Detection of Bioactive Compounds of *Vitex agnus-castus* and *Citrus sinensis* Using Fourier-transform infrared spectroscopic profile and Evaluation of Its Anti-microbial Activity

Ahmed Hadi Abdal Sahib[1], Ekhlas Al-Shareefi[2], Imad Hadi Hameed[3]

[1]Ecological Sciences, AL-Qasim Green University, Hillah City, 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

Vitex agnus-castus is a supplement derived from berries. It is also called Vitex, Chaste Tree, or Chasteberry. The objectives of this study were analysis of the secondary metabolite products using Fourier-transform infrared spectroscopic profile and evaluation of its anti-microbial activity. In the current study, the anti-microbial activity of *Vitex agnus-castus* and *Citrus sinensis* methanolic extract was evaluated by determining the zone of inhibition against nine bacteria and eight fungi and yeast. Maximum zone formation was against *E.coli* (5.26±0.19) and very highly active against *Aspergillus terreus* (5.01±0.17). The FTIR analysis of *Vitex agnus-castus* proved the presence of Alkenes, alkyl halides, and Aromatic which shows major peaks at 715.59, 1014.56, 1047.35, 1095.57, 1234.44, 1242.16, 1317.38 and 1597.06. The FTIR analysis of *Citrus sinensis* proved the presence of alkyl halides, Alkenes, alkyl halides, and Amide which shows major peaks at 675.09, 692.44, 738.74, 813.96, 974.05, 1008.77, 1049.28, 1093.64, 1232.51, 1276.88, 1606.70, and 1647.21

Keywords: *Vitex agnus-castus, Citrus sinensis, FT-IR, Anti-Bacterial, Anti-Fungal Activity.*

INTRODUCTION

Infrared spectroscopy provides a useful method for herbal analysis and elucidate the compounds structures as well as for quantitative analysis of drugs. Fourier transform infrared spectrometry is a physico-chemical analytical technique and one of the most widely used methods to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated with molecular composition or content of the chemical group. The present study involves an assessment using FT-IR spectroscopic techniques to investigate the authenticity of commercial sample of the herbal drug by analyzing their fingerprints. *Vitex agnus-castus* (Verbenaceae), commonly called "chasteberry", a small deciduous tree that grows in Asia, Europe (especially in Mediterranean region) and North America. It bears slender spikes of violet blue, 8-10 cm flowers. It is popularly used in folk medicine to treat ovarian insufficiency, uterine bleeding, premenstrual syndrome, fibroid cysts, infertility and acne in teenagers [1-3]. It has also been traditionally used as a digestive aid, sedative and anti-infective. There have been several reports on its chemical constituents. It includes iridoid glycosides (agnuside, aucubin); flavonoids (vitexin, kaempferol, casticin, quercetagetin); progestins (progesterone, hydroxy progesterone, androstenedione); alkaloids (viticin); volatile oil (1,8-cineol, limes, α-pinenes, β-pinenes) and essential fatty acids (palmitic acid, oleic acid, stearic acid) [4-9]. Several other Vitex species are also reported to possess biological activities Viz. Vitex rotundifolia has repelling activity against Aedes aegypti mosquitoes, Vitex negundo L act as a larvicidal agent of mosquito's and antioxidant. V. pinramidata, V. pubescens, V. gaumeri are folk remedies to treat diarrhea, gastro intestinal affections, malaria, colds and cough spells. The aims of our research were analysis of the secondary metabolite products using Fourier-transform infrared spectroscopic profile and evaluation of its anti-microbial activity.

MATERIALS AND METHOD

Collection and preparation of plant material

The leaves were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal foreign materials, the leaves were stored in airtight container to avoid the effect of humidity [10-17] and then stored at room temperature until further use.

Preparation of sample

About 20 grams of the plant sample powdered were soaked in 100 ml methanol for 16 hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture [18-23].

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *Vitex agnus-castus* and *Citrus sinensis* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm [24-31].

Determination of antimicrobial activity of crude bioactive compounds of *Vitex agnus-castus* and *Citrus sinensis*

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty m*L* of plant extract was loaded on the bored wells. Antifungal activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control [32-39]. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

RESULTS AND DISCUSSION

Identification of biochemical compounds

Analysis of compounds was carried out in methanolic extract of *Vitex agnus-castus* and *Citrus sinensis*, shown in **Table 1** and **Table 2** respectively. The FTIR analysis of *Vitex agnus-castus* proved the presence of alkyl halides, Alkenes, and Amide which shows major peaks at Alkenes, alkyl halides, and Aromatic which shows major peaks at 715.59, 1014.56, 1047.35, 1095.57, 1234.44, 1242.16, 1317.38 and 1597.06. The FTIR analysis of *Citrus sinensis* proved the presence of alkyl halides, Alkenes, alkyl halides, and Amide which shows major peaks at 675.09, 692.44, 738.74, 813.96, 974.05, 1008.77, 1049.28, 1093.64, 1232.51, 1276.88, 1606.70, and 1647.21. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. WHO encourages countries to provide safe and effective traditional remedies and practices in public and private health services and it also published two monographs on medicinal plants with information on pharmacopoeial summaries for quality assurance: botanical features, distribution, identity tests, purity requirements, chemical assays, and active or major chemical constituents, clinical applications, pharmacology, contraindications, warnings, precautions, potential adverse reactions, and posology [42-49]. The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc.. Recently, a number of plants have been reported for antibacterial properties across the world. Based on the present study, it is concluded that the whole plants of A. lanata contains various bioactive components with high degree of antibacterial activity against various pathogens. It is hoped that this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin [50-53]. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

Table 1. FT-IR peak values of solid analysis of *Vitex agnus-castus*.

