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Research Article

Analysis of Bioactive Compounds of Tribolium Castaneum and Evaluation of Anti-Bacterial Activity

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ABSTRACT

The aims of this research were analysis of methanolic extract of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity against *Streptococcus pneumonia*, *Pseudomonas eurogenosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Klebsiella pneumonia*. GC-MS analysis of *Tribolium castaneum* revealed the existence of the Oxirane, [(hexadecyloxy)methyl], 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione, Cis,cis,cis-7,10,13-hexadecatrienal, Methyl N-cyclohexyl-3-phenylpropanimidate, Ethyl(1-adamantylamino)carbothioylcarbamate, (-)-Norephedine, 1-Dimethyl(pentafluorophenyl)silyloxycyclopentane, 2-Cyclopropyl-2-nitro-1-phenyl-ethanol, 3-Chloropropionic acid ,nonyl ester, Decane, 1-chloro, 2,5-Dimethylhexane-2,5-dihydroperoxide, Ethanamine, 2-(2,6-dimethylphenoxy)-N-methyl, D-(+)-Ribonic acidγ-lactone, Methanone, 1,3-dithian-2-ylphenyl, Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene, Phenylethyl alcohol, 2,5-dihydroxy-α-methyl, 1-Hexadecanol, 3,6-Diazahomoadamantan-9-one Hydrazone, 3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-α, Spiro[5.5]undecane-1,7-dione, 4-Cyclopropylcarbonyloxytridecane, Eicosanoic acid, phenylmethyl ester, 3,6-Dinitro-4-cyclohexene-1,2-dicarboxylic acid, 1-Propyl-3,6-diazahomoadamantan-9-ol, 2(1H)-Benzocyclooctenone , decahydro-4a-methyl-,trans, Heptanoic acid, 4,5-dimethoxy-2-nitrobenzyl ester, Phthalic acid, butyl undecyl ester, Pterin-6-carboxylic acid, 2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy, Cis-9-hexadecenoic acid, 9,12-Octadecadienoic acid (Z,Z), Octadec-9-enoic acid, Methyl 18-fluoro-octadec-9-enoate, Dasycarpidan-1-methanol, acetate(ester), 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'tetra. The results of anti-bacterial activity produced by *Tribolium castaneum* showed that the volatile compounds were highly effective to suppress the growth of *Escherichia coli*.

Keywords: *Tribolium castaneum*, Anti-bacterial activity, GC/MS, Bioactive compounds.

INTRODUCTION

The red flour beetle (*Tribolium castaneum*) is a species of beetle in the Tenebrionidae, is considered as a major pest of stored grains[1]. It is a worldwide pest of stored products, particularly food grains, and a model organism for ethological and food safety research. Polygamy in red flour beetles is a behavior common to both males and females of this species. Multiple mating events can ensure that females obtain a greater net amount of sperm, resulting in an increased likelihood of successful fertilization[2]. The red flour beetle attacks stored grain and other food products including flour, cereals, pasta, biscuits, beans, and nuts, causing loss and damage. Female red flour beetles are polyandrous in mating behavior. Within a single copulation period, a single female will mate with multiple different males[4]. In red flour beetles, females that engage in polygamous behavior produce more offspring than those that are less polygamous. Polygamy is mostly seen in populations that lack genetic diversity[5]. Female red flour beetles engage in polyandrous mating behavior in order to increase their fertility assurance. By mating with an increased number of males, female beetles obtain a greater amount of sperm. Obtaining a greater amount of sperm is especially important since many sexually active male red flour beetles are non-virgins and may be sperm-depleted[6]. It is important to note that red flour beetles engage in polyandry to obtain a greater amount of sperm from males, not to increase the likelihood of finding genetically compatible sperm. Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides[7,8], toxic residues on stored grain, toxicity to consumers and increasing costs of application. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides[9,10].

