Antimicrobial Activity of Extracts of some Plants Collected from the Kingdom of Saudi Arabia

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Abstract. The antimicrobial activity of twenty wild plants growing in the western regions of the Kingdom of Saudi Arabia was tested. The methanolic extracts from the aerial parts was determined using the agar diffusion method. Eight microorganisms were used in this study, namely, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Sarcina lutea, Bacillus subtilis, Mycobacterium phlei and Candida albicans. The results revealed that thirteen extracts exhibited a significant broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. On the other hand, seven extracts showed only a narrow spectrum activity against Gram-positive bacteria. All the tested extracts with the exception of one plant showed a significant anti-mycobacterium effect, while they have variable antifungal effects.

Keywords: Antimicrobial activity, Antibacterial, Antifungal, Wild plants.

Introduction
Over the past decade, herbal medicine has become a topic of augmented global importance, having impacts on both world health and international trade. In terms of world health, traditional medicinal plants continue to play a central role in the healthcare systems of large proportions of the
world's population\textsuperscript{[1]}. This is particularly true in developing countries, where traditional systems of medicine have a long and uninterrupted history of use. Recognition and development of the medicinal and economic benefits of traditional medicinal plants is on the increase in both developing and industrialized countries, although it varies greatly from region to region\textsuperscript{[2]}. Since their discovery, antimicrobial drugs have proved remarkably effective for the control of bacterial infections. However, it was soon evident that bacterial pathogens were unlikely to surrender unconditionally, because some pathogens rapidly become resistant to many of the first discovered effective drugs\textsuperscript{[3]}. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used as antimicrobials and may have a significant clinical value in the treatment of resistant microbial strains\textsuperscript{[4]}.

**Materials and Methods**

**Plant Materials**

Twenty plant species were collected from Jeddah-Al-Taif Road, Al-Hadda and Al-Taif district. The collected plants were identified by Dr. Abdulaziz Fayed, Professor of Plant Taxonomy, College of Science, Assuit University, Egypt. Plants were air-dried at room temperature, powdered and stored in dark colored bottles.

**Preparation of Crude Extracts**

Fifty grams of each plant material was extracted by reflux twice with methanol (150 ml) for 15 min followed by evaporation of combined extracts using rotary evaporator under vacuum. The residue of each extract was kept in refrigerator until use.

**Test Organisms**

Seven strains of bacteria and one yeast were used in all antimicrobial screening. The microorganisms were: *Escherichia coli* (ATCC10536), *Proteus vulgaris* (NCTC4175), *Pseudomonas aeruginosa* (CNCMA21), *Staphylococcus aureus* (ATCC4175), *Sarcina lutea* (laboratory collection strain), *Bacillus subtilis* (NCTC6633), *Mycobacterium phlei* (laboratory collection strain) and *Candida albicans* (ATCC60193).
**Agar-Diffusion Method**

The agar-diffusion method was used\(^5\) to assess the antimicrobial activities of the methanolic extracts of the plants against representatives of acid fast bacilli (*M. phlei*), gram-positive bacteria (*B. subtilis*, *S. aureus* and *Sarcina lutea*), Gram-negative bacteria (*E. coli*, *Proteus vulgaris* and *P. aeruginosa*) and yeast (*C. albicans*). Applying the agar diffusion method, cups (0.5 cm in diameter) were made using No. 3 cork borer using trypticase soy agar (Difco). Extracts were dissolved in dimethylsulfoxide (DMSO) at concentration of 100 mg/mL then 50µl (containing 5 mg of the extract under test) were aseptically added to the cups (5 mg/cup). Plates were incubated inverted at 37°C for 24-48 hr. After incubation, the inhibition zones were recorded in mm. Diameters less than 5 mm indicated no effect. Fifty microlitre of DMSO were used as a negative control, while ofoxacine (Ofx, Oxoid) and amphotericine B (Amp.B, Oxoid); 5 µg/disc each were used as positive controls.

**Results**

Table 1 lists plant extracts and their activities against the various organisms. In the present investigation, extracts of 20 plants belonging to different families were screened, all of which showed activity against at least four of the test organisms. *Sarcina lutea* was the most inhibited microorganism by 12 of the tested plant extracts and weakly inhibited by 8 plant extracts. *S. aureus* was the second most inhibited microorganism with 10 plant extracts, while 10 others showed a weak activity. *Bacillus subtilis* was partially inhibited by 15 plant extracts and weakly inhibited by the others.

