Biological Study from Ruta Plants Extracts Growing in Tunisia

Yosra, Bejaoui•; Manef, Abderrabba

Laboratoire Matériaux Molécules et Applications (LMMA), IPEST, BP51, La Marsa 2070, TUNISIE

Sameh, Ayadi*+

Institut National des Sciences et Technologies de la Mer (INSTM), Laboratoire Milieu Marin, Centre la Goulette, TUNISIE

ABSTRACT: *Ruta species are known as a potential source of natural products with biological activities. They are used in several fields such as in therapeutic and traditional medicine. In order to contribuate to the valorization of these plants, this work investigated the chemical composition and antibacterial activity of the essential oils of Ruta montana and Ruta gravelons growing in tunisia (north of tunisia). The total phenolic content of these two essential oils was also studied. The antibacterial activities of essential oils were assessed against Escherichia coli (ATCC7625), Staphylococcus aureus (ATCC76110), Pseudomonas aeruginosa (ATCC 7624), Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa. Results show that the chemical composition of essential oils was dominated by 2-undecanone (86.77%), followed by 2-decanone (4.91%) and 2-nonanone (23.62%). Furthermore, the total phenolic content in essential oil of Ruta gravelons is more important than the total phenolic content in essential oil of Ruta montana. Indeed, the value of total phenolic content is 41.70 mg Gallic acid equivalents per gram of dry extract, in essential oil of Ruta gravelons but the total phenolic content in essential oil of Ruta montana is a 7.50 mg Gallic acid equivalents per gram of dry extract. Besides, the ruta montana essential oil has the most important antibacterial activity than the Ruta gravelons essential oil especially against Staphylococcus aureus (ATCC76110) and Pseudomonas aeruginosa (ATCC 7624).*

KEYWORDS: *Ruta montana; Ruta gravelons; Essential oil; Antibacterial.*

INTRODUCTION

Ruta species have a wide distribution in the word. They are more distributed in the tropical and temperature countries, such as tropical america, south africa, mediterranean region, and australia. The Rutaceae family has about 150 genders and over 1600 species [1-3]. The ruta species present a strongly aromatic due to the presence of essential oils [4]. In fact, many research groups are interested in the several therapeutic and pharmacological properties of ruta species. Indeed, Ruta species are used in traditional medicine for the treatment of variety diseases such as menstrual disorders, skin inflammations, cramps and earaches [5-7]. Thus, Ruta has several therapeutic properties such as anti-inflammatory, antiulcer,anti-diabetics, anti-diarrheic,

***** *To whom correspondence should be addressed.*

+ E-mail: sameh_ayadi2003@yahoo.fr ; sameh.ayadi@instm.rnrt.tn

• Other Address: Faculté des Sciences de Bizerte, Université de Carthage, TUNISIE

1021-9986/2019/2/85-89 5/$/5.05

anti-rheumatism and antimicrobial properties [2,6,8] Besides, Ruta essential oil has antimicrobial activities which interest scientist for the treatment of resistant microbial strains [9]. Therefore, the composition of Ruta species extracts is characterised by the presence of alkaloïds, flavonoïds, coumarins, volatile oil, sterols, amino acids and sponins [10-12]. In this paper, we report the chemical composition and the total phenolic content of the essential oils from Ruta montana and Ruta gravelons growing in the north of tunisia. The antibacterial activity of the two essential oils is studied against standard bacteria such as *Escherichia coli* (ATCC7625), *Staphylococcus aureus* (ATCC76110) and *Pseudomonas aeruginosa* (ATCC 7624) and against bacteria such as *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeruginosa*.

EXPERIMENTAL SECTION

Plant material

The arial parts of plants such as ruta montana and Ruta gravelons collected respectively from Nathor mountain near Bizerte and from Tunis. Arial parts of Ruta montana and Ruta gravelons were collected during the flowering stage of the plant, in July 2015.

The essential oils isolation procedure

Two hundred grams of aerial parts from each species such as Ruta montana and Ruta gravelons were submitted to hydro-distillation for 3h using a clevenger apparatus [13]. The volatile distillate was collected over anhydrous sodium sulfate, filtered and stored at 4°C.

Essential oil analysis

The essential oils were analysed by GC/MS. A Hewlett-packard G1800A GCD System, equiped with an HP-Innowax silica capillary column (60 cm × 0.25 cm, film thickness 0.25 μm) was used for GC/MS analysis. Heluim was the carrier gas at a flow rate of 0.7 ml/L and the split ratio was 50:1. Mass units were monitored from 35 to 425 m/z at 70 eV. The GC analysis applied the same column temperate programme. Basing in their relative retention times and the data from the baser library of essential oil constituents, wiley, Mass-Finder and Adams GC/MS libraries, the components of essential oils were identified by comparing GC retention indices, mass spectra with publishing data [14].

