Evaluation of antibacterial activity of some wild plants of Algeria

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Abstract: The current study was undertaken to assess in vitro antibacterial effects of some plants well known in Algerian traditional medicine. Hydroalcoholic extracts of bark of Pinus pinaster (PPI), flowering tops of Marrubium vulgare (MUV) and aerial part of Artemesia herba-alba (AHA) were tested on four bacterial strains viz. Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Staphylococcus aureus. All plants were found to be active on one or more bacteria at 25 mg/ml, the effects of the extracts were compared to that of ampicillin, as a positive control. By the diffusion method in solid medium, and the diffusion method in liquid, both PPI and MUV extracts showed higher antibacterial activity on E. coli than AHA extract, the areas of inhibition were 14.33 ± 3.51 mm and 15.33 ± 8.2 mm respectively. This important activity has been demonstrated on E. coli with a MIC of 0.195mg/ml. The hydroalcoholic extract of AHA showed significant activity when compared to positive control (AMPIC) with a MIC of 0.097mg/ml against P. aeruginosa. An inhibition diameter of 10 ± 1.00 mm was observed with hydroalcoholic extract of MUV against P. vulgaris with a MIC equal to 0.048 mg/ml.

Keywords: antibacterial activity; Pinus pinaster; Marrubium vulgare; Artemesia herba-alba.

Introduction

The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled and the increase of bacterial resistance have created the need for studies directed towards the development of new antimicrobials (Recio and Rios 1989; Silver and Bostian 1993; Koné et al. 2004; Tra Bi Tra Bi et al. 2008). The situation is more worrying more because of the emergence of strains of microorganisms antibioresistants and the emergence of uncommon infections that compromise treatment with existing drugs (Vandan and Vlietinck 1991).

Faced with these many challenges posed by the use of antibacterial agents available, it is necessary to search for new antibacterial substances effective with broad spectrum of action. One strategy for this research is to explore the plants used in traditional medicine.

*Marrubium vulgare* L. is a perennial herb of the Labiatae family which is commonly known as “horehound” in Europe, or “Merriouet” in Algeria. According to Zarai et al. (2011), *M. vulgare* is frequently used in Mediterranean region folk medicine; it is reported to possess hypoglycemic, vasorelaxant, antihypertensive, analgesic, anti-inflammatory, antioxidant, antiedematogenic, and many other biological activities. Some biological properties of essential oils extracted from this plant and their constituents have been recently investigated: antibacterial, antifungal, and antioxidant properties (Baratta et al. 1998; Bounatirou et al. 2007; Cosentino et al. 1999; Zarai et al. 2011).

*Pinus pinaster* Aiton (maritime pine) is a western Mediterranean species reaching the High Atlas and Tunisia in North Africa. It is particularly abundant in Spain where it attains a cover of over 1 300 000 ha (Gil et al., 1990).
The main uses of the maritime pine species are related to wood and resin production, recreation and soil protection (Alía and Martín 2003). This plant has been traditionally used for timber and as a source of turpentine (Devesa 1997).

Artemisia herba-alba (Asso), known also as desert wormwood (known in Arabic as shih) (Segal et al. 1987) has been used in folk medicine by many cultures since ancient times as antihypertensive, antidiabetic (Zeggwagh et al. 2008; Ziyyat et al. 1987), analgesic, antibacterial, antispasmodic, and hemostatic agents (Laid et al. 2008). It has been used also for treatment of gastric disturbances, such as diarrhoea, abdominal cramps and for healing external wounds (Feuerstein et al. 1986).

The current study was undertaken to investigate antibacterial properties of these three plants known for their use in traditional medicine in Algeria: P. pinaster, M. vulgar and A. herba alba. The extracts of these plants were tested on four bacterial strains: Staphylococcus aureus, Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa.

Material and Methods

Plant material

The plant material was obtained from plants growing in their natural habitat or from herbists. Their identification was done by a taxonomist confirmed, and vouchers specimens (No. AB04-xx) were deposited (Table 1) in the herbarium of the Laboratory of medical botany, pharmacy department, Faculty of Medicine, Mentouri Constantine University (Algeria).

Table 1: Information about the studied plants.