No.	Peak (Wave number cm-1)	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	715.59	67.897	Strong	=C–H	Bending	Alkenes	650-1000
2.	1014.56	58.136	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1047.35	58.483	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1095.57	63.618	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1234.44	78.418	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1242.16	78.354	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1317.38	80.864	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1597.06	76.023	Medium	C=C	Stretch	Aromatic	1400-1600

Table 2. FT-IR peak values of solid analysis of *Citrus sinensis*.

No.	Peak (Wave number cm-1)	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	675.09	67.825	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	692.44	69.075	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	738.74	72.075	Strong	=C–H	Bending	Alkenes	650-1000
4.	813.96	76.441	Strong	=C–H	Bending	Alkenes	650-1000
5.	974.05	65.287	Strong	=C–H	Bending	Alkenes	650-1000
6.	1008.77	54.765	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1049.28	58.347	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1093.64	64.409	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1232.51	80.641	Strong	C-F	Stretch	alkyl halides	1000-1400
10.	1276.88	80.140	Strong	C-F	Stretch	alkyl halides	1000-1400
11.	1606.70	79.503	Bending	N-H	Stretch	Amide	1550-1640
12.	1647.21	79.220	Variable	C=C	Stretch	Alkene	1620–1680

CONCLUSION

Medicinal property of *Vitex agnus-castus* and *Citrus sinensis* methanolic extract is due to presence of secondary metabolites. Twenty phytoconstituents were identified by (FT-IR) analysis. This plant derived bioactive compounds used as source of antibiotic properties and pharmaceutical industries used for drug formulation.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

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

REFERENCES

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. 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.
3. 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.
4. 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.
5. Hussein HM, Ubaid JM, Hameed IH. Inscticidal 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.
6. Ubaid JM, Hussein HM, Hameed IH. Determination of bioactive chemical composition of Callosobruchus maculutus and investigation of its anti-fungal activity. International journal of pharmcognosy and phytochemical research. 2016; 8(8): 1293-1299.
7. 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.
8. 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.
9. 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.
10. 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.
11. Kadhim MJ, Sosa AA, Hameed IH. Evaluation of anti-bacterial activity and bioactive chemical analysis of Ocimum basilicum using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 127-146.
12. Mohammed GJ, Kadhim MJ, Hussein HM. Characterization of bioactive chemical compounds from Aspergillus terreus and evaluation of antibacterial and antifungal activity. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 889-905.
13. 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
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. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of Saccharomyces cerievisiae and Evaluation of Antibacterial Activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(5): 304-315.
20. 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.
21. 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.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. 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.
31. Ubaid JM, Hussein HM, Hameed IH. Analysis of bioactive compounds of Tribolium castaneum and evaluation of anti-bacterial activity. International Journal of Pharmaceutical and Clinical Research. 2016; 8(7): 655-670.
32. Hameed, I.H., Al-Rubaye A.F. and Kadhim, M.J. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. International Journal of Pharmaceutical and Clinical Research. 2017; 8(11): 44-54.
33. Kadhim WA, Kadhim, M.J., Hameed, I.H. Antibacterial Activity of Several Plant Extracts Against Proteus Species. International Journal of Pharmaceutical and Clinical Research. 2017; 8(11): 88-94.
34. 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.
35. 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.

36. 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.
37. 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.
38. Al-Rubaye AF, Hameed IH, Kadhim MJ. A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants. International Journal of Toxicological and Pharmacological Research. 2017; 9(1); 81-85.
39. 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.
40. 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.
41. 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.
42. Hussein HM, Ubaid JM, Hameed IH. Inscticidal 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.
43. Ubaid JM, Hussein HM, Hameed IH. Determination of bioactive chemical composition of Callosobruchus maculutus and investigation of its anti-fungal activity. International journal of pharmcognosy and phytochemical research. 2016; 8(8): 1293-1299.
44. 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.
45. 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.
46. 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.
47. 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.
48. 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.
49. 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.
50. Khudhair ME, Hameed IH, Mekhlef AK. A Prospective and Retrospective Study of Acute Bronchitis in Hillah City-Iraq. Research Journal of Pharmacy and Technology. 2017; 10 (11): 3839-3844.
51. Kamal SA, Hamza LF, 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.