Phytochemical investigations and antibacterial activity of selected medicinal plants from Jordan

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ABSTRACT

Aims: To determine the antibacterial effect of crude methanolic extracts of six medicinal important plants grown in Jordan (Paronychia argentea Lam., Inula viscosa L., Arbutus andrachne L, Asphodelus microcarpus Salzm et Vivi, Peganum harmala L and Aloysia citriodora Palau) against Bacillus subtilis, Staphylococcus aureus and Escherichia coli.

Study Design: In vitro assessment antibacterial study
Place and Duration of Study: Department of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy, University of Jordan, Amman, Jordan. Between (December 2012 and January 2013).

Methodology: In-vitro Laboratory experimental tests; preparation of plant Extracts, phytochemical screening; susceptibility tests (zones of inhibition) and minimum inhibitory concentration (MIC)s determination.

Results: While the methanolic extract of P. argentea, A. andrachne, A. microcarpus Salzm et Vivi had no antibacterial activity. The crude methanol extract of P. harmala showed significant antibacterial activities against all the tested bacterial strains. MIC values for the seed and root extract of against S.aureus were (0.375 mg/ ml and 1.5 mg/ml) respectively while MIC values for seed and root extracts against B. subtilis were (0.375 and 6.25 mg/ml), respectively with less significant activity against negative bacteria. The crude extract of I.viscosa & A. citriodora was significantly active against bacterial strains S.aureus and B. subtilis but was inactive against Ecoli. Minimal inhibitory concentration value for I. viscosa L. extract against S.aureus were 6.25 mg/ ml and against B.subtilis 0.375 mg/ ml. Meanwhile, Minimal inhibitory concentration value for A. citriodora against S.aureus were 12.5 mg/ ml and against B.subtilis 1.5 mg/ ml.

Conclusion: Results indicate the potential antibacterial activity of I.viscosa L and A. citriodora towards gram positive bacteria such as B.subtilis, S.aureus. The extracts
phytochemical screening revealed the presence of terpenoids, flavonoids and phenolics. These preliminary results would be a guide in the selection of potential candidates for further pharmacological study and in search of new drug candidate for treatment of infections caused by gram positive bacteria.

Keywords: Antibacterial activity; Medicinal plants, methanolic extract, phytochemical screening, Jordan.

1. INTRODUCTION

During the last decades, there is increasing interest to unlock the secrets of ancient herbal remedies. For this purpose, various strategies have been developed e.g., biological screening, isolation as well as clinical trials for a variety of plants [1].

The bacterial organisms including gram positive and gram negative like different species of Bacillus, Staphylococcus, Salmonella and Pseudomonas are the main source to cause severe infections in humans [2]. Resistance to antimicrobials is a significant and growing problem, limiting treatment options, especially for serious Gram-positive infections, among them Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa etc. Furthermore, methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant Pneumococcus, vancomycin-resistant Enterococcus faecalis (VRE), and multidrug-resistant Mycobacterium tuberculosis (MDRTB). These are the major cause of worldwide outbreaks of both hospitals and the community infections [3]. The spread of multidrug-resistant (MDR) strains of bacteria necessitates the discovery of new classes of anti-bacterials and compounds that inhibit these resistance mechanisms [4, 5].

At present, there are no single chemical entity plant-derived anti-bacterials used clinically, and this chemically diverse group deserves consideration as a source for two major reasons. First, plants have exceptional ability to produce cytotoxic agents and second there is an ecological rationale that antimicrobial natural products should be present or synthesised de novo in plants following microbial attack to protect the producer from pathogenic microbes in its environment [6, 7]. Plant-derived antibacterials are always a source of novel therapeutics. Historically, plants have been placed at top among the sources of novel drugs with antimicrobial activity, as traditional medicines based on plants and plant extracts have made considerable contributions to human health and well-being.

