

# Phytochemical Screening by FTIR Spectroscopic Analysis and Anti-Fungal Activity of Fruit Extract of Selected Medicinal Plant of *Ruta graveolens*

Ekhlash Al-Shareefi<sup>1</sup>, Ahmed Hadi Abdal Sahib<sup>2</sup>, Imad Hadi Hameed<sup>3</sup>

<sup>1</sup>Department of Biology, College of Science for Women, University of Babylon, Hillah City, Iraq,

<sup>2</sup>Ecological Sciences, AL-Qasim Green University, Hillah City, Iraq, <sup>3</sup>Biomedical Science Department, University of Babylon, College of Nursing, Hillah City, Iraq

## ABSTRACT

*Ruta graveolens* (the common rue) has been used for various therapeutic purposes, including relief of rheumatism and treatment of circulatory disorder. The purpose of our research were analysis of the bioactive chemical compounds of selected medicinal plant of *Ruta graveolens* using fourier transform infrared spectrophotometer analysis and evaluation of anti-fungal activity. Thirteen bioactive compounds were identified in the methanolic extract of *Ruta graveolens*. The Fourier transform infrared spectrophotometer analysis of *Ruta graveolens* proved the presence of alkenes, alkanes, and alkyl halides which shows major peaks at 667.37, 873.75, 921.97, 1004.91, 1014.56, 1242.16, 1317.38, 1595.13, 1614.42, 2330.01, 2357.01, 3228.84 and 3282.84. In the current study, the anti-fungal activity of *Ruta graveolens* methanolic extract was evaluated by determining the zone of inhibition against fungi. *Ruta graveolens* was very highly active against *Aspergillus fumigatus* (5.39±0.21).

**Keywords:** Phytochemical screening, FTIR spectroscopic, Medicinal plant, *Ruta graveolens*

## INTRODUCTION

In recent years there has been a great scientific advance regarding chemical and pharmacological studies of medicinal plants aimed at obtaining new compounds with biological properties. Among the countless species of medicinal interest, there are plants belonging to the Rutaceae family, which has species of economic, ecological and therapeutic importance. The Rutaceae family, also named as Rutaceae, belongs to the order of Sapindales with about 150 genders and over 1600 species. In recent years, there has been a growing interest in researches looking for possible uses of plant products as antimicrobial instead of several synthetic antibacterial which can cause several

side effects. Historically, natural products and their derivatives have been an invaluable source of therapeutic agents. When in vitro, antimicrobial assays have effectively served as reliable methods to detect several classes of secondary metabolites with high antimicrobial activity<sup>1-4</sup>. *Ruta graveolens* commonly known as rue, is a dicot herb, belongs to Rutaceae family and native to Mediterranean region but widely distributed all over the tropical regions. The leaves are bipinnate or tripinnate with a feathery appearance and green to strongly glaucous blue-green in colour<sup>5-9</sup>. This plant is used by the Iraqi populations, systemically for its antispasmodic and analgesic effects and externally for its anti-rheumatic activity. The methanol, petroleum ether, ethyl acetate and water-methanol extracts of *R. graveolens* were found to possess antimicrobial and cytotoxic activities. *Ruta* in combination with Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> is found to be effective in treatment of brain cancers, particularly glioma. Leaf extracts also reported to possess strong anti-inflammatory activity<sup>10-16</sup>. However, no significant reports are available about the antimicrobial activity of *Ruta graveolens* stem; therefore, present investigation

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### Corresponding author:

**Imad Hadi Hameed.**

Biomedical Science Department, University of Babylon, College of Nursing, Hillah city, Iraq; Phone number: 009647716150716;

E-mail: imad\_dna@yahoo.com

was undertaken to examine the antimicrobial activities of stem extract of *Ruta graveolens* using various pathogenic microbial strains.

## MATERIALS AND METHOD

### Collection and preparation of plant material

The leaves were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal foreign materials, the leaves were stored in airtight container to avoid the effect of humidity<sup>17-25</sup> and then stored at room temperature until further use.

### Preparation of sample

About 20 grams of the plant sample powdered were soaked in 100 ml methanol for 16 hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture<sup>26-31</sup>.

### Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *Ruta graveolens* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm<sup>32-39</sup>.