<table>
<thead>
<tr>
<th>Plant species (family)</th>
<th>Part used</th>
<th>Code</th>
<th>Location</th>
<th>Voucher specimen No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia herba-alba Asso. (Asteraceae)</td>
<td>Aerial parts</td>
<td>AHA</td>
<td>Meskiana (Oum el-Bouaghi)</td>
<td>AB04 – 103</td>
</tr>
<tr>
<td>Marrubium vulgare L. (Labiateae)</td>
<td>Flowering tops</td>
<td>MVU</td>
<td>Chataba – Ain Smara (Constantine)</td>
<td>AB04 – 112</td>
</tr>
<tr>
<td>Pinus pinaster L. (Pinaceae)</td>
<td>Bark</td>
<td>PPI</td>
<td>Djebel el-Ouahch (Constantine)</td>
<td>AB04 – 115</td>
</tr>
</tbody>
</table>

Microorganisms tested

The strains tested were provided by the bacteriology laboratory of the University Hospital of Constantine: Staphylococcus aureus (Micrococccaceae), Escherichia coli (Enterobacteriaceae), Proteus vulgaris (Enterobacteriaceae) and Pseudomonas aeruginosa (Pseudomonadaceae).

Preparation of the plant extract

The plant material was cut and dried in the shade at room temperature (25 °C) for three weeks. Finally, the various organs (aerial parts, flowering tops and bark) were sprayed to the mill to a fine powder. The preparation of this extract is made according to the method described by Zirihi et al. (2003). 100 g of plant powder were stirred vigorously on a magnetic plate heater for 24 hours in a methanol : water (70: 30 v / v, 500ml x3 times). After filtration, the filtrates were combined and then concentrated under reduced vacuum at a temperature not exceeding 45 °C. Then the aqueous residue obtained was lyophilized using a freeze dryer.

Antibacterial activity

Diffusion Method in a solid medium

The assay was conducted as described by Bauer et al. (1966) and adopted by Ananil et al. (2000). From colonies of 18 to 24 h, a bacterial suspension is carried out in sterile distilled water for each strain. The turbidity of this suspension is adjusted to 0.5 Mc Farland and then diluted 1/100. This gives an inoculum estimated at $10^6$ colony forming units per milliliter (Muc/ml). This inoculum is seeded by flooding on Petri dishes containing Mueller-Hinton agar (NCCLS 2002).

Concentrated stock solutions of 25 mg/ml were prepared, then sterilized by autoclaving
(121°C for 15 minutes). Discs of Whatman filter paper 6 mm in diameter were impregnated with 25 µl of the stock solution. Finally we prepare disks impregnated with sterile distilled water and solvent methanol (v/v). This last category of disk served as negative controls. Disks of ampicillin (10 g) were also used as reference antibiotic positive control.

The Petri dishes were first left for 1 h at room temperature for a pre-release of substances before being incubated at 37 °C in an oven for 24 h. The antibacterial activity was determined by measuring the diameter of the inhibition zone around each disk.

**Diffusion Method in liquid medium**

Determination of Minimum Inhibitory Concentration (MIC) for each plant extract was prepared by the method of double dilution, a sterile concentration, ranging from 25 to 0.024 mg/ml with distilled water. Also prepared for each bacterial strain, an inoculum with a turbidity adjusted to 0.5 Mc Farland (10⁸ cfu / ml) and returned a 10⁶ cfu / ml in Mueller-Hinton broth double concentrated. Then added in hemolysis tubes, 1 ml of each concentration and 1 ml of bacterial inoculum. The range of concentration of each extract is then subjected to a dilution in half and spread as follows: 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.097, 0.048, 0.024 mg/ml. Also prepared a growth control tube containing 1 ml of sterile distilled water and 1 ml of solvent. The concentration range is seeded incubated at 37 °C for 24 hours. After incubation, examine bacterial growth in each tube, resulting in turbidity.

The MIC of an extract versus a given strain will be the smallest concentration showing no visible growth of organism (NCCLS 1999).

**Statistical Analysis**

Results are expressed as mean of three replicates with standard deviation (mean ± SD), they were analyzed by Origin software, version 6.0 by applying the Student t-test to compare populations. Values were considered significant at p < 0.05.

### Results and Discussion

The results showed a significant antibacterial activity of plant extracts PPI, MUV and AHA against bacterial strains tested.

