Propolis from the Mediterranean Region: Chemical Composition and Antimicrobial Activity

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Propolis, \textit{Populus}, Antimicrobial Activity

The chemical composition of propolis from Bulgaria, Turkey, Greece and Algeria was investigated by GC-MS. All of them contained mainly flavonoids and esters of caffeic and ferulic acids, which indicated that their main source are buds of poplars of the taxonomic section \textit{Aegieros}. Some Turkish samples contained a low percent of diterpenic acids, while in Algerian samples significant amounts of a hydroxyditerpenic acid (M=322, its structure not determined by its MS) were found. All samples showed significant antibacterial and weak to moderate antifungal activity.

Introduction

Propolis (bee glue) is well known for its valuable biological activities (antibacterial, antifungal, immunostimulating, antitumor, antiinflammatory, etc.) (Marcucci, 1995; Burdock, 1998). Its chemical composition is variable, depending on the site of collection, because in different ecosystems different plant exudates and secretions could serve as a source of propolis. Numerous investigations have established that in Central Europe, as well as in North America and the non-tropical regions of Asia, the main propolis source is the exudate of poplar buds of \textit{Populus} spp. (Greenaway et al., 1990; Bankova et al., 2000). These exudates contain typical phenolic compounds: flavanones and esters of caffeic and ferulic acids. In “border regions”, however, (Sonoran Desert, Tunisia, Egypt) some other plants interfere and new components appear in propolis (Wollenweber and Buchmann, 1997; Martos et al., 1997; Christov et al., 1998). The objective of this work is to study the chemical composition of bee glue from the Mediterranean region in order to find out the importance of poplar trees and other plants as its source. This information would be of importance for a future elaboration of propolis standards, for this reason antibacterial and antifungal tests were also carried out.

Experimental

Propolis samples

The geographic origin of the samples and time of collection are listed in Table I.

Extraction and sample preparation

Propolis, grated after cooling, was extracted for 24h with 70\% EtOH at room temperature (1:10, w/v). The extract was evaporated to dryness. About 5 mg of the residue were mixed with 75 \mu l of dry pyridine and 50 \mu l bis(trimethylsilyl)trifluoracacetamide (BSTFA), heated at 80 °C for 20 min and analysed by GC-MS.

Table I. Geographical origin and time of collection of propolis samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Year</th>
</tr>
</thead>
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<tr>
<td>LOV</td>
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<td>1999</td>
</tr>
<tr>
<td>BUR</td>
<td>Aitos, Bulgaria</td>
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</tr>
<tr>
<td>GR</td>
<td>Nigrita, Greece</td>
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</tr>
<tr>
<td>BU-7</td>
<td>Bursa, Turkey</td>
<td>1997</td>
</tr>
<tr>
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<td>Bursa, Turkey</td>
<td>1998</td>
</tr>
<tr>
<td>MU</td>
<td>Mugla, Turkey</td>
<td>1998</td>
</tr>
<tr>
<td>IZ</td>
<td>Izmir, Turkey</td>
<td>1998</td>
</tr>
<tr>
<td>AN</td>
<td>Beytepe, Turkey</td>
<td>1998</td>
</tr>
<tr>
<td>AL-1</td>
<td>M’Sila, Algeria</td>
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</tr>
<tr>
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GC-MS analysis

The GC-MS analysis was performed with a Hewlett Packard Gas Chromatograph 5890 Series II Plus linked to Hewlett Packard 5972 mass spectrometer system equipped with a 30 m x 0.25 mm ID SPB-1 column, film thickness 25 μm. A fused silica capillary column was used, mass selective detector, with He as carrier gas, linear velocity 35 cm/min, split ratio 1:50, temperature program 100–300 °C at 5 °C/min, injector temperature 300 °C.

Identification of compounds

The identification was accomplished using computer searches on commercial libraries. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. Reference compounds were co-chromatographed when possible to confirm GC retention times.

Antibacterial activity

For the investigation of the antibacterial activity the agar cup method was used with Staphylococcus aureus 209 and Escherichia coli WF+ as test strains. An inhibitory zone with a diameter less than 10 mm corresponds to lack of activity (10 mm is the diameter of the cup), 0.1 ml of test solution containing 0.4 mg propolis extract in ethanol was applied to every cup.

Antifungal activity

For the investigation of the antifungal activity the agar cup method was used (Spooner and Sykes, 1972). As test microorganism, Candida albicans 562 was used. The antifungal activity was measured as diameter of the inhibitory zones. An inhibitory zone with a diameter less than 10 mm corresponds to lack of activity (10 mm is the diameter of the cup), 0.1 ml of test solution containing 0.5 mg propolis extract in ethanol was applied to every cup. Control experiments with ethanol showed that solvent does not have any activity.

Results and Discussion

The chemical composition of two Bulgarian, one Greek, five Turkish and two Algerian samples were investigated by GC-MS after silylation. The results obtained are presented in Table II.

All samples display the typical pattern of “poplar type” propolis. They contain the combination of secondary metabolites characteristic for the buds of Populus spp. of the section Aigeiros: pinocembrin, pinobanksin and its acetate, prenyl esters of caffeic and ferulic acids (Greenaway et al., 1990; Wollenweber and Buchmann, 1997). The poplar origin of Bulgarian bee glue is well documented (Bankova et al., 1992). Obviously the main source of the other samples investigated were also poplar buds. This is an expected result as far as the locations where propolis was collected are within the area of poplar species. On the other hand, several differences between the samples are evident. Some of these differences are quantitative and this could be explained by the well known fact that each species and even each clone of poplar has its own characteristic mixture of compounds in its bud exudate (Greenaway et al., 1990).

More important are qualitative differences: samples from Algeria and Turkey contained diterpenic acids. Till now, diterpenic acids have only been found in Brazilian propolis (Bankova et al., 1996; Banskota et al., 1998). In two Turkish samples small amounts of pimaric and isopimaric (sample from Mugla); abietic and dihydroabietic acid (sample from Beytepe) were detected. In Algerian samples however, diterpenic acids appear to be among the main components, and especially a compound with M=322 (hydroxyditerpenic acid). Its structure could not be determined by its MS only. The plant source of these compounds remains unknown and is a question of future investigations.

These chemical differences, however, did not result in a significant change of the biological activity of the samples investigated. All samples showed good effect against S. aureus. The activity against E. coli was weak or lacking, which corresponds to the literature data (Marcucci, