Evaluation of Biological Activity and Chemical Content of Some Medicinal Plants Using Different Methods

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Abstract:
This study was conducted to evaluate the biological and chemical effectiveness of some medicinal plants using different methods. Five species of medicinal plants (\textit{Origanum majorana}, \textit{Lawsonia inermis}, \textit{Salvia officinolis}, \textit{Conocarpus erectus} and \textit{Zizphus spina-christi}) were used as antimicrobial for three types of bacteria (\textit{Staphylococcus aureus}, \textit{Proteus mirabilis} and \textit{Pseudomonas aeruginosa}) and antioxidants agents. The study included the use of four organic solvents (ethanol, methanol, acetone) and water for the purpose of determining the efficiency of each solvent in the separation of active compounds. Secondary metabolism compounds, were detected by qualitative detection. Antioxidants were tested by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test and total phenolic content (TPC) was calculated. Antibiotics (Amikacin, Gentamicin, Imipenem, Norflaxacin, Tetracycline, Tobramycin, Trimethoprim, Vancomycin) were used. Imipenem antibiotic was better than all plant extracts.

Key words: \textit{Conocarpus erectus}, \textit{Lawsonia inermis}, Antioxidants, Antibiotics.

1. Introduction:
Medicinal plant is defined as a plant that contains one or more of its chemical substances, in low or high concentration, which has the ability to treat a particular disease or at least it reduces the symptoms of infection (Hamza, 2006). Today, the biggest problem facing the world is the so-called undeclared war that takes place between bacteria and antibiotics. Most germs are characterized by their great resistance to antibiotics because they have many factors of ferocity (Hansen \textit{et al}. 2004). Humans have used herbs to treat some of the diseases that afflict them or their pets since about 6000 years (Farid and Rachid, 2013). The inhabitants of Iraq and for thousands of years BC had used plants in the treatment of diseases and can be considered the oldest tablets of medicine in the world (Hadi, 2007). The use of medicinal plants in the pharmaceutical field has increased significantly in the present time. Therefore, many researchers in this field focused on the study of medicinal plants spread around the world, which are used for therapeutic purposes, including the treatment of colds, fever, diarrhea and others where the use of medicinal plants is common in the population of the regions where these plants grow is known as folk medicine (Khafagi and Dewedar, 2000). Medicinal plants contain various compounds that play a key role in protecting the plant.
itself from disease by its activity against the regeneration of pathogenic bacteria (Abu-Shanab et al., 2005). The components of medicinal plants are divided according to their effectiveness to active ingredients and non-effective ingredients, as the active ingredients are the substances that have therapeutic values such as phenols, alkaloids, volatile oils, turbines and tannins. The ineffective ingredients are substances that have no therapeutic value such as cellulose (Aburjai et al., 2001). The plant kingdom contains many plant varieties, which are an essential source of natural products with inhibitory effect of various types of microscopic organisms (Nascimento et al., 2000). The Prophet's Hadiths mentioned many plants and their reasons for use and their nutritional benefits. These include henna, figs, dates, garlic, etc. (Barham, 2007). The reason for the use of natural herbs in treatment is to overcome the side effects of manufactured drugs (Poole, 2001). The aim of this study to evaluate the inhibitory effectiveness of some plant extracts on some types pathogenic bacteria.

2. Materials and Methods

a) Plants collection and preparation

The leaves collected from each of *Origanum majorana*, *Lawsonia inermis* and *Salvia officinolis* from the local markets of AL-Nasiriyah city (south of Iraq) these plants were classified by the professor of Plant taxonomist at the College of Education for Pure Sciences, Dr. Yass Khudair, than, leaves were cleaned from impurities and grinded by the electric grinder and placed in the bottles of opaque and kept in the refrigerator until the preparation of extracts. The bark of both *Zizphus spina-christi* and *Conocarpus erectus* were collected from one of the orchards located west of AL-Nasiriyah city, It was cleaned from impurities and dried by the sun with continuous stirring. After that it was grinded and then placed in darkened bottles and kept in the refrigerator until preparation of the extracts, alcoholic, acetone and water.

b) Extraction process of antioxidants

Preparation of plant extracts plant materials were extracted using the methods described previously (Musa et al., 2011). Briefly, 0.1 g dried plant powder and 10 ml 50% aqueous acetone were stirred for 1 h in a 25-mL universal bottle at 1,000 rpm using a magnetic stirrer (IKA, Staufen, Germany). Samples were then centrifuged at 4,750 g for 10 min using a mini centrifuge (Thermo-line, China) and the supernatants were used for further analyses.

c) Total Phenol Content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of (Musa et al. 2011). About 100 μL plant extract was added with 0.5 mL diluted Folin Ciocalteu reagent. The samples extracts with Folin Ciocalteu reagent) were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dry sample (mg GA/100 g of DW).

d) DPPH Radical Scavenging Activity

The determination of antioxidant activity through 2,2- diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of (Musa et al. 2011). Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100 μL sample extract with 1 ml methanolic
DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow:

