

# *Harpagophytum procumbens* and *Cordia myxa*: In vitro Antibacterial Activity and Bioactive Compounds of Methanolic Fruit Extract Using Fourier-Transform Infrared Spectroscopic Technique

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## ABSTRACT

*Harpagophytum Procumbens* (commonly called Devil's Claw) is a tuber vegetable that is used for combatting lower back pain as well as arthritis (both osteoarthritis and rheumatoid arthritis). The objectives of this study were analysis of the secondary metabolite products and evaluation of antibacterial activity. The FTIR analysis of *Harpagophytum Procumbens* proved the presence of alkyl halides, Alkenes, and Amide which shows major peaks at 669.30, 831.32, 918.12, 1016.49, 1029.99, 1240.23, 1608.63, 2358.94, and 3251.98. The FTIR analysis of *Cordia myxa* proved the presence of alkyl halides, Alkenes, Aromatic, Amide, and Alkane which shows major peaks at 669.30, 684.73, 827.46, 873.75, 927.76, 1010.70, 1236.37, 1313.52, 1417.68, 1604.77, 2358.94, 2918.30, and 3269.34. Antibacterial activity was evaluated by determining the zone of inhibition. Maximum zone formation was against *Staphylococcus aureus* (5.13±0.19) and (6.59±0.21) for *Harpagophytum Procumbens* and *Cordia myxa* respectively.

**Keywords:** FT-IR analysis, *Harpagophytum procumbens*, *Cordia myxa*, Anti-Bacterial Activity

## INTRODUCTION

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. *Harpagophytum procumbens* (devil's claw) has been used as an analgesic, a remedy for fever and allergies.

The major chemical constituents of *Harpagophytum* are iridoid glycosides<sup>1-13</sup>, phytosterols, aromatic acids, and flavonoids. Glycosides found in the tubers of the plant appear to be the most therapeutically important constituents. Whole-plant extracts appear to have a better therapeutic effect than those prepared from isolated parts. Devil's claw (*Harpagophytum procumbens*) is reported to have an anti-inflammatory effect in humans and laboratory animals. The active ingredients are various iridoid glycosides, acetylated phenolic glycosides, and terpenoids. Devil's Claw is primarily marketed for its painkilling and anti-inflammatory properties, and has many testimonials claiming relief from rheumatism and other joint disorders. The plants of genus *Cordia* comprise of trees and shrubs which are widely<sup>14-22</sup> distributed in warmer regions. Various compounds like flavonoids, triterpenes, tannins, alkaloids and fatty acids possessing wide range of bioactivities were isolated from different plant parts of *Cordia* species. *Cordia myxa* fruit (family: Boraginaceae), is popularly used

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for the treatment of chest and urinary infections, and as an anthelmintic, diuretic, astringent, demulcent and expectorant agent. The aims of this study were analysis of the secondary metabolite products and evaluation of antibacterial 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<sup>23-29</sup>, the leaves were stored in airtight container to avoid the effect of humidity 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<sup>30-37</sup>.

### Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of *Harpagophytum procumbens* and *Cordia myxa* was treated for FTIR spectroscopy (Shimadzu, IR Affinity, Japan). The sample was run at infrared region between 400 nm and 4000 nm.

### Determination of antimicrobial activity of crude bioactive compounds of *Harpagophytum procumbens* and *Cordia myxa*

The test pathogens were swabbed in Müller-Hinton agar plates. Sixty  $\mu$ 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<sup>51-55</sup>. Amphotericin B and fluconazole were used as reference antifungal agent<sup>38-49</sup>. 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 *Harpagophytum procumbens* and *Cordia myxa*, shown in Table 1 and Table 2. The FTIR analysis of *Harpagophytum Procumbens* proved the presence of alkyl halides, Alkenes, and Amide which shows major peaks at 669.30, 831.32, 918.12, 1016.49, 1029.99, 1240.23, 1608.63, 2358.94, and 3251.98 Table 1. The FTIR analysis of *Cordia myxa* proved the presence of alkyl halides, Alkenes, Aromatic, Amide, and Alkane which shows major peaks at 669.30, 684.73, 827.46, 873.75, 927.76, 1010.70, 1236.37, 1313.52, 1417.68, 1604.77, 2358.94, 2918.30, and 3269.34 Table 2. The very strong absorption band observed around 669.30-1240.23  $\text{cm}^{-1}$  may be due to the presence of bonded C-H/O-H stretching of alkyl halides Alkenes. 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. 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. 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 *Harpagophytum procumbens*.**

