EVALUATION OF INHIBITORY EFFECTS OF IRANIAN PROPOLIS AGAINST FILAMENTOUS BACTERIA

Saeed Eshraghi 1, Shirin Valafar 2

ABSTRACT
Objective: To investigate the antibacterial activities of propolis in samples collected from Zanjan province IRAN, against 25 pathogenic strains of bacteria.
Methodology: In order to evaluate the biological properties of methanol extract of propolis using agar distribution methods (disk and drop plate). Seven concentrations of methanolic extract of propolis were prepared and added drop wise to the bacterial seed layer cultured agar media individually. The diameter of the clear zone formed in each concentration was measured and correlated to the ability of the extracts to inhibit the growth of bacteria.
Results: Nocardia asteroides and N. brasiliensis has nearly shown the same susceptibility to various concentrations of propolis extract, and the complete clear zones revealed that this effect was quite remarkable. For other bacteria, different degrees of susceptibility to propolis were observed.
Conclusions: We came to this conclusion that zones formed by 50mg/ml Amikacin in agar was similar to that of 5% concentration of propolis, and that the potency of propolis is 80% of Amikacin potency, which is the most effective antibiotic against Nocardia.

KEYWORDS: Propolis, Nocardia, Antibacterial activity, Flavonoids, Caffeic acid.

INTRODUCTION
One of the most useful products of the bee-hive is propolis (bee glue), a brownish and resinous substance that is collected by worker bees from the leaf buds and cracks in the bark of numerous tree species. Bees mix the original propolis with beeswax and 6-glucosidase they secrete during the propolis collection. Since ancient time, propolis was employed as an antiseptic2,3 and cicatrizant in wound treatment,4,5 and as mouth disinfectant,5 an antipyretic agent.6,7 Flavonoids content and caffeic acid phenethyl esters (CAPE), active component of propolis,2,4,8 may be responsible for most of this biological activities.9-11 At a concentration of 10 µM, CAPE completely blocks the production of reactive oxygen species in human neutrophils and in the xanthine/xanthine oxidase (X/XO) system.12 These observations attracted the attentions to the antibacterial properties of this substance. The main purpose of the present study was to evaluate the antibacterial activity of methanolic extract of propolis.

Propolis Compounds: Propolis is composed of 50% resin (composed of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds.6 Polyphenols due to their proven abil-
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Enzymes are considered as the main pharmacologically active molecules in propolis. Clinical pharmacological studies show the potential beneficial effects of propolis product as an adjuvant to treat asthmatic and gastro duodenal ulcers. Propolis contains a high concentration of flavonoids, which are used in a wide range of cosmetics and health food preparations for their antimicrobial properties. At present, propolis is one of the most popular health food products and is available commercially through all over the world.

Microbiology: Nocardia, a gram positive variably acid-fast aerobic bacterium is an opportunistic pathogen in immunocompromised hosts. The species have pathogenic potential to human, including Nocardia asteroides, N. farcinica and N. brasiliensis. Because of influence of propolis in the treatment of both cutaneous and respiratory disorders, the efficacious and antibacterial properties of propolis on the above bacteria have been studied.

MATERIALS AND METHODS

Microorganisms: In this study a total number of 25 different bacterial strains were provided from School of Public Health, Teheran University of Medical Sciences, Iran. The bacteria used were included of six strains Nocardia asteroides, seven strains Nocardia brasiliensis as original bacteria and two strains of each Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter cloacae, Shigella flexneri and Staphylococcus aureus as elective bacteria. The cultures were stored at –70ºC in brain heart infusion (BHI) broth containing 20% glycerol.

Media and Sensi-Disk: The following microbiological media and antimicrobial susceptibility test discs were supplied from Becton Dickinson Microbiology Systems Cockeysville Maryland (BBL products):

- Glycerol Yeast Extract agar (GYEA), Brain Heart Infusion Broth (BHI) containing 20% glycerol, Sabouraud’s Dextrose Agar (SDA), Mueller Hinton Agar (MHA), Blood Agar base (BA) and test disks including; Amikacin (AN-30) (31597), Amoxicillin(AMC-30)(31629), Ceftizoxime (ZOX-30)(31623), Cephalothin (CF-30) (31271), Chloramphenicol (C-30) (31274), Gentamicin (GM-10)(31299), Kanamycin (K-30) (31301), Neomycin (N-30) (31313), Ceftazidime (CAZ-30) (31633), Streptomycin (S-10) (31328), (Co-trimoxazole) Sulfamethoxazole with Trimethoprim (SXT-23.75) (31539), Tetracycline (Te-30) (31344).