Plants are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids, polyphenols) are generally superior in their biological activities suggesting that this strength is dependent on the diversity and quantity of such constituents [8]. For example, Quinine (Cinchona) and berberine (Berberis) are the antibiotics obtained from plants which are highly effective against microbes (Staphylococcus aureus, Escherichia coli) [1]. Therefore, the determination of the compounds responsible for any biological activity would facilitate the selection of the plants for future investigation.

Jordan is a land of biodiversity in terms of plant species. Various plants have been mentioned in traditional medicine literature, for their therapeutic advantages [9, 10]. Many herbs used by herbalists show promising results in the treatment of various ailments and these herbs could be appropriate for large randomized trials. Many potent drugs have been
purified from medicinal plants having anti-rheumatic, antithrombotic, antimalarial, anticancer, antidiabetic and antimicrobial properties [11].

The present study aimed to investigate the susceptibility of three clinically significant bacterial strains against six naturally growing plants crude extracts (*Paronychia argentea* Lam., *Inula viscosa* L., *Arbutus andrachne* L, *Asphodelus microcarpus* Salzm et Vivi, *Peganum harmala* L and *Aloysia citriodora* Palau). The minimum inhibitory concentrations (MICs) were also determined for the crude extracts showing significant activity against the bacterial strains selected for the susceptibility assay. The family name, different part and also the uses of these plants are tabulated in Table 1.

Table 1: Ethnobotanical data of the studied Jordanian medicinal plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>Systematic name</th>
<th>Common name</th>
<th>Family</th>
<th>Part(s) used</th>
<th>Traditional uses</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paronychia argentea</em> L.</td>
<td>Silver nail root, Silvery Whitlow Wort, 'rejel el-hamama' or shoishet el-raei</td>
<td>Caryophyllaceae</td>
<td>Aerial part</td>
<td>Diuretic, dissolve kidney stones, urinary tract infections, Gastric analgesic, bladder and prostate abdominal stomach ulcers ailments.</td>
<td></td>
</tr>
<tr>
<td><em>Inula viscosa</em> L.</td>
<td>Taioon, Tribe Inula</td>
<td>Compositae (Asteraceae)</td>
<td>Aerial part</td>
<td>Wound and ulcer treatment, bleeding Joints pain, Respiratory tract infections, Athlete's foot, Hemorrhoids and Intestinal worms. Bone repair, Blood pressure diabetes, Back ache, Gum disorder and Skin fungi treatment</td>
<td></td>
</tr>
<tr>
<td><em>Asphodelus microcarpus</em> Salzm et Vivi</td>
<td>Tall Asphodel</td>
<td>Liliaceae</td>
<td>Bulb &amp; Root</td>
<td>Ectoderm parasites and jaundice</td>
<td></td>
</tr>
<tr>
<td><em>Peganum harmala</em> L</td>
<td>Syrian rue, harmel</td>
<td>Zygophyllaceae Or Nitrariaceae</td>
<td>Root&amp; seeds</td>
<td>Analgesic, anti-inflammatory agent. Harmaline, an active ingredient in <em>P. harmala</em>, is a central nervous system stimulant and a reversible inhibitor of MAO-A, a category of antidepressant. Antibacterial activity against drug-resistant bacteria. The root is applied to kill lice and insects. It is also used as an antihelmintic, abortifacient, and in large quantities, it can reduce spermatogenesis and male fertility in rats. Has antioxidant and antimutagenic properties.</td>
<td></td>
</tr>
<tr>
<td><em>Aloysia citriodora</em> Palau</td>
<td>Lemon Verbana</td>
<td>Verbenaceae</td>
<td>Aerial part</td>
<td>Asthma, spasms, cold, fever, flatulence, colic, diarrhea, indigestion, insomnia and anxiety.</td>
<td></td>
</tr>
</tbody>
</table>

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2. MATERIALS AND METHODS

2.1 Plant materials

The plant materials Paronychia argentea Lam. [2 CARYO PA], Inula viscosa L. [7 COMP IV, Arbutus andrachne L. [3 Erica AA], Asphodelus microcarpus Salzm et Vivi. [4 LILIA AM], Peganum harmala L. [5 ZYGO PH] and Aloysia citriodora Palau. [AC-V1] were collected from different geographical regions of Jordan. The collected plants were identified by Prof. Suleiman Al-Olmat, Dept. of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman, Jordan and a specimen voucher was deposited in the Herbarium of the University of Jordan for future reference. Each plant material was thoroughly washed under running tap water and dried under shade. The dried plant materials were ground to powder form for extraction.

