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The Chemical Composition and the Antibacterial Properties of *Ruta graveolens* L. Essential Oil Grown in Northern Jordan

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Abstract

Very little has been published on the essential oil composition of *Ruta graveolens* worldwide. Herein, we report on the essential oil composition of *Ruta graveolens* growing in Jordan. The essential oil was isolated using hydrodistillation from the aerial parts of *Ruta graveolens* L and a chemical composition analysis was conducted using Gas Chromatography/Mass Spectrometry (GC-MS). The antibacterial activity was evaluated using a disc diffusion method. Thirty components, accounting for 98.1% of the oil, were identified. Ketones (43.02%), aldehydes (37.12%), esters (9.33%) and sesquiterpene hydrocarbons (5.22%), were the major constituents. The major compounds identified were 2-nonanone (37.13%), undecanal (34.69%), 2-acetoxydodecane (5.0%), and 2-decanone (3.31%). The essential oil of *Ruta graveolens* showed a good antibacterial activity against all the tested bacterial isolates. The oil proved to be more effective on Gram-positive than Gram-negative bacterial species.

Keywords: *Ruta graveolens*, Gas Chromatography-Mass Spectrometry, Disc diffusion, Essential oil.

1. Introduction

For thousands of years, nature has been serving as a rich source of medicines, to which a large number of modern drugs owe their origin. At present, natural products and their derivatives represent over 50% of all drugs in clinical use, with plant-derived natural products representing about 25% of the total (Ellof, 1998; Thomas and Devi, 2013).

Plant-derived traditional medicines continue to play an essential role in health care, with about 80% of the world's population using traditional medicine at some time or other (Owolabi *et al.,* 2007). Needless to say, such plants should be investigated to better understand their properties, their safety, and efficacy.

The emergence of bacterial resistance to the currently available antimicrobial agents is an increasing concern since the treatment options available for infected patients are severely limited (Carlet *et al.,* 2014). To make the matter even worse, many pharmaceutical companies seem to have lost interest in developing new antimicrobial agents due to their reduced profitability. It may be noted that the development of new antimicrobial agents costs between $0.8 and $1.7 billion (Stanton, 2013; Spellberg, 2012). Thus, there is a need for other strategies, such as the use of plant-derived essential oils, to fight against resistant bacteria.

Ruta graveolens L. belongs to the Rutaceae family, originally native to the Mediterranean region (Asgarpanah and Khoshkam, 2012). *Ruta graveolens* is an evergreen shrub with bluish-green leaves that emit a powerful odor and have a bitter taste. *Ruta graveolens* is an ornamental, aromatic, and medicinal plant. In Jordan, it is used as a flavoring agent in foods and beverages. In traditional medicine, it is used for its antispasmodic, diuretic, sedative, and analgesic effects, and externally for its anti-rheumatic effect (Khouri and El-Akawi, 2005). In addition, *Ruta graveolens* promotes menstruation, and is used as a contraceptive (Browner, 1985; Steenkamp, 2003).

The chemical composition of the oil of *Ruta graveolens*, growing in Jordan, has never been established. The aim of the present study is to determine the chemical composition of the essential oil from the aerial parts of *Ruta graveolens* grown in northern Jordan and its antibacterial activity.

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2. Materials and Methods

2.1. Collection and Authentication of Plants

Fresh *Ruta graveolens* plants were collected before flowering from Qumeim town, Irbid, northern Jordan. The plants were taxonomically identified and authenticated by the Botanical Survey Division of Yarmouk University, Jordan.

2.2. Isolation of Essential Oil

Fresh aerial parts of *Ruta graveolens* were finely chopped and subjected to hydrodistillation for 4 h using a Clevenger-type apparatus, yielding 0.27% (v/w) pale yellowish oil. Subsequently, oil was dried over anhydrous sodium sulfate and immediately stored in GC-grade hexane at 4ºC until analysed by Gas Chromatography/ Mass Spectrometry (GC-MS).

