of water by microwave energy at 460 W in the multimode reactor fixed at 2450 MHz and equipped by a Clevenger apparatus. The extraction oil was performed at atmospheric pressure for 80 min. The essential oils were dried over anhydrous sodium sulphate yielding 1.47 % and stored in the dark at 5°C until analysis.

## 3. Analytical procedures

### *3.1. Analytical gas chromatography (GC/FID)*

GC analysis was carried out using an Agilent 6890 gas chromatography equipped with a flame ionisation detector (FID) and a 30 m x 0.25 mm HP-5 (cross-linked Phenyl-Methyl Siloxane) column with 0.25 µm film thickness (Agilent). Helium was used as carrier gas, the flow through the column was 1 ml min$^{-1}$, and the splitless mode was used. The column was initially at 35°C, increased to 85°C at rate of 20°C/min then increased to 300°C at rate of 5°C/min and held for 10 min for a total run time of 55.5 min. The relative proportions of the essential oil constituents were expressed as percentage obtained by peak area normalization.

### *3.2. Gas chromatography/mass spectrometry (GC/MS)*

The oils were analysed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 5973N mass selective detector coupled with an Agilent 6890N gas chromatograph. The MS operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass units were monitored from 30 to 450 m/z. The components of the oils were identified by matching their mass spectra and retention indices relatives to n-alkanes from C-5 to C-20 with those of the Wiley 275 library (Wiley, New York) in the computer library and literature [27-30]. The chromatographic conditions were identical to those used for GC analysis.

## 4. Results and discussion

### *4.1. Chemical composition of the essential oil*

The chemical composition of the volatile fractions obtained from *R. Eriocalyx* during the HD and MAH extraction processes are represented together with the retention indices in Table 1. The GC–MS analysis of the oil samples revealed the presence of a total of 27 components.

The total yield of the volatile fractions obtained through HD and MAH was 1.21% and 1.47%, respectively. Twenty-two compounds were identified in the hydrodistilled oil which accounted for 97.97% of the total oil composition. This oil was dominated by monoterpenoids such as tricyclene, α-pinene, camphene, o-cymene and limonene (39.04%). However, twenty-six compounds were identified from the microwave extracted oil which accounted for 97.63% of the total oil composition. This oil contained only (34.45%) of monoterpenoids. The two oils recovered from HD and MAH extraction contained the same dominant components: camphor (35.92%, 35.33%); camphene (19.74%, 19.07%); α-pinene (14.53%, 12.87%); 1,8-cineol (6.52%, 6.73%) and β-pinene (4.60%, 4.63%), respectively.

The oil compositions revealed that higher amounts of oxygenated monoterpenes such as borneol, 1,8-cineol, isobornyl acetate, α-terpeneol, caryophyllene oxide (13.59%) are present in the essential oil isolated by MAH in comparison with the oil extracted by HD (12.36%). This difference in the essential oil composition of rosemary could be attributed to the hay absorption of microwave by polar compounds in MAH more than in HD extraction. This supports the hypothesis that the nature and composition of essential oils may vary depending on the methods of extraction used [14,31]. This constitutes the advantage of microwave haiting. According to Presti et al. [10] the method of essential oil extraction affects their chemical compositions and biological activities. Recently, some studies [14,15] showed that MAH oil was more active against microorganisms than the oil obtained through HD. This may be partly due to the fact that the microwave extracted oil and these clases of compounds have been proved to possess strong antibacterial and antifungal activities [32-34].