Determination of total phenolic content

The total phenolic content of each extract of essential oils was determined using the Folin-ciocalteu method [15]. Each extract of essential oils (0.5 mL) was mixed with Folin-ciocalteu reagent (0.2 mol/L; 2.5mL). The mixture was kept for 5min. Then was added with sodium carbonate solution (75 g/L in water; 2mL). After incubation during 1h, we determined the absorbance at 760 nm against water blank. A standard calibration curve was plotted using gallic acid (0 - 300 mg/L). The curve absorbance versus concentration is described by this equation: $Y = 0.001\ X + 0.014$ ($R^2 = 0.999$). 1mg of gallic acid is equivalent to (GAE)/kg of dry plant material.

Determination of antibacterial activity by disc diffusion method

The antibacterial activity of the essential oils was determined by the disc diffusion method [16] against the bacteria such as (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* (ATCC7625), *Staphylococcus aureus* (ATCC76110) and *Pseudomonas aeruginosa* (ATCC 7624). The filter paper discs (6 mm in diameter) were impregnated with 10μL of the oil and then placed onto the gar plates. The plates were incubated at 37°C for 24h. The diameters of the inhibition zones (mm) were measured including the diameter of discs. All the tests were performed in duplicate. Gentamycin served as positive control.

RESULTS AND DISCUSSION

Essential oil extraction and analysis

The essential oil was obtained by hydrodistillation of the two Ruta species (Ruta montana and Ruta gravelons), produced a yield color were 1.21% (V/W) and 1.67% (V/W) respectively. The chemical compositions of the two essential oils were analyzed by GC/MS. Table 1 shows the chemical composition of the two essential oils with the retention times. Nine compounds were identified from the essential oil of Ruta montana which represented 91.14% of the oil extracted. Nevertheless, seven compounds were identified from essential oil of Ruta montana that represented 93.95%. The major compounds of essential oil from Ruta montana were 2-undecanone (86.77%), followed by 2-decanone (4.91%). The essential

Table 1: Essential oils composition of Ruta montana (R.M) and Ruta gravelons (R.G).

Rt	compounds	RM	RG
9.799	2-nonanone	0.23	23.62
10.091	Nonanal	0.20	--
11.327	5,6-diethenyl-1-methyl-cyclohexene	--	2.13
12.923	2-decanone	4.91	0.43
14.388	1-nonene	--	4.35
16.35	2-undecanone	86.77	56.92
19.457	2-dodecanone	0.51	1.02
20.636	1-tetradecanol methyacrylate	--	0.98
22.57	2-tridecanone	0.31	0.23
31.411	NI	---	1.46
31.662	NI	1.02	--
total		93.95%	91.14%

oil from Ruta gravelons was characterized by 2-undecanone (56.92%) and 2-nonanone (23.62%) followed by 1-nonene (4.35%).

In this work, Ruta gravelons essential oil is similar to others results reported in the literature [16-18]. However, *Nascimento et al*. [5] reported the identification of seven compounds of the essential oil of Ruta gravelons growing in Brazil. These compounds presented aliphatic compounds, especially ketones. But there are no terpene compounds. The major compounds in this essential oil are 2-undecanone (47.21%) and 2-nonanone (39.17%). We also notice that the major compounds of Ruta gravelons essential oil from algeria are ketones such as undecanone and 2-nonanone [19], which is similar to the composition of our essential oils of Ruta species. For the two ruta species, the major products are 2-undecanone followed by 2-decanone and 2-nonanone. This variation of compounds depended on climate, genotype and growth location, which can affect the total essential oil.

Total phenolic content

Results show that the total phenolic content in essential oils, Ruta gravelons and Ruta montana were 41.7 mg Gallic acid equivalents per gram of dry extract and 7.5 mg Gallic acid equivalents per gram of dry extract respectively. This phenomenon presented high content of polyphenols for Ruta gravelons essential oil. By contrest, Ruta montana essential oil exhibited weak content. Our results are closed with others works [20]. However, a lower content of polyphenols was reported in Ruta gravelons shoots and leaves were (37 mg Gallic acid equivalents per gram of dry extract) [21] and (4.3 mg Gallic acid equivalents per gram of dry extract) [22], respectively. For the ruta montana extracts the content of polyphenols is very weak (3.13 mg gallic acid equivalents per gram of dry extract [23]).

Antibacterial activity

Results obtained show that the essential oils of the two Ruta species had the low potential of antibacterial activity against seven bacteria tested (Table 2). The highest activity was observed against *Staphylococcus aureus* with the strongest inhibition zones (14 and 17 mm, respectively) recorded for Ruta gravelons and ruta Montana essential oils, respectively. Tested bacteria were more sensitive to Gentamycin (17-26 mm) than to essential oils tested, except for *Staphylococcus aureus* (ATCC76110) which is more susceptible to Ruta montana.