2.3. Essential Oil Composition

2.3.1. Gas Chromatograph-Flame Ionization Detector (GC–FID) analysis

The oil was analyzed in an Agilent (Palo Alto, USA) 6890N gas chromatograph fitted with a 5% phenyl–95% methylsilicone (HP5, 30 m × 0.25 mm × 0.25 μm) fused silica capillary column. The oven was programmed to run from 60°C to 240°C at 3°C/min with hydrogen being used as the carrier gas (1.4 mL/min). One microliter of a 1% solution of the oil in hexane was injected in split mode (1:50). The injector was kept at 250°C and the flame ionization detector (FID) was kept at 280°C. Concentrations (% content) of oil constituents of *Ruta graveolens* were determined using their relative area percentages obtained from the GC chromatogram, assuming a unity response by all components.

2.3.2. Gas Chromatography-Mass Spectrometry (GC–MS) analysis

The chemical analysis of the essential oils was carried out using GC-MS (Agilent 6890N gas chromatograph, Palo Alto, USA). The chromatographic conditions were as follows: column oven program; 60ºC (1 min, isothermal) to 246ºC (3 min, isothermal) at 3ºC/min, the injector and detector temperatures were 250ºC and 300ºC, respectively. Helium was the carrier gas (flow rate 0.90 mL/min), and the ionization voltage was maintained at 70 eV. An HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thicknesses) was used. A hydrocarbon mixture of *n*-alkanes (C_8-C_{20}) was analyzed separately by GC-MS under the same chromatographic conditions using the same HP-5 column. Kovats Retention Indexes (KRIs) were calculated by injection of a series of *n*-alkanes (C_8–C_{20}) in the same column and conditions as above for gas chromatography analyses.

The identification of the oil constituents was based on a computer search using the library of mass spectral data (http://www.massbank.jp) and a comparison of the calculated KRIs with those of the available authentic standards and literature data was drawn.

2.4. Maintenance and Preparation of Cultures

Six clinical isolates of bacteria were used in the present study; three strains of Gram-positive bacteria (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, and *Bacillus subtilis*) and three strains of Gram-negative bacteria (*Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*). Pure isolates were obtained by subculture on nutrient agar plates and characterized by standard microbiological and biochemical methods, including Gram-stain, catalase test, coagulase test, and the API system (bioMerieux, France).

The bacteria were inoculated into Tryptone Soy Broth (TSB) (Oxoid, Hampshire, England) and incubated at 37°C for 24 h. One milliliter of the broth, containing 1 X 10^8 CFU/mL, was used as inoculums and spread on each Mueller Hinton agar plate (Oxoid, Hampshire, England).

2.5. Disc Diffusion Assay

The antibacterial activity of the *Ruta graveolens* essential oil was determined by the disc diffusion method according to the Clinical Laboratory Standards Institute guidelines (2012). Sterile paper discs (Oxoid, Hampshire, England) of 6 mm diameter were impregnated with 10 μL of essential oil and deposited on the agar surface. Petri dishes were placed at 4°C for 2 h to facilitate the dissemination of extract on the culture medium, followed by incubation at 37°C for 24 h. For each sample, a negative water control and a positive antimicrobial agents disc control (Oxoid, Hampshire, England) were used. At the end of the period, inhibition zones (if any), formed on the medium, were evaluated in mm. Studies were performed in triplicate in three independent experiments.

3. Results

3.1. Chemical Composition of the Essential Oil

Hydrodistillation of the aerial parts of the *Ruta graveolens* sample gave pale yellowish oil with a yield of 0.27%. The chemical composition of the oil was investigated using the GC-MS techniques.

The identified component groups are shown in Table 1 with ketones (43.02%), aldehydes (37.12%), esters (9.33%), and sesquiterpene hydrocarbons (5.22%), being the major constituents.

Table 1. Constituents groups of the essential oil of *Ruta graveolens*

Compounds	Peak area %
Monoterpene hydrocarbons	0.61
Oxygenated monoterpenes	1.49
Sesquiterpene hydrocarbons	5.22
Oxygenated sesquiterpenes	0.2
Nitrogen-containing compounds	0.21
Aldehydes	37.12
Ketones	43.02
Alcohols	0.9
Esters	9.33

The identified components of the essential oil, their percentages and retention indices are given in Table 2. Thirty components, accounting for 98.1% of the oil, were identified. The major identified compounds were 2-nonanone (37.13%), undecanal (34.69%), 2-acetoxydodecane (5.0%), and 2-decanone (3.31%).

