Journal of Ornamental Plants
www.jornamental.iaurasht.ac.ir
ISSN (Print): 2821-0093 ISSN (Online): 2783-5219

Research Article
Volume 12, Number 4: 259-266, December, 2022
DOR: https://dorl.net/dor/20.1001.1.28210093.2022.12.4.3.3

Detecting the Active Ingredients in the Extract and Essential Oil of the Ornamnetal and Medicinal Plant Species Rue (*Ruta graveolens* L.) and Studying their Antimicrobial Activity

Fatemeh Torabi Chafjiri[1], Davood Hashemabadi[1*], Behzad Kaviani[1], Leila Asadpour[2]
[1]Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran
[2]Department of Microbiology, Rasht Branch, Islamic Azad University, Rasht, Iran

Received: 20 June 2022 **Accepted:** 03 July 2022

*Corresponding author's email: davoodhashemabadi@yahoo.com

Abstract

The research aimed to explore the active ingredients of rue extract and essential oil as an ornamental and medicinal plant species and to assess their antimicrobial activity. So, rue shoots were collected from the natural habitat of this species in the elevations of Chaboksar, Guilan province in October and November 2019. The leaves and stems were oven-dried at 75 °C for 24 hours, and their extracts and essential oils were extracted with a Clevenger. The active ingredients of the extracts and essential oils were detected with GC-MS. The most abundant compounds in the leaf essential oil, stem essential oil, leaf extract, and stem extract were found to be hexadecanoic acid, isomaturnin, 2-ethyl-1,3,4,5,6,7,8-heptamethy, and 12-methoxy-19-norpodocarpa, respectively. The microbial assay by the disk diffusion method on the strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii,* and *Escherichia coli* showed that the leaf and stem extracts were most influential in inhibiting *A. baumannii*. The assessment of MIC and MBC revealed that *S. aureus* was the most sensitive bacterium to the leaf and stem essential oils with the lowest MIC and MBC and *A. baumannii* was the most sensitive bacterium to the leaf extract with the lowest MBC. So, rue extract and essential oil can be used as an antimicrobial compound against human pathogenic bacteria.

Keywords: *Acinetobacter baumannii*, Clevenger, Disk diffusion, GC-MS, Microbial resistance.

INTRODUCTION

Rue (*Ruta graveolens* L.) is a famous ornamental and medicinal species from the family of Rutaceae that is used to produce natural insecticides and pesticides in addition to its ornamental and medicinal applications in the cosmetic, health, and food industries. The plant is native to the Mediterranean area and grows in northern Iran wildly (Naghibi Harat *et al*., 2009; Sharma, 2022). Rue is an aromatic plant whose strong unpleasant odor is related to the presence of active ingredients or essential oil in all of its parts. So far, over 120 natural compounds have been identified in the roots and shoots of rue. The most important compounds include volatile oils, quinolone alkaloids, coumarins, lignins, glycosides, and alcohols with a wide range of therapeutic activities (Naghibi Harat *et al*., 2009; Kannan and Babu, 2012; Barceloux *et al*., 2018; Sharma, 2022). There are reports about the antioxidant, antibacterial, and antifungal effects and, in general, the inhibitory effects of rue extract and essential oil on various harmful microorganisms and their effectiveness in a wide range of diseases (Naghibi Harat *et al.,* 2009; Reddy and Al-Rajab, 2016; Barceloux *et al*., 2018).

Azalework *et al*. (2017) identified 26 compounds in the leaf extract of rue by the GC-MS method. They, then, used the disk diffusion method to examine the antimicrobial effect of 200 mg/ml of the extract on microbial strains of *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Candida albicans*, *Candida tropicalis*, and *Micrococcus luteus*. The results showed that the methanol leaf extract had a high inhibitory effect (with a halo diameter of 20 mm) on *B. subtilis* whereas its ethanol leaf extract was more effective in suppressing *P. vulgaris* (with a halo diameter of 16 mm). Also, *C. tropicalis*, *C. albicans*, and *E. coli* lacked a growth halo and were resistant to the extract. Reddy and Al-Rajab (2016) detected 13 compounds in rue essential oil by the GC-MS method. The main ones were 2-undecanone and 2-nonanone. The essential oil had strong antimicrobial activity against the bacterium *Staphylococcus* and the yeast *C. albicans*. The researchers reported the minimum inhibitory concentration (MIC) against these bacteria to be 0.7-1.58 µg/ml. Saderi *et al*. (2006) reported that the aquatic extract of rue stem was significantly influential in suppressing *Staphylococcus* growth.

