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ORIGINAL ARTICLE

Essential Oil Composition of Algerian *Ruta Montana* (Clus.) L. and its Antibacterial Effects on Microorganisms Responsible for Respiratory Infections

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Belkassam Abdelwahab, Zellagui Amar, Gherraf Noureddine, Lahouel Mesbah and Rhouati Salah: Essential Oil Composition of Algerian *Ruta Montana* (Clus.) L. and its Antibacterial Effects on Microorganisms Responsible for Respiratory Infections

ABSTRACT

Essential oil components of the aerial parts of *Ruta Montana* have been studied by gas chromatography-mass spectrometry to afford 24 compounds. The major components were found to be: 2-Undecanone (60.19 %), 2-Nonanone (08.63 %), Monoethylhexyl phthalate (6.46 %) Decanone (06.26%), 2-Acetoxytridecane (3.38 %), and 2-Tridecanol(3.37 %). Some other compounds were only present in minor amounts. In total, volatile oil composition of *Ruta Montana* was considered as a rich source of ketones and esters **.** Moreover, the antibacterial essay of these essential oils was conducted against two microorganisms responsible for respiratory infections and found to be effective against *Staphylococcus aureus.*

Key words: *Ruta montana* (Clus.) L.*;* Essential oil*; GC-MS.* antibacterial effects; *Staphylococcus aureus; Klebsiela pneumoniae.*

Introduction

Essential oils are aromatic oily liquids, volatile, characterized by a strong odour, rarely coloured, and generally less dense than water. They can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root) and therefore extracted from these parts, where they are stored in secretory cells, cavities, canals, epidermic cells orglandular trichomes (Rubiolo *et al.,* 2010). Essential oils have a complex composition, containing from a dozen to several hundred components. The great majority of components identified in essential oils includes terpenes (oxygenated or not), with monoterpenes and sesquiterpenes prevailing. Nevertheless, allyl- andpropenylphenols (phenylpropanoids) are also important components of some essential oils (Burt *et al*., 2004).

In Nature, essential oils play an important role in the attraction of insects to promote the dispersion of pollens and seeds or to repel other ones. In addition, essential oils may also act as antibacterials (Derwich et al, 2010) antivirals (Ramy *et al*.,), antifungals, antioxidant (El-Hela *et al*.,), insecticides (Naveen *et al*., 2011), herbicides, or have feeding deterrent effects against herbivores by reducing their appetite for such plants. Essential oils have also an important role in allelopathic communication between plants (Bakkali *et al*., 2008). The detection of some of these biological properties needed for the survival of plants has also been the base for searching similar properties for the combat of several microorganisms responsible for some infectious diseases in humans and animals. This search intends to respond to the increasing resistance of pathogenic microbes to antibiotics. Reichling *et al.,* (2009) have compiled the most important results about antibacterial and antiviral properties of essential oils published in the last decade. The Genus *Ruta*. (Rutaceae) is represented in Algeria by three species: *R. montana* (Clus.) L., *R. chalpensis* L.(Quezel et santa, 1963), *R. Tuberculata*,(Ozenda). *Ruta montana* (Clus.) L. is a perennial aromatic herb distributed in the north of Algeria (Quezel et Santa, 1963), *Ruta montana* (Clus.) L. is used in Algeria as a remedy for emmenagogue, antispasmodic rubéfiant, echarrotic powder. It has also been used in Spain as a remedy for Fever, emmenagogue, abortive, antispasmodic, against

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intestinal worms (Forment et Roques, 1941). *Ruta* species are sources of diverse classes of natural products such as flavonoids, alkaloids, essential oils, coumarins, phenols, saponins lignans, and triterpenes, with biological activities including antifungal, antioxidant, phytotoxic, abortive, depressant, antidotal and anti-inflammatory (Mohhr, 1982; Juan, 1984; Raghav *et al.,* 2006; Kuzovkina *et al.,* 2009; Mejrib *et al.,* 2010).

The aim of the present investigation was to study the essential oil composition of aerial parts of *Ruta montana* (Clus.) L. from Grarem - Mila region located in east of Algeria and its antibacterial effects on two microorganisms responsible for respiratory infections.

Materials and Methods

Plant Material:

The aerial parts of *Ruta montana* (Clus.) L. were collected in March 2009 (flowering stage) in Mila, Algeria. The plant was identified by Dr. Zellagui amar, department of life sciences and nature, University Larbi Ben M'hidi, Oum el Bouaghi Algeria. A voucher specimen was deposited at the life sciences and nature Department, University Larbi Ben M'hidi, Oum el Bouaghi, Algeria under the code number ZA 116.