Preparation of Propolis extracts: Crude propolis samples collected by Apis mellifera were obtained from hive bee’s located in the province of Zanjan (Northwestern IRAN). About 2 g of propolis was dissolved in an appropriate amount of Merck ethanol. The extract was evaporated and filtrated aseptically under flow cabinet. The sticky extract yielded (specific gravity of 1.3), was used to prepare appropriate concentrations for tests. Seven concentrations of methanolic extract of propolis were prepared using 80% methanol.

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the plates were examined and the clear zones of inhibitory surrounding of the bacterial strains were investigated using standard antibiotic clear zones.

RESULTS

According to the results, *Nocardia* strains have nearly shown the same susceptibility to various concentrations of propolis extracts. The comparison of inhibitory effects of propolis on glycerol yeast extract agar plate, against *Nocardia asteroides* using two different distribution methods are demonstrated in Fig-1. The comparison of antibacterial activity, revealed the clearly inhibitory effects of the propolis extracts against all the bacterial strains using drop and disk plate procedures (Table-I). The antibacterial susceptibility test against *Nocar-
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Components depend on various factors such as season, and vegetation of the area, geographical origin and the state of propolis (fresh or aged). There were about 180 kinds of polyphenols isolated from propolis. Among these compounds, rutin, ferulic acid, apigenin, luteolin, quercetin, caffeic acid, flavonoids and phenolic acids are the important active ingredients in propolis. The chemical composition of propolis is variable depending on the geographical origin of this natural substance. In this descriptive study, we report a new variety of Iranian propolis which is collected from the Northwestern of the country (Zanjan). As the tables indicate that the inhibitory effect of propolis was complete against \textit{E. coli} and \textit{S. aureus}, while for the other four bacteria the effect was the same and also less than the two mentioned. These results indicate that the propolis undoubtedly has antibacterial effects as reported in the previous studies.

The results demonstrated that the propolis, especially Iranian sample, could be a promising anti-bacterial agent; however, further \textit{in-vitro} and \textit{in-vivo} studies need to be performed. Although the flavonoids content and caffeic acid phenethyl esters (CAPE), active component of propolis, may be responsible for most of these biological activities, but the biological effects of propolis cannot be attributed solely to these contents.

Table-II: The comparison of clean zone diameter of standard antibiotic disks against \textit{Nocardia} strains

<table>
<thead>
<tr>
<th>No</th>
<th>Antibiotic disk</th>
<th>Media</th>
<th>\textit{N. brasiliensis}</th>
<th>\textit{N. asteroides}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ceftazidime</td>
<td>GYEA, BHIA, BA</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>Ceftizoxime</td>
<td>GYEA, BHIA, SDA</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Cephalothin</td>
<td>GYEA, BA</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Cotrimoxazole</td>
<td>MHA, SDA</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>Amikacin</td>
<td>GYEA, BHIA</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>Amoxicillin</td>
<td>GYEA, BHIA</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>Tetracycline</td>
<td>GYEA, BHIA, MHA</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>Neomycin</td>
<td>GYEA, BA, BHIA</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>Streptomycin</td>
<td>GYEA</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>Chloramphenicol</td>
<td>GYEA, BHIA</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>11</td>
<td>Gentamicin</td>
<td>GYEA, BA, BHIA</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>Kanamycin</td>
<td>BHIA</td>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

BA = Blood Agar, BHIA = Brain Heart Infusion Agar, MHA = Mueller Hinton, SDA = Sabouraud’s Dextrose Agar, GYEA = Glucose Yeast Extract Agar

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2}
\caption{The comparison of antibacterial activity of propolis against \textit{Nocardia} species using Muller Hinton agar}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure3}
\caption{The comparison of antibacterial activity of propolis against \textit{Nocardia} species using glucose yeast extract agar}
\end{figure}
CONCLUSIONS

From the present study it is concluded that:
1. Zones formed by 50mg/ml Amikacin in agar was similar to that of 5% concentration of propolis.
2. The potency of propolis is 80% of Amikacin potency, which is the most effective antibiotic against Nocardia.

REFERENCES