Synergistic effect of mountain honey and *Argemone mexicana* plant on bacteria associated with wound infections

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Abstract: Fortyfive wound specimens were collected from patients suffering from wound infections and taken from various hospitals in Ibb city, Yemen. The study was to determine synergic antibacterial activity of between mountain honey and *Argemone mexicana* plant. Isolation, identification of bacterial isolates and antibiotic sensitivity test were done. Agar-disc and agar-well diffusion method were carried to determine antibacterial activity of honey, *Argemone mexicana* plant and a mixture of them against bacterial isolates. Out of 45 specimens, 29 (64.4%) gave positive cultures. *Staphylococcus aureus* was the predominant bacterial pathogens with percentage (72.4%) followed by *Pseudomonas aeruginosa* (17.2%) and *Staphylococcus epidermidis* (10.4%). The highest concentration (70%) of honey was more effective than other concentrations and crude milky sap of *A.mexicana* plant had higher antibacterial activity against pathogenic bacteria than any milky sap dilutions. The mixture of honey and *A.mexicana* plant gave an excellent inhibitor effect against bacterial growth. Mixture of honey (70%) and crude milky sap of *A.mexicana* revealed higher antibacterial activity against pathogenic bacteria comparing with honey and plant crude extract alone.

Keywords: *Staphylococcus spp; Pseudomonas aeruginosa; Argemone mexicana; honey; wound infection*

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health and all kinds of antibiotics, including the major last-resort drugs, as the frequencies of resistance are increased worldwide [1]. The increase in consumer use of complementary medicines has prompted an increasing interest in traditional and nonconventional medical treatments. One treatment that has received much interest is honey. Honey has a long tradition of use within various medical systems and over the past decade several research groups have focused their attention to this product. Whereas honey has a number of uses therapeutically and as a food preservation agent, it is most well known for its beneficial actions within the wound environment. Honey maintains a moist wound environment that promotes healing, and its high viscosity helps to provide a protective barrier to prevent infection. In addition, the mild acidity and low-level hydrogen peroxide release assists both tissue repair and contributes to the antibacterial activity of honey. This antibacterial activity is a major factor in promoting wound healing where infection is present [2]. There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics [3].

Medicinal plants are important to the health of many peoples in developing countries. According to World Health Organization (WHO), approximately 80% of people in developing countries still rely on traditional medicine for their primary health care needs. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharamgological activities, have been extensively investigated as a source of medicinal agents. The plant *Argemone mexicana* belongs to family Papaveraceae is commonly known as Mexican poppy or prickly poppy is used as medicinal herbs [4]. It is a prickly, glabrous, branching herb with yellow juice and showy yellow flowers [5].

The aim of this work included detection of antibacterial activity of honey and *Argemone mexicana* plant against bacteria associated with wound infection, also it focused on the synergic effect between honey and plant extract.

MATERIALS AND METHODS

Bacteriological study

A total of 45 specimens were collected from patients suffering from wound infections and taken from various hospitals in Ibb city, Yemen. By using sterilized cotton swabs, they were placed on blood agar and MacConkey agar, and then incubated aerobically at 37°C for 18 hr. For the isolation and identification of isolates, each specimen was identified depending on the morphology, cultural characteristics and biochemical activity of bacteria comparing with honey and the antibacterial activity of *Argemone mexicana* plant and a mixture of them against bacterial isolates.
reaction [6]. The antibiotic susceptibility patterns of all isolated were determined by the modified Kirby-Bauer disk diffusion technique [7]. The diameter of the zone of inhibition produced by each antibiotic disc was measured and recorded, and the isolates were classified as “resistant” or “sensitive” based on the standard interpretative according to CLSI (formerly NCCLS) guidelines.

Evaluation of activity:

**Honey:**
Mountain honey used in this study was diluted with sterile distilled water with the following concentrations; 10, 20, 40, 50 and 70% (v/v). Agar-Well Diffusion (100µ for each well) was applied to determine the antibacterial activity [8].

