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# Determination of Anti-microbial Activity and Characterization of Metabolites Produced by *Neisseria gonorrhea*

**Rafid Hadi Hameed[1], Ekhlas Al-Shareefi[2], Imad Hadi Hameed[3]**

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

## ABSTRACT

*Neisseria gonorrhoeae*, also known as gonococci, or gonococcus, is a species of gram-negative coffee bean-shaped diplococci bacteria responsible for the sexually transmitted infection gonorrhea. The objectives of this study were analysis of the secondary metabolite products and evaluation antimicrobial activity. Thirty nine bioactive compounds were identified in the methanolic extract of *Neisseria gonorrhea*. GC-MS analysis of *Neisseria gonorrhea* revealed the existence of the Butanoic acid , 2-methyl, Propane , 2-methoxy, Hexanoic acid, 2-methyl, 1-Propaneamine, 3-(methylthio), 7-Methylenebicyclo[3.2.0]hept-3-en-2-one, 1,2-Benzisothiazol-3-amine tbdms, Propanoic acid, Benzeneethanamine , N-methyl, Phenelzine, Hexanediamide, N,N'-di-benzoyloxy, Benzeneacetic acid, Silane, methylenebis, Ethanamine, N-ethyl- N-[(1-methylethoxy)methyl], 2-Pentanamine, Butanal, 4-hydroxy-3-methyl, o-Allylhydroxylamine, 2-Butanamine, 3-methyl, Hexanal , 2-chloro, Hexanoic acid, 4-octyl ester, Pyrrolidine , 2,5-dimethyl-1-nitroso, 3-Pentanone , dimethylhydrazone, 1-Pentanol, 2-ethyl, 3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, Pyrrolo[1.2-a]pyrazine-1,4-dione,hexahydro. *Nerium olender* (Alkaloids) was very highly active 6.800±0.24 mm. The results of anti-fungal activity produced by *Neisseria gonorrhea* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus flavus* (6.800±0.24).

***Keywords*:** Anti-Microbial, *Neisseria gonorrhea*, GC-MS, Secondary metabolites.

## INTRODUCTION

Neisseria species are fastidious gram-negative cocci that require nutrient supplementation to grow inlaboratory cultures[1]. To be specific, they grow on chocolate agar with carbon dioxide. Symptoms of infection with *N. gonorrhoeae* differ, depending on the site of infection. Note also that 10% of infected males and 80% of infected females are asymptomatic. Men who have had a gonorrhea infection have a significantly increased risk of having prostate cancer. Specific culture of a swab from the site of infection is a criterion standard for diagnosis at all potential sites of gonococcal infection. Cultures are particularly useful when the clinical diagnosis is unclear, when a failure of treatment has occurred, when contact tracing is problematic, and when legal questions arise [2]. Recently, a high-level ceftriaxone-resistant strain of gonorrhea, called H041, was discovered in Japan. Lab tests found it to be resistant to high concentrations of ceftriaxone, as well as most of the other antibiotics tested. Within *N. gonorrhoeae*, there are genes that confer resistance to every single antibiotic used to cure gonorrhea, but thus far they do not coexist within a single gonococcus[3]. Because of N. gonorrhoeae's high affinity for horizontal gene transfer, however, antibiotic-resistant gonorrhea is seen as an emerging public health threat. Transmission can be reduced by the usage of latex barriers, such as condoms or dental dams, during intercourse, oral and anal sex, and by limiting sexual partners. Due to the relative frequency of infection and the emerging development of antibiotic resistance in

**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

strains of *N. gonorrhoeae*, vaccines are thought to be an important goal in the prevention of infection[4].

## MATERIALS AND METHOD

### Detection of secondary metabolites

Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45ºC. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for gas chromatography mass spectrometry [5-11].

### Gas chromatography – Mass Spectrum analysis

*Neisseria gonorrhea* GC–MS analysis were carried out in a GC system (Agilent 7890A series, USA). The flow rate of the carrier gas, helium (He) was set to beat 1 mL min−1, split ratio was 1:50. The injector temperature was adjusted at 250◦C, while the detector temperature was fixed to280◦C [12-19]. The column temperature was kept at 40◦C for 1 min followed by linear programming to raise the temperature from 40◦to 120◦C (at 4 Cº min−1with 2 min hold time), 120 Cº to 170 Cº (at 6 Cº min−1with 1 min hold time) and 170 Cº to 200 Cº (at10◦C min−1with 1 min hold time). The transfer line was heated at 280 Cº. Two microliter of FAME sample was injected for analysis. The components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library [20-26].