### Determination of antimicrobial activity of crude bioactive compounds of *Ruta graveolens*

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty  $\mu\text{L}$  of plant extract was loaded on the bored wells. Antifungal activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference

antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

## RESULTS AND DISCUSSION

Analysis of compounds was carried out in methanolic extract of *Ruta graveolens*, shown in **Table 1**. Chromatogram GC-MS analysis of the methanol extract of *Ruta graveolens* showed the presence of thirteen major peaks and the components corresponding to the peaks were determined as follows. The Fourier transform infrared spectrophotometer analysis of *Ruta graveolens* proved the presence of alkenes, alkanes, and alkyl halides which shows major peaks at 667.37, 873.75, 921.97, 1004.91, 1014.56, 1242.16, 1317.38, 1595.13, 1614.42, 2330.01, 2357.01, 3228.84 and 3282.84. In the current study, the anti-fungal activity of *Ruta graveolens* methanolic extract was evaluated by determining the zone of inhibition against fungi. *Ruta graveolens* was very highly active against *Aspergillus fumigatus* (5.39 $\pm$ 0.21). Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. WHO encourages countries to provide safe and effective traditional remedies and practices in public and private health services and it also published two monographs on medicinal plants with information on pharmacopoeial summaries for quality assurance: botanical features, distribution, identity tests, purity requirements, chemical assays, and active or major chemical constituents, clinical applications, pharmacology, contraindications, warnings, precautions, potential adverse reactions, and posology. The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc.

**Table 1. FT-IR peak values of *Ruta graveolens* methanolic extract.**

No.	Peak (Wave number $\text{cm}^{-1}$ )	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.37	56.899	Strong	C-Cl	Stretch	alkyl halides	600-800
2.	873.75	73.673	Strong	=C-H	Bending	Alkenes	650-1000
3.	921.97	71.567	Strong	=C-H	Bending	Alkenes	650-1000
4.	1004.91	52.162	Strong	C-F	Stretch	alkyl halides	1000-1400

Cont... Table 1. FT-IR peak values of *Ruta graveolens* methanolic extract.

5.	1014.56	51.019	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1242.16	77.325	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1317.38	77.311	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1595.13	73.531	Medium	C=C	Stretch	Aromatic	1400-1600
9.	1614.42	72.276	Bending	N-H	Stretch	Amide	1550-1640
10.	2330.01	83.341	Unknown	-	-	-	-
11.	2357.01	77.583	Unknown	-	-	-	-
12.	3228.84	73.851	Bending	N-H	Stretch	Amide	3100-3500
13.	3282.84	72.556	Bending	N-H	Stretch	Amide	3100-3500

### CONCLUSION

The Fourier transform infrared spectrophotometer analysis of *Ruta graveolens* proved the presence of alkenes, alkanes, and alkyl halides which shows major peaks at 667.37, 873.75, 921.97, 1004.91, 1014.56, 1242.16, 1317.38, 1595.13, 1614.42, 2330.01, 2357.01, 3228.84 and 3282.84. In the current study, the anti-fungal activity of *Ruta graveolens* methanolic extract was evaluated by determining the zone of inhibition against fungi. *Ruta graveolens* was very highly active against *Aspergillus fumigatus* (5.39±0.21).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** In our research, all protocols were approved under the Department of Biology, College of Science for women, University of Babylon, Hillah city, Iraq and all methods were carried out in accordance with approved guidelines.

### REFERENCES

- Altaee N, Kadhim MJ, Hameed IH. Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. *International Journal of Toxicological and Pharmacological Research*. 2016; 8(6): 462-470.
- Hussein HM, Hameed IH, Ubaid JM. Analysis of the secondary metabolite products of *Ammi majus* and evaluation anti-insect activity. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1192-1189.
- Hussein HM, Ubaid JM, Hameed IH. Insecticidal activity of methanolic seeds extract of *Ricinus communis* on adult of *Callosobruchus maculatus* (coleopteran:brauchidae) and analysis of its phytochemical composition. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1385-1397.
- Ubaid JM, Hussein HM, Hameed IH. Determination of bioactive chemical composition of *Callosobruchus maculatus* and investigation of its anti-fungal activity. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1293-1299.
- Ibraheem IA, Hussein HM, Hameed IH. *Cyclamen persicum*: Methanolic Extract Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique. *International Journal of Pharmaceutical Quality Assurance*. 2017; 8(4): 200-213.
- Ibraheem IA, Hadi MY, Hameed IH. Analysis of Bioactive Compounds of Methanolic Leaves extract of *Mentha pulegium* Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique. *International Journal of Pharmaceutical Quality Assurance*. 2017; 8(4): 174-182.
- Hadi MY, Hameed IH, Ibraheem IA. *Ceratoniasiliqua*: Characterization, Pharmaceutical Products and Analysis of Bioactive Compounds: A Review. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3585-3589.
- Hadi MY, Hameed IH, Ibraheem IA. *Mentha pulegium*: Medicinal uses, Anti-Hepatic, Antibacterial, Antioxidant effect and Analysis of Bioactive Natural Compounds: A Review. *Research Journal of Pharmacy and Technology*. 2017; 10(10):