MATERIALS AND METHODS

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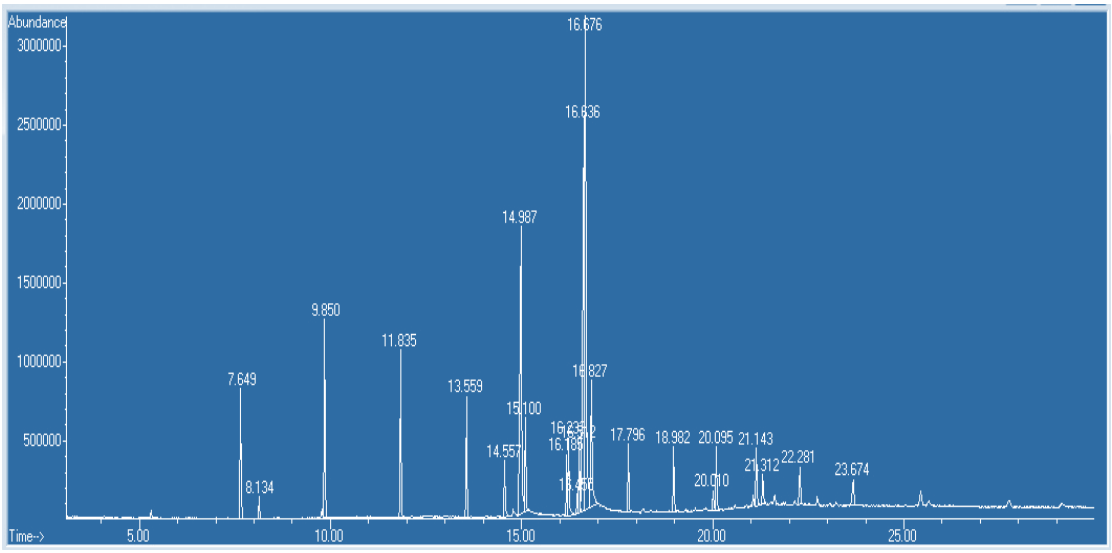


Figure 1: GC-MS chromatogram of methanolic extract of *Tribolium castaneum*

Insect

Tribolium castaneum was obtained from laboratory cultures maintained in the dark in incubators at 26 ± 1°C. This insect was reared on wheat flour mixed with yeast (10:1, w: w).

Determination of antibacterial activity

The test pathogens (*Streptococcus pneumonia*, *Pseudomonas eurogenosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Klebsiella pneumonia*) were swabbed in Muller-Hinton agar plates[11-16]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control[17].

Gas chromatography – Mass Spectrum analysis

Interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The database consists of more than 62,000 patterns of known compounds. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library. *Tribolium castaneum* 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 [18-23]. The column temperature was kept at 40◦C for 1 min fol-lowed 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. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z[24-32].

Statistical analysis

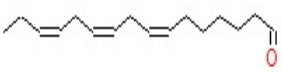
Results of the study were based on analysis of variance (ANOVA) using Statistica Software. A significance level of 0.05 was used for all statistical tests.

RESULTS AND DISCUSSION

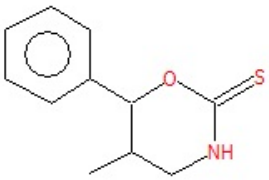
Identification of biochemical compounds

Analysis of compounds was carried out in methanolic extract of *Tribolium castaneum*, shown in Table 1. The GC-MS chromatogram of the peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Tribolium castaneum* showed the presence of thirty-one major peaks and the components corresponding to the peaks were determined as follows. All peaks were determined to be Oxirane, [(hexadecyloxy)methyl], 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione, Cis,cis,cis-7,10,13-hexadecatrienal, Methyl N-cyclohexyl-3-phenylpropanimidate, Ethyl(1-adamantylamino) carbothioylcarbamate, (-)-Norephedine, 1-Dimethyl(pentafluorophenyl) silyloxycyclopentane, 2-Cyclopropyl-2-nitro-1-phenyl-ethanol, 3-Chloropropionic acid ,nonyl ester, Decane,1-chloro, 2,5-Dimethylhexane - 2,5-dihydroperoxide, Ethanamine, 2-(2,6-dimethylphenoxy)-N-methyl, D-(+)-Ribonic acidγ-lactone, Methanone, 1,3-dithian-2-ylphenyl, Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene, Phenylethyl alcohol, 2,5-dihydroxy-α-methyl, 1-Hexadecanol, 3,6-Diazahomoadamantan-9-one Hydrazone, 3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-α, Spiro[5.5]undecane-1,7-dione, 4-Cyclopropylcarbonyloxytridecane, Eicosanoic acid, phenylmethyl ester, 3,6-Dinitro-4-cyclohexene-1,2-dicarboxylic acid, 1-Propyl-3,6-diazahomoadamantan-9-ol, 2(1H)-Benzocyclooctenone , decahydro-4a-methyl-,trans, Heptanoic acid , ,4,5-dimethoxy-2-nitrobenzyl ester, Phthalic acid, butyl undecyl ester, Pterin-6-carboxylic acid, 2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy, Cis-9-hexadecenoic acid, 9,12-Octadecadienoic acid (Z,Z), Octadec-9-enoic acid, Methyl 18-fluoro-octadec-9-enoate, Dasycarpidan-1-methanol, acetate(ester), 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'tetra. Solvent extract was found to exhibit significant inhibitory effects on the fungi

