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Chemical Composition and Antimicrobial Activity of *Ruta chalepensis* L. Essential Oil from Algeria

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The essential oil from aerial parts of *Ruta chalepensis* L. was analyzed by GC and GC/MS. 20 Compounds were identified representing 93.99-98.58 % of the oil. 2-Undecanone (79.06-82.74 %) was the major constituent, followed by 2-decanone (3.53 - 4.38 %). Variation in essential oil composition, physico-chemical properties and yields was studied according to state of material used (fresh or dry) and years of collection of the plant. The essential oil of aerial parts was tested for its anti-microbial activity using paper disc diffusion method. The oil was ineffective on the inactivation of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Listeria monocytogens*. In the contrast the essential oil demonstrated significantly antifungal activities against *Aspergillus niger, Aspergillus flavus, Alternaria* Sp., *Trichoderma* Sp. and *Candida albicans*.

Key Words: *Ruta chalepensis*, Rutaceae, Essential oil composition, 2-Undecanone, Antimicrobial activity.

INTRODUCTION

Natural products contribute in great extent to the fight against pathogenic micro-organisms. Several plants or parts of them are used in food as spices and are thought to display some therapeutic activity or to provide a natural conservation by inhibiting the microbial growth.

The antimicrobial activity is another widely studied feature of essential oils[1]. Recently, many studies on plants revealed antibacterial and anti-inflammatory activity and therefore, have been made in order to understand their antimicrobial properties[2-4].

Rutaceae constitutes a systemically difficult taxon that is widely distributed and consists of *ca.* 160 genera and over 1600 species[5-7].

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Ruta chalepensis L., locally known as Fidjel is indigenous to the Mediterranean. It is a small shrub measuring between 0.5 and 1m in height that grows naturally. It is a member of the rutaceae family. It is an aromatic plant, widely diffused in the Mediterranean area[8]. It grows well at the cool climates of higher altitudes but can also be grown in plains. It prefers a well-drained, calcareous clayey soil[9]. In Algeria the plant is often cultivated in gardens.

Ruta chalepensis L. is an ancient medicinal plant still used in the traditional medicine of many countries as a laxative, antiinflammatory, analgesic, antispasmodic, abortifacient and for dermatopathy treatment[10]. Pharmacological investigations clearly indicated that the ethanol extract of the aerial part of *Ruta chalepensis* shares the antiinflammatory and antipyretic activities[11]. Phytochemical screening showed that the aerial part of *Ruta chalepensis* yielded coumarins (chalepensis, chalepin, rutamarin) as well as alkaloids (kokusaginine, skimmianine, arborinine)[12,13].

The literature reports some works on the composition of *Ruta chalepensis* oil from Argentine, Saudi Arabia, Turkey, Italia, Greece, Iran and India[14-21].

To our knowledge, there is no report is available on chemical composition of *Ruta chalepensis* oil from Algeria and there is not previous study on the antimicrobial activity of *Ruta chalepensis* using a paper disc diffusion method.

The aim of this paper is to present qualitative and semi quantitative analysis of the essential oil of this rutaceae specie growing in the north west of Algeria, to compare their chemical composition according to state of plant material (fresh or dry) and year of cultivation and we elucidate its antimicrobial effects (antibacterial and antifungal effects).

EXPERIMENTAL

The aerial part of *Ruta chalepensis* were collected in April 2004 and 2005 from Ouzidane Mountains, Tlemcen in northern Algeria. A voucher specimen has been deposited in the Herbarium of the Laboratory of Botany, Department of Biology, Tlemcen University, Tlemcen, Algeria.

A part of the plants material, were extended by ground, in one layer, in an open room protected from the sun. During drying time, plants were turned over to allow homogeneous drying.

Essential oil isolation: Aerial part fresh or dried at room temperature for two weeks, were hydrodistilled for 5 h, using Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate and stored at low temperature prior to analysis. The oils obtained were yellow, with a strong deterrent odour.

Physicochemical analysis: The physico-chemical properties of the oils were determined according to AFNOR standards[22].

Chemical analysis: Identification of volatile compounds was performed using gas chromatography-mass spectrometry (GC-MS).

Analytical GC: GC analyses were carried out using two GC apparatus.