Presently, with the increasing microbial resistance to antibiotics, the application of plant essential oils and extracts has emerged as a promising solution for coping with infections owing to their antimicrobial activities. So, it is imperative to recognize the active ingredients and study the antimicrobial and antioxidant properties of different plants, especially those that are native to Iran (Ahmadi *et al.*, 2011). In this regard, since rue grows in the northern parts of Iran wildly and the conditions are ready for its cultivation as an ornamental and medicinal plant, the present study aimed to extract the leaf and stem extract and essential oil of rue and identify its active ingredients using the GC-MS method. The research also investigates the antimicrobial effects of the extract and essential oil on four pathogenic bacterial strains.

MATERIALS AND METHODS

Plant collection and extract and essential oil preparation

The rue plants were collected at the end of their vegetative growth (before flower emergence) from the elevations of Chaboksar County, Guilan province in October and November of 2019. The leaves and stems were detached from the plants and oven-dried at 75 °C for 24 hours. To derive the extract, 100 g of dry plant powder was mixed with 100 ml of 70 % ethanol gently with a shaker in darkness for 24 hours. The extract was infiltrated through Whatman No. 2 paper and was put in a distillation device for 2 hours for the evaporation of the solvent. To remove the likely bacteria in the extract, it was exposed to UV radiation at 280-400 nm for 20 minutes. It was, then, transferred to a dark container and stored in a refrigerator until they were subjected to the assays. To derive the essential oil of the rue leaves and stems, the plant's dry powder was subjected to the water distillation method using a Clevenger for 2 hours.

Detection of chemical compounds in rue leaf and stem extract and essential oil

After the extract and essential oils were prepared, their chemical compounds were detected using a gas chromatography-mass spectrometry (GC-MS) device. The device column was of the Varian VF-1ms type with a length of 30 m, an internal diameter of 0.25 mm, and a thickness of 0.25 µm. The temperature program of the column was as an initial temperature of 50 °C, a final temperature of 280 °C, and a temperature gradient of 7 °C. The injection spot had a temperature of 280°C and a volume of 1 µm. Helium (99.999%) was used at a rate of 1 ml/min as the carrier gas. The mass spectrometer used was an Agilent 5973 Network, Mass Selective Detector with an ionization voltage of 70 eV. The ionization method of electron impact (EI) and the ionization source temperature was 230 °C. The spectra were identified using the inhibition index and time, and this component was compared with the mass spectra of standard compounds and the data of the NIST Ms Spectral Search Program and Wiley Mass Spectral Library.

Studied bacteria

To study the antibacterial effect of the rue leaf and stem extract and essential oil, the strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumanii*, and *Escherichia coli* were used.

Determination of antimicrobial effect of rue extract and essential oil by the disk diffusion method

The antibacterial effect of the leaf and stem extract and essential oil was studied on the target bacteria using the method recommended in NCCLS (2003). First, disks with a diameter of 6 mm were prepared. After bacterial inoculation on the plate surface, paper blank disks (made by PadtanTeb, Iran) were poured with 30 µl of the extract or essential oil. Then, the disks were placed on the plate surface with tweezers. The plates were then incubated at 37 °C for 24 hours. Then, the halo of bacteria non-growth around the disks was measured in mm.

Determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the rue extract and essential oil

NCCLS's (2003) method was used to determine MIC and MBC of the leaf and stem extract and essential oil. To this end, 3.12, 6.25, 12.5, 20, 25, 50, and 100 ml of the extracts and essential oils were poured into test tubes containing Mueller Hinton broth culture media made in Canada. A test tube lacking the extract and essential oil was considered the control. Then, 20 µl of the suspension of the four studied bacterial strains was added to each tube. The tubes were incubated at 37°C for 24 hours. Then, the bacteria in 1 ml of the initial bacterial suspension were counted. Next, ten-fold serial dilutions of each tube were prepared in 0.85 % physiological serum, and 50 µl of each dilution was cultured in separate plates and put in a hot chamber at 37 °C for 24 hours. At this stage, the number of bacterial colonies was counted. The lowest concentration of each extract or essential oil at which the number of bacteria was fixed was reported as MIC, and the lowest concentration of each extract or essential oil that reduced the number of bacteria to less than 0.001 of the initial count was reported as MBC.

Statistical analysis

Data were analyzed in the SPSS 19 software package. The differences of the variables were analyzed by one-way analysis of variance and the Tukey test was applied at the $P < 0.05$ probability level.

RESULTS

Compounds detected in rue leaf and stem extract and essential oil by the GC-MS method

Table 1 presents the compounds identified in the leaf and stem essential oils of rue. According to the results, nine compounds were identified in the leaf essential oil and stem essential oil. The most dominant compound in the leaf essential oil was hexadecanoic acid, which accounted for 10.09% of the essential oil. Isomaturnin (16.57%) was the most abundant compound in the stem essential oil (Table 1).

The compounds identified in the leaf and stem extracts are listed in table 2. Based on the results, five compounds were detected in the leaf extract and nine compounds in the stem extract. The most abundant compound was 2-ethyl-1,3,4,5,6,7,8-heptamethy (26.74%) in the leaf extract and 12-methoxy-19-norpodocarpa (24.44%) in the stem extract.

Table 1. The chemical compounds detected in the leaf and stem essential oils of *Ruta graveolens* L.

Essential oil	Compound	Percentage	The minute identified
Leaf essential oil	Toluene	2.43	3.57
	Phenol	3.84	7.03
	Phenol, 2-methyl	4.34	8.22
	Phenol, 4-methyl-	6.17	5.55
	Indole	4.47	11.71
	9-Octadecyne	7.41	18.40
	Hexadecanoic acid	10.09	19.52
	PHYTOL	5.74	20.96
	9-Pentadecadien-1	5.49	21.14
Stem essential oil	Phenol, 3,5-dimethyl	2.80	10.00
	Phenol, 4-ethyl-2-methoxy	2.58	11.58
	2-Undecanone	12.18	11.78
	Hexadecanoic acid	3.42	19.51
	Isomaturnin	16.57	21.56
	Heneicosane	3.65	30.06
	Morphinan-4-ol-6,7-dione	3.54	32.72
	N-Eicosane	5.69	33.79
	Dodecamethylnaphthacene	3.96	34.63

Table 2. The chemical compounds detected in the leaf and stem extracts of *Ruta graveolens* L.

Extract	Compound	Percentage	The minute identified
Leaf extract	Psoralene	9.62	15.10
	Furo[2,3-b]quinolone	7.61	16.24
	Xanthotoxin	10.11	17.41
	2-Ethyl-1,3,4,5,6,7,8-heptamethy	26.74	18.55
	20-Methyl-Pregna-5,17-Dien-3	25.62	22.33
Stem extract	Propanal	4.98	4.09
	Cytidine	12.62	13.32
	Dodecanamide	7.73	15.95
	Psoralene	5.88	18.11
	Furo[2,3-b]quinolone	6.57	19.25
	Isobergapten	5.01	20.42
	12-methoxy-19-norpodocarpa	24.44	21.56
	4-Acetyl-3-(2-oxocyclohexyl)-N	8.55	23.68
	Ethoxycarbamoyl	7.07	25.33