DPPH scavenging activity (%) = [(A blank – A sample) / A blank] × 100. Where A is the absorbance.

e) Antimicrobial Assay

*Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis* were used in experiment. The bacterial isolates were obtained from the Microbiology Laboratory, College of Education for Pure Sciences, Thi-Qar University. Mueller Hinton agar was used in antimicrobial assay. Plant extracts were dissolved in (acetone, ethanol, methanol and water) to obtain a concentration of (75,100,200 and 300)μg/10μL. Antimicrobial assays were conducted using the disc diffusion method as previously described by (Kusuma et al. 2010). Negative controls were prepared using the same solvent employed to dissolve the plant extract. Gentamicin discs (10 μg/ disc, Oxoid, UK) were used as control and positive controls. Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

f) Phytochemical Analysis

One gram of the plant methanol extracts was dissolved in 100 mL methanol and subjected to preliminary phytochemical screening following standard methods(Harborne, 1998; Kokate 2001).

3. Results and Discussion

3.1 - Effect of organic solvents on the effectiveness of *Conocarpus erectus* extract against *Pseudomonas aeruginosa*

Natural products may be a particularly rich source of anti-infective agents. The antimicrobial activity on pathogenic strains of Gram-positive *Pseudomonas aeruginosa* bacteria of *Conocarpus erectus* bark extracts was evaluated in the present study (Table 1). The antimicrobial activity of *Conocarpus erectus* bark and As it can be observed from this Table, all extracts exhibited antibacterial action against *Pseudomonas aeruginosa*. The diameter of the zone of inhibition varied ranging from (11mm) to (17mm) for bark extract. The results of the present study disagree with the study by Hajar and Gumgumjee (2013) where they found that the ethanol extract of *Conocarpus erectus* inhibits *Pseudomonas aeruginosa* at 24.00mm at 100mg/mL at 33.33mm at 200mg/mL concentration, the reason may be variation in the extraction method used or because of the agricultural location of the plant and different weather conditions in terms of temperature. The results of this study are similar to the study carried out by Abdel-Hammed et al. (2012), where they found during their studies that the average rate of inhibition of the methanol extract of the conocarps stalks against *Pseudomonas aeruginosa* is (15.00 ± 0.11) mm. Figure (1).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Concentrations mg/mL</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Ethanol</td>
<td>7.33±3.78</td>
<td>12.00±2.64</td>
</tr>
<tr>
<td>Methanol</td>
<td>11.00±1.00</td>
<td>13.00±1.00</td>
</tr>
<tr>
<td>Acetone</td>
<td>13.66±1.52</td>
<td>16.66±0.57</td>
</tr>
<tr>
<td>Water</td>
<td>11.33±2.51</td>
<td>17.00±1.00</td>
</tr>
<tr>
<td>Mean</td>
<td>10.83</td>
<td>14.66</td>
</tr>
</tbody>
</table>

L.S.D=3.12
3-2-Antibiotics against the pathogenic bacterial isolates used

From Table(2) *P. aeruginosa* and *S. aureus* were sensitive to the antibiotic (GN and IPE) while *Proteus mirabilis* was not sensitive to the antibiotic (AK). Results agree with Jaloob and Gafile (2012), they found that the antibiotic (AK) resistance ratio of bacteria (*Proteus mirabilis*) was 100%, but different from the study conducted by Dulaimi *et al* (2016), they found that *Proteus mirabilis* was sensitive to the antibiotic (AK). The antibiotic (AK) inhibits the synthesis of the bacterial cell wall and the antibody (GN) interferes with protein synthesis in bacteria by altering the ribosome portion (Susan *et al*., 2003). The antibiotic (IPE) inhibits the formation of peptidoglycan as it has the ability to penetrate the protein channels and thus destroys the Gram-negative bacteria (Bagge *et al*., 2004). It also disrupts the permeability barrier to the membrane of the Gram-positive bacteria and thus destroys the cell (Carson *et al*., 2002). All bacterial isolates were sensitive to antagonist (NOR), this was consistent with Hussein *et al.* (2017). Figure (2).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>P. aeruginosa</em></th>
<th><em>S. aureus</em></th>
<th><em>P. mirabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin (AK)</td>
<td>8</td>
<td>18</td>
<td>0.00</td>
</tr>
<tr>
<td>Gentamicin (GN)</td>
<td>4</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Imipenem (IPE)</td>
<td>26</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>Norflaxacin (NOR)</td>
<td>30</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Tetracycline (TE)</td>
<td>22</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Tobramycin (TOB)</td>
<td>18</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

