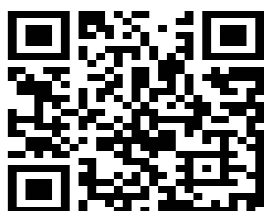




Ruta graveolens in vitro Anti-fungal Activity and Screening of Bioactive Chemical Compounds Using Fourier transform infrared spectroscopic Technique

Fatima Moeen Abbas

College of Science for
Women, University of
Babylon, Babylon, Iraq



Abstract:

The objectives of this study were analysis of the secondary metabolite products and evaluation of Anti-fungal activity. The FTIR analysis of *Ruta graveolens* proved the presence of functional group assignment Alkyl halides, Alkenes, Aromatic, and Amide, with Intensity 56.899 (Strong), 73.673 (Strong), 71.567 (Strong), 52.162 (Strong), 51.019 (Strong), 77.325 (Strong), 77.311 (Strong), 73.531 (Medium), 72.276 (Bending), 83.341 (Unknown), 77.583 (Unknown), 73.851 (Bending), 72.556 (Bending). and Peak (Wave number cm^{-1}) 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. anti-fungal activity of *R. graveolens* methanolic extract was evaluated by determining the zone of inhibition against fungi. *R. graveolens* was very highly active against *A. fumigatus* (3.71 ± 0.19).

Keywords: *Ruta graveolens*, Anti-fungal Activity, Bioactive Chemical Compounds.

Copyright : © 2023 The Authors. Published by Publisher. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction:

The interest in herbal remedies has grown because they have an intriguing chemical makeup and are widely utilized in folk medicine. One of the potential sources for finding new agents and/or medications is plant-based natural products. The Rutaceae family of plants, or the citrus family, are well-known for their economic significance as well as for the cultivated citrus fruits, timber, and aromatic oils that have the potential to yield a variety of therapeutic compounds [1,2]. As a result of their anti-bacterial, anti-fungal, anti-

leishmanial, and anti-plasmodial properties, the natural products in this family have shown promise in the treatment of a variety of ailments, including depression, cancer, Alzheimer's disease, and other diseases [3]. The genus *Ruta* is one of the Rutaceae family plants researched. *Ruta*, sometimes known as rue, is the type genus of the subfamily Rutoideae and a member of the tribe Ruteae [4,5]. *Ruta* is a subshrub with a powerful aroma that is indigenous to the Mediterranean region [6,7]. Ten species of perennial shrubs make

up the genus *Ruta*, with *R. chalepensis* and *R. graveolens* being the most widely distributed [8]. The analysis of secondary metabolite products and assessment of anti-fungal action were the objectives of this study.

Materials and Methods:

Collection and preparation of plant material:

In the center of Iraq's Hilla city, *Ruta graveolens* were acquired at a local market. Once thoroughly cleaned and all foreign contaminants have been removed, the item should be stored in an airtight container to minimize the impact of humidity before being kept at room temperature [9].

Preparation of sample:

In a rotatory shaker, 20 grams of the plant sample *Ruta graveolens* powder were steeped in 100 ml of methanol for 16 hours. Whatman The plant extract was separated using No. 1 filter paper [10,11]. The filtrates were used to conduct additional phytochemical research. It was once more filtered through sodium sulphate to get rid of any remaining moisture.

Fourier transform infrared spectrophotometer (FTIR):

For FTIR spectroscopy, the powdered *Ruta graveolens* sample was prepared (Shimadzu, IR Affinity, Japan). The sample was run in the 400–4000 nm infrared range [12,13].

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

Müller-Hinton agar plates were swabbed with the test microorganisms. On the bored wells, 70µL of plant extract were loaded. By assessing the zone of inhibition against the test microorganisms, antifungal activity was assessed. Methanol was utilized to regulate the solvent. As a standard antifungal agent, fluconazole and amphotericin B were used. The experiments were conducted in triplicate [14]. By evaluating the inhibition-zone diameter seen after 48 hours of incubation, the antifungal activity was assessed.

Statistical analysis:

The study used a triple design, and the results were reported as means. The study was performed with the help of a variance test ANOVA (SPSS).