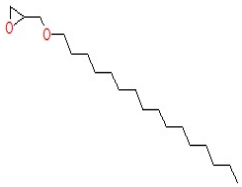
Table 1: Bioactive chemical compounds identified in methanolic extract of *Tribolium castaneum*



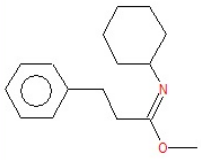
Cis,cis, cis-7,10,13 hexadecatrienal RT=4.878 MW=234.198365



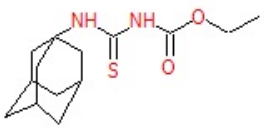
5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione RT= 4.271 Mw= 207.071785



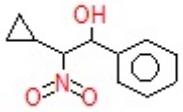
Oxirane [(hexadecyloxy) methyl] RT= 3.367 Mw=298.28718



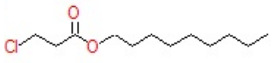
Methyl N-cyclohexyl-3-phenylpropanimidate RT=5.862 MW=245.177964



Ethyl(1-adamantylamino) carbothioylcarbamateRT=6 .326 MW=282.1402

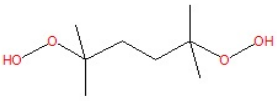


2-Cyclopropyl-2-nitro-1-phenyl-ethanol RT=7.853 MW=207.089543

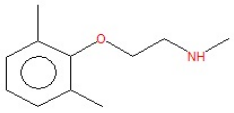


3-Chloropropionic acid, nonyl ester RT=7.968 MW=234.138658

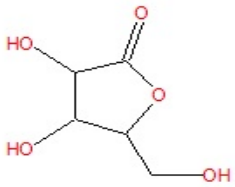
Decane ,1-chloro- RT=8.054 MW=176.133179



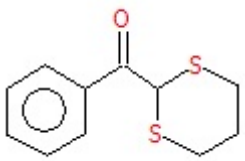
2,5-Dimethylhexane -2,5 dihydroperoxide RT=8.122 MW=178.120509



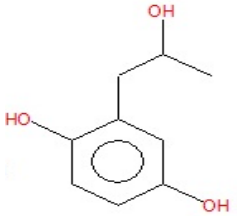
Ethanamine, 2-(2,6-dimethylphenoxy)-N-methyl-RT=8.225 MW=179.131014



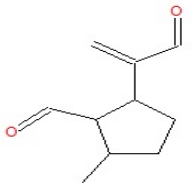
D- (+)-Ribonic acidγ-lactone RT=8.466 MW=148.037173



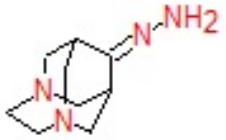
Methanone, 1,3-dithian-2-ylphenyl- RT=9.158 MW=224.032957



Phenylethyl alcohol, 2,5-dihydroxy-α-methyl-, (-)- RT=9.696 MW=168.078644

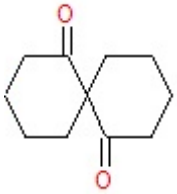


Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene- RT=9.593 MW=166.09938

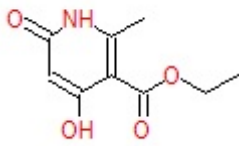


3,6-Diazahomoadamantan-9-one Hydrazone Rt=10.016 MW=180.137497

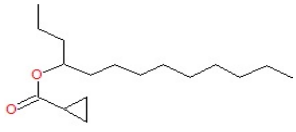
1-Hexadecanol Rt=9.770 Mw=242.260965



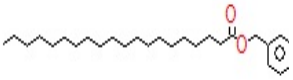
Spiro [5.5] undecane-1,7-dione RT=11.750 MW=180.115029



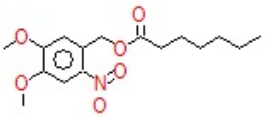
3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-ethyl-6-α RT=11.464 MW=197.068808



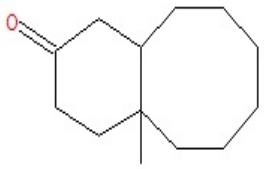
4 Cyclopropylcarbonyloxytridecane TR=12.093 MW=268.24023