Extraction:

Essential oils were obtained by hydrodistillation of 100g of dried aerial parts using a Clevenger-type apparatus for 3 h. diethyl ether (10 ml) was used as the collector solvent as reported in literature. After evaporation of the solvent, the oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at 4 °C before analyses. The oil sample was subsequently analyzed by GC-MS.

Identification of Components:

Gas Chromatography/Mass Spectroscopy:

The oil was analyzed by GC/MS using a Agilent 5973EI mass selective detector coupled with an Agilent GC6890A gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m · 0.25 mm · film thickness 0.25 μm). Operating conditions: The carrier gas flow was 1.6 ml He/min, column pressure was 100 Kpa. The injector and detector temperatures were 220°C and 250°C respectively. The column temperature was held at 60°C for 1 min, then raised from 60°C to 200°C at 10°C/min and held there for 5 min and from 200°C to 240°C at 10°C /min and held there for 6 min. The program was run in the splitless mode with a mass range of 50–400 u, and the scan interval was 0.5 s. Detector voltage was set at 1.5 kV.

Identification of Components:

Identification of oil components was achieved on the basis of their retention indices RI, (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, 2007) and stored on the MS library (NIST database). The concentration of the identified compounds was computed from the GC peak total area without any correction factor.

Antibacterial Activity:

Microorganism Strains:

The clinical bacteria strains *Staphylococcus aureus and klebsella pneumoniae* were obtained from Bacteriology Laboratory Constantine Hospital University (C.H.U).

Antimicrobial Assay:

The Anti-microbial assay was carried out on essential oil using agar diffusion method [NCCLS], against two human pathogenic bacteria: *klebsella pneumoniae* and *Staphylococcus aureus*. The bacterial strains were first grown on Muller Hinton medium (MHI) at 37 ˚C for 24 h prior to seeding onto the nutrient agar. The essential oil was mounted on sterile filter paper discs (6 mm in diameter) with the following concentrations 2, 1, 0.5 , and 0.25 mg/ml. The discs were placed on the inoculated agar media. The treated Petri discs were kept at 4 ◦C for 1 h, and incubated at 37 ◦C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. Each experiment was carried out in triplicate.

Results and Discussion

Prior to carrying out the hydrodistillation, a phytoscreening study has been conducted focussing on seven chemical groups. The results revealed the presence of essential oils, flavonoids, saponins, tannins, and Coumarins. (Table: 1).

Table 1: Phytochemical survey of *Ruta Montana* L.

Chemical Groups	R	L	St	Fl	F&S
Volatile oils	++	+++	+++	+++	+++
Alkaloids	++	++	++	++	++
Flavone aglycones	+	+	+	+	+
Coumarins	++	+++	+++	+++	+++
Tannins	++	++	++	++	++
Saponins	±	±	±	±	±
Flavone glycosides	±	++	++	++	++

(+) present, (++) present, (+++) present, (±) Traces, (-) absent
R: Roots, L : Leaves, St : Steams, Fl : Flowers, F&S: Fruits and Seeds

Hydrodistillation of the dry aerial parts of *Ruta montana* (Clus.) L. furnished 4.5 % of a yellowish essential oil, having an intense and penetrating odour. GC-MS analysis afforded the identification of twenty-four compounds, representing 94.64 % of the oil. Table 2 shows the percentage composition of the essential oils that is characterized by the great prevalence of ketones mainly: Undecan-2-one (60.19 %) , 2-Nonanone (08.63 %) and Decanone (6.26 %). Monoethylhexyl phthalate, 2-Acetoxytridecane and 2-Tridecanol are also present in considerable amounts. Essential oils from the aerial part of *Ruta montana* L. growing in Tipaza (North Central Algeria) revealed the presence of a higher content of ketones, about 95% of total oils (Moulay *et al.*,2009). Noneless, the same plant growing in Oran, west of Algeria, gave 1.63% yield of essential oils in which twenty compounds were identified and composed mainly of undecan-2-one (32.8%), nonan-2-one (29.5%), and nonanol-2-acetate (18.2%).

The comparison of the essential oil composition between *Ruta montana* L. and other *Ruta* species showed that the aerial parts oils of *R. chalpensis* growing in Italy yielded 0.74% and were dominated by undecan-2-one (46.8%) and nonan-2-one (18.8%). However, the same plant growing in Tunisia yielded 5.51% and the main constituents were 2-undecanone (77.18%), 2-decanone (8.96%) and 2-dodecanone (2.37%) (Mejrib *et al.,* 2010).