- 3580-3584.
9. Kadhim MJ, Sosa AA, Hameed IH. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 127-146.
  10. Mohammed GJ, Kadhim MJ, Hussein HM. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 889-905.
  11. Hameed IH, Altameme HJ, Idan SA. *Artemisia annua*: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2016; 7(2): 1843- 1868
  12. Mohammed GJ, Omran AM, Hussein HM. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 977-996.
  13. Jasim H, Hussein AO, Hameed IH, Kareem MA. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(4): 56-72.
  14. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(2): 8-24.
  15. Hameed IH, Ibraheem IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus officinalis* leaves. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(6): 90-106.
  16. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. *Oriental Journal of Chemistry*. 2016; 32(2): 20-40.
  17. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of *Saccharomyces cerevisiae* and Evaluation of Antibacterial Activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(5): 304-315.
  18. Mohammed GJ, Al-Jassani MJ, Hameed IH. Antibacterial, Antifungal Activity and Chemical analysis of *Punica grantanum* (Pomegranate peel) using GC-MS and FTIR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3): 480-494.
  19. Dhahir BM, Hameed IH, Jaber AR. Prospective and Retrospective Study of Fractures According to Trauma Mechanism and Type of Bone Fracture. *Research Journal of Pharmacy and Technology*. 2017; 10(10):1827-1835.
  20. Hapeep MA, Hameed IH, Jasim AA. Risk Factors, Cause and Site of Firearm Injuries: A Prospective and Retrospective Study. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3420-3425.
  21. Jasim AA, Hameed IH, Hapeep MA. Traumatic Events in an Urban and Rural Population of Children, Adolescents and Adults in Babylon Governorate - Iraq. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3429-3434.
  22. Altameme HJ, Hameed IH, Abu-Serag NA. Analysis of bioactive phytochemical compounds of two medicinal plants, *Equisetum arvense* and *Alchemilla vulgaris* seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Malays. Appl. Biol*. 2015; 44(4): 47-58.
  23. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial Activity and spectral chemical analysis of methanolic leaves extract of *Adiantum Capillus-Veneris* using GC-MS and FT-IR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3): 369-385.
  24. Kadhim MJ, Mohammed GJ, Hameed IH. In vitro antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. *Oriental Journal of Chemistry*. 2016; 32(2): 10-30.
  25. Hussein HM. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and fourier-

