Investigation of the Antibacterial Effect of Native Peganum harmala, Mentha pulegium and Alcea rosea Hydro-alcoholic Extracts on Antibiotic Resistant Streptococcus pneumoniae and Klebsiella pneumonia Isolated from Baku, Azerbaijan

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Background: Pneumonia and respiratory tract infections, is associated with high mortality and complications in humans. Current antibiotics are used to treat this infectious disease, but may lead to many problems such as unwanted side effects and resistance to antibiotics. This study investigated the antibacterial activity of the hydro alcoholic extracts of the native medicinal plants Peganum harmala, Mentha pulegium and Alcea rosea, in Baku, as a natural alternative to antibiotics, on antibiotic-resistant Streptococcus pneumoniae and Klebsiella pneumoniae, the main bacteria that cause pneumonia.

Materials and Methods: Antibacterial activity of the hydro alcoholic extracts of medicinal part of these plants was evaluated by the disk diffusion susceptibility test method and the broth dilution test method on bacteria.

Results: The rate of MIC of P. harmala, M. pulegium and A. rosea extracts of S. pneumoniae were 80, 110 and 375μgL⁻¹ respectively and for K. pneumoniae were 150, 230 and 680μgL⁻¹ respectively, and the rate of MBC were 120, 165 and 550μgL⁻¹ for S. pneumoniae and 210, 315 and 800μgL⁻¹ for K. pneumonia respectively. The maximum amount of inhibition zone diameter in50μgL⁻¹ concentration of P. harmala, M. pulegium and A. rosea extracts for S. pneumoniae were 21.2mm, 17.2mm, 6.9mm and for K. pneumonia were 10.1mm, 8.1mm, 3.2mm respectively.

Conclusion: This work showed that substances in the hydro-alcoholic extracts of medicinal plants prevented the growth of bacteria. So these plants with having effective ingredients can be used as an affordable and available source for medicinal purposes.

Keywords: Pneumonia, Peganum harmala, Mentha pulegium, Alcea rosea, Baku, Azerbaijan

1. Background

Respiratory diseases include pathological conditions affecting the organs and tissues that are part of gas exchange process in higher organisms, and involves both upper and lower regions consisting of the nose, pharynx, and other structures such as the middle ear and sinuses. The lower portion of the system comprises of the respiratory tubes and alveoli of the lungs, the excessive moisture, and rich supply of nutrients in this area, makes it ideal for infections to grow. Streptococcus pneumoniae, a diplococcus, gram-positive, alpha-hemolytic with polysaccharide capsule, and Klebsiella pneumoniae, gram-negative, non-motile, encapsulated, lactose fermenting, rod shaped bacteria, are the main facultative bacteria which causes pneumonia in humans. Respiratory tract infections caused by these pathogenic bacteria are associated with high mortality and complications (1-3).

Although antibiotics are used to treat common infectious diseases, but these treatments are associated with many problems such as unwanted side effects and development of resistance. Plants can be considered as a substitute for chemical drugs, since they have fewer side effects. Today, extensive researches have been carried out in traditional medicine, including herbal medicine in different fields of medical sciences (4).

Investigations on the essential oil and extract of Mentha pulegium (English), belonging to family Lamiaceae, shows significant impacts in preventing the growth of several species of spoilage and pathogenic agents due to its anti-bacterial, anti-inflammatory and anti-spasmodic activity (5-7). Alcea rosea (English name= Russian Hollyhock), belonging to family Malvaceae, is herbaceous and perennial plant that reaches a height of about 2 meters. The leaves are wide, serrated, like the heart. It has large yellow flowers (8). Peganum harmala (English name= harmala), from Zygophyllaceae family, has been one of the popular herb in traditional medicine and has been effective due to its anti-bacterial, anti-fungal, anti-parasitic, sleeping, sweating, aborting the fetus, anti-cancer, immune system stimulating and mono amino oxidase enzyme inhibitors (9).

2. Objectives

This study aimed to determine the antibacterial in vitro effects of native Mentha pulegium, Alcea rosea and Peganum harmala hydro-alcoholic extracts as natural alternatives to antibiotics, on K. pneumoniae and S. pneumoniae, isolated from Baku, Azerbaijan.

3. Materials and Methods

3.1. Preparation of plant extracts

Hydro-alcoholic extraction was performed by maceration method. First of all, 50g of the leaves of pennyroyal, the seeds of harmala and the flower of Russian Hollyhock were prepared and dried in oven and then powdered. The powders of plants were poured into the flasks, separately. For each sample 1500mL solvent [50% Water and 50% ethanol (96%)] was added, so as to cover the powder completely. Then, the flasks were covered with aluminum foil. Flasks were shaken for 48 h at 90rpm. Then, the homogeneous solutions were filtered by filter paper and finally rotary evaporator was used to separate solvent from the extract. The purified extracts were stored in the refrigerator for further experiment (4).
3.2. Preparation of microorganisms
Antibiotic-resistant bacteria were prepared from cultures isolated in several hospitals in Baku. The lyophilized standard strains S. pneumoniae ATCC49619 and K. pneumoniae ATCC13883 were prepared from ATCC reference center. In order to preparation of bacteria from lyophilized samples, first samples were cultured in a nutrient broth overnight at 30-35°C. After the turbidity samples were isolated and purified on blood agar and MacConkey agar, respectively (10, 11).