Table 2. Constituents compounds of the essential oil of *Ruta graveolens*

No.	Kovats Index	Compound	Peak area %
1	844	2E-Hexenal	0.28
2	847	3Z-Hexenol	0.28
3	860	Tetrahydro-3,6-dimethyl - 2H-pyran-2-one	0.1
4	970	Sabinene	0.10
5	987	2-Octanone	0.73
6	1026	d-Limonene	0.45
7	1080	3-Ethyl-2,2-dimethyloxazoldine	0.11
8	1107	2-Nonanone	37.13
9	1109	2-Nonanol	0.62
10	1110	Nonanal	0.35
11	1135	Geijerene	0.11
12	1139	2-Octanol acetate	0.36
13	1142	E-Myroxide	0.77
14	1160	2E-Nonen-1-al	0.1
15	1193	2-Decanone	3.31
16	1210	Octanol acetate	0.06
17	1239	2-Acetoxydodecane	5.0
18	1256	Cis- piperitone	0.37
19	1288	Pereijerene	5.22
20	1307	Undecanal	34.69
21	1314	2-Nonanyl acetate	0.63
22	1364	2-Methylundecanal	1.7
23	1393	3-Dodecanone	0.95
24	1433	Methylundecanoate	1.27
25	1457	2-Pentadecanone	0.05
26	1494	2-Tridecanone	0.8
27	1532	Citronellyl butanoate	0.1
28	1545	Elemol	0.35
29	1555	Alpha-agarofuran	0.2
30	1805	2-Ethyl hexyl salicylate	1.87
		Total	98.1

3.2. Antimicrobial Activity

The disc diffusion results, presented in Table 3, provide data on the activity of *Ruta graveolens* essential oil against several bacterial clinical isolates. The results show that the essential oil has a very potent activity against all the tested species. The *Ruta graveolens* essential oil was more active than the tested antimicrobial agents on MRSA, *S. epidermidis*, *B. subtilis*, and *E. aerogenes*. The essential oil was more active than neomycin, but less active than nitrofurantoin, against *E. coli*. It was more active than ceftazidime, and was similarly active to cefotaxime, against *P. aeruginosa*. Overall, the essential oil of *Ruta graveolens* was more active against Gram-positive than Gram-negative bacteria.

Table 3. Antibacterial activity of *Ruta graveolens* essential oil.

Bacterial species	*Ruta graveolens* essential oil zone of inhibition (mm); Mean± SD	Antimicrobial agent	Antimicrobial agent zone of inhibition (mm); Mean± SD
MRSA	20±0.20	Vancomycin	5±0.15
		Rifampicin	16±0.19
S. epidermidis	22±0.23	Cefuroxime	5±0.11
		Cefotaxime	11±0. 17
B. subtilis	28±0.65	Vancomycin	20±0.45
		Chloramphenicol	26±0.21
E. coli	14±0.2	Neomycin	12±0.29
		Nitrofurantoin	16±0.15
E. aerogenes	26±0.26	Neomycin	20±0.40
		Nitrofurantoin	22±0.25
P. aeruginosa	10±0.11	Ceftazidime	6±0.10
		Cefotaxime	10±0.14

4. Discussion

Hydrodistillation of the aerial parts of *Ruta graveolens* yielded pale yellowish oil. The chemical composition of the oil was investigated using GC-MS techniques, leading to the identification of thirty components, accounting for 98.1% of the oil content. The major constituent groups, given in Table 1, were ketones (43.02%), aldehydes (37.12%), esters (9.33%) and sesquiterpene hydrocarbons (5.22%). The major identified compounds, as shown in Table 2, were 2-nonanone (37.13%), undecanal (34.69%), 2-acetoxydodecane (5.0%), and 2-decanone (3.31%). A point to note is that the essential oil content of plants of a given species can vary depending on a number of factors, including the harvest time and the stage of growth at the time of picking (Adzet *et al.*, 1992). Table 4 summarise the essential oil content of *Ruta graveolens* according to previously published research.