Results of microbial test by the disk method

Table 3 presents the results of the microbial test by the disk diffusion method, which shows the antibacterial activity of the leaf and stem essential oil and extract of rue against *P. aeruginosa, A. baumannii, E. coli*, and *S. aureus*. As is observed in table 3, the highest non-growth halo was related to the leaf extract (55 mm) and stem extract (41.5 mm) against *A. baumannii*, respectively. The lowest non-growth halo among all bacteria and the leaf and stem extract and essential oil of rue was related to *S. aureus*, reflecting the high resistance of this bacterium against rue extract and essential oil. In total, it can be said that the leaf and stem extracts of rue outperformed its essential oils in suppressing the bacteria. The leaf extract of rue was more effective against *P. aeruginosa* and *A. baumannii*, and its stem extract was more effective against *E. coli*. The leaf and stem extracts were equally effective in suppressing *S. aureus* (Table 3).

Table 3. The antimicrobial effect of the leaf and stem essential oils and extracts of rue on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli* by disk diffusion method.

	Stem essential oil	Leaf essential oil	Stem extract	Leaf extract
	(mm)			
E. coli	22.5	22	37.5	36
P. aeroginusa	29	34	32.5	36
A. bumanii	24	20	41.5	55
S. aureus	15.5	14	20	20

Determining MIC and MBC of the rue extract and essential oil against *P. aeruginosa*, *A. bumanii, E. coli,* and *S. aureus*

The MIC and MBC results of the rue extracts and essential oils against the target bacteria are presented in table 4. The MIC results revealed that the minimum concentration of leaf and stem extracts for inhibiting the growth of *P. aeruginosa*, *A. baumannii*, *E. coli*, and *S. aureus* was 25 mg/ml. The minimum concentration of the stem and leaf essential oil required to suppress the growth of *P. aeruginosa* and *A. baumannii* was 25 mg/ml whereas the minimum concentration of leaf and shoot essential oil for the suppression of S. aureus growth was 3.12 mg/ml (Table 4).

Table 4. The MIC and MBC of the leaf and stem essential oils and extracts of rue for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Escherichia coli.*

	E. coli		*P. aeruginosa*		*A. bumanii*		*S. aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	(mg/ml)							
Leaf essential oil	50	50	25	50	25	50	3.12	6.25
Stem essential oil	25	50	25	50	25	50	3.12	6.25
Leaf extract	25	50	25	50	25	12.50	25	50
Stem extract	25	50	25	50	25	50	25	50

The results of MBC (Table 4) showed that the MBC of the leaf and stem extracts and essential oils was 50 mg/ml against *P. aeruginosa* and *E. coli*. The highest sensitivity of *A. baumannii* to the leaf extract was recorded at a rate of 12.5 mg/ml. The MBC of the leaf and stem essential oil against *S. aureus* was found to be 6.25 mg/ml. In general, *S. aureus* with the lowest MIC and MBC was determined to be the most sensitive bacteria to the leaf and stem essential oil whereas *A. baumannii* with the lowest MBC exhibited the highest sensitivity to the leaf extract (Table 4).