2.2 Extraction

The crude ethanolic extracts were prepared by refluxing each 10 g of the dried coarsely powdered plant material with 100 ml ethanol (95%) for 15 min and keeping the extract overnight at room temperature. After filtering twice through filter paper, the volume of the filtered solution was increased to 100 ml with ethanol (95%) to obtain 10% (equivalent to 100 mg/ml) crude ethanolic extracts.

2.3 Phytochemical Screening

The extracts were subjected to phytochemical tests for plant secondary metabolites alkaloids, Terpenoids, phenolics, coumarins and flavonoids using methods of [12,13].

2.4 Experimental procedure:

2.4.1 Antibacterial activity

2.4.1.1 Bacteria strains

In the present study, overnight cultures of three bacteria strains were used. These were two Gram positive strains (Staphylococcus aureus, ATCC25923 & Bacillus subtilis, ATCC441) and one Gram negative (Escherichia coli, ATCC8739). All bacteria were cultured on nutrient agar (Oxoid).

2.4.1.2 Preparation of inoculums

One single colony of each type of microorganism (from the nutrient agar stock culture) was inoculated with a sterile loop, and was transferred into 10 mL sterile nutrient broth (Oxoid). The broth cultures were incubated in a shaking incubator at 37°C for 16 - 20 hours.
2.4.1.3 Antibacterial susceptibility test: Disc diffusion assay

The antimicrobial activity of the plants Methanol extracts were initially assessed against all tested microorganisms using the agar diffusion method as recommended by the Clinical Laboratory Institute (CLSI) [14] (NCCLS; 2003). Nutrient agar medium was prepared by suspending nutrient agar (Merk) 20 g/L in distilled water. The pH value of the media was adjusted to 7.0, autoclaved, and allowed to cool up to 45°C. The media was seeded with 10 mL prepared inocula. Subsequently, the seeded medium (75-80 mL) was poured into pre-labelled Petri plates (diameter = 14 cm) and allowed to solidify. Impregnated disks were prepared by the addition of 20 µl plant extract (10% w/v; 100 mg/ml) to “susceptibility blank disks” (Oxoid). These were subsequently applied to the inoculated agar plates and then incubated 24 hours at 37°C. Antibacterial activity was indicated when clear inhibition zones were noted around the discs. The diameter of the inhibition zones was measured and the results were expressed as mean of three independent experiments. The test was repeated three times. 30 µg amoxicillin and blank disc impregnated with dimethylsulphoxide (DMSO) without extract were served as positive and negative controls, respectively.

2.4.1.4 Determination of minimum inhibitory Concentration (MIC)

Minimal inhibitory Concentration (MIC) performed on extracts which showed positive activity in the preliminary screening using the microdilution method in 96-well plates (Cellstar®, Greiner Bio-One, Germany) (National Committee for Clinical Laboratory Standards NCCLS, 2008). Double-strength medium (100 µl) of the Mueller Hinton broth (Oxoid) (bacterial culture) were used to fill the first experimental well. The other wells were filled with single-strength medium (100 µl). A volume of 100 µl of the plant extract (10%, w/v). Double-fold serial dilution was then carried out across the plate. The overnight batch culture of the microorganisms (10 µl) was used to inoculate each well to achieve an inoculum size of approximately 1 × 106 CFU/ml. The plates were incubated for 24 h at 37°C. The MIC (Minimal inhibitory Concentration) was calculated. For each bacterial strain, controls were maintained where pure solvents were used. Amoxicillin 30 ug positive controls were used. Each MIC determination was carried out in triplicate.