Results and Discussion:

Numerous species of *Ruta* are known to have naturally occurring chemicals with a wide range of biological activities [15], including antifungal, phytotoxic, and antidotal effects. The currently available antifungal medicines can cause a wide variety of unwanted side effects, such as recurrence or the development of resistance in the fungal infection. The FTIR analysis of *Ruta graveolens* proved the presence of functional group assignment Alkyl halides, Alkenes, Aromatic, and Amide, with Intensity 56.899 (Strong), 73.673 (Strong), 71.567 (Strong), 52.162 (Strong), 51.019 (Strong), 77.325 (Strong), 77.311 (Strong), 73.531 (Medium), 72.276 (Bending), 83.341 (Unknown), 77.583 (Unknown), 73.851 (Bending), 72.556 (Bending). and Peak (Wave number cm^{-1}) 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. (Table 1; Figure 1). *R. graveolens* was very highly active against *A. fumigatus* (3.71 ± 0.19). The existence of functional group assignment was demonstrated by the FTIR analysis of *R. graveolens*. In the present investigation, the zone of inhibition against fungi was used to assess the anti-fungal activity of the methanolic extract of *R. graveolens*. *R. graveolens* was extremely active (3.71 ± 0.19) against *A. fumigatus*. Due to the plant's putative antifungal, antibacterial [16], anti-inflammatory, and cytotoxic qualities, it has been used medicinally since the beginning of time to treat a wide variety of clinical illnesses. Rue has a long history of usage in traditional medicine, particularly in the treatment of lumbago, neuralgia, migraines, epistaxis, asthenopia, and gastrointestinal problems [17]. In addition to this, it has been demonstrated that glioma responds favorably to it [18]. Everyone is in agreement that there is an immediate need for novel antifungal drugs that do not have these downsides.