DISCUSSION

The tendency of humans toward naturally occurring and plant compounds and the emergence of the phenomenon of microbial resistance to antibiotics have drawn researchers' interest in studying the potential of plants in removing and suppressing microbial and fungal pathogens. Research has shown that the extracts and essential oils of different plants have an inhibitory effect on a wide range of microorganisms and can be used, alone or along with chemical compounds, to remove and control pathogens (Alzoreky and Nakahara, 2003; Ahmadi *et al*., 2011; Althaher *et al*., 2022). In this respect, it is of high importance to exploit the potential of indigenous and available plants. Rue is a wild ornamental and medicinal plant species in the north of Iran, which has been interested as a medicinal plant since centuries ago and has been subject to research to detect its active ingredients (Naghibi Harat *et al*., 2009; Azalework *et al*., 2017; Althaher *et al*., 2022). This research used a GC-MS device to detect the active ingredients of the leaf and stem extract and essential oil of this plant species extracted with a Clevenger. As was already reported in the results section, we identified nine compounds in the leaf and stem essential oil, nine compounds in the stem extract, and five compounds in the leaf extract of rue. Yaacob *et al*. (1989) studied the shoot essential oil of rue plants grown in Malaysia by the GC-MS method and detected 20 compounds, mainly including 2-undecanone, 2-nonanone, 2-nonyl acetate, psoralen, xanthotoxin, and bergaptene. Soleimani *et al*. (2009) reported that ketons, sesquiterpenoids, and monoterpenoids were the main constituents of the rue essential oil. These researchers identified 19 compounds in the essential oil of native rue of Northern Iran. The main compounds included 2-undecanone, 2-heptanol acetate, 1-dodwcanol, geyrene, and 2-nonanone. In Fredj *et al*.'s (2007) study, 15 compounds were identified in the rue leaf and stem essential oil. The most abundant compounds were 2-nonanone, 2-nonanol, and 2-undecanone. These authors stated that the essential oil of the rue leaf and stem had antimicrobial effects on *E. coli, P. aeruginosa, S. aurens*, and *E. faecalis.*

Bacteria, especially antibiotic-resistant bacteria, are a major pathogenic factor in humans. *P. aeruginosa, S. aureus*, *A. baumannii,* and *E. coli* are prevailing antibiotic-resistant bacteria in hospitals (Soltani *et al*., 2016; Chiemchaisri *et al*., 2022). The disk diffusion method is a well-known method for determining bacteria sensitivity to antibiotics. The present research, in which the disk diffusion method was used, revealed the antimicrobial effect of the rue leaf extract and essential oil on the studied bacteria. However, the non-growth halo showed that Acinetobacter, which had the largest non-growth halo, was the most sensitive bacterium to the leaf and stem extract of this plant. *S. aureus* was the most resistant bacterium with the shortest non-growth halo against the leaf and stem extract and essential oil. In general, the rue extract showed stronger antimicrobial activity than its essential oil against the studied bacteria, which can be attributed to the higher concentrations of active ingredients in the essential oil and their adverse impact, which needs further research.

As was already reported, the minimum concentration of the rue leaf and stem essential oil for growth inhibition and bactericidal was reported for *S. aureus* (MIC = 3.12; MBC = 6.25), and the minimum concentration of leaf extract for bacterial removal was recorded for *Acinetobacter* (MIC = 25; MBC = 12.5). In Saderi *et al*.'s (2006) research, MIC and MBC of the aquatic extract of rue stem for suppressing *S. aureus* were estimated at 10% and 20%, respectively. It was reported to be more effective than the aquatic extract of dry seeds. Alzoreky and Nakahara (2003) recorded the MIC of rue extract at 2.6 mg/ml for *S. aureus*, a prevailing bacterium involved in human infections. According to the results, it can be said that gram-negative bacteria were more resistant to the rue essential oil and extract and the gram-positive bacterium (*S. aureus*) was more sensitive to the rue leaf and stem essential oil. It can be claimed that the high concentration of active ingredients is the reason for the higher efficiency of the essential oil than the extract in controlling and eliminating *S. aureus.*

CONCLUSIONS

Based on the results, the leaf and stem essential oil and extract of rue have inhibitory effects on the activity of *P. aeruginosa, A. baumannii, E. coli*, and *S. aureus.* Therefore, it can be said that rue as a wild ornamental plant in the northern regions of Iran is an essential source of medicinal compounds with antimicrobial activities, so it can be used as a natural and available alternative for antibiotics and chemical disinfectants to control prevalent pathogenic bacteria in healthcare centers.

ACKNOWLEDGMENT

The authors should express their gratitude to the Research Deputy of Islamic Azad University-Rasht Branch.

Literature Cited

Ahmadi, Z., Sattari, M., Tabarraee, B. and Bigdeli, M. 2011. Identification of the constituents of *Achillea santolina* essential oil and evaluation of the anti-microbial effects of its extract and essential oil. Journal of Arak University of Medical Sciences, 14 (3): 1-10.