2.5 Statistical analysis

Analysis of variance (ANOVA) and Least Significant Difference (LSD) test at p < 0.0001 was carried out using Prism 5 software (Graphpad, La Jolla, CA, USA) to determine the significance of percentage inhibition values between the extracts against bacterial strains.

3. RESULTS AND DISCUSSION

The medical world is on an immense requirement to discover novel antibiotics due to widespread emergence of resistance among microbial pathogens against currently available antibiotics. Traditional plants have been proved to be better source in the search for novel antimicrobial compounds. In such effort, we accessed the susceptibilities of some clinically significant bacterial species against various extracts made up from six Jordanian medicinal plants. It has been reported that biological activities in the selected plants were exhibited by different class of phytochemicals [15]. Therefore, it is important to screen for phytochemical group in these plants.

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The results of phytochemical screening of the selected plants species are given in Table 2. Flavonoids, terpenoids, phenolics and coumarins were identified in all tested plants, while the presence of alkaloids could be only detected in Harmal.

Table 2: Classes of phytochemicals present in the plant extracts

<table>
<thead>
<tr>
<th>Plant botanical name</th>
<th>Paronychia argentea Lam</th>
<th>Inula viscosa L.</th>
<th>Arbutus andrachne L</th>
<th>Asphodelus microcarpus Salzm et Vivi.</th>
<th>Peganum harmala L (Root)</th>
<th>Peganum harmala L (Seed)</th>
<th>Aloysia citriodora Palau</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</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>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Phenolics</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>±</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**KEY:** - = absence; + = presence; ++ = abundant; +++ = abundant in appreciable quantity

The susceptibility of bacteria towards the plant extracts was assessed. All six extracts were tested on selected Gram positive and negative bacteria. The screening showed that crude extract of *Inula viscosa* L. *Peganum harmala* L and *Aloysia citriodora* Palau are the most active extracts showed antibacterial activity against Gram positive bacteria. These three plant extracts produced the largest zones of inhibition against *Bacillus subtilis* and *Staphylococcus aureus*, mean inhibition zone diameter.

The crude methanol extract of *P. harmala* showed significant antibacterial activities against all the tested bacterial strains. Maximum activity was conferred against *S. aureus* (13.66, 12.66 mm) for root and seed respectively (Figure 1, 2 and 3) in addition both extracts caused significant *B. subtilis*, mean inhibition zone diameter (14.23, 12.66 mm), respectively while lower activity was observed against *E coll* with mean inhibition zone diameter (8.16 and 7.16 mm), respectively. Results of MIC determination (Table 3) showed that MIC values for the seed and root extract of *S. aureus* were (0.375 mg/ml and 1.5 mg/ml) respectively while MIC values for seed and root extracts against *B. subtilis* were 0.375 and 6.25 mg/ml, respectively.

On the basis of the obtained results that are shown in three the figures, the seed and root extracts of *P. harmala* have a broad antibacterial activity so that all of the tested bacteria were sensitive. This is in agreement with previous reports by the several workers about harmal with antibacterial activity of root and seed extracts against most of the tested gram positive bacteria was better gram negative bacteria [16,17,18,19,20]. The crude extract of *Inula viscosa* was significantly active against gram positive bacterial strains *S. aureus* and *B. subtilis* but was inactive against *E coli*. Maximum inhibition zone (11.66 and 14.36 mm) was shown against *S. aureus* and *B. subtilis* respectively (Figure 1, 2 and 3). In addition, *A. citriodora* methanolic extract caused significant antibacterial activity against gram positive bacterial strains *S. aureus* and *B. subtilis* but was also inactive against *E coli* with maximum inhibition zone (11.0 and 12.4 mm) was shown against *S. aureus* and *B. subtilis* respectively. Minimal inhibitory concentration value for *Inula viscosa* L. extract against *S. aureus* were 6.25 mg/ml and against *B. subtilis* 0.375 mg/ml. Meanwhile, Minimal inhibitory concentration value for *Aloysia citriodora* Palau against *S. aureus* were 12.5 mg/ml and against *B. subtilis* 1.5 mg/ml (Table 3).