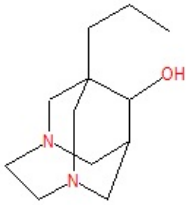
Eicosanoic acid, phenylmethyl ester RT=12.534 MW=402.349781



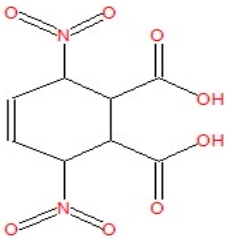
Heptanoic acid,4,5-dimethoxy-2-nitrobenzyl ester RT=13.770 MW=



2(1H)-Benzocyclooctenone, decahydro-4a-methyl-, trans-(-)- RT=13.678 MW=194.167066

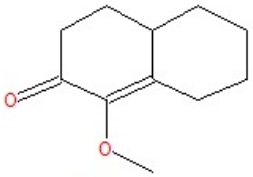


1-Propyl-3,6-diazahomoadamantan-9-ol RT=13.192 MW=210.173213

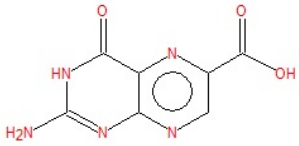


3,6-Dinitro-4-cyclohexene-1,2-dicarboxylic acid RT=12.831 MW=260.028065

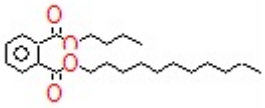
Cis-9-hexadecenoic acid RT=14.800 MW=254.22458



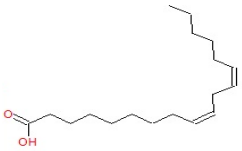
2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy- RT=14.468 MW=180.115029



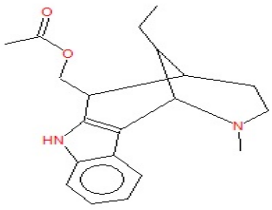
Pterin-6-carboxylic acid RT=14.188 MW=207.039239



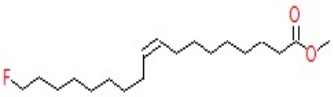
Phthalic acid, butyl undecyl ester RT=14.062 MW=376.26136



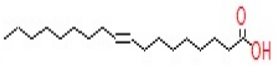
9,12-Octadecadienoic acid (Z, Z)- RT=17.558 MW=280.24023



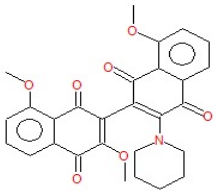
Dasycarpidan-1-methanol, acetate(ester) RT=19.572 MW=326.199429



Methyl 18-fluoro-octadec-9-enoate RT=18.439 MW=314.262108



Octadec-9-enoic acid RT=17.884 MW=282.25588



3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'tetra RT=20.001 MW=487.163101

($p < 0.05$). The results of anti-bacterial activity produced by *Tribolium castaneum* showed that the volatile compounds were the largest zone inhibition in *Escherichia coli* (5.09±0.22 mm), *Streptococcus pneumonia* (4.03±0.10 mm), *Pseudomonas eurogenosa* (5.00±0.20 mm), *Staphylococcus epidermidis* (4.07±0.18 mm), *Proteus mirabilis* (5.00±0.20 mm), *Streptococcus pyogenes* (3.07±0.10 mm), *Staphylococcus aureus* (2.52±0.12 mm), *Streptococcus faecalis* (3.09±0.20 mm) and *Klebsiella pneumonia* (4.95±0.20 mm). *Tribolium castaneum* 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

Table 2: Zone of inhibition (mm) of test bacterial strains to Tribolium castaneum bioactive compounds and standard antibiotics.

Bacteria	Antibiotics /(Tribolium castaneum) products				
	Metabolites of insect	Cefotoxime	Kanamycin	Rifambin	Streptomycin
Streptococcus pneumonia	4.03±0.10	2.00±0.10	1.76±0.21	1.27±0.19	3.00±0.25
Pseudomonas eurogenosa	5.00±0.20	1.99±0.10	2.43±0.21	1.60±0.20	1.00±0.09
Staphylococcus epidermidis	4.07±0.18	1.72±0.11	0.98±0.10	1.98±0.20	1.69±0.22
Escherichia coli	5.09±0.22	1.00±0.10	1.09±0.20	0.05±0.03	2.13±0.11
Proteus mirabilis	5.00±0.20	2.95±0.21	1.99±0.20	1.72±0.18	1.94±0.20
Streptococcus pyogenes	3.07±0.10	1.88±0.20	2.02±0.21	1.00±0.09	2.94±0.26
Staphylococcus aureus	2.52±0.12	1.01±0.10	0.58±0.09	2.86±0.21	1.00±0.09
Streptococcus faecalis	3.09±0.20	2.07±0.22	0.76±0.09	1.01±0.10	1.99±0.25
Klebsiella pneumonia	4.95±0.20	2.04±0.20	1.94±0.20	1.99±0.14	2.36±0.23