Semi quantitative analysis: The component relative percentages in each essential oil were calculated from GC peak areas, using a Shimadzu-GC 17A gas chromatograph, under the following operating conditions: vector gas, N_2 (1.8 mL/min); injector and detector (FID) temperatures, 270 °C; DB35-MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness); temperature program 60-220 °C at 5 °C/min and 220 °C for 2 min.

Qualitative analysis: GC analyses were carried out using a Dani 8521 gas chromatograph with DB5-MS capillary column (50 m × 0.32 mm × 0.25 μm film thickness). Oven temperature was programmed from 50 to 228 °C at 4 °C/min with initial hold of 5 min. Injector and detector (FID) temperatures were maintained at 230 and 235 °C, respectively. Nitrogen was used as carrier gas (1.2 mL/min).

The retention indices (RI) were calculated for all volatile constituents using an *n*-alkanes homologous series (C_9-C_{20}) at the same GC conditions.

GC-MS analyses: The essential oils were analyzed by GC/MS using a Varian CP 3800/Varian Saturn 2000, with a DB5-MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Oven temperature was programmed from 80 to 300 °C at 10 °C/min with initial and final hold of 1 min and 30 min, respectively. Injector temperature was maintained at 220 °C. Helium was used as carrier gas (1 mL/min) with a split ratio of 1/50 and the MS were taken at 70 eV. Mass spectral data were analyzed by Nist 98r.lbr, Mainlib and Replib libraries search.

Components identification was based on a comparison of retention indices and mass spectra with those of authentic sample and with literature data[18,23-29]. Others identifications were made by comparison of mass spectra with those in the data system libraries (Nist 98r.lbr, Mainlib and Replib).

Microbial strains, inoculation and media

Bacteria: The strains were obtained from Institut de Pasteur de Paris. It concerns two Gram-negative: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and two Gram-positive: *Staphylococcus aureus* (ATCC 25923) and *Listeria monocytogens* (ATCC 19115).

Yeasts: One strains of *Candida albicans* was used throughout this study (Ca 444). It was also obtained from Institut de Pasteur de Paris.

Fungi: The four tested strains have been isolated and purified at the laboratory of microbiology, Department of Biology, Tlemcen University: *Aspergillus flavus, Aspergillus niger, Alternaria* Sp. and *Trichoderma* Sp.

Preparation of the inoculum

Bacteria: The strains preserved in nutrient agar at 4 °C, were revivified in nutrient solution and incubated at 37 ± 1 °C during 18 to 24 h. 0.1 mL of each culture was added to 10 mL BHIB (Brain Heart Infusion Broth, Pronadisa Hispanalab, S.A.).

Yeasts: The strains preserved at 4 °C in the Sabouraud agar supplemented with chloramphenicol, were revivified in nutrient solution and incubated at 30 ± 1 °C during 24 to 48 h. 0.1 mL of each culture was added to 10 mL sterile physiological water.

Fungi: The inoculum was presented[30] in the form of spores' suspension in sterile physiological water at 0.1 % of Tween 80.

The inoculum used for all the assays reached the microbial density of the order of 10^6 to 10^7 UFC.mL^{-1} for the bacteria and yeasts and 10^8 to 10^9 spores.mL^{-1} for the moulds.

Culture media: Muller Hinton for the bacteria. Sabouraud dextrose Agar + chloramphenicol for the yeasts. Sabouraud dextrose Agar for the moulds (Pronadisa Hispanalab, S.A.).

Antimicrobial assay: The paper disc diffusion method was used to test the microbial activity.

Paper disc diffusion: The agar plate containing the appropriate medium was spread with the inoculum previously adjusted to the microbial densities cited above. Several discs (6 mm diameter) have been impregnated with 10 mL of essential oil. After incubation (18 to 24 h at 37 ± 1 °C for the bacteria, 24 to 48 h at 30 ± 1 °C for the yeasts and 10 to 12 h at 25 ± 1 °C for the moulds), the diameters of inhibition zones were measured with a calliper.

The antibiotics, gentamycin (100 UI/disc) and piperacillin (100 µg/disc) served as positive control for the bacteria. For the yeasts and moulds, we used amphothericine (100 µg/disc) and econazole (50 µg/disc).

