

Analysis of Methanolic Fruit Extract of *Citrus aurantifolia* Using Gas Chromatography – Mass Spectrum and FT-IR Techniques and Evaluation of Its Anti-bacterial Activity

Rafid Hadi Hameed¹, Ekhlas Al Shareefi², Imad Hadi Hameed³

¹Ministry of Public Health, Maysan Health Department, Mesan Governorate, Iraq, ²Department of Biology, College of Science for Women, University of Babylon, Hillah City, Iraq, ³Biomedical Science Department, University of Babylon, College of Nursing, Hillah City, Iraq

ABSTRACT

The objectives of this study were analysis of the secondary metabolite products and evaluation antibacterial and antifungal activity. Bioactives are chemical compounds often referred to as secondary metabolites. Twenty nine bioactive compounds were identified in the methanolic extract of *Citrus aurantifolia*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Citrus aurantifolia* revealed the existence of the Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6, Furfural, 2-Vinyl-9-[3-deoxy-β-d-ribofuranosyl] hypoxanthine, 2-Myristynoyl pantetheine, 2,5-Furandione, dihydro-3-methylene-, Cyclohexene,1-methyl-4-(1-methylethenyl)-,(S)-, O-Acetyl-4-hydroxyproline, 1,5,5-Trimethyl-6-methylene-cyclohexene, Acetic acid, 2-(1-buten-3-yl)-2-nitro-,ethyl ester, Methyl 3-hydroxytetradecanoate, L-α-Terpineol, 4-Methyl itaconate Glycyl-D-asparagine, 2(3H)-Benzofuranone, hexahydro-7a-methyl-, 7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,8)]decan-4-ol, Cholestan-3-ol, 2-methylene-,(3β,5α)-, Formic acid, 3,7,11,-trimethyl-1,6,10-dodecatrien-3-yl ester, 7-epi-cis-sesquisabinene hydrate, 2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl-2-(1-oxo-, Pyrrolidin-2-one-3β-(propanoic acid, methyl ester), 5-methylen, D-Fructose, diethyl mercaptal, pentaacetate, n-Hexadecanoic acid, 2H-1-Benzopyran-2-one, 5,7-dimethoxy-, Dihydroxanthin, Oleic acid, Octadecanoic acid, Phorbol, 9-Octadecenamide, (Z)- and 9-Octadecenamide. Clinical pathogens were selected for antibacterial activity namely, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, and *Pseudomonas eurogenosa*. *Citrus aurantifolia* has maximum zone against *Escherichia coli* 5.66±0.21.

Keywords: Antimicrobial activity, Bioactive compounds, Fruit, GC-MS, *Citrus aurantifolia*

INTRODUCTION

Fruit a globose to ovoid berry, 3-6 cm in diameter, sometimes with apical papillae, greenish-yellow; peel very thin, very densely glandular; segments with yellow-green pulp-vesicles, very acid, juicy and fragrant. Seeds small, plump, ovoid, pale, and smooth with white

embryos (polyembryonic). The fruit is used in nearly every home in the tropics, mainly to flavour food, but also to prepare drinks and for a variety of medicinal applications. The antifungal activity of the plant have been attributed to the presence of monoterpenes and the plant is currently used as a fungicide for citrus fruit crop, and it has also been suggested that the plant may be a potential candidate used for the protection of food and feeds from toxigenic fungal growth as well as their aflatoxin contamination¹⁻⁹. It has been traditionally used in the management of several diseases and has the prospects of being developed into useful drugs. Citrus fruits are highly recommended for persons suffering from kidney stones, gout and arthritis¹⁰⁻¹⁸. *C. aurantifolia* juice

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

contains potassium citrate which prevents the formation of kidney stones and eases their dissolution. Due to the high content of vitamin C, citrus fruits are used in the treatment of scurvy¹⁹⁻²³. The aims of our study were analysis of the metabolite products and determination of antimicrobial activity.

MATERIALS AND METHOD

Collection and Preparation of Plant Material

In this research, *Citrus aurantifolia* fruit was dried at room temperature for fifteen days and the fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature²³⁻³⁷.

Gas Chromatography-Mass Spectroscopy (GC-MS) and Fourier Transform Infrared Spectrophotometer (FTIR) analysis

GC-MS analysis of the ethanol extract of *Citrus aurantifolia* was carried out using a (Agilent 7890A series, USA)³⁸⁻⁴¹. The powdered sample of *Citrus aurantifolia* was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 nm and 4000 nm⁴²⁻⁴⁴.

Determination of antimicrobial activity of crude bioactive compounds of *Citrus aurantifolia*

Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control⁴⁵⁻⁴⁷.

Table 1. Major phytochemical compounds identified in methanolic extract of *Citrus aurantifolia*.