The extracts of *Achillea biebersteinii Afan.*, *Argemone ochroleuca Sweet*, *Rumex nervosus* and *Vernonia schimperii* strongly inhibited the growth of *Mycobacterium phlei*, while 5 plant extracts had a moderate effect and three showed a weak effect, while eight plant extracts were inactive. Gram-negative bacteria were weakly inhibited by 14 of the plant extracts. *Candida albicans* was inhibited by 9 tested plant extracts and 6 showed a weak activity.

In general, the antibacterial activity of plant extracts appears to be more inhibitory to Gram-positive bacteria than Gram-negative bacteria. It should be remembered that penicillin and some of the other prominent
Table 1. Inhibition zones obtained from the tested plant extracts.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Family</th>
<th>SN</th>
<th>Place of collection</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5*</th>
<th>6</th>
<th>7*</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea biebersteinii</em> Afan.</td>
<td>Asteraeae</td>
<td>AB1064</td>
<td>BTR</td>
<td>11</td>
<td>10</td>
<td>17</td>
<td>18</td>
<td>22</td>
<td>18</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td><em>Argemone ochroleuca</em> Sweet</td>
<td>Papavaraeae</td>
<td>AO1128</td>
<td>ED</td>
<td>11</td>
<td>12</td>
<td>18</td>
<td>18</td>
<td>22</td>
<td>18</td>
<td>25</td>
<td>22</td>
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<tr>
<td><em>Maerua crassifolia</em></td>
<td>Capparaceae</td>
<td>MC1034</td>
<td>HR</td>
<td>10</td>
<td>15</td>
<td>13</td>
<td>12</td>
<td>10</td>
<td>12</td>
<td>–</td>
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</tr>
<tr>
<td><em>Commicarpus grandiflorus</em></td>
<td>Nyctaginaceae</td>
<td>CG1126</td>
<td>T</td>
<td>9</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>11</td>
<td>10</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><em>Commicarpus plumbagineus</em></td>
<td>Nyctaginaceae</td>
<td>CP1127</td>
<td>HR</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>9</td>
<td>12</td>
<td>10</td>
<td>10</td>
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<tr>
<td><em>Crotalaria emarginella</em></td>
<td>Fabaceae</td>
<td>CE1119</td>
<td>HR</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>12</td>
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<td>15</td>
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<tr>
<td><em>Ochradenus baccatus</em> Delile</td>
<td>Resedaceae</td>
<td>OB1131</td>
<td>T</td>
<td>10</td>
<td>12</td>
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<td>19</td>
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<tr>
<td><em>Rumex nervosus</em></td>
<td>Polygonaceae</td>
<td>RN1129</td>
<td>HR</td>
<td>10</td>
<td>10</td>
<td>19</td>
<td>14</td>
<td>22</td>
<td>17</td>
<td>22</td>
<td>18</td>
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<tr>
<td><em>Ecbolium viride</em> (Forssk.)</td>
<td>Acanthaceae</td>
<td>EV1006</td>
<td>S, T</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>9</td>
<td>14</td>
<td>17</td>
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<td><em>Diptygium glaucum</em> Decne.</td>
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<td>DG1037</td>
<td>J</td>
<td>12</td>
<td>10</td>
<td>12</td>
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<td>12</td>
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<td><em>Jasminum grandiflorum L.Subsp.</em></td>
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<td>–</td>
<td>22</td>
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<tr>
<td><em>Cleome ramosissima</em> Webb ex Parl</td>
<td>Capparaceae</td>
<td>CR1040</td>
<td>HR</td>
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<td>–</td>
<td>13</td>
</tr>
<tr>
<td><em>Lavandula pubescent</em> Decne.</td>
<td>Lamiaeae</td>
<td>LP1089</td>
<td>HR</td>
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<td>CS1049</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>18</td>
<td>15</td>
<td>–</td>
<td>14</td>
</tr>
<tr>
<td><em>Fioria dictyocarpa</em> (Webb)</td>
<td>Malveae</td>
<td>FD1124</td>
<td>T</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>19</td>
<td>14</td>
<td>–</td>
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<td><em>Echium arabicum</em></td>
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<td>EA1045</td>
<td>B</td>
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<td>–</td>
<td>–</td>
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<tr>
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<td>Astreaceae</td>
<td>PG1063</td>
<td>MR</td>
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<td>12</td>
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<td><em>Plectranthus asirensis</em> J.R.I</td>
<td>Lamiaeae</td>
<td>PA1093</td>
<td>B</td>
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<td>11</td>
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<td>19</td>
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<tr>
<td><em>Vernonia schimperi</em></td>
<td>Asteraeae</td>
<td>VS1054</td>
<td>HR</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>16</td>
<td>19</td>
<td>22</td>
<td>10</td>
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</tr>
</tbody>
</table>

1. *E. coli*; 2 *Pseudomonas aeruginosa*; 3 *Proteus vulgaris*; 4 *Staphylococcus aureus*; 5 *Sarcina lutea*; 6 *Bacillus subtilis*; 7 *Mycobacterium phlei*; 8 *Candida albican*.