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No inhibition was observed in Gram negative bacteria *E.coli* at the same concentration for all the plant extracts, except Harmal root & seed extracts these were found to be the only effective extract but, with comparatively lower activity than Gram positive bacteria.

Furthermore, *Paronychia argentea, Arbutus andrachne, Asphodelus microcarpus Salzm et Vivi.* extracts were found completely inactive against all the organisms tested.

Data of antibacterial activity of active crude extracts of these plants are shown in figures: 1, 2 and 3 for *S. aureus, B. subtilis* and *Ecoli* respectively in the range of 10 mm to 14 mm size of inhibition zone for active extracts.

**Figure 1:** Zone of inhibition (in mm diameter) against *S.aureus* by crude plants extracts showing susceptibility. DMSO was used as negative control and Amoxicillin as positive control. Each bar indicates the mean ± S.E.M. of three determinations; p< 0.0001

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Figure 2: Zone of inhibition (in mm diameter) against *B. subtilis* by plants extracts showing susceptibility. DMSO was used as negative control and Amoxicillin as positive control. Each bar indicates the mean ± S.E.M. of three determinations; p < 0.0001

Figure 3: Zone of inhibition (in mm diameter) against *Escherichia coli* by Harmal extracts (Roots& Seeds) DMSO was used as negative control and Amoxicillin as positive control. Each bar indicates the mean ± S.E.M. of three determinations; p < 0.0001

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Table 3: The MIC values of selected plant extracts against two bacterial pathogens *(Staphylococcus aureus & Bacillus subtilis)*

<table>
<thead>
<tr>
<th>Plant name</th>
<th>MIC (mg/ml) against S. aureus</th>
<th>MIC (mg/ml) against B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Inula viscosa</em> L.</td>
<td>6.25</td>
<td>0.375</td>
</tr>
<tr>
<td><em>Peganum harmala</em> L (Root)</td>
<td>1.5</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Peganum harmala</em> L (Seed)</td>
<td>0.375</td>
<td>0.375</td>
</tr>
<tr>
<td><em>Aloysia citriodora</em> Palau</td>
<td>12.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The current scenario of antibiotics is very threatening with significant emergence of resistance among bacterial pathogens against available antibiotics. In the present study six Jordanian medicinal plants used for different remedies by local communities were tested against ATCC bacterial cultures to determine and investigate their antibacterial potential.

We observed that the crude methanolic extract of *Inula viscosa* L. *Peganum harmala* L and *Aloysia citriodora* Palau showed high activity toward Gram positive bacteria but not Gram negative species except harmal. Therefore, the extracts of these plants were considered as suitable candidates for antibacterial drug discovery. Other extracts *Paronychia argentea*, *Arbutus andrachne* and *Asphodelus microcarpus* Salzm et Vivi, methanolic extracts showed no activity against all tested bacterial strains this might suggest the lack of bio-active components and/or insufficient quantities in the extract.

The antibacterial activity of five plants extract *Inula viscosa* L. and *Aloysia citriodora* Palau *Paronychia argentea*, *Arbutus andrachne*, *Asphodelus microcarpus* Salzm et Vivi are reported for the first time. No previous report on the antibacterial activity of these species could be found in the literature. The effectiveness of *Inula viscosa* L. and *Aloysia citriodora* Palau as antibacterial activity towards gram-positive species might warrant fruitful studies in the future.

The potential for developing antimicrobials from higher plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within these plants with their full spectrum of efficacy. However, the present study of *in vitro* antibacterial activity of some plants forms primary platform for further phytochemical and pharmacological studies. Since, the present study was focused only to examine the antibacterial potential of crude extracts; therefore further study can be made to isolate the pure compounds responsible for the activity from the extracts with the help of numerous advanced technologies such as GC-MS, LC-MS, IR and NMR.
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author SA designed the study, wrote the protocol and the first draft of the manuscript. Authors SA and SO conducted the experimental works. Author RA performed the statistical analysis. Author PC managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

REFERENCES


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