Researchers showed that there is a relationship between chemical composition essential oil and antibacterial activity [24, 25]. However, the major compounds of Ruta essential oil are aliphatic ketones, which have an antiseptic property [5, 18]. These results are in agreement with our study. Therefore, the two ruta essential oils have a weak antispectic activity. Furthermore, the major compounds of the two ruta essential oils are also ketones

Table 2: antibacterial activity of essentials oils.

Inhibition diameters (mm)			
Essential oils strains	RG (10µl/disc)	RM (10µl/disc)	Gentamycin (15µl/disc)
Gram negative			
Escherichia coli	6	7	19
Pseudomonas aeruginosa	6	6	18
Klebsiella pneumoniae	6	6	17
Escherichia coli (ATCC7625)	7	9	24
Pseudomonas aeruginosa (ATCC 7624)	12	21	21
Gram positive			
Staphylococcus aureus	14	17	17
Staphylococcus aureus (ATCC76110)	16	21	22

such as 2-undecanone and 2-nonanone. Other studies showed that the essential oils of ruta species are among the less potent essential oils with regard to antibacterial activities [26].

CONCLUSIONS

We study the chemical composition and the biological activities of the two Ruta species essential oils growing in tunisia such as Ruta gravelons and ruta montana. Results show that the major compounds of Ruta essential oils are aliphatic ketones like 2-undecanone, 2-decanone, and 2-nonanone. Furthermore, Ruta gravelons essential oil contents terpene 1-nonene (4.35%). But there is no terpene compound in Ruta montana essential oil. Besides the essential oil of Ruta gravelons has a high phenolic content (41.7 mg Gallic acid equivalents per gram of dry extract). The antibacterial study shows that ruta species essential oils have antibacterial activities against *Escherichia coli* (ATCC7625), *Staphylococcus aureus* (ATCC76110), *Pseudomonas aeruginosa* (ATCC 7624) and *Staphylococcus aureus.* Besides Ruta montana essential oil has the most important antibacterial activity especially against *Staphylococcus aureus* (ATCC76110).

Received : Jul. 23, 2017 ; Accepted : Feb. 26, 2018

REFERENCES

[1] Zeichen de sa. R., Rey A., Arganaraz E., Bindstein E., Perinatal Toxicology of Ruta Chalepensis (Rutaceae) in Mice, *J Ethnopharmacol*, **69**(2): 93-98 (2000).

[2] Gonzalez-trujano M.E., Carrera D., Ventura-martinez R., Cedillo-Portugal E., Navarrete A., Neuropharmacological Profile of an Ethanol Extract of *Ruta chalepensis* L. in Mice, *J. Ethnopharmacological*, **106**(1): 129-135 (2006).

[3] Albarici T.R., Vieira P.C., Fernandes J.B., Silva M.F.G., Pirani J.R., Cumarinas e Alcaloides de Rauia Resinosa (Rutaceae) [Coumarins and Alkaloids of Rauia Resinosa (Rutaceae)] *Quimica Nova*, **33**: 2130-2134 (2010).

[4] França orlanda J.F., Nascimento A.R., Chemical Composition and Antibacterial Activity of *Ruta graveolens* L. (Rutaceae) Volatile Oils, from São Luís, Maranhão, *Brazil South African Journal of Botany*, **99**: 103-106 (2015).

[5] Mejri J., Abderrabba M., Mejri M., Chemical Composition of the Essential Oil of *Ruta chalepensis* L: Influence of Drying, Hydro-Distillation Duration and Plant Parts, *Industrial Crops and Products*, **32**(3): 671-673 (2010).

[6] Lauk L, Mangano K, Rapisarda A, Ragusa S, Maiolino L, Musumeci R, Costanzo R, Serra A, Speciale A, Protection Against Murine Endotoxemia by Treatment with *Ruta Chalepensis* L., a Plant with Anti-Inflammatory Properties *J Ethnopharmacol*, **90** (2-3): 267-272 (2004).

[7] Ratheesh M, Shyni G.I, Sindhu G, Helen A, Inhibitory Effect of *Ruta graveolens* L. on Oxidative Damage, Inflammation and Aortic Pathology in Hypercholesteromic Rats, Experimental and Toxicologic Pathology, **63**(3): 285-290 (2011).

[8] Gardete S., Tomasz J., Mechanisms of Vancomycin Resistance in Staphylococcus Aureus, *Journal of Clinical Investigation*, **124**(7): 2836-2840 (2014).

[9] Hnatyszyn O., Arenas P., Moreno A.R., Rondina R., Coussio J.D., Plantas Reguladoras de la Fecundided Segun la Medicina Folklorica, *Rev. Soc. Cient. Paraguai*, **14**: 23-57 (1974).