### Determination of antibacterial and antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions *Nerium olender* (Alkaloids), *Ricinus communis* (Alkaloids), *Datura stramonium* (Alkaloids), *Linum usitatissimum* (Crude), *Cassia angustifolia* (Crude), *Euphorbia lathyrus* (Crude), *Citrullus colocynthis* (Crude), *Althaea rosea* (Crude), *Coriandrum sativum* (Crude), *Origanum vulgare* (Crude), *Urtica dioica* (Crude), *Foeniculum vulgare* (Crude), and *Punica granatum* (Crude) were delivered into the wells. The plates were incubated for 48 h at room temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms [27-32]. The studied fungi, *Microsporum canis*, *Streptococcus faecalis*, *Aspergillus flavus*, *Penicillium expansum*, *Trichoderma viride*, *Trichoderma horzianum*, *Aspergillus niger* and *Aspergillus terreus* were isolated and maintained in potato dextrose agar slants. Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for 16 days at 130 rpm. The extraction was performed by adding 25 ml methanol to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture. 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 [33-37]. All the measurements were replicated three times for each assay and the results are presented as mean ± SD and mean ± SE. IBM SPSS 20 version statistical software package was used for statistical analysis of percentage inhibition and disease incidence and disease severity in each case.

**Table 1. Major chemical compounds identified in methanolic extract of *Neisseria gonorrhoeae*.**

| Serial No. | Phytochemical compound | RT (min) | Molecular Weight |
|---|---|---|---|
| 1. | Butanoic acid , 2-methyl- | 3.173 | 102.06807 |
| 2. | Propane , 2-methoxy- | 3.367 | 74.073165 |
| 3. | Hexanoic acid , 2-methyl- | 3.533 | 130.09938 |
| 4. | 1-Propaneamine , 3-(methylthio) | 4.134 | 105.06122 |
| 5. | 7-Methylenebicyclo[3.2.0]hept-3-en-2-one | 3.808 | 120.057514 |
| 6. | 1,2-Benzisothiazol-3-amine tbdms | 4.517 | 264.11164 |
| 7. | Propanoic acid | 4.912 | 74.0367794 |
| 8. | Benzeneethanamine , N-methyl | 5.324 | 135.104799 |
| 9. | Phenelzine | 5.364 | 136.100048 |
| 10. | Hexanediamide , N,N’-di-benzoyloxy | 5.845 | 384.132137 |
| 11. | Benzeneacetic acid | 6.777 | 136.052429 |

***Cont...*** **Table 1. Major chemical compounds identified in methanolic extract of *Neisseria gonorrhoeae*.**

| | | | |
|---|---|---|---|
| 12. | Silane , methylenebis | 7.315 | 76.0164533 |
| 13. | Ethanamine , N-ethyl-N-[(1-methylethoxy)methyl] | 7.922 | 145.146665 |
| 14. | 2-Pentanamine | 8.013 | 87.1047993 |
| 15. | Butanal , 4-hydroxy-3-methyl- | 7.819 | 102.068079 |
| 16. | o-Allylhydroxylamine | 9.072 | 73.052764 |
| 17. | 2-Butanamine , 3-methyl | 8.357 | 87.1047993 |
| 18. | Hexanal , 2-chloro- | 9.032 | 134.049843 |
| 19. | Hexanoic acid , 4-octyl ester | 10.399 | 228.20893 |
| 20. | Pyrrolidine , 2,5-dimethyl-1-nitroso- | 11.550 | 128.094963 |
| 21. | 3-Pentanone , dimethylhydrazone | 11.527 | 128.131349 |
| 22. | 1-Pentanol , 2-ethyl- | 11.979 | 116.120115 |
| 23. | 3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione , | 12.196 | 210.100442 |
| 24. | Pyrrolo[1.2-a]pyrazine-1,4-dione,hexahydro- | 12.848 | 154.074227 |
| 25. | 2-Undecene , (Z) | 13.323 | 154.172151 |
| 26. | 3,5,5-Trimethylhexyl acetate | 13.432 | 186.16198 |
| 27. | Pentanedinitrile , 2-methyl- | 13.936 | 108.068748 |
| 28. | Decanoic acid , 2-methyl- | 14.302 | 186.16198 |
| 29. | trans-2,3-Epoxynonane | 14.931 | 142.135765 |
| 30. | Cyclopentane , butyl | 15.973 | 126.140850 |
| 31. | Oleic acid | 16.339 | 282.25588 |
| 32. | Octadecanoic acid | 16.533 | 284.27153 |
| 33. | DL-Leucine , N-DL-leucyl | 17.077 | 244.178693 |
| 34. | 5-Chlorovaleric acid ,morpholide | 17.357 | 205.086956 |
| 35. | 2-Propanamine,2-methyl-N2-[1-tetrahydro-1h-1-pyr | 17.380 | 154.146998 |
| 36. | Fumaric acid , 3-heptyl propyl ester | 20.504 | 256.16746 |
| 37. | Benzene ,[1-(methoxymethoxy)-2-propynyl] | 21.414 | 176.08373 |
| 38. | Benzenepropanoic acid , 3,5-bis(1,1-dimethyethyl) | 24.802 | 530.469894 |
| 39. | Propanoic acid, 2,2-dimethyl-,2,6-bis(1methylethyl | 25.036 | 262.19328 |