Serial No.	Phytochemical compound	RT (min)	Exact Mass
1.	Thieno[2,3-c]furan-3-carbonitrile ,2-amino-4,6-dihydro	3.196	222.0826845
2.	Furfural	3.465	96.021129
3.	2-Vinyl-9-[3-deoxy-β-d-ribofuranosyl]hypoxanthine	3.596	278.101505
4.	2-Myristynoyl pantetheine	3.751	484.297094
5.	2,5-Furandione , dihydro-3-methylene-	4.437	112.016044
6.	Cyclohexene,1-methyl-4-(1-methylethenyl)-,(S)-	4.649	136.1252
7.	O-Acetyl-4-hydroxyproline	4.861	173.068808
8.	1,5,5-Trimethyl-6-methylene-cyclohexene	4.969	136.1252
9.	Acetic acid , 2-(1-buten-3-yl)-2-nitro-,ethyl ester	5.336	187.084458
10.	Methyl 3-hydroxytetradecanoate	5.467	258.219496
11.	L-α-Terpineol	5.891	154.135765
12.	4-Methyl itaconate	6.263	144.042258
13.	Glycyl-D-asparagine	6.549	189.074956
14.	2(3H)-Benzofuranone , hexahydro-7a-methyl-	6.972	154.09938
15.	7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,8)]decan-4-ol	7.235	188.050715
16.	Cholestan-3-ol , 2-methylene-,(3β,5α)-	7.687	400.370516
17.	Formic acid , 3,7,11,-trimethyl-1,6,10-dodecatrien-3-yl ester	8.214	250.19328
18.	7-epi-cis-sesquisabinene hydrate	8.980	222.198365
19.	2,5-Cyclohexadien-1-one,3,5-dihydroxy-4,4-dimethyl-2-(1-oxo	11.275	238.120509
20.	Pyrrrolidin-2-one-3β-(propanoic acid, methyl ester), 5-methylen	11.252	311.173273
21.	D-Fructose, diethyl mercaptal , pentaacetate	11.224	496.14369
22.	n-Hexadecanoic acid	13.730	256.24023
23.	2H-1-Benzopyran-2-one , 5,7-dimethoxy-	14.102	206.057909
24.	Dihydroxanthin	14.393	308.162374
25.	Oleic acid	15.423	282.25588
26.	Octadecanoic acid	15.578	284.27153
27.	Phorbol	15.978	364.18859
28.	9-Octadecenamide , (Z)-	17.272	281.271864
29.	9-Octadecenamide	17.323	281.271864

Table 2. Fourier-transform infrared spectroscopic profile solid analysis of *Citrus aurantifolia*.

No.	Peak (Wave number cm ⁻¹)	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	661.5	58.395	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	688.5	58.541	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	873.7	73.620	Strong	=C–H	Bending	Alkenes	650-1000
4.	921.9	71.540	Strong	=C–H	Bending	Alkenes	650-1000
5.	1016.1	50.097	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1047.4	52.070	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1095.2	60.041	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1244.7	73.963	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1317.1	75.349	Strong	C-F	Stretch	alkyl halides	1000-1400
10.	1361.6	73.525	Strong	C-F	Stretch	alkyl halides	1000-1400
11.	1373.3	72.091	Strong	C-F	Stretch	alkyl halides	1000-1400
12.	1394.2	71.347	Strong	C-F	Stretch	alkyl halides	1000-1400
13.	1608.3	69.356	Bending	N-H	Stretch	Amide	1550-1640
14.	1645.9	71.054	Variable	C=C	Stretch	Alkene	1620-1680
15.	2335.2	82.034	Unknown	-	-	-	-
16.	2358.5	75.576	Unknown	-	-	-	-

Table 3. Zone of inhibition (mm) of test bacterial strains to *Citrus aurantifolia* bioactive compounds and standard antibiotics.

/ <i>Citrus aurantifolia</i> Antibiotics	Bacteria				
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Proteus mirabilis</i>	<i>Klebsiella pneumonia</i>	<i>Pseudomonas eurogenosa</i>
<i>Citrus aurantifolia</i>	4.97±0.20	5.66±0.21	3.98±0.19	3.98±0.20	3.99±0.19
Rifambin	1.09±0.21	1.05±0.19	0.99±0.18	1.03±0.19	1.95±0.18
Streptomycin	0.99±0.18	1.71±0.20	1.07±0.18	0.94±0.17	1.72±0.19
Kanamycin	0.43±0.16	1.00±0.18	1.94±0.14	0.77±0.14	1.63±0.18
Cefotaxime	2.06±0.19	2.05±0.19	1.06±0.15	1.18±0.19	1.03±0.16

Table 4. Zone of inhibition (mm) of fungal strains test to *Citrus aurantifolia* bioactive compounds and standard antibiotics.