diseases, the purification of compounds produced by *Tribolium castaneum* can be useful. Maximum zone formation against *Escherichia coli*, Table 2. It may cause an allergic response, but is not known to spread disease or cause damage to structures or furniture[33]. The United Nations, in a recent post-harvest compendium, estimated that *Tribolium castaneum* & *Tribolium confusum*, the confused flour beetle, are "the two most common secondary pests of all plant commodities in store throughout the world." The red flour beetle is of Indo-Australian origin and less able to survive outdoors than the closely related species *Tribolium confusum*. It has, as a consequence, a more southern distribution, though both species are worldwide in heated environments[34]. The adult is long-lived, sometimes living more than three years. Although previously regarded as a relatively sedentary insect, it has been shown in molecular and ecological research to disperse considerable distances by flight[35-40].

CONCLUSION

Thirty-three bioactive chemical constituents have been identified from methanolic extract of the *Tribolium castaneum* by GC-MS technique. *In vitro* antibacterial (*Streptococcus pneumonia*, *Pseudomonas eurogenosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Klebsiella pneumonia*) evaluation of secondary metabolite products of *Tribolium castaneum* forms a primary platform for further pharmacological investigation for the development of new potential antifungal compounds.

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REFERENCES

1. Matthews, G.A. Insecticide application in stores. In: Matthews GA, Hislop EC (Eds) Application Technology for Crop Protection. CAB International, Wallingford, UK pp. 305-315 (1993).
2. Fedina, T. Y.; Lewis, S. M. "Female influence over offspring paternity in the red flour beetle Tribolium castaneum". *Proceedings of the Royal Society B: Biological Sciences*. 271 (1546), 1393–1399 (2004).
3. Lewis, Jutkiewicz. "Sperm Precedence and sperm storage in multiply mates red flour beetles". *Behavioral ecology and Socialbiology*. 43, 365–369 (1998).
4. Arnaud, L; Gage, M; Haubruge, E. "The dynamics of second- and third-male fertilization precedence in *Tribolium castaneum*". *Entomologia Experimentalis et Applicata*. 99, 55–64 (2001).
5. Grünwald, S. "The Red Flour Beetle Tribolium castaneum as a Model to Monitor Food Safety and Functionality". *Adv Biochem Eng Biotechnol*. 135, 111–122 (2013).
6. Pai, Aditi; Bennett, Lauren; Yan, Guiyun. "Female multiple mating for fertility assurance in red flour beetles (*Tribolium castaneum*)". *Canadian Journal of Zoology*. 83(7), 913–919 (2005).
7. Boake, R.B. "Genetic Consequences of Mate Choice: A Quantitative Genetic Method for Testing Sexual Selection Theory". *Science*. 227(4690), 1061–1063 (1985).
8. Riebeiro, B.M., Guedes, R.N.; Oliveira, E.E.; Santos JP. Insecticide resistance and synergism in Brasilian populations of *Sitophilus zeamais* (Coleoptera: Curculionidae). *J. Stored Prod. Res*. 39, 21-31 (2003).
9. Arnaud, L.E. "Mating Behavior and Male Mate Choice in Tribolium castaneum (Coleoptera, Tenebrionidae)". Behaviour. 136, 67–77 (1999).
10. Pai, A.; Feil, S.; Yan, G. "Variation in polyandry and its fitness consequences among populations of the red flour beetle, Tribolium castaneum". *Evolutionary Ecology*. 21 (5), 687–702 (2007).
11. Mohammed, G.J.; Omran, A.M.; Hussein, H.M. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 8(6), 977-996 (2016).
12. Sosa, A.A.; Bagi, S.H.; Hameed, I.H. Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *International*