When the inhibitory zone diameter is lower or equal to 6 mm, the sample tested was considered as not active.

RESULTS AND DISCUSSION

The yields and physico-chemical properties of essential oils of aerial part of *Ruta chalepensis* were presented in Table-1. Comparing the essential oil yields of the fresh and dry material (April 2004), no much difference was found. The decline is certainly due to the evaporation of the volatile compounds during drying times (2 weeks). These results suggest that we can consider the effective time of drying two weeks corresponding to 16.67 % water content.

A significant effect on yields was observed when the essential oil was obtained from the aerial part of plants collected in April 2004 and from the aerial part of plants collected in April 2005. The local climatic conditions

of 2004 and 2005 years were different. The winter 2004 was characterized by mild climate. On the contrary, winter 2005 has been very cold, with many rain and snow. The yields of the essential oil of plant collected in April 2004, was found to be more relevant than that of essential oil plant collected in April 2005.

TABLE-1
YIELDS AND PHYSICO-CHEMICAL PROPERTIES OF THE ESSENTIAL OILS OF *Ruta chalepensis*

	April 2004		April 2005
	Fresh material	Dry material	Dry material
H[a] (% H_2O)	64.00	16.67	16.00
R[b] (%)	2.86	2.82	1.28
d_4^{20}	0.8131	0.8141	0.8193
n_D^{20}	1.4309	1.4305	1.4328
α_D^{25}	+6	+6	+10
m.p. (°C)	+12	+11.5	+5
Ethanol 70% solubility at 20 °C	1V/2V	1V/2V	1V/2V
Acid indice	2.91	2.48	3.78

[a]water content, [b]yields (%) of the aerial part essential oils of *Ruta chalepensis* with respect to the dry matter (H= 0 %).

However, some physico-chemical properties of the analyzed essential oils showed a variation by the influence of local climatic condition of years of collection.

The components of the essential oil, the percentage of each constituent and the retention indices are listed in Table-2 in relation to their elution order on DB5-MS column. Identifications have carried out by means of GC (retention indices) and GC-MS analyses. Chromatographic profiles of the essential oil from aerial part of the plant revealed 20 identified constituents, which represented 93.99-98.58 % of the total GC area for the essential oil.

The oil was characterized by large amount of aliphatic ketones (87.42-90.62 %), with 2-undecanone (79.06-82.74 %) and 2-decanone (3.53-4.38 %) being the major constituents found. The composition of the oil produced from plants growing in Argentina, Turkey, Iran and India were similar in that 2-undecanone was the major component (38.1, 66.5, 52.5 and 41.3-67.8, respectively)[14,16,19-21]. In the Turkish, Italian, Iranian and Indian oils, 2-nonanone was a major constituent (16.2, 49.9, 24.1 and 5.2-33.6 %, respectively)[16,17,19-21]. In the Saudi oil, 2-undecanone, 2-tridecanone, elemol and β-eudesmol were the main components[15]. In the Greek oil, 2-methyl octyl acetate (44.0 %) and β-phellandrine (10.7 %) were the main components[18].