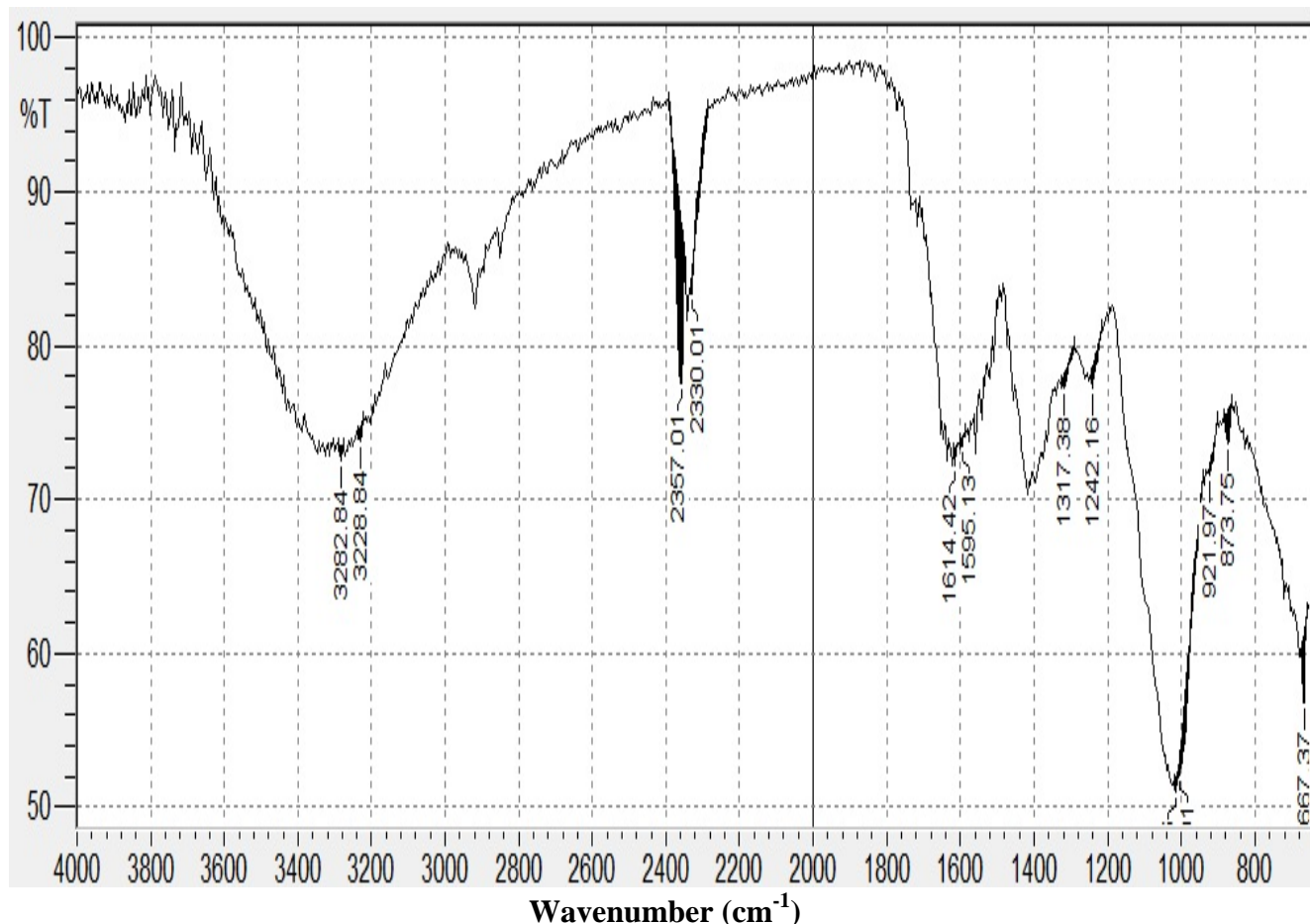


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Ruta graveolens* .

Table: FT-IR peak values of solid analysis of *Ruta graveolens*.

No.	Peak (Wave number cm ⁻¹)	Intensity	Base (H)	Base (L)	Type of Intensity	Bond	Functional group assignment	Group frequency
1.	667.37	56.899	673.16	657.73	Strong	C-Cl	alkyl halides	600–800
2.	873.75	73.673	883.40	862.18	Strong	=C–H	Alkenes	650-1000
3.	921.97	71.567	923.90	900.76	Strong	=C–H	Alkenes	650-1000
4.	1004.91	52.162	1006.84	941.26	Strong	C-F	alkyl halides	1000-1400
5.	1014.56	51.019	1020.34	1008.77	Strong	C-F	alkyl halides	1000-1400
6.	1242.16	77.325	1246.02	1215.15	Strong	C-F	alkyl halides	1000-1400
7.	1317.38	77.311	1325.10	1290.38	Strong	C-F	alkyl halides	1000-1400
8.	1595.13	73.531	1600.92	1585.49	Medium	C=C	Aromatic	1400-1600
9.	1614.42	72.276	1618.28	1602.85	Bending	N-H	Amide	1550-1640
10.	2330.01	83.341	2333.87	2285.65	Unknown	-	-	-
11.	2357.01	77.583	2393.66	2349.30	Unknown	-	-	-
12.	3228.84	73.851	3238.48	3221.12	Bending	N-H	Amide	3100-3500
13.	3282.84	72.556	3290.56	3275.13	Bending	N-H	Amide	3100-3500

