Chemistry of Natural Compounds, Vol. 43, No. 4, 2007

COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Rosmarinus officinalis* FROM ALGERIA

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UDC 547.913

Rosmarinus is one of the oldest known medicinal plants in Algeria, it is used as an antispasmodic and as a flavor and fragrance ingredient in food [1]. The aim of this study was first to investigate the chemical composition of the essential oil of *R. officinalis* from Algeria and then to evaluate its potential antimicrobial activity using the agar diffusion disc method [2].

Microscopic observations have been carried out in order to correlate the kinetic behavior with the localization of the essential oil in the vegetable.

The vegetable matter was harvested at the flowering stage in March 2005 in El Hamma National Park (North Algeria) having a sub-humid climate; the soil is clay-muddy without fertilization.

The evolution of oil yield according to the extraction time is shown in Fig. 1. After 90 min, 60.41% of the essential oil was recovered, and it was totally recovered after 240 min of extraction.

The shapes of the kinetic curves suggest that the essential oil is localized in endogenous and exogenous sites. In addition, cross section observations on the aerial parts (leaf and steam) have been made on a photonic microscope using the double coloring technique (Methyl green and Congo red).

The essential oil is white in color, its yield was 0.82%. The physico-chemical properties have been measured as follows [3]: acid number: 2.80, ester number: 102.66, optical rotations: [α] +0.73 (*c* 30.0, CH_2Cl_2).

The identified volatile components are listed in Table 1. The essential oil consisted of a complex mixture of different substances, with oxygenated monoterpenes being the dominating constituents. Among them, camphor (14.6%) and 1,8-cineole (12.2%) were the main compounds.

When the chemical profile of the studied essential oils is compared to the previously studied essential oil of *R. officinalis* from the Algerian Sahara [4], it appears different as 1,8-cineole is present in higher amounts in the later sample (29.5% vs 12.2%), while 2-ethyl-4,5-dimethylphenol (12.0%) is totally absent in our sample. In addition, 1,8-cineole was abundant (60.9%) in rosemary essential oil from Turkey [5].

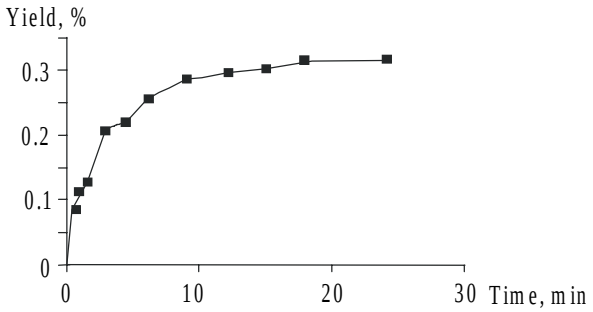

Fig. 1. *Rosmarinus officinalis* essential oil yield.

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0009-3130/07/4304-0487

TABLE 1. Composition of *R. officinalis* Essential Oil

Compound	KI*	Percentage, %	Compound	KI*	Percentage, %
α-Pinene	913	5.4	β-Caryophyllene	1395	10.9
Camphene	930	7.2	Aromadendrene	1406	0.3
β-Pinene	965	8.5	α-Humulene	1419	3.0
1-Octen-3-ol	994	0.6	α-Amorphene	1436	1.3
1,8-Cineole	1008	12.2	β-Selinene	1442	0.2
γ-Terpinene	1041	1.4	α-Zingiberene	1449	0.5
Camphor	1126	14.6	α-Muurolene	1454	0.4
Borneol	1155	10.6	γ-Cadinene	1465	1.1
Terpinolene	1164	0.7	δ-Cadinene	1474	2.0
Linalool	1175	2.2	Caryophyllene oxide	1493	3.1
α-Terpineol	1175	5.2	*cis*-α-Bisabolene	1540	Tr.
cis-Piperitol	1184	0.1	Caryophylla-4(12),8(13)-dien-5β-ol	1581	0.1
Citronellol	1220	0.1	α-Bisabolol	1646	Tr.
Bornyl acetate	1257	5.3	Total		99.3
Carvacrol	1277	0.2	Grouped components		
α-Cubebene	1312	0.3	Terpenoids		
α-Copaene	1340	1.3	Monoterpene hydrocarbons		23.4
β-Bourbonene	1348	0.1	Oxygenated monoterpenes		45.0
α-Cubebene	1355	0.1	Sesquiterpene hydrocarbons		21.8
α-Cadinene	1365	0.2	Oxygenated sesquiterpenes		3.2
β-Funebrene	1373	0.1			

*KI: Kovats index.

Order of elution from a DB 5 column, including their Kovats indices calculated against C_9-C_{24} *n*-alkanes on the DB 5 column and their percent contribution.

Tr.: trace.