**Table 2: Inhibitory effect of vegetable extracts on Staphylococcus aureus growth.**

<table>
<thead>
<tr>
<th>Staphylococcus aureus</th>
<th>D₁ (mm)</th>
<th>D₂ (mm)</th>
<th>D₃ (mm)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI*</td>
<td>15</td>
<td>10</td>
<td>12</td>
<td>12.33 ±2.52³³</td>
</tr>
<tr>
<td>MUV*</td>
<td>17</td>
<td>15</td>
<td>18</td>
<td>16.66 ±1.53³³</td>
</tr>
<tr>
<td>AHA*</td>
<td>10</td>
<td>15</td>
<td>16</td>
<td>13.66 ±3.21³³</td>
</tr>
<tr>
<td>CONT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>00 ±0</td>
</tr>
<tr>
<td>AMPIC*</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32± 0</td>
</tr>
</tbody>
</table>

PPI: Pinus pinaster, MUV: Marrubium vulgare, AHA: Artemisia herba alba, CONT: Control [Methanol/Water (70 %, 30 %)], AMPIC : Ampicillin, D 1, 2, 3 : Inhibition diameter measured, SD: standard deviation, ☑ : 25 mg/ml, • :10 µg/disc, ⊱ ⊱(P<0.05) [vegetable extracts vs control].

At a concentration of 25 mg/ml of the plant extracts PPI, MUV, AHA and AMPIC at 10 mg/disc have a significant effect on the strain of Staphylococcus aureus tested against the control (Table 2). In the presence of extracts of PPI (P <0.05), MUV (P <0.05) and AHA (P <0.05) Staphylococcus aureus has developed diameters of inhibition of 12.33, 16.66 and 13.66 mm respectively. No significant differences were noted between the extracts of PPI, MUV and AHA.

**Table 3: Inhibitory effect of vegetable extracts on Escherichia coli.**

<table>
<thead>
<tr>
<th>Escherichia coli</th>
<th>D₁ (mm)</th>
<th>D₂ (mm)</th>
<th>D₃ (mm)</th>
<th>Mean ± SD</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI*</td>
<td>18</td>
<td>11</td>
<td>14</td>
<td>14.33 ±2.51♀♀,♀♀</td>
<td>0.2</td>
</tr>
<tr>
<td>MUV*</td>
<td>13</td>
<td>16</td>
<td>17</td>
<td>15.33 ±2.08♀♀</td>
<td>0.2</td>
</tr>
<tr>
<td>AHA*</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>7.33a ±5.0♀♀</td>
<td>0.0</td>
</tr>
<tr>
<td>CONT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>00a 0</td>
<td>0.0</td>
</tr>
<tr>
<td>AMPIC*</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25a 0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

PPI: Pinus pinaster, MUV: Marrubium vulgare, AHA: Artemisia herba alba, CONT: Control [Methanol/Water (70 %, 30 %)], AMPIC : Ampicillin, D 1, 2, 3 : Inhibition diameter measured, SD: standard deviation, MIC : Minimum Inhibitory Concentration, ☑ : 25 mg/ml, • :10 µg/disc, ⊱ ⊱(P<0.05) [vegetable extracts vs control], ⊱ ⊱(P <0.05) [extracts vs control], ⊱ ⊱(P<0.01) [extrait PPI vs AHA], [[(P <0.01) [MUV vs AHA]].
At a concentration of 25 mg/ml of plant extracts PPI, MUV, AHA and AMPIC at 10 mg/disc have a significant effect on the strain of *Escherichia coli* tested against the control (Table 3). In the presence of extracts of PPI (P <0.05), MUV (P <0.05) and AHA (P <0.05) *Escherichia coli* has developed, in 24 hours, the inhibition diameters of 14.33, 15.33 and 7.33 mm respectively. A significant difference in PPI extract (P <0.05) compared to the AHA extract and significantly difference of MUV extracts (P <0.01) compared to the AHA extract, by cons no significant differences between the extracts of PPI and MUV were noted.