No.	Peak (Wave number cm <sup>-1</sup> )	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	669.30	59.096	Strong	C-Cl	Stretch	alkyl halides	600-800
2.	831.32	75.631	Strong	=C-H	Bending	Alkenes	650-1000
3.	918.12	74.543	Strong	=C-H	Bending	Alkenes	650-1000
4.	1016.49	53.947	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1029.99	53.825	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1240.23	77.627	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1608.63	72.518	Bending	N-H	Stretch	Amide	1550-1640
8.	2358.94	73.485	Unknown	-	-	-	-
9.	3251.98	73.653	Bending	N-H	Stretch	Amide	3100-3500

**Table 2. FT-IR peak values of solid analysis of *Cordia myxa*.**

No.	Peak (Wave number cm <sup>-1</sup> )	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	669.30	59.416	Strong	C-Cl	Stretch	alkyl halides	600-800
2.	684.73	63.115	Strong	C-Cl	Stretch	alkyl halides	600-800
3.	827.46	74.505	Strong	=C-H	Bending	Alkenes	650-1000
4.	873.75	72.300	Strong	=C-H	Bending	Alkenes	650-1000
5.	927.76	69.360	Strong	=C-H	Bending	Alkenes	650-1000
6.	1010.70	48.730	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1236.37	79.328	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1313.52	79.285	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1417.68	73.681	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1604.77	77.448	Bending	N-H	Stretch	Amide	1550-1640
11.	2358.94	79.466	Unknown	-	-	-	-
12.	2918.30	81.850	Strong	C-H	Stretch	Alkane	2850-3000
13.	3269.34	74.844	Bending	N-H	Stretch	Amide	3100-3500

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

<i>Harpagophytum procumbens</i> Antibiotics	Bacteria				
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Proteus mirabilis</i>	<i>Klebsiella pneumonia</i>	<i>Pseudomonas eurogenosa</i>
<i>Harpagophytum procumbens</i>	4.00±0.31	4.90±0.13	5.00±0.16	4.63±0.41	4.12±0.11
Rifambin	1.01±0.10	0.77±0.41	0.98±0.11	1.00±0.30	1.05±0.42
Streptomycin	0.91±0.27	1.60±0.29	1.90±0.10	0.96±0.47	0.87±0.20
Kanamycin	0.42±0.18	1.12±0.46	0.40±0.12	1.00±0.10	0.90±0.47
Cefotaxime	0.87±0.95	0.96±0.27	0.93±0.25	0.92±0.18	0.71±0.13

## CONCLUSION

Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. Infrared spectroscopy provides a useful method for herbal analysis and elucidate the compounds structures as well as for quantitative analysis of drugs. Twenty two phyto-constituents were identified by (FTIR) analysis.

**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. 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.
2. Altaee N, Kadhim MJ, Hameed IH. Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. *International Journal of Toxicological and Pharmacological Research*. 2016; 8(6): 462-470.
3. 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.
4. Hussein HM, Ubaid JM, Hameed IH. Insecticidal activity of methanolic seeds extract of *Ricinus communis* on adult of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and analysis of its phytochemical composition. *International journal of pharmacognosy and phytochemical research*. 2016; 8(8): 1385-1397.
5. 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.
6. Ibraheem 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.
7. Ibraheem 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.
8. Hadi MY, Hameed IH, Ibraheem IA. *Ceratonia siliqua*: Characterization, Pharmaceutical Products and Analysis of Bioactive Compounds: A Review. *Research Journal of Pharmacy and Technology*. 2017; 10(10): 3585-3589.
9. Hadi MY, Hameed IH, Ibraheem 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.
10. 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.
11. 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.
12. 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
13. 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.
14. 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.
15. 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.
  16. Hameed IH, Ibraheem 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.
  17. 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.
  18. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of *Saccharomyces cerevisiae* and Evaluation of Antibacterial Activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(5): 304-315.
  19. 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.
  20. 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.
  21. 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.
  22. 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.
  23. 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.
  24. 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.
  25. 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.
  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. 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.
  31. 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.
32. 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.
33. 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.
34. 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.
35. Mekhleif 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.
36. 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.
37. 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.
38. 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.
39. 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.
40. 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.
41. 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.
42. 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.
43. 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.
44. 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.
45. 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.
46. 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.
47. 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.
48. 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.
49. Khudhair ME, Hameed IH, Mekhleif AK. A Prospective and Retrospective Study of Acute Bronchitis in Hillah City-Iraq. *Research Journal of*

- Pharmacy and Technology. 2017; 10 (11): 3839-3844.
50. 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.