TABLE 2. Antimicrobial Activity of *R. officinalis* Essential Oil Expressed as the Diameter of the Inhibition Zone in mm in the Disk Sensitivity Assay

Test microorganisms	Oil (20% solution)	Sensitivity*	Saline sol. 2% of Tween 80	Amikacine
Staphylococcus aureus	34.1±0.03	+++	10.2±0.01	27.0±0.01
Staphylococcus epidermis	24.1±0.02	+++	5.1±0.01	20.8±0.00
Enterococcus faecalis	25.0±0.02	+++	4.6±0.02	17.6±0.02
Pseudomonas aeruginosa	29.1±0.02	+++	8.4±0.02	16.5±0.02
Escherichia coli	19.3±0.01	++	5.1±0.01	19.6±0.01
Klebsiella pneumonia	25.1±0.02	+++	4.4±0.02	21.0±0.02
Salmonella bruneii	10.6±0.02	+	8.2±0.03	19.2±0.02
Candida albicans	0.00±0.00	-	0.0±0.00	N.t.

*The sensitivity to the different strains was classified by the diameter of the inhibition zone as follows [16]:

-: diameter less than 8 mm, not sensitive; +: sensitive, diameter 9-14 mm; ++: very sensitive, diameter 15-19 mm; +++: extremely sensitive for diameter larger than 20 mm.

Myconasol: *Candida albicans* - 23.8±0.02.

It is also reported [6] that two major types of rosemary oil can be distinguished with respect to their major constituents: with over 40% of 1,8-cineole, which is characteristic of oils from Morocco, Tunisia, Turkey, Greece, Serbia, Italy, and France [7, 8], and oils with approximately equal ratios (20–30%) of 1,8-cineole, α-pinene, and camphor (oils from France, Spain, Italy, Greece, Bulgaria) [7, 9].

This difference could be due to the different climates between south Europe and North Africa Mediterranean areas: higher mean monthly temperatures and longer sunshine duration.

The essential oil of *R. officinalis* showed strong antimicrobial activity against: *S. aureus, S. epidermis, P. aeruginosa, E. faecalis, K. pneumonia,* and *E. coli,* moderate effect against *S. bruneii,* and no effect against *C. albicans* (Table 2).

It was previously mentioned that the essential oil of *R. officinalis* exhibit a significant antibacterial activity only against *K. pneumoniae* and *P. aeruginosa* [4], although its activity against *C. albicans* is lower compared to the essential oils of *Thymus* sp. and *Calamintha* sp. [10].

Fresh aerial parts (leaf, stem, and flower) of *R. officinalis* have been collected from national parks in Algiers (North of Algeria), called El Hamma, during the flowering period in March 2005.

Fresh aerial parts have been hydrodistilled for 3 h, using a Clevenger apparatus according to standard procedures [11]. The essential oil has been dried over anhydrous sodium sulfate and stored at 2–4°C. The yield was 0.82%.

GC analyses were performed on a Perkin–Elmer 8500 gas chromatograph with FID, fitted with a fused silica DB-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 μm). The column was temperature programmed from 60°C to 250°C at a rate of 3°C /min. The injector and detector temperatures were programmed at 230°C and 280°C, respectively.

GC-MS analyses were performed on a Hewlett-Packard 5973–6890 system operating in EI mode (70 eV) equipped with a split/splitless injector (220°C), a split ratio 1/10, using two different columns: a fused silica HP-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 μm) and an HP-Innowax capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.50 μm). The temperature program for the HP-5 MS column was from 60°C (5 min) to 280°C at a rate of 4°C/min, and for the HP-Innowax column from 60°C to 260°C at a rate of 3°C/min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Injection volume of the sample was 2 μL. Retention indices for all compounds were determined according to the Van der Dool approach [12], using *n*-alkanes as standards. The identification of the components was conducted in [13]. Optical rotation was measured on a Perkin–Elmer 341 polarimeter.

The agar disc diffusion method was used for the determination of antimicrobial activity of the essential oil [2].

The following Gram-negative bacteria were used: *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 4157), and *Klebsiella pneumonia* (ATCC 4352), as well as Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermis* (ATCC 12228), *Enterococcus faecalis* (ATCC 6569), *Salmonella bruneii* (clinical strains isolated directly from patients at the "Centre Hospitalier Universitaire," Algiers, Algeria), and the fungus *Candida albicans* (ATCC 2601).

The bacterial species were cultured overnight at 37°C in Mueller-Hinton medium (Bio-Rad), while *C. albicans* was maintained on Sabouraud agar (SDA).

Suspensions of the tested microorganisms (0.1 mL of 10^7–10^8 cells/mL) were spread over the surface of Petri plates. The inocula were stored at +4°C for further use.

The samples were dissolved in a final concentration of 20% in saline solution using up to 2.0% Tween 80 (0.9% NaCl and 20% Tween 80, ratio 1:10) (in order to facilitate the essential oil dispersion) [14]. Filter paper discs (Whatman n 1; 6.0 mm in diameter) were impregnated with 20 μL of the sample and placed on the inoculated agar plates. Standard antibiotics (Amikacine 1 mg/mL and Myconasol 10 mg/mL) were used in order to control the sensitivity of the test organisms.

The plates containing the bacteria were incubated for 24 h at 37°C, while that of fungi for 48 h at 30°C. The resulting diameter of the inhibition zones have been measured in millimeters [15]. All experiments were performed in triplicate.

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