3-3- Specific data on active compounds in medicinal plants used

Table(3) shows that the water extract of *Conocarpus erectus* contains phenols, carotenoids, saponins, alkaloids, flavonoids and does not contain tannins, carotenoids and terpenoids. These results are consistent with the study of Abdel-Hameed *et al.* (2012), found that *Conocarpus erectus* bark contains phenols and saponins. The current study disagrees with Shohayeb *et al.* (2013), where it confirmed that antibacterial activity against microorganisms is due to the presence of tannins. This may be due to different methods of detection of materials or because of different plant source and country of plant cultivation. The results showed that the water extract of *Lawsonia inermis* leaves contains phenols, glycosides, saponins, turines, carotenoids and flavonoids and did not contain alkaloids and tannins. The results of the present study agree with the study carried out by AL-Hamdani, and Al-Mahna (2009), they found that *Lawsonia inermis* leaves contain phenols, glycoside, soap, dyes, comarins, and resins. However, the results of this study did not agree with Raja *et al.* (2013). The results showed that the water extract of *Origanum majorana* leaves contains both the following compounds phenols, flavonoids, alkaloids, carotenoids and tannins, and...
did not contain glycosides, saponins and terpenoids, these results disagree with the study conducted by Farid and Rashid (2013), they found that the leaves of *Origanum majorana* did not contain alkaloids. The phytochemicals tested are known to exhibit medicinal activity and physiological activity. Flavonoids have been reported to possess antibacterial and antioxidant, saponins showed hypocholesterolemic and antidiabetic properties (Miller, 1996).

**Table 3:** Phytochemicals analysis of plant extracts

<table>
<thead>
<tr>
<th>Compounds</th>
<th><em>Conocarpus erectus</em></th>
<th><em>Lawsonia inermis</em></th>
<th><em>Origanum majorana</em></th>
<th><em>Salvia officinolis</em></th>
<th><em>Zizhus spina-christi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tanins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Where (+) represents the positive result (-) represents the negative result

**3-4- Total phenol content determination (TPC)**

The TPC varied markedly among the tested plant extracts ranging from 132.00 to 62.40 mg/GAE. Among the plants in this study, *Conocarpus erectus* showed the highest value (132.00 mg/GAE). Also note significant differences between *Conocarpus erectus* and *Lawsonia inermis* and the results of the current study consistent with Al-Fikki and Rikabi (2013), which confirmed that the methanol extract of *Lawsonia inermis* leaves contained phenolic compounds (25 mg/GAE), this does not agree with Hosein and Zinab (2007), they found that the amount of phenols in *Lawsonia inermis* leaves is (145 mg/GAE). The cause of the difference is the difference of plant source. Abdel-Hameed et al. (2012), found that *Conocarpus erectus* contain large quantities of phenols, they also found that the ethyl acetate extract of *Conocarpus erectus* contains amount of phenols (181.61 ± 3.98 mg/GAE), while the butanol extract contains amount of phenols (416.09 ± 14.35 mg/GAE). Olajuyigbe and Afolayan (2011) found that the ethanol extract of *Zizhus spina-christi* contains a large amount of phenol compared with the water extract of *Zizhus spina-christi*. Figure (3).

**3-5- DPPH radical scavenging activity**

DPPH radical scavenging activity varied from 78 to 37 % representing an approximately 41%-fold variation between the lowest and highest activities. *Origanum majorana* showed the highest antioxidant activity, followed by *Salvia officinolis, Zizhus spina-christi, Lawsonia inermis* and *Conocarpus erectus*(76,55,51 and 37 % respectively). *Conocarpus erectus* showed the lowest antioxidant activity (46 %) in the DPPH assay. Comparing antioxidant activity from this study and other published data is difficult due to the fact that content of antioxidant compounds can be influenced by extracting solvent, cultivar and location extraction methods used (Uma and Wan Aida .,2010). Figure (4).
Figure 1: The areas of inhibition of the acetone extract of *Conocarpus erectus* against *P. aeruginosa*.

Figure 2: The effect of antibiotics against bacteria (*P. aeruginosa*).

Figure 3: The total phenol content of plants used in the study.

\[ \begin{array}{ccccc}
\text{Conocarpus erectus} & \text{Lawsonia inermis} & \text{Origanum majorana} & \text{Salvia officinolis} & \text{Zizphus spina-christi} \\
(a) & (b) & (c) & (d) & (e) \\
\end{array} \]

\text{mg GAE/100 g DW}

\( a-d \) Mean with different letters are significantly different (\( P<0.05 \))
Conclusion
These results showed that the different medicinal plants contained significantly different amount of antioxidant capacity and antibacterial activity. According to this results, *Origanum majorana*, *Lawsonia inermis*, *Salvia officinolis*, *Conocarpus erectus*, and *Zizphus spina-christi* extracts with higher phenolic content, antioxidant capacity and antibacterial activity. Further study using bioassay guided of crude extracts of *Origanum majorana*, *Lawsonia inermis*, *Salvia officinolis*, *Conocarpus erectus*, and *Zizphus spina-christi* extracts are needed to isolate and identify the active compounds. Their active constituents may be potential candidates with therapeutic value in the treatment of bacterial and oxidation.

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