Al-Baha-Al Taif Road: BTR; Al-Hadda Road: HR; Al-Baha: B, Al-Sheffa: S, Al-Taif: T; ED: Ekrema dame; Jeddah: J; Jeddah-Al-Hadda Road: JHR; Al-Maddina Road: MR; Specimen number: SN.

*Laboratory collection strains*
antibiotic agents of fungal origin are also rather selective in their inhibitory action, most of them being inhibitory to Gram-positive bacteria. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria[6]. The outer lipopolysaccharide layer hinders access of most compounds to the peptidoglycan layer of the cell wall. This explains the resistance of Gram-negative strains to the lytic action of most extracts exhibiting activity. The negative results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria[7]. The antimicrobial extracts of tested plants can be assumed to be useful to the producing plant in warding off infectious diseases and there is therefore a compelling reason to suppose that anti-infective agents could be active against human pathogens as was suggested by folkloric and historical accounts[8].

Infections caused by Mycobacterium phlei are among the most difficult to treat with conventional antibiotics. The growth of Mycobacterium phlei was completely inhibited by four plants extracts and partially by five extracts. Bacillus species are common microbes found in most natural environments including soil, water, plant and animal tissues. While most Bacillus species are regarded as having a little pathogenic potential, both Bacillus cereus and Bacillus subtilis have been known to act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning[9]. Figures 1-4 illustrated the results of some selected plant extracts, which have a significant antimicrobial activity.

**Conclusion**

Our results allow us to conclude that the crude extracts of Achillea biebersteinii Afan. Ecbolium viride (Forssk) and Rumex nervosus exhibited a significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents[10]. This probably explains the use of these plants by the indigenous people against a number of infections since generations[11]. Researches have some reports on the chemical nature of the antimicrobial active ingredients of different species belonging to the same genera listed above, which could be helpful in future investigations targeting the identification of the chemical nature of the antimicrobial
active ingredients present in the most efficacious indigenous species used in this study.

Fig. 1. Antimicrobial activity of plant extracts showing the highest activity against Gram-positive bacteria.

Antimicrobial activity against Gram negative bacteria

Fig. 2. Antimicrobial activity of plant extracts showing the highest activity against Gram-negative bacteria.
Fig. 3. Antimicrobial activity of plant extracts showing the highest activity against *Mycobacterium phlei*.

Fig. 4. Antifungal activity of plant extracts showing the highest activity against *Candida albicans*.

**Acknowledgement**

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References


دراسة التأثير المضاد للميكروبات لخلاصات بعض النباتات التي تنمو بالمملكة العربية السعودية

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المستخلص: في هذه الدراسة تم اختيار التأثير المضاد للميكروبا للخلاصات الكحولية المحضرة من الأعشاب البرية، التي تنمو في المنطقة الغربية من المملكة العربية السعودية. تم قياس هذا التأثير باستخدام طريقة الأجار المنتشر. تم استخدام ثمانية ميكروبات مختلفة في هذه الدراسة، هي: الوشي شياكولاي، والبروتيس فالجاس، والسودومونس، والميكرسب العنقودي المكور الذهبي، والميكرسب الأحمر، والسروشيا لوتيا، والسبسكيزا العصوية، والميكروسبية فيلارا، وخميرة الكنديدا البيكانس. تم اختبار عشرين خلاصة نباتية، وقيمت مناطق تثبيط النمو الميكروبي بالميليمتر.

وقد أظهرت النتائج أن ثلاثة عشر من النباتات المختبرة، لها تأثير مضاد واسع المدى للميكروبا الموجبة والسلبية الجرام. ومن ناحية أخرى أظهرت النتائج أن الخلاصات النباتية السبعة المتبقية، لها تأثير مضاد ضيق المدى على البكتيريا الموجبة الجرام فقط. ولقد لوحظ أن تسعة عشر من النباتات المختبرة، لها تأثير إيجابيا على ميكروسب الميكروسبية، ولوحظ أيضا تأثيرات مختلفة لخميرة الكنديدا.