Journal of Pharmacognosy and Phytochemical Research. 8(5), 109-126 (2016).
13. Kadhim, M.J.; Sosa, A.A.; Hameed, I.H. 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*. 8(6), 127-146 (2016).
14. Mohammed, G.J.; Kadhim, M.J.; Hussein, H.M. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. *International Journal of Pharmacognosy and Phytochemical Research*. 8(6), 889-905 (2016).
15. Altameme, H.J.; Hameed, I.H.; Abu-Serag, N.A. 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*. 44(4), 47–58 (2015).
16. Hamza, L.F.; Kamal, S.A.; Hameed, I.H. Determination of metabolites products by *Penicillium expansum* and evaluating antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 7(9), 194-220 (2015).
17. Hameed, I.H.; Altameme, H.J.; Idan, S.A. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 7(2), 1843- 1868 (2016).
18. Hameed, I.H.; Hamza, L.F.; Kamal, S.A. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy*. 7(8), 132-163 (2015).
19. Hameed, I.H.; Hussein, H.J.; Kareem, M.A.; Hamad, N.S. 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*. 7(7), 107-125 (2015).
20. Altameme, H.J.; Hadi, M.Y.; Hameed, I.H. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 7(10), 238-252 (2015).
21. Hameed, I.H.; Ibraheam, I.A.; Kadhim, H.J. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. *Journal of Pharmacognosy and Phytotherapy*. 7(6), 90-106 (2015).
22. Al-Marzoqi, A.H.; Hadi, M.Y.; Hameed, I.H. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 8(2), 25-48 (2016).
23. Hussein, A.O.; Mohammed, G.J.; Hadi, M.Y.; Hameed, I.H. 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*. 8(3), 49-59 (2016).
24. Altameme, H.J., Hameed, I.H.; Idan, S.A.; Hadi, M.Y. 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*. 7(9), 221-237 (2015).
25. Jasim, H.; Hussein, A.O.; Hameed, I.H.; Kareem, M.A. 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).
26. Hussein, H.J.; Hadi, M.Y.; Hameed, I.H. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. *Journal of Pharmacognosy and Phytotherapy.* 8(3), 60-89 (2016).
27. Hadi, M.Y.; Mohammed, G.J.; Hameed, I.H. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 8(2), 8-24 (2016).
28. Mohammed, G.J.; Al-Jassani, M.J.; Hameed, I.H. 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*. 8(3), 480-494 (2016).
29. Hussein, H.M.; Hameed, I.H.; Ibraheem, O.A. 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*. 8(3), 369-385 (2016).
30. Kadhim, M.J.; Mohammed, G.J.; Hameed, I.H. In *vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. *Oriental Journal of Chemistry*. 32(2), 10-30 (2016).
31. Shareef, H.K.; Muhammed, H.J.; Hussein, H.M.; Hameed, I.H. 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).
32. Al-Jassaci, M.J.; Mohammed, G.J.; Hameed, I.H. Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity. *International Journal of Pharmaceutical and Clinical Research*. 8(5), 304-315 (2016).
33. Michalczyk, L; Martin, O; Millard, a; Emerson, B; Gage, M. "Inbreeding depresses sperm competitiveness, but not fertilization or mating success in male Tribolium castaneum". *Proceedings of the royal society B* 333, 1739–1742 (2010).

34. Tyler, F; Tregenza, T. "Why do so many flour beetle copulations fail?". Entomologia Experimentalis et Applicata. 146, 199–206 (2012).
35. Ridley, A. "The spatiotemporal dynamics of Tribolium castaneum (Herbst): adult flight and gene flow". *Molecular Ecology*. 20(8), 1635–1646 (2011).
36. Zettler, J.L.; Cuperus, G.W. Pesticide resistance in *Tribolium castaneum* (Coleopteran: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. *J. Econ. Entomol*. 83, 1677-1681 (1990).
37. Hussein, H.M. Determination of phytochemical composition and ten elements content (CD, CA, CR, CO, FE, PB, MG, MN, NI AND ZN) of *CARDARIA DRABA* by GC-MS, FT-IR and AAS technique. *Int. J Pharm Bio Sci*. 7(3), (B) 1009 – 1017 (2016).
38. Hussein, H.M. 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.* 7(4), 2529 – 2555 (2016).
39. Jaddoa, H.H.; Hameed, I.H.; Mohammed G.J. Analysis of Volatile Metabolites Released by Staphylococcus Aureus using Gas Chromatography-Mass Spectrometry and Determination of its Antifungal Activity. *Orient J Chem*. 32(4) (2016).
40. Kadhim, M.J.; Mohammed, G.J.; Hussein, H.M. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. *International Journal of Pharmaceutical and Clinical Research*. 8(7), 655-670 (2016).