**Table 4:** Inhibitory effect of vegetable extracts on *Pseudomonas aeruginosa*.

<table>
<thead>
<tr>
<th></th>
<th>D₁ (mm)</th>
<th>D₂ (mm)</th>
<th>D₃ (mm)</th>
<th>Mean ± SD</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI</td>
<td>8</td>
<td>10</td>
<td>7</td>
<td>8.33 ±1.53*</td>
<td>-</td>
</tr>
<tr>
<td>MUV</td>
<td>15</td>
<td>10</td>
<td>9</td>
<td>11.33 ±3.21**</td>
<td>-</td>
</tr>
<tr>
<td>AHA</td>
<td>20</td>
<td>18</td>
<td>19</td>
<td>19±1.00**</td>
<td>0.1</td>
</tr>
<tr>
<td>CONT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0</td>
<td>-</td>
</tr>
<tr>
<td>AMPIC</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24±0</td>
<td>-</td>
</tr>
</tbody>
</table>

PPI: *Pinus pinaster*, MUV: *Marrubium vulgare*, AHA: *Artemisia herba alba*, CONT: Control [Methanol/Water (70 %, 30 %)], AMPIC: Ampicillin, D 1, 2, 3: Inhibition diameter measured, SD: standard deviation, MIC: Minimum Inhibitory Concentration, *P<0.05* [vegetable extracts vs control], **P<0.01** [AHA vs PPI & MUV].

At a concentration of 25 mg/ml of MUV extract and AMPIC to 10 mg / disc have a significant effect on the strain of *Proteus vulgaris* tested against control, however any significant difference was noted between the PPI and AHA extracts compared with the control (Table 5). In the presence of the extract of MUV (P <0.05), *Proteus vulgaris* developed diameters of inhibition within 24 h of 10 mm. A significant difference of the extract MUV (P<0.01) compared to extracts PPI and AHA, for against any significant difference between the PPI and AHA extract.

**Table 5:** Inhibitory effect of vegetable extracts on *Proteus vulgaris*.

<table>
<thead>
<tr>
<th></th>
<th>D₁ (mm)</th>
<th>D₂ (mm)</th>
<th>D₃ (mm)</th>
<th>Mean ± SD</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0</td>
<td>-</td>
</tr>
<tr>
<td>MUV</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>10±1.00*</td>
<td>0.05</td>
</tr>
<tr>
<td>AHA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0</td>
<td>-</td>
</tr>
<tr>
<td>CONT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0±0</td>
<td>-</td>
</tr>
<tr>
<td>AMPIC</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25±0</td>
<td>-</td>
</tr>
</tbody>
</table>

PPI: *Pinus pinaster*, MUV: *Marrubium vulgare*, AHA: *Artemisia herba alba*, CONT: Control [Methanol/Water (70 %, 30 %)], AMPIC: Ampicillin, D 1, 2, 3: Inhibition diameter measured, SD: standard deviation, MIC: Minimum Inhibitory Concentration, *P<0.05* [vegetable extracts vs control], **P<0.01** [MUV vs PPI & AHA].

Given the above results, we have highlighted that the extract of AHA shows a significant effect against *Pseudomonas aeruginosa* and MUV extract against *Proteus vulgaris*. Extracts PPI, MUV and AHA have a similar effect on these strains *Staphylococcus aureus* and *Escherichia coli*.

The effectiveness of the aerial part of AHA against *Pseudomonas aeruginosa* may be explained by the dominance of sesquiterpene lactones; in fact, bacteria have sensitivity to these metabolites (Laid 2008).

The sensitivity of the flowering tops of MUV against *Proteus vulgaris* and *Escherichia coli* is probably due to the presence of terpenoids reported from this plant (Schlemper et al. 1996; Meyre-Silva et al. 2005). The activity of the bark extract of PPI against *Staphylococcus aureus* and *Escherichia coli* may be attributed to polyphenols content (> 90%) as reported by some authors (Royer et al. 2010).
Conclusion

The present study has highlighted the antibacterial properties of extracts of the three plants studied. This important activity has been demonstrated in *Escherichia coli* with an MIC of 0.195 mg/ml. The hydroalcoholic extract AHA revealed significant activity with MIC of 0.097 mg/ml against *Pseudomonas aeruginosa*. An MIC equal to 0.048 mg/ml was observed with the hydroalcoholic extract of MUV against *Proteus vulgaris*.

References


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