/ Plant Antibiotics	Fungal strains			
	<i>Aspergillus niger</i>	<i>Penicillium expansum</i>	<i>Aspergillus flavus</i>	<i>Trichophyton mentagrophytes</i>
<i>Citrus aurantifolia</i>	2.860±0.16	5.000±0.22	6.160±0.24	4.972±0.21
Amphotericin B	2.771±0.14	3.931±0.21	3.951±0.21	3.813±0.19
Fluconazol	4.655±0.19	2.869±0.23	2.904±0.20	4.614±0.20
Control	0.00	0.00	0.00	0.00

RESULTS AND DISCUSSION

Chromatogram GC-MS analysis of the methanol extract of *Citrus aurantifolia* showed the presence of twenty nine major peaks and the components corresponding to the peaks were determined Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6, Furfural, 2-Vinyl-9-[3-deoxy- β -D-ribofuranosyl] hypoxanthine, 2-Myristinoyl pantetheine, 2,5-Furandione, dihydro-3-methylene-, Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-, O-Acetyl-4-hydroxyproline, 1,5,5-Trimethyl-1,6-methylene-cyclohexene, Acetic acid, 2-(1-buten-3-yl)-2-nitro-, ethyl ester, Methyl 3-hydroxytetradecanoate, L- α -Terpineol, 4-Methyl itaconate Glycyl-D- asparagine, 2(3H)-Benzofuranone, hexahydro-7a-methyl-, 7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,8)] decan-4-ol, Cholestan-3-ol, 2-methylene-, ($3\beta,5\alpha$)-, Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester, 7-epi-cis-sesquisabinene hydrate, 2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl-2-(1-oxo-, Pyrrolidin-2-one- 3β -(propanoic acid, methyl ester), 5-methylen, D-Fructose, diethyl mercaptal, pentaacetate, n-Hexadecanoic acid, 2H-1-Benzopyran-2-one, 5,7-dimethoxy-, Dihydroxanthin, Oleic acid, Octadecanoic acid, Phorbol, 9-Octadecenamide, (Z)- and 9-Octadecenamide **Table 1**. The FTIR analysis of *Citrus aurantifolia* leaves proved the presence of alkyl halides, Alkenes and Amide which shows major peaks at 661.5, 688.5, 873.7, 921.9, 1016.1, 1047.4, 1095.2, 1244.7, 1317.1, 1361.6, 1373.3, 1394.2, 1608.3, 1645.9, 2335.2 and 2358.5 **Table 2**. In the current study, the anti-microbial activity of *Citrus aurantifolia* methanolic extract was evaluated by determining the zone of inhibition against five bacteria and four fungi. Clinical pathogens were selected for antibacterial activity namely, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, and *Pseudomonas eurogenosa*. *Citrus aurantifolia* has maximum zone against *Escherichia coli* (5.66 \pm 0.21) **Table 3**. Antifungal activities against *Aspergillus niger*, *Penicillium expansum*, *Aspergillus flavus* and *Trichophyton mentagrophytes*. *Citrus aurantifolia* was very highly active against *Aspergillus flavus* (6.160 \pm 0.24) **Table 4**. In comparison to the antibiotics used in this study, the plants extracts were far more active against the test bacterial strains. However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *Citrus aurantifolia*

extracts looking toward a pharmaceutical use.

CONCLUSION

Twenty nine major chemical constituents have been identified from methanolic extract of the *Citrus aurantifolia* by gas chromatogram mass spectrometry (GC-MS). In vitro antimicrobial evaluation of *Citrus aurantifolia* forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Biology, College of Science FOR Women, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

REFERENCES

1. Mohammed GJ, Kadhim MJ, Hameed IH. Proteus species: Characterization and herbal antibacterial: A review. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(11): 1844-1854.
2. Huda JA, Hameed IH, Hamza LF. Anethum graveolens: Physicochemical properties, medicinal uses, antimicrobial effects, antioxidant effect, anti-inflammatory and analgesic effects: A review. International Journal of Pharmaceutical Quality Assurance. 2017; 8(3): 88-91.
3. 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.
4. Ibraheam 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.
5. Ibraheam 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.
6. Hadi MY, Hameed IH, Ibraheam IA. *Ceratonia siliqua*: Characterization, Pharmaceutical Products and Analysis of Bioactive Compounds: A Review. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3585-3589.
 7. Hadi MY, Hameed IH, Ibraheam 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.
 8. 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
 9. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(3): 49-59.
 10. Altameme HJ, Hadi MY, Hameed IH. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(10): 238-252.
 11. 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.
 12. 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.
 13. 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.
 14. 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*. 2015; 7 (6): 90-106.
 15. 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.
 16. 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.
 17. 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.
 18. 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.
 19. 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.
 20. 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.
 21. Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy*. 2015;7(8): 132-163.
 22. Hameed IH, Hussein HJ, Kareem MA, Hamad NS. Identification of five newly described bioactive

- chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7 (7): 107-125.
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. Altameme HJ, Hameed IH, Idan SA, Hadi MY. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(9): 221-237.
 26. 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.
 27. 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.
 28. 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.
 29. 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.
 30. 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.
 31. 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.
 32. Mekhlief 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.
 33. Kadhim MJ. In Vitro antifungal potential of *Acinetobacter baumannii* and determination of its chemical composition by gas chromatography-mass spectrometry. *Der Pharma Chemica*. 2016; 8(19): 657-665.
 34. 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.
 35. Kadhim MJ, Kaizal 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.
 36. 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.
 37. 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.
 38. 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.
 39. 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.
 40. 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.
41. 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.
42. 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.
43. 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.
44. 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.
45. 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.
46. 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.
47. 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.
48. Kamal SA, Hamza LF, Ibraheam 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.