TABLE-2
CHEMICAL COMPOSITION OF AERIAL PART ESSENTIAL OILS OF *Ruta chalepensis*

Compounds[a]	M_w[b]	RI[c]	RI_{lit}[c]	April 2004		April 2005	I.M[d]
				Fresh matter	Dry matter	Dry matter	
Limonene	136	1030	1031	0.05	0.09	0.06	MS, RI
2-Nonanone	142	1086	1083	0.34	0.57	0.26	MS, RI
2-Nonanol	144	1097	1099	0.32	0.71	0.16	MS, RI
2-Decanone	156	1192	1194	3.53	4.38	3.67	MS, RI
1-Decanol	158	1272	1273	0.05	0.03	0.05	MS, RI
2-Undecanone	170	1297	1297	80.11	79.06	82.74	MS, RI
2-Undecanol	172	1305	1308	2.68	1.18	0.31	MS, RI
1,7Octadiene2,7dimethyl 3,6 bis (methylene)[e]	162	1311	-	tr	tr	0.93	MS
1-Undecanol	172	1364	1367	0.76	0.42	0.15	MS, RI
2-Dodecanone[f]	184	1365	-	0.69	0.50	0.54	MS
2-Dodecanone	184	1390	1388	1.76	1.78	2.11	MS, RI
1-Dodecanol	186	1463	1466	0.11	0.04	0.12	MS, RI
Unknown[g]	284	1470	-	0.13	0.10	0.16	-
2-Tridecanone	198	1491	1489	0.99	1.42	1.30	MS, RI
E-11, 13-dimethyl-12-tetradecen-1-ol acetate[e]	282	1525	-	0.03	0.58	0.37	MS
Elemol	222	1553	1554	3.02	0.43	0.33	MS, RI
Elemicin	208	1565	-	0.35	0.69	0.19	MS
2-Butyl 4-(3',5'-benzo-dioxyl)-acetate [h]	236	1700	-	1.39	2.19	tr	MS
6-(3',5'-Benzo-dioxyl)-3,3-dimethyl-1-hexene [h]	232	1753	-	1.79	2.81	tr	MS
Chalepensin [h]	254	1890	-	tr	tr	0.29	MS
Clausindin [h]	254	1898	-	0.61	0.93	0.41	MS
Identified compound (%)				98.58	97.81	93.99	

[a]Compounds are listed in order of their elution from DB5 column. [b] Molecular weight (g/mole). [c] Retention indices are determined on DB5-MS column using the homologous series of *n*-alkanes. [d] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; RI_{lit}[18,23-29]. [e]Tentative identification based on mass spectrum. [f]Branched form. [g]Unknown (RI = 1470): 284(2), 251(1), 225(1), 211(2), 193(2), 178(4), 160(24), 145(64), 120(45), 105(90), 91(100), 43(48). [h]Tentative identification based on mass spectrum and literature[28-29]. tr: Trace (< 0.01 %).

In conclusion, the chemical composition of the analyzed essential oils showed quantitative variation by the influence of state of plant (fresh or dry) and local climate conditions of years of collection.

It is known that natural products can contribute to the fight against pathogenic micro organisms. *Ruta chalepensis* is used in traditional medicine of many countries, in particular antiinflammatory and antispasmodic activities. For evaluation of the antimicrobial activity of the essential oil from the aerial part of plant, 9 strains of micro organisms were studied in this assay. Table -3 summarizes the antimicrobial activities on various germs. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Listeria monocytogens* were resistant and do not show any inhibitory zone.

TABLE-3
in vitro ANTIMICROBIAL ACTIVITY OF AERIAL PART ESSENTIAL OIL OF *Ruta chalepensis*

Tested strains	Diameter of inhibition zone (mm)				
	E.O.	Gentamycine	Piperacillin	Amphothericine	Econazole
Bacteria:	–	–	–	–	–
E. coli	n.a	22	13	–	–
P. aeruginosa	n.a	14	20	–	–
S. aureus	n.a	23	n.a	–	–
L. monocytogens	n.a	20	n.a	–	–
Yeast:	–	–	–	–	–
C. albicans	12	–	–	15	14
Fungi:	–	–	–	–	–
A. flavus	21	–	–	n.a	20
A. niger	22	–	–	n.a	35
Alternaria Sp.	21	–	–	n.a	39
Trichoderma Sp.	20	–	–	n.a	42

E.O. = Essential oil (10 µL/disc) of aerial part of *Ruta chalepensis* which was collected in April 2005; n.a.: not active.

The fungus *Aspergillus flavus, Aspergillus niger, Alternaria* Sp and *Trichoderma* Sp. were insensitive to the amphothericine. In accordance with previous antifungal properties of essential oils and as show in Table-3, the essential oil of plant demonstrated significantly antifungal activities. The essential oil was more active against *Aspergillus niger* than *Aspergillus flavus, Alternaria* Sp. and *Trichoderma* Sp. with inhibitory zones 22, 21, 21 and 20 mm, respectively. On the other hand, the yeast *Candida albicans* showed a lower inhibition with the inhibitory zones of 12 mm.

The results presented here for the antimicrobial activity study demonstrate the antifungal activity of *Ruta chalepensis* L. and support the use of this plant in traditional medicine.

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