**Argemone mexicana plant**
Milky sap extract was obtained from cutting the stem of the plant and collected in sterile container by using sterile syringe. Agar-Disc Diffusion method and serial twofold dilutions of the crude extract (100 μl per disc) by using sterile distilled water to evaluate the antibacterial activity [9].

**Mixture of honey and A.maxicana plant**
The two methods that mentioned above (Agar-well diffusion and Agar-disc diffusion method) were used to determine antibacterial activity of this mixture. Crude milky sap and (70%) concentration of honey were mixed well in equal volumes, and then the two methods were applied against pathogenic bacteria.

**RESULTS and DISCUSSION**
Out of A total of 45 wound specimens, 29 specimens (46.4%) yielded positive culture. The bacterial types were distributed into 21 isolates with a ratio (72.4%) belonged to *Staphylococcus aureus*, 5 isolates (17.2%) belonged to *Pseudomonas aeruginosa* and 3 isolates (10.4%) belonged to *Staphylococcus epidermidis*. Exposed subcutaneous tissue provides a favorable medium for microorganisms to contaminate and colonize. The conditions become optimal for microbial growth if the involved tissue is devitalized and/or the host immunity is compromised [10]. Wound infection may be accidental or postoperative and many organisms can cause sepsis. The source of infection may be exogenous (from environment) or endogenous (from commensal of body). *Staphylococcus aureus* is the most frequently isolated wound pathogen [11]. This bacterium possesses large number of toxins and enzymes which contribute to the ability of the organism to overcome the body's defense and to invade survive and produce disease in the host. It's an opportunistic pathogen in that it causes infection most commonly at sites of lowered host resistance, e.g. damaged skin [12].

*Staphylococcus aureus* and *Pseudomonas aeruginosa* are among the most common organisms isolated from both acute and chronic wounds of various etiologies. Their prevalence has been demonstrated in surgical site infections as well as in the military setting where they have been attributed to causing infections of combat related injuries such as penetrating trauma and burn wounds [13].

The results of antibiotic sensitivity test were shown in Tables (1,2). Multiple antibiotic resistances among bacterial isolates were observed and this may be belonged to the intrinsic or acquired resistance, so antibiotic resistance is becoming an extremely serious public health problem [14], emphasizing the need of using antibacterial agent other than antibiotics.

### Table 1: Percentages of resistance and susceptibility of Bacterial isolates for antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Staphylococcus epidermidis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R%</td>
<td>S%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>77.7</td>
<td>22.3</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Cefadroxil</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>
The efficiency of crude milky sap to inhibit bacterial growth was assessed. No adverse effects were observed.

The importance of crude milky sap in inhibiting bacterial growth is attributed to its high osmolarity, acidity (low pH) and content of hydrogen peroxide and non-peroxide components, i.e., the presence of phytochemical components like methylglyoxal. Honey is an effective broad-spectrum antibacterial agent that has no adverse effects on wound tissues, therapeutic use of honey is often referred to as “alternative” medicine.

Evaluation of antibacterial activity of *Argemone mexicana*

The antibacterial activity of the *Argemone mexicana* plant was studied against the bacterial isolates *Staph. aureus, Staph. epidermidis* and *Pseudomonas aeruginosa* using agar disc diffusion method (Figure 2). Table 4 illustrates the broad spectrum antimicrobial results of plant against the clinical isolates. The results confirmed the efficiency of crude milky sap to inhibit growth of pathogenic bacteria and the antibacterial activity of crude milky sap was decreased while the dilution was increased. Higher IZD for *Staph. aureus* were (25, 20, 20, 19, 15, 7 mm) for crude extract and dilutions (1:2, 1:4, 1:8, 1:16, 1:32); while for *Staph. epidermidis* were (25, 20, 20, 17, 8, 7 mm). The antibacterial activity against *P. aeruginosa* was lowered comparing with Staphylococcus species, higher IZD were (15, 10 mm) for the crude extract and dilution 1:2, and the other dilutions had no effect on these isolates. *Argemone mexicana* plant confirmed that it has antibacterial activity against Gram +ve and Gram −ve bacteria. Sanguinarine, the main *Argemone* alkaloid, has a strong bacteriocidal effect on Gram-positive bacteria. The antimicrobial and physiological effects of sanguinarine suggest that it may confer protection against diverse pathogens [17]. *Argemone mexicana* is widely known around the world for its medicinal property.
Table 4: Effect of *Argmone maxicana* plant against bacteria evaluated by determination of inhibition zone diameter.