In Turkey, the oils gave Twenty-four compounds representing 93.4% of the oil with 2-undecanone (66.4%) and 2-nonanone (16.24%) as major constituents (Baser *et al.,* 1996) while the main components of essential oils of *Ruta chalepensis* L from Greece were β-phellandrene (10.7%) and 2-methyloctyl acetate (44.0%) (Olga *et al.,* 2001).

The oil yield from *Ruta chalepe*nsis L. subsp. *Angustifolia* (Pers.) P. Cout. growing in Algeria was 0.27%, and made up mainly of 2-undecanone (28.2%), 2-nonanone (20.0%), 2-methyloctyl acetate (12.7%) and 2-methyldecyl acetate (5.8%) (Dob *et al.,* 2008).

The essential oil of *R. graveolens* produced in Malaysia gave thirty compounds representing 82% of the oil. The major compounds were 2-undecanone 30.73, 2-nonanone 18.06, and 2-nonyl acetate 11.03%. Psoralen (1.2%) and bergaptene (7.2%) also were identified in the oil (Yaacob *et al.,* 1989). In Iranian *Ruta graveolens* L. oils gave nineteen compounds. The major constituents were 2-undecanone (33.9%), 2-Heptanol acetate (17.5%), 1-dodecanol (11.0%), geyrene (10.4%) and 2-nonanone (8.8% (Mojtaba *et al.,* 2009). The essential oils from the fresh herb of *R. graveolens* (Rutaceae) growing in Egypt contains: 81.6% ketones, mainly 2-undecanone (49.2%) and 2-nonanone (24.7%), and 6.92% esters, mainly 2-nonyl acetate (6.2%) (Aboutabl *et al.*,1988). Cedric *et al.,* (2003) reported in the aerial parts of *Ruta corsica* DC, thirty-six compounds with 2-nonyl acetate (42.9%) as the major constituent.

The present results show high percentages of Undecan-2-one (60.19 %) in the aerial parts, which are completely in accordance with the other studied species of *Ruta genus* except *R. Montana* growing in Tunisia.

A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active constituents, mainly attributable to isoprenes such as monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols.

The lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of main importance in the antimicrobial action of essential oil components Griffin *et al.,* (1999).

Table 2: Essential oil composition from *Ruta montana* (Clus.) L.

Peak	Chemical constituents	Rt	%

01	Ethylbenzene	2.675	0.11
02	o-Xylene	2.781	0.19
03	2-Octanone	2.981	0.15
04	α-Phellandrene	4.986	0.62
05	Octanal	5.600	0.13
06	1,2-Diisopropenylcyclobutane	7.042	0.51
07	2-Nonanone	10.292	08.63
08	Isopropyl salicylate	14.892	0.10
09	Decanone	15.520	06.26
10	2-Undecanone	19.446	60.19
11	2-Tridecanol	19.802	03.27
12	2-Dodecanone	22.321	01.43
13	α -Caryophyllene	22.979	0.97
14	2-Acetoxytridecane	23.609	03.38
15	2-Tridecanone	25.016	0.41
16	Cyclopentane1,2,3,4,5pentamethyl	25.779	0.43
17	Caryophyllene oxide	26.794	0.11
18	Tridecane-2,4-dione	27.003	0.27
19	1-Dodecanone, 1-cyclopropyl-	31.106	0.45
20	Palatinol 1C	32.743	0.09
21	trans-Oleic acid	38.345	0.08
22	Monoethylhexyl phthalate	39.873	6.46
23	Nonacosane	40.506	0.17
24	Palatinol D10	45.340	0.22
	Total	-	94.46 %

Table 3: main classes of essential oils components of *R. montana* (Clus.) L.

ketones	81.06
esters	10.25
Sesquiterpenes hydrocarbons	0.97
hydrocarbons	0.73
Monoterpene Hydrocarbons	0.62
Oxygenated Monoterpenes	0.51
Aldehydes	0.13
Oxygenated Sesquiterpenes	0.11
acids	0.08

Due to these data, we were interested to study the antibacterial activity of the essential oils. The results were summarized in Table 3, which showed that essential oil extracted from *Ruta montana* (Clus.) L. prevented the growth of the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentrations of the tested samples. The obtained inhibition on bacteria strains varied from 9.33 to 24 mm with a highest inhibition zone recorded with *Staphylococcus aureus* at 2 mg/ml, and a considerable inhibition effect with the same concentration at *Klebsiela pneumoniae*.

Table 3: Antibacterial effects of essential oils of *Ruta montana* (Clus.) L. at two strain bacteria

Microorganisms	Oil concentration mg /mL)			
	0.025	0.100	0.5	2
Staphylococcus aureus	9.33±1.15	15.66±1.15	22.66±1.15	24±1
Klebsiela pneumoniae	..	18.33±1.15	21.66±1.15	22.00±2

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