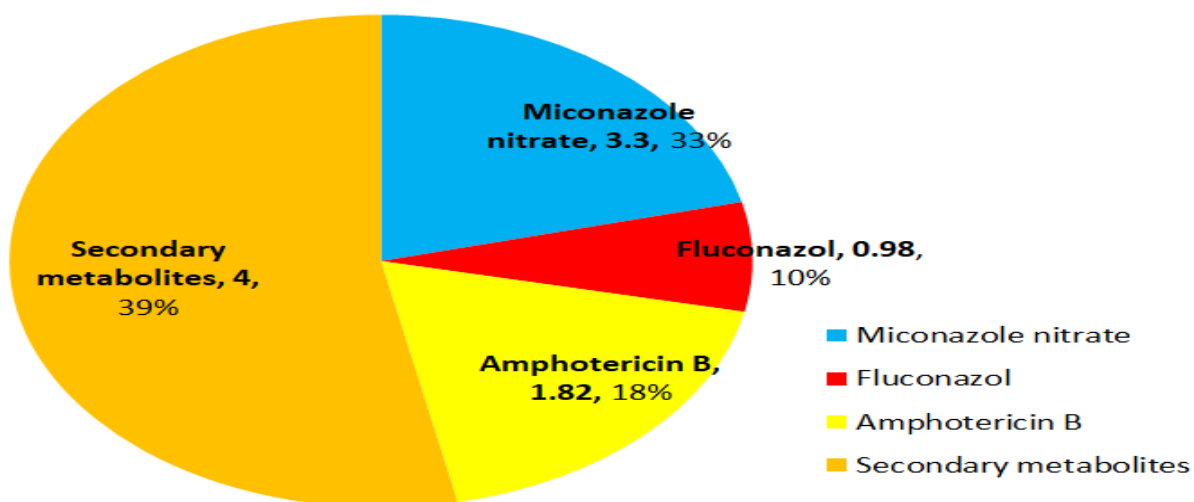


Figure 2. Metabolite products *R. graveolens* , Amphotericin B, Fluconazol and Miconazole nitrate as anti- Fungal activity against *A. terreus*

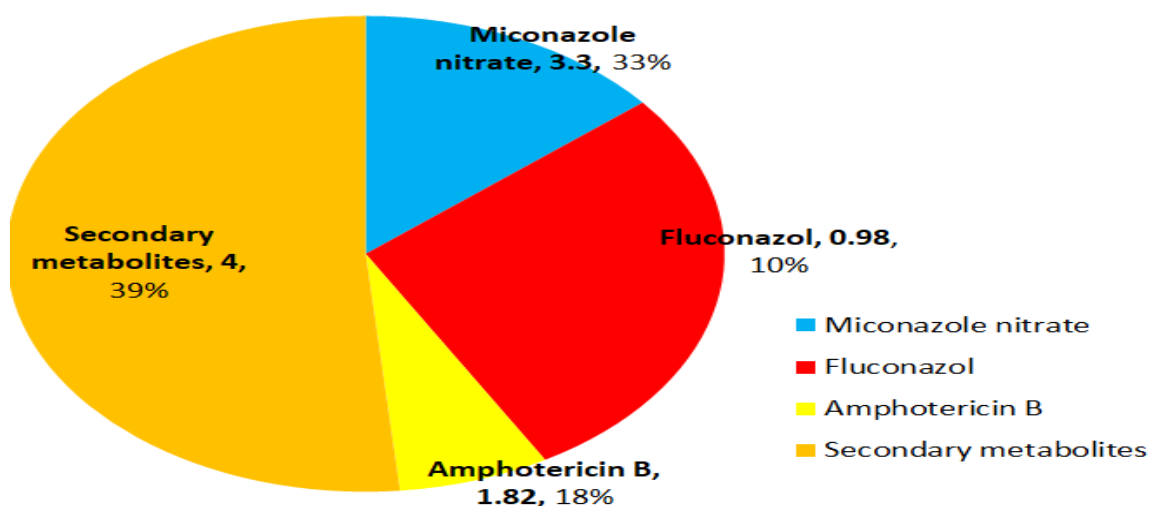


Figure 3. Metabolite products *R. graveolens* , Amphotericin B, Fluconazol and Miconazole nitrate as anti- Fungal activity against *A. fumigatus*