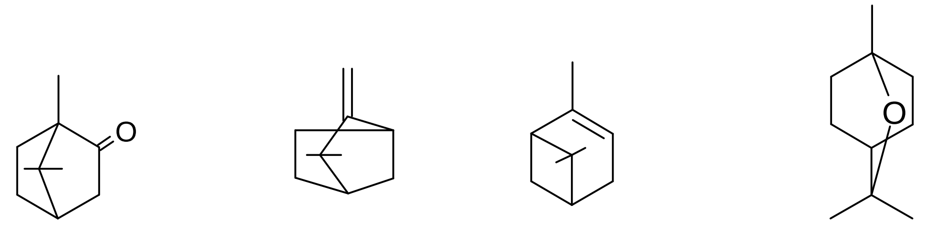
The same chemotype described for the *R. Eriocalyx* Algerian essential oil [26] was found for our essential oil respectively: camphor (37.0, 35.92%), followed by camphene (20.0, 19.74%), α-pinene (18.2, 14.53%) and 1,8-cineole (11.4, 6.52%).

Compared with the *R. officinalis* oil harvested by Hammouti and al [9] from the Eastern Morocco, 1,8-cineol (54.1%) was the major component followed by camphor (14%). we confirmed the presence of the two species: *R. Eriocalyx* and *R. officinalis* in the Eastern Morocco.

**Table 1** Chemical composition of *R. Eriocalyx* essential oils obtained by hydrodistillation HD and microwave extraction MAH.

| No | Compounds | KI | HD % | MAH % |
|---|---|---|---|---|
| 1 | tricyclene | 919 | 0.86 | 0.73 |
| **2** | **α-pinene** | **930** | **14.53** | **12.87** |
| **3** | **camphene** | **946** | **19.74** | **17.07** |
| 4 | β-pinene | 974 | 4.60 | 4.63 |
| 5 | o-cymene | 1019 | 1.55 | 1.54 |
| 6 | limonene | 1024 | 3.22 | 2.97 |
| 7 | **1,8-cineole** | **1028** | **6.52** | **6.73** |
| 8 | trans sabinene hydrat | 1095 | 0.47 | 0.36 |
| 9 | alloocimene | 1122 | 0.73 | 0.66 |
| **10** | **camphor** | **1147** | **35.92** | **35.33** |
| 11 | pinacarvone | 1154 | 0.41 | - |
| 12 | 1-borneol | 1163 | 3.38 | 3.47 |
| 13 | terpinene-4-ol | 1173 | 1.20 | 1.13 |
| 14 | p-cymen-8-ol | 1178 | - | 0.12 |
| 15 | α-terpineol | 1185 | 0.75 | 0.77 |
| 16 | myrtenol | 1196 | 0.40 | 0.37 |
| 17 | verbenone | 1205 | 0.25 | 0.20 |
| 18 | isobornyl acetate | 1278 | 0.85 | 1.06 |
| 19 | α-capaene | 1370 | - | 0.29 |
| 20 | β-caryophyllene | 1417 | 0.99 | 3.25 |
| 21 | α-humulene | 1450 | 0.28 | 0.75 |
| 22 | γ-muurolene | 1471 | 0.13 | 0.42 |
| 23 | AR-curcumene | 1502 | - | 0.23 |
| 24 | α-muurolene | 1506 | 0.21 | 0.22 |
| 25 | β-bisabolene | 1509 | - | 0.30 |
| 26 | δ-cadinene | 1518 | 0.13 | 0.58 |
| 27 | caryophyllene oxide | 1578 | 0.86 | 1.56 |
|  | Total identified |  | 97.97 | 97.63 |
|  | Monoterpene hydrocarbons |  | 39.04 | 34.45 |
|  | Oxygenated monoterpenes |  | 12.36 | 13.59 |
|  | Sesquiterpenes |  | 0.13 | 0.58 |

KI: Kovat's index.



camphor camphene α-pinene 1,8 cineole

**Figure 1.** Four major compounds of *R. Eriocalyx*

## Conclusion

This study has revealed that the oil obtained from *R. Eriocalyx* by microwave-assisted hydrodistillation extraction contained more oxygenated compounds than conventional hydrodistillation. However, the two oils recovered from HD and MAH extraction contained the same dominant components. We reported the presence of *R. Eriocalyx* and *R. officinalis* in the Eastern Morocco. The two species presented different chemotype.

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