**Table 2.** Antifungal activity of *Neisseria gonorrhoeae* metabolite products.

| **Fungi** | **Antibiotics /** *Neisseria gonorrhoeae* **metabolite products** | | | |
|---|---|---|---|---|
| | *Neisseria gonorrhoeae* **metabolite products** | **Amphotericin B** | **Fluconazol** | **Miconazole nitrate** |
| ***Microsporum canis*** | 2.906±0.18 [a] | 2.006±0.10 | 3.014±0.12 | 2.881±0.19 |
| ***Streptococcus faecalis*** | 2.900±0.19 | 3.013±0.14 | 2.881±0.13 | 1.794±0.11 |
| ***Aspergillus flavus*** | 6.007±0.22 | 3.006±0.14 | 3.792±0.17 | 2.995±0.16 |
| ***Penicillium expansum*** | 4.009±0.16 | 3.025±0.18 | 2.839±0.13 | 1.892±0.15 |
| ***Trichoderma viride*** | 4.939±0.20 | 1.974±0.11 | 2.005±0.12 | 3.485±0.19 |
| ***Trichoderma horzianum*** | 3.992±0.20 | 1.001±0.02 | 4.001±0.19 | 2.992±0.16 |
| ***Aspergillus niger*** | 5.011±0.19 | 1.968±0.12 | 3.751±0.17 | 2.016±0.15 |
| ***Aspergillus terreus*** | 4.980±0.20 | 3.061±0.15 | 2.957±0.18 | 3.079±0.18 |

[a] The values ( average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract and 30 µg/mL of (Amphotericin B; Fluconazol and Miconazole nitrate).

**Table 3. Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of medicinal plants to *Neisseria gonorrhoeae*.**

| S. No. | Plants | Zone of inhibition (mm) |
|---|---|---|
| **1.** | *Nerium olender* (Alkaloids) | 6.800±0.24 |
| **2.** | *Ricinus communis* (Alkaloids) | 3.904±0.18 |
| **3.** | *Datura stramonium*(Alkaloids) | 4.005±0.19 |
| **4.** | *Linum usitatissimum* (Crude) | 4.891±0.20 |
| **5.** | *Cassia angustifolia* (Crude) | 5.407±0.23 |
| **6.** | *Euphorbia lathyrus* (Crude) | 5.966±0.23 |
| **7.** | *Citrullus colocynthis* (Crude) | 3.893±0.17 |
| **8.** | *Althaea rosea* (Crude) | 5.933±0.22 |
| **9.** | *Coriandrum sativum* (Crude) | 6.004±0.23 |
| **10.** | *Origanum vulgare* (Crude) | 6.010±0.23 |
| **11.** | *Urtica dioica* (Crude) | 3.881±0.20 |
| **12.** | *Foeniculum vulgare* (Crude) | 3.005±0.19 |
| **13.** | *Punica granatum* (Crude) | 6.015±0.23 |
| **14.** | Control | 0.00 |

## RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Neisseria gonorrhea*, shown in **Table 1.** The GC-MS chromatogram of the twenty nine peaks of the compounds detected were determined to be Butanoic acid , 2-methyl, Propane , 2-methoxy, Hexanoic acid , 2-methyl, 1-Propaneamine , 3-(methylthio), 7-Methylenebicyclo[3.2.0]hept-3-en- 2-one, 1,2-Benzisothiazol-3-amine tbdms, Propanoic acid, Benzeneethanamine , N-methyl, Phenelzine, Hexanediamide , N,N'-di-benzoyloxy, Benzeneacetic acid, Silane , methylenebis, Ethanamine , N-ethyl-N-[(1-methylethoxy)methyl], 2-Pentanamine, Butanal , 4-hydroxy-3-methyl, o-Allylhydroxylamine, 2-Butanamine , 3-methyl, Hexanal , 2-chloro, Hexanoic acid , 4-octyl ester, Pyrrolidine , 2,5-dimethyl-1-nitroso, 3-Pentanone , dimethylhydrazone, 1-Pentanol , 2-ethyl, 3-Methyl-1,4-diazabicyclo[4.3.0]nonan-2,5-dione, Pyrrolo[1.2-a]pyrazine-1,4-dione,hexahydro, 2-Undecene , (Z), 3,5,5-Trimethylhexyl acetate, Pentanedinitrile , 2-methyl, Decanoic acid , 2-methyl, trans-2,3-Epoxynonane, Cyclopentane , butyl, Oleic acid, Octadecanoic acid, L-Leucine, N-DL-leucyl, 5-Chlorovaleric acid ,morpholide, 2-Propanamine,2-methyl-N2-[1-tetrahydro-1h-1-pyr, Fumaric acid , 3-heptyl propyl ester, Benzene ,[1-(methoxymethoxy)- 2-propynyl], Benzenepropanoic acid, 3,5-bis (1,1-dimethyethyl), Propanoic acid, 2,2-dimethyl-,2,6-bis(1methylethyl. The results of anti-fungal activity produced by *Neisseria gonorrhea* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus flavus*. *Neisseria gonorrhea* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Neisseria gonorrhea* can be useful. Maximum zone formation against *Aspergillus flavus* (6.007±0.22) mm, **Table 2**. In agar well diffusion method the selected medicinal plants *Nerium olender* (Alkaloids), *Ricinus communis* (Alkaloids), *Datura stramonium*(Alkaloids), *Linum usitatissimum* (Crude), *Cassia angustifolia* (Crude), *Euphorbia lathyrus* (Crude), *Citrullus colocynthis* (Crude), *Althaea rosea* (Crude), *Coriandrum sativum* (Crude), *Origanum vulgare* (Crude), *Urtica dioica* (Crude), *Foeniculum vulgare* (Crude), and *Punica granatum* (Crude) were effective against *Staphylococcus aureus*, **Table 3**. *Nerium olender* (Alkaloids) was very highly active (6.800±0.24) mm against *Neisseria gonorrhea*.

## CONCLUSION

Thirty nine bioactive chemical constituents have been identified from methanolic extract of the *Neisseria gonorrhea* by gas chromatogram mass spectrometry (GC-MS). In vitro antifungal evaluation of secondary metabolite products of *Neisseria gonorrhea* forms a primary platform for further chemical and pharmacological investigation for the development of new potential antimicrobial compounds. *Neisseria gonorrhea* produce many important secondary metabolites with high biological activities.

**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. Detels R, Green M, Klausner Jeffrey D. The Incidence and Correlates of Symptomatic and Asymptomatic Chlamydia trachomatis and Neisseria gonorrhoeae Infections in Selected Populations in Five Countries. Sex. Transm. Dis. 2011; 38(6): 503–509.
2. Caini S, Gandini S, Dudas M, Bremer V, Severi E, Gherasim A. Sexually transmitted infections and prostate cancer risk: A systematic review and meta-analysis. Cancer Epidemiology. 2014; 38(4): 329–338.
3. Anderson, M, Dewenter L, Maier B, Seifert H. Seminal Plasma Initiates a Neisseria gonorrhoeae Transmission State. MBio. 2014; 5(2): e01004–13.
4. Simons M, Nauseef W, Apicella M. Interactions of Neisseria gonorrhoeae with Adherent Polymorphonuclear Leukocytes. Infection and Immunity. 2005; 73(4): 1971–7
5. Michod E, Bernstein H, Nedelcu M. Adaptive value of sex in microbial pathogens. Infection, Genetics and Evolution. 2008; 8(3): 267–85
6. Shireen SK, Hameed IH, Hamza LF. Acorus calamus: Parts used, insecticidal, anti-fungal, antitumour and anti-inflammatory activity: A review. International Journal of Pharmaceutical Quality Assurance. 2017; 8(3): 153-157.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. 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.
13. 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.
14. 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.
15. 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.
16. 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.
17. Hameed IH, Ibraheam IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-

transform infrared spectroscopy analysis of methanolic extract of Rosmarinus oficinalis leaves. Journal of Pharmacognosy and Phytotherapy. 2015; 7 (6): 90-106.

18. 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.

19. Mohammed GJ, Al-Jassani MJ, Hameed IH. Anti-bacterial, 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.

20. 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.

21. Altameme HJ, Hameed IH, Abu-Serag NA. Analysis of bioactive phytochemical compounds of two medicinal plants, Equisetum arvense and Alchemila valgaris seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. Malays. Appl. Biol. 2015; 44(4): 47–58.

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 chemicalanalysis 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. 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.

31. 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.

32. 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.

33. 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.

34. 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.

35. 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.

36. 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.

37. 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.