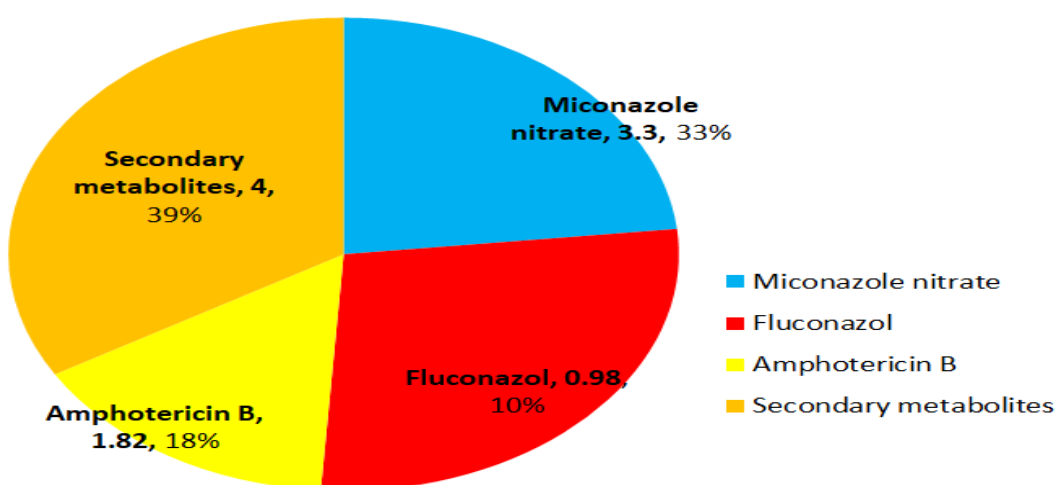


Figure 4. Metabolite products *R. graveolens* , Amphotericin B, Fluconazol and Miconazole nitrate as anti- Fungal activity against *S. cerevisiae*

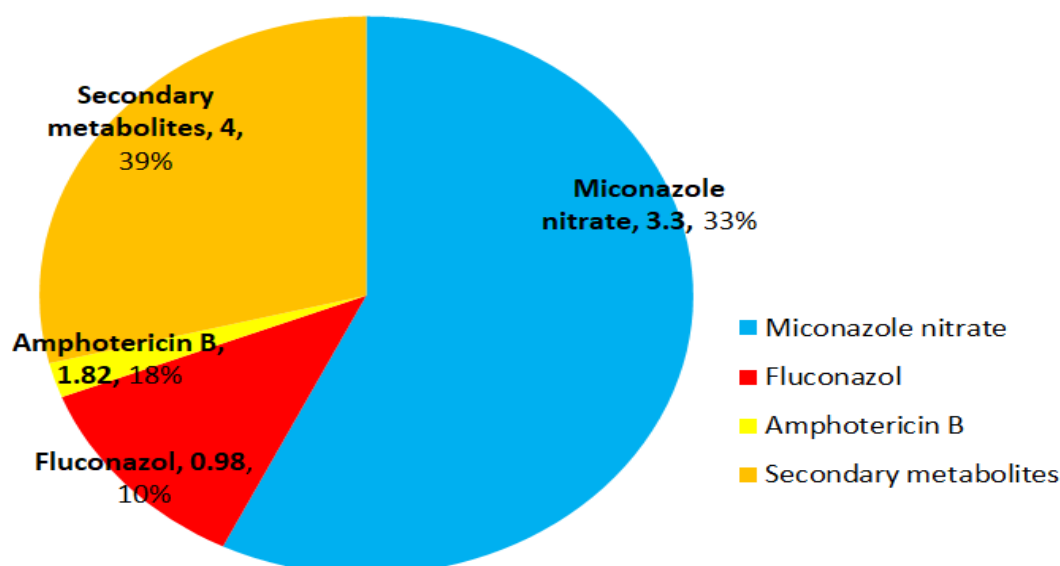


Figure 5. Metabolite products *R. graveolens* , Amphotericin B, Fluconazol and Miconazole nitrate as anti- Fungal activity against *C. albicans*