3.3. Antibacterial susceptibility testing
Antibacterial activity of extracts was evaluated by the agar-disk diffusion method. Overnight bacterial suspensions were first adjusted to 0.5 McFarland standard turbidity. The mixtures of bacteria (one loop full) and dilutions of the extracts were incubated at 37°C for 24 hours in 1mL Muller Hinton broth. After this period, the concentration of the first tube without turbidity was considered as MIC. In the next stage, the contents (one swab) of the non-growth tubes were cultured in Muller Hinton agar plates. After incubation at 37°C for 24 hours the first non-growth plate was considered as minimum bactericidal concentration (MBC). Controls were as follows: i) Medium and extract without bacteria: non-growth, ii) medium and distilled water with bacteria: growth, iii) medium and chlorhexidine (positive control) with bacteria: non-growth (3, 10, 11, 13).

Moreover, binary combination as well as the sum of all three plants was prepared with an equal volume of dilution 500μg/mL\(^{-1}\) in order to observe the synergy of plants and tested with mentioned similar experiments.

4. Results
The results of antibacterial activity of native M. pulegium, A. rosea and P. harmala hydro-alcoholic extracts on antibiotic-resistant K. pneumonia and S. pneumoniae by the disk diffusion agar susceptibility test and broth macrodilution test methods is presented below (P-value <0.05). The results of the inhibition zone diameter obtained from effect of different concentrations of hydro-alcoholic extracts of medicinal plants on antibiotic-resistant bacteria are shown in Table1. The standard diameter of inhibition zone of antibiotics ampicillin, ciprofloxacin, nitrofurantoin and vancomycin are 26≤, 21≤, 17≤ and 12≤ mm for sensitive S. pneumoniae ATCC49619 and that of ciprofloxacin, tetracycline, amoxicillin and nitrofurantoin are 21≤, 19≤, 18≤ and 17≤ mm for sensitive K. pneumonia ATCC13883. The amount of all controls was Ø=0.0mm.

The results of broth macrolidilution test method is presented in Table 2 which shows the difference between rates of MIC and MBC with level of significance (P-value <0.05) native medicinal plant extracts in Baku on S. pneumoniae and K. pneumoniae. Table 3 shows the results of the different combined effect of plant extracts on bacteria.

### Table 1. The diameter of non-growth zone of different concentrations of plants hydro-alcoholic extracts on antibiotic-resistant bacteria.

<table>
<thead>
<tr>
<th>Concentration (μg/mL(^{-1}))</th>
<th>P. harmala</th>
<th>M. pulegium</th>
<th>A. rosea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. pneumonia</td>
<td>K. pneumonia</td>
<td>S. pneumonia</td>
</tr>
<tr>
<td>62.5</td>
<td>7.1±0.7</td>
<td>3±4.0</td>
<td>5.9±0.9</td>
</tr>
<tr>
<td>125</td>
<td>11±1±8</td>
<td>6±8±1.2</td>
<td>10±9±2.2</td>
</tr>
<tr>
<td>250</td>
<td>16±2±2</td>
<td>8±9±1.8</td>
<td>13±2±6.6</td>
</tr>
<tr>
<td>500</td>
<td>27±2±1.9</td>
<td>10±1±2.2</td>
<td>17±2±8</td>
</tr>
</tbody>
</table>

### Table 2. The amount of (MIC) and (MBC) of medicinal plants extracts on bacterial species (μg/mL\(^{-1}\)).

<table>
<thead>
<tr>
<th>P. harmala</th>
<th>M. pulegium</th>
<th>A. rosea</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumonia</td>
<td>K. pneumonia</td>
<td>S. pneumonia</td>
</tr>
<tr>
<td>MIC</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>MBC</td>
<td>210</td>
<td>110</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>MIC</td>
<td>MIC</td>
</tr>
<tr>
<td>MBC</td>
<td>230</td>
<td>315</td>
</tr>
<tr>
<td>S. pneumonia</td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td>MBC</td>
<td>680</td>
<td>800</td>
</tr>
</tbody>
</table>