- transform infrared spectroscopy. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2016; 7(4): 2529 – 2555.
26. Jaddoa HH, Hameed IH, Mohammed GJ. Analysis of volatile metabolites released by *Staphylococcus aureus* using gas chromatography-Mass spectrometry and determination of its antifungal activity. *Oriental Journal of Chemistry*. 2016; 32(4): 8-24.
  27. Hameed IH, Salman HD, Mohammed GJ. Evaluation of antifungal and antibacterial activity and analysis of bioactive phytochemical compounds of *Cinnamomum zeylanicum* (Cinnamon bark) using gas chromatography-mass spectrometry. *Oriental Journal of Chemistry*. 2016; 32(4): 16-25.
  28. Kadhim MJ, Mohammed GJ, Hussein HM. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(7): 655-670.
  29. Ubaid JM, Hussein HM, Hameed IH. Analysis of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(7): 655-670.
  30. Hameed, I.H., Al-Rubaye A.F. and Kadhim, M.J. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. *International Journal of Pharmaceutical and Clinical Research*. 2017; 8(11): 44-54.
  31. Kadhim WA, Kadhim, M.J., Hameed, I.H. Antibacterial Activity of Several Plant Extracts Against *Proteus* Species. *International Journal of Pharmaceutical and Clinical Research*. 2017; 8(11): 88-94.
  32. Ahmed MD, Hameed IH, Abd-Ali MQ. Prospective and Retrospective Study of the Acute Heart Attack Cases in Marjan Hospital-Hillah City-Iraq. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3408-3416.
  33. Fakhir DF, Hameed IH, Flayyih SS. Burns Injuries: A Prospective Statistical Study of 112 patients. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3401-3407.
  34. Mekhlef AK, Hameed IH, Khudhair ME. Prevalence of Physical Injuries on the Head, Neck and Entire Body in, Hilla, Iraq. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3276-3282.
  35. Hameed IH, Al-Rubaye AF, Kadhim MJ. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. *International Journal of Pharmaceutical and Clinical Research*. 2017; 9(1): 44-50.
  36. Al-Rubaye AF, Hameed IH, Kadhim MJ. A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. *International Journal of Toxicological and Pharmacological Research*. 2017; 9(1): 81-85.
  37. Kadhim MJ, Kaizat AF, Hameed IH. Medicinal Plants Used for Treatment of Rheumatoid Arthritis: A Review. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(12): 1685-1694.
  38. Ubaid JM, Kadhim MJ, Hameed IH. Study of Bioactive Methanolic Extract of *Camponotus fellah* Using Gas Chromatography – Mass Spectrum. *International Journal of Toxicological and Pharmacological Research*. 2016; 8(6): 434-439.
  39. Hussein HM, Hameed IH, Ubaid JM. Analysis of the secondary metabolite products of *Ammi majus* and evaluation anti-insect activity. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1192-1189.
  40. Hussein HM, Ubaid JM, Hameed IH. Insecticidal activity of methanolic seeds extract of *Ricinus communis* on adult of *callosobruchus maculatus* (coleopteran:brauchidae) and analysis of its phytochemical composition. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1385-1397.
  41. Ubaid JM, Hussein HM, Hameed IH. Determination of bioactive chemical composition of *Callosobruchus maculatus* and investigation of its anti-fungal activity. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1293-1299.
  42. Hussein JH, Hameed IH, Hadi MY. Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Compounds of Methanolic Leaves extract of *Lepidium sativum*. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 3981-3989.
  43. Hadi MY, Hameed IH. Uses of Gas Chromatography-

- Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Chemical Compounds of *Lepidium sativum*: A Review. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 4039-4042.
44. Ubaid JM, Hadi MY, Hameed IH. Bioactive Chemical Compounds Identified in Methanolic Extract of *Trogoderma granarium*. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 3997-4004.
45. Hameed IH, Calixto MR, Hadi MY. Antimicrobial, Antioxidant, Hemolytic, Anti-anxiety, and Antihypertensive activity of *Passiflora* species. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 4079-4084.
46. Hameed IH, Calixto MR, Hadi MY. A Review: *Solanum nigrum* L. Antimicrobial, Antioxidant properties, Hepatoprotective effects and Analysis of Bioactive Natural Compounds. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 4063-4068.
47. Hussein JH, Hameed IH, Hadi MY. A Review: Anti-microbial, Anti-inflammatory effect and Cardiovascular effects of Garlic: *Allium sativum*. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 4069-4078.
48. Flayyih SS, Hameed IH, Fakhir FD. Road Traffic Accident Coming to Hillah Teaching Hospital: Prospective and Retrospective Study. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 3819-3825.
49. Fakhir DF, Hameed IH, Flayyih SS. Retrospective Study: Burn Injury from 2010 to 2015 in a Burn Unit-Hillah Teaching Hospital-Iraq. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 3831-3838.
50. Khudhair ME, Hameed IH, Mekhleef AK. A Prospective and Retrospective Study of Acute Bronchitis in Hillah City-Iraq. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 3839-3844.
51. Kamal SA, Hamza LF, Ibraheem IA. Characterization of Antifungal Metabolites Produced by *Aeromonas hydrophila* and Analysis of its Chemical Compounds Using GC-MS. *Research Journal of Pharmacy and Technology*. 2017; 10 (11): 3845-3851.