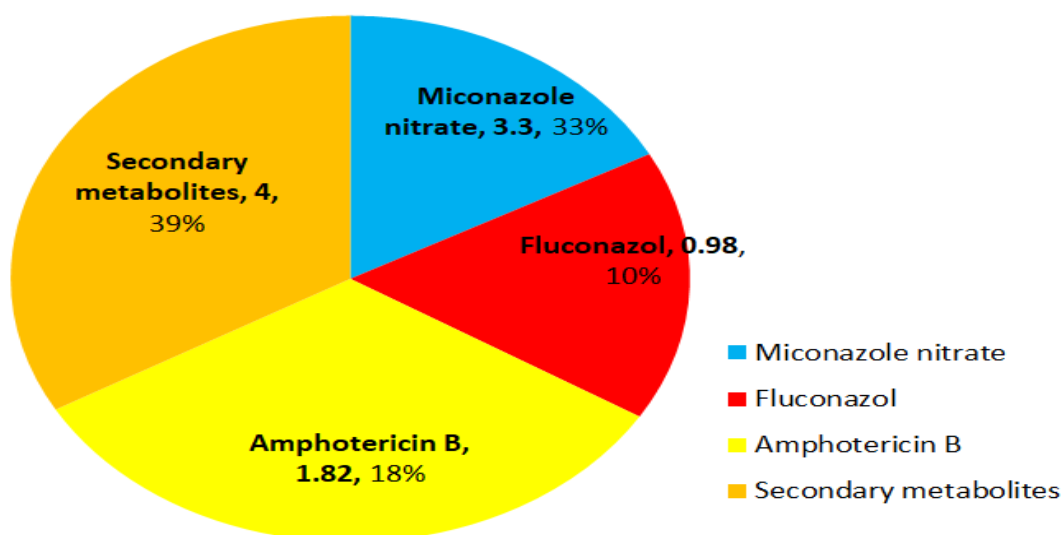


Figure 6. Metabolite products *R. graveolens* , Amphotericin B, Fluconazol and Miconazole nitrate as anti- Fungal activity against *T. viride*

Conclusion:

The results of this investigation demonstrated that the chemical components found in *R. graveolens* L. possess substantial antibacterial and antifungal activity capabilities. (FT-IR) research revealed the presence of thirteen different phytoconstituents. Our research has led us to believe that the essential oils obtained from the *Ruta graveolens* L. plant could be exploited as a natural source of therapeutic application, specifically for antibacterial and antifungal activity. However, additional research on other species in the Rutaceae family is strongly urged in order to

determine the possible anti-agent activities of those other species.

Compliance with ethical standards

Acknowledgments

In the course of our investigation, each and every protocol was authorized by the Department of Biology at the College of Science for Women at the University of Babylon in Hillah City, Iraq, and each and every approach was carried out in accordance with the accepted recommendations.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References:

1. H. Akkari et al. Chemical composition, insecticidal and in vitro anthelmintic activities of *Ruta chalepensis* (Rutaceae) essential oil Ind. Crop. Prod. (2015)
2. S. Al-Massarani et al. Assessment of selected Saudi and Yemeni plants for mosquitocidal activities against the yellow fever mosquito *Aedes aegypti* Saudi Pharmaceut. J. (2019)
3. A. Adsersen et al. Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity J. Ethnopharmacol. (2006)
4. S.M. Alotaibi et al. Phytochemical contents and biological evaluation of *Ruta chalepensis* L. growing in Saudi Arabia Saudi Pharmaceut. J. (2018)
5. N.S. Alzoreky et al. Antibacterial activity of extracts from some edible plants commonly consumed in Asia Int. J. Food Microbiol. (2003)
6. S. Arora. Anti-proliferative effects of homeopathic medicines on human kidney, colon and breast cancer cells Homeopathy (2013).
7. Z. Bennaoum et al. Chemical variability in essential oils from *Ruta* species among seasons, and its taxonomic and ecological significance. Nat. Prod. Res. (2017)
8. W. Bouabidi. Chemical composition, phytotoxic and antifungal properties of *Ruta chalepensis* L. essential oils. Nat. Prod. Res. (2015)
9. Kadhim MJ, Mohammed GJ, Hameed IH. In vitro antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. Oriental Journal of Chemistry. 32(2): 10-30.(2016)
10. 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. 8(8): 1293-1299.(2016)
11. 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. 7(4): 56-72.(2015)
12. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. Journal of Pharmacognosy and Phytotherapy. 8(2): 8-24.(2016)
13. Hameed IH, Ibraheam 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. 7 (6): 90-106.(2015)
14. 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. 32(2): 20-40.(2016)
15. N.S. Alzoreky et al. Antibacterial activity of extracts from some edible plants commonly consumed in Asia Int. J. Food Microbiol. (2003)
16. J. Mejri et al. Chemical composition of the essential oil of *Ruta chalepensis* L: influence of drying, hydro-distillation duration and plant parts Ind. Crops Prod. (2010).
17. A. Ivanova et al. Antimicrobial and cytotoxic activity of *Ruta graveolens* Fitoterapia (2005)
18. S.K. Raghav et al. Anti-Inflammatory effect of *Ruta graveolens* L. in murine macrophage cells J. Ethnopharmacol. (2006)