[10] Chen C.C., Huang Y.L., Hunang F.I., Wang C.W., Water-Soluble Glycosides from Ruta Graveolens *Journal of Natural Products*, **64**: 990-992 (2001).

[11] Ramezanpour S., Ardestani F., Asadollahzadeh M.J, Combination Effects of Zataria Multiflora, Laurus Nobilis and Chamaemelum Nobile Essences on Pathogenic E. coli and Determination of Optimum Formulation Using Fraction and Factorial Statistical Method, *Iran. J. Med. Microbiol.*, **10**(2):56-65 (2016).

[12] Marcous A., Rasouli S., Ardestani F., Low-Density Polyethylene Films Loaded by Titanium Dioxide and Zinc Oxide Nanoparticles as a New Active Packaging System against Escherichia coli O157:H7 in Fresh Calf Minced Meat, *Packaging Technology and Science*, **30**(11): 693-701 (2017).

[3] Viuda-Martos M., Mohamady M.A., Fermandez-Lopez J., Abd Elrazik K.A., Omer E.A., Perez-Alvarez J.A., *In vitro* Antioxidant and Antibacterial Activities of Essentials Oils Obtained from Egyptian Aromatic Plants, *Food Control*, **22**(11): 1715-1722 (2011)

[14] Adams R.P, Identification of Essential Oil Components by Gas Chromatography /Mass Spectroscopy, *J. American Society for Mass Spectrometry* (2007).

[15] a) NCCLS, "Quality Control Values for Veterinary-Use Fluoroquinolones", National Committee for Clinical Laboratory Stnadards, M100-511, Wayne, PA, USA (2001).
b) Singleton.V.L, Rossi.J.A, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents American, *Journal of Enology and viticulture*, **16**: 144-158 (1965)

[16] Dob T., Dahmane D., Gauriat-desrdy B., Daligault V., Volatile Constituents of the Essential Oil of Ruta chalepensis L. subsp. Angustifolia (Pers.) P. Cout. *Journal of Essential Oil Research*, **20**(30): 306-309 (2008).

[17] Merghache S., Hamza M., Tabti B., Etude Physicochimique de l'huile Essentielle de Ruta Chalepensis L. de Tlemcen, Algérie, *Afrique Science*, **05** (1): 67-81 (2009).

[18] Haddouchi F., Zaouli Y., Ksouri R., Attou A., Chemical Composition and Antimicrobial Activity of the Essential Oils from Four *Ruta* Species *Growing in Algeria Food Chemistry*, **141**(1): 253-258 (2013)

[19] Lucia B., Renata A., Ernesto R., Concentration of *Ruta graveolens* Active Compounds Using SC-CO_2 Extraction Coupled with Fractional Separation, *The Journal of Supercritical Fluids*, **131**: 82-86 (2018).

[20] Ouerghemmi I., Bettaieb Rebey I., Rahali F.Z., Bourgou S., Pistelli L., Ksouri R., Marzouk B., Tounsi Saidani M., Antioxidant and Antimicrobial Phenolic Compounds from Extracts of Cultivated and Wild-Grown Tunisian *Ruta chalepensis*, *Journal of Food and Drug Analysis*, **25**(2): 350-359 (2017).

[21] Diwan R., Shinde A., Malpathak N., Phytochemical Composition and Antioxidant Potential of Ruta graveolens L. In Vitro Culture Lines, *J. Bot.*, **17**: 1-6 (2012).

[22] Proestos C., Komaitis M., Ultrasonically Assisted Extraction of Phenolic Compounds From Aromatic Plants: Comparison with Conventional Extraction Technics, *J. Food Qual.*, **29**: 567-582 (2006).

[23]Djerdane A., Yousf M., Nadjemi B., Boutassouna D., Stocker P., Vidal N., Antioxidant Activity of some Algerian Medicinal Plants Extracts Containing Phenolic Compounds, *Food Chem.*, **97**(4): 654-660 (2006).

[24] Delaquis P.J., Stanich K., Girard B., Mazza G., Antimicrobial Activity of Individual and Mixed Fractions of Dill, Cilantro, Coriander and Eucalyptus Essential Oils, *International Journal of Food Microbiology*,**74**(1-2): 306-309 (2002).

[25] Dorman H.J.D., Deans S.G, Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils, *Journal of Applied Microbiology*, **88** (2): 308-316 (2000).

[26] Ben-Bnina E., Hammami S., Daamii-remadi M., Ben jannet H., Mighiri Z., Chemical Composition and Antimicrobial Effects of Tunisian Ruta Chalepensis L. Essential Oils, *Journal de la Société Chimique de Tunisie*, **12**: 1-9 (2010).