<table>
<thead>
<tr>
<th>Bacterial Types</th>
<th>Milky sap dilution</th>
<th>Crude</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M I Z mm (IZD)mm</td>
<td>M I Z mm (IZD)mm</td>
<td>M I Z mm (IZD)mm</td>
<td>M I Z mm (IZD)mm</td>
<td>M I Z mm (IZD)mm</td>
<td>M I Z mm (IZD)mm</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11.5(25)</td>
<td>8.6(20)</td>
<td>7.4(20)</td>
<td>6.4(19)</td>
<td>5.6(15)</td>
<td>5.1(7)</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>21.6(25)</td>
<td>18.3(20)</td>
<td>17.20)</td>
<td>10.3(17)</td>
<td>6.6 (8)</td>
<td>5.6(7)</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>9(15)</td>
<td>6.8(10)</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
</tbody>
</table>

M I Z: Mean of inhibition zone diameters. IZD: Inhibition zone diameter. mm: millimeter. NE: Not effective.

Table 5: Effect of mixture (honey and *A. maxicana* plant) against bacteria evaluated by determination of inhibition zone diameter.

<table>
<thead>
<tr>
<th>Bacterial types</th>
<th>Disc method</th>
<th>Hole method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M I Z mm (IZD)mm</td>
<td>M I Z mm (IZD)mm</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>14.1(25)</td>
<td>11.2(20)</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>20.6(25)</td>
<td>11(12)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>12.2(16)</td>
<td>9.8(12)</td>
</tr>
</tbody>
</table>

Figure-1 Effect of various concentrations of honey on *Staph. aureus*.

Figure-2 Effect of various dilution of *Argemone maxicana* on *Staph. aureus*. 
**Synergic effect of Honey and Argemone mexicana**

Mixture of honey and crude milky sap revealed antibacterial activity against bacterial isolates and the agar-disc diffusion method gave the best results comparing with agar-well diffusion method, (Table 5). The antibacterial activity of mixture of honey and milky sap of *Argemone mexicana* was higher than the activity of honey and milky sap separately (Figure 3). The mean of inhibition zone diameters (MIZ) by using agar-disc method of mixture for the bacterial isolates *Staph. aureus*, *Staph. epidermidis* and *P. aeroginosa* were (14.1, 20.6, 12.2) mm respectively, while MIZ of the same isolates were (13.6, 15.6, 10.8) mm for honey with conc. 70% and (11.5, 21.6, 9) mm for crude milky sap. We observed that there was synergic effect between honey and crude extract of plant according to increase in the diameters of inhibition zone. Agar-disc diffusion method revealed better results than agar-well diffusion method, this may be due to the highest diffusion of the mixture when using disc on the surface of agar comparing with well. In this study, mean of inhibition zones of mixture of mountain honey and *Argemone mexicana* against all tested pathogens was greater than those of the two substances alone. The synergic antimicrobial effect of *Argemone mexicana* extract and honey mixture against all pathogenic bacteria was found to be greater than other antimicrobial agents. That may be the reason why the local society or community widely uses this plant and mountain honey to treat different pathogenic bacterial infections associated with skin. This study was relatively resembled with other researches like [15].

**CONCLUSIONS**

The results confirmed the efficiency of honey and *Argemone mexicana* to inhibit the growth of pathogenic bacteria. Synergic effect of mixture of honey conc. 70% and crude milky sap of *A. mexicana* revealed higher antibacterial activity comparing with honey and *Argemone mexicana* alone.

**References**


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