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, 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 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...
for the treatment of chest and urinary infections, and as an anthelminthic, 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, 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.

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 Müllner-Hinton agar plates. Sixty ml 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. 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 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 cm⁻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 cm⁻¹. The very strong absorption band observed around 669.30-1240.23 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_.

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

Table 2. FT-IR peak values of solid analysis of _Cordia myxa_.

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

Table 3. Zone of inhibition (mm) of test bacterial strains to _Harpagophytum procumbens_ bioactive compounds and standard antibiotics.

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

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