Table 4. Reported major components of *Ruta graveolens* essential oil

Compound	Zhu *et al.* (1993)	Formacek and Kueczka (1982)	Aboutab *et al.* (1988)	El-Sherbeny *et al.* (2007)	Fredj *et al* (2007)	Soleimani *et al.* (2009)
2-Undecanone	36.5%	90.42%	49.2%	51%	27.34%	33.9%
2- Nonanone	23.17%	4.27%	24.7%	10.15%	38.66%	8.8%
2-Nonyl acetate	22.03%	-	6.2%	-	-	-
Limonene	-	-	6.06%	-	-	-
2-Nonanol	-	-	-	-	12.25%	-
2-Octyl acetate	-	-	-	-	7.71%	-
2-Heptanol acetate	-	-	-	-	-	17.5%
1-Dodecanoln	-	-	-	-	-	11%
Geyrene 10.4%	-	-	-	-	-	10.4%

The increase of the bacterial resistance to commonly used antimicrobial agents' presents itself as a major challenge for therapy. The essential oil of *Ruta graveolens* showed a very potent activity against all the tested bacteria being more effective against Gram-positive than Gram-negative bacteria. The results indicate that the essential oil of *Ruta graveolens* may have a potential as an effective and affordable means of combating bacterial infection. The complex mixtures of different compounds of the essential oil may provide multiple mechanisms for an antimicrobial activity. This could explain why this essential oil, and indeed other essential oils, has such a potent activity. The modes of action of only a limited number of the essential oil components were studied. The major components of an essential oil are thought to play a more significant role in the antimicrobial activity, while the minor constituents are thought to result in synergistic outcomes (Li *et al.,* 2014). Published data show that the most active essential oil components are phenols, followed by cinnamic aldehyde, alcohols, aldehydes and ketones, and ethers, while the least active being hydrocarbons (Kalemba and Kunicka, 2003).

The major components of *Ruta graveolens* essential oil were ketones (43.02%) and aldehydes (37.12%). These two components normally show a moderate antimicrobial activity (Fredj *et al.,* 2007). Li *et al.* (2014) reported that 70% of the *Litsea cubeba* essential oil, which has a very good antibacterial activity, was aldehydes. The antibacterial activity of *Ruta graveolens* essential oil may be similarly attributed to its aldehydes and ketones content, while the minor constituents could be responsible for other synergistic effects.

The main target of the essential oil compounds is the cell membrane. They lead to a cell membrane damage causing increased membrane permeability, ions leakage, and inhibition of different enzymes and proteins (Saad *et al.,* 2013; Hyldgaard *et al.,* 2012; Cox *et al.,* 2000). As to why the essential oil of *Ruta graveolens* was more effective on Gram-positive than on Gram-negative bacteria, it may be noted that the cell wall structures of the Gram-positive and the Gram-negative bacteria are different, being more complex in the latter. The cell wall of Gram-negative bacteria has an outer membrane that lies outside and is linked to the peptidoglycan layer below it (Nazzaro *et al.,* 2013; Saad *et al.,* 2013; Hyldgaard *et al.,* 2012). This outer membrane possesses pores that allow only small hydrophilic molecules to pass through and is almost impermeable to hydrophobic molecules. This outer membrane is absent in Gram-positive bacteria. This may explain why the hydrophobic essential oil is more active against Gram-positive than Gram-negative bacteria (Nazzaro *et al.,* 2013; Saad *et al.,* 2013; Hyldgaard *et al.,* 2012; Kavanaugh and Ribbeck, 2012; Gao *et al.,* 1999). However, the susceptibility of Gram-negative bacteria may vary according to genus and species. For example, the essential oil of cinnamon inhibits the growth of *E. aerogenes* through the interaction with different amino acid decarboxylases. Thus, the activity of essential oils may vary depending on the presence or absence of some targets (Boire *et al.,* 2013). This may explain why *Ruta graveolens* essential oil yielded a high antimicrobial activity toward *E. aerogenes*.

5. Conclusions

This study is the first to report on the essential oil composition of *Ruta graveolens* from Jordan. The essential oil of *Ruta graveolens* had an antimicrobial activity to several bacterial clinical isolates including MRSA and *P. aeruginosa*.

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