### Table 3. The mean of concentration 500 μg/mL\(^{-1}\) of combined with an equal volume of medicinal plants hydro-alcoholic extracts on the non-growth zone (mm), MIC and MBC (μg/mL\(^{-1}\)).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>P. harmala and M. pulegium</th>
<th>P. harmala and A. rosea</th>
<th>M. pulegium and A. rosea</th>
<th>All three plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumonia</td>
<td>Non-growth zone (mm)</td>
<td>22±2±1</td>
<td>18±2±6</td>
<td>14±8±4</td>
</tr>
<tr>
<td>MIC (μg/mL(^{-1}))</td>
<td>95</td>
<td>100</td>
<td>135</td>
<td>95</td>
</tr>
<tr>
<td>MBC (μg/mL(^{-1}))</td>
<td>135</td>
<td>150</td>
<td>185</td>
<td>135</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>Non-growth zone</td>
<td>9±2±1</td>
<td>7±9±1</td>
<td>6±1±5</td>
</tr>
<tr>
<td>MIC</td>
<td>160</td>
<td>170</td>
<td>280</td>
<td>165</td>
</tr>
<tr>
<td>MBC</td>
<td>230</td>
<td>250</td>
<td>390</td>
<td>255</td>
</tr>
</tbody>
</table>

5. Discussion

The use of herbs at various eras and nations has played important role in the treatment of different diseases (2, 4, 5, 7, 9, 11). The native medicinal plants extract is provided as a source of antimicrobial compounds. Hence, in this work the antibacterial effects of native M. pulegium, A. rosea and P. harmala, in Baku; hydro-alcoholic extracts were investigated on bacteria that cause pneumonia. In antibacterial susceptibility test the inhibitory diameters raised with increasing of extracts concentration, significantly; the controls in these experiments had not any antimicrobial effect. The maximum effects involved in 500μg mL⁻¹ that were 27.2mm (P. harmala), 13.2mm (M. pulegium) and 6.9mm (A. rosea) in S. pneumoniae and 10.1mm (P. harmala), 8.1mm (M. pulegium) and 3.2mm (A. rosea) in K. pneumonia. The rates of MIC and MBC of extracts were 80μg mL⁻¹ and 120μg mL⁻¹ (P. harmala), 110μg mL⁻¹ and 165μg mL⁻¹ (M. pulegium) and 375μg mL⁻¹ and 550μg mL⁻¹ (A. rosea) in S. pneumoniae, 150μg mL⁻¹ and 210μg mL⁻¹ (P. harmala), 230μg mL⁻¹ and 315μg mL⁻¹ (M. pulegium) and 680μg mL⁻¹ and 800μg mL⁻¹ (A. rosea) in K. pneumoniae, respectively. The appropriate concentration of P. harmala extract against S. pneumoniae could be really effective in comparison with antibiotics; although other extracts, contrary to local belief, had minor effects. Observations showed that the combination of these extracts did not create special synergy effect. Sensitivity of S. pneumoniae against all 3 plant extracts was the most prominent compared with K. pneumonia. The P. harmala had the greatest impact; and in the next step were M. pulegium and A. rosea, respectively.

Essential oils obtained by hydrodistillation from the leaves of Mentha pulegium L. and Mentha rotundifolia (L.) Huds. from Uruguay have been analyzed by GC-FID and GC-MS. Oxygen-containing monoterpens had the main group of constituents in both oils. Pulegone, isomenthone and menthone comprised the major components in the oil of M. pulegium, whereas piperitone oxide and (Z)-sabinene hydrate included the major ones in M. rotundifolia. Enantiomerically pure (+)-menthone, (+)-isomenthone, (+)-isomenthol, (-)-menthol and (+)-pulegone had been detected by multidimensional gas chromatography in the case of M. pulegium oil (12). Harmala as a plant with medicinal properties of having active compounds such as harmaline, harmine and harmalol alkaloids have traditionally been considered. Most studies on the therapeutic properties of the harmala seed have been associated with parasitic infections. Based on findings alcoholic extract of seeds of Peganum showed the growth measurable inhibitory and fatal activity on yeast Candida measurable (13). The research has suggested that the ethanol extract of P. harmala, as an antioxidant, reduces free radicals as a result of the use of silver nanoparticles (9). This work was also concluded that the appropriate concentration of P. harmala extract against S. pneumoniae could be really effective than antibiotics; although the other extracts, contrary to local belief, had minor effects. Observations showed that the composition of these extracts did not create a special synergy effect.

6. Conclusion

Each year in the mountainous areas in Baku, the large amount of variety of herbs grew self-propelled, particularly our plants in this work, and later destroyed without any use. With regard to the effective ingredients of these plants in our study and other researches in the growth inhibition of bacteria, simply, could be exploited as an affordable and available source of biopharmaceuticals.

Conflict of Interests

Authors declare they have no conflict of interests.

Acknowledgments

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Authors’ Contributions

All authors contributed extensively to the work presented in this paper.

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