Althaher, A.R., Oran, S.A. and Bustanji, Y.K. 2022. Induction of apoptosis by *Ruta chalepensis* L. essential oil in human breast cancer cells (MCF-7). Journal of Pharmacy and Pharmacognosy Research, 10 (1): 73-83.

Alzoreky, N.S. and Nakahara, K. 2003. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology, 80 (3): 223-230.

Azalework, H.G., Sahabjada, A.J. and Arshad, T.M. 2017. Phytochemical investigation, GC-MS profile and antimicrobial activity of a medicinal plant *Ruta graveolens* L. from Ethiopia. International Journal of Pharmacy and Pharmaceutical Sciences, 9 (6): 29-34.

Barceloux, D.G. 2008. Medical toxicology of natural substances: Foods, fungi, medicinal herbs, plants, and venomous animals. Chapter 85: Rue (*Ruta graveolens* L.). John Wiley and Sons Inc. pp. 579- 581.

Chiemchaisri, W., Chiemchaisri, C., Witthayaphirom, C., Saengam, C. and Mahavee, K. 2022. Reduction of antibiotic-resistant *E. coli*, *K. pneumoniae*, *A. baumannii* in aged-sludge of membrane bioreactor treating hospital wastewater. Science of the Total Environment, 812: 152470.

Fredj, M.B.H., Marzouk, B., Chraief, I., Boukef, K. and Marzouk, Z. 2007. Analysis of Tunisian *Ruta graveolens* L. oils from Jemmel. Journal of Food Agriculture and Environment, 5 (1): p. 52.

Kannan, R. and Babu, U.V. 2012. Identity and pharmacognosy of *Ruta graveolens* Linn. Ancient Science of Life, 32 (1): 15-19.

Naghibi Harat, Z., Kamalinejad, M., Sadeghi, M.R., Sadeghipour, H.R. and Eshraghian, M.R. 2009. A review on *Ruta graveolens* L. its usage in traditional medicine and modern research data. Journal of Medicinal Plants, 8 (30): 1-19.

National Committee for Clinical Laboratory Standards (NCCLS). 2003. National committee for clinical laboratory standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 6th Edition, Wayne, Pennsylvania, 30 page.

Reddy, D.N. and Al-Rajab, A.J. 2016. Chemical composition, antibacterial and antifungal activities of *Ruta graveolens* L. volatile oils. Cogent Chemistry, 2 (1): 1220055.

Saderi, H., Oulia, P. and Radmanesh, M. 2006. Antimicrobial effect of hydro-alcoholic and aqueous extracts of *Ruta graveolens* L. against Staphylococcus aureus. Iranian Journal of Medicinal and Aromatic Plants, 22 (4): 366-372.

Sharma, D. 2022. A brief review of Ruta graveolens plant in homoeopathic system of medicine.

Indian Journal of Integrative Medicine, pp.1-5.

Soleimani, M., Azar, P.A., Saber-Tehranil, M. and Rustaiyan, A. 2009. Volatile composition of *Ruta graveolens* L. of North of Iran. World Applied Sciences Journal, 7 (1): 124-126.

Soltani, R., Fazeli, H., Bahri Najafi, R. and Jelokhanian, A. 2017. Evaluation of the synergistic effect of tomatidine with several antibiotics against standard and clinical isolates of *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*. Iranian Journal of Pharmaceutical Research, 16 (1): 290-296.

Yaacob, K.B., Abdullah, C.M. and Joulain, D. 1989. Essential oil of *Ruta graveolens* L. Journal of Essential Oil Research, 1 (5): 203-207.

How to cite this article:

Torabi Chafjiri, F., Hashemabadi, D., Kaviani, B., & Asadpour, L. (2022). Detecting the Active Ingredients in the Extract and Essential Oil of the Ornamnetal and Medicinal Plant Species Rue (*Ruta graveolens* L.) and Studying their Antimicrobial Activity. Journal of Ornamental Plants, 12(4), 259-266.
https://jornamental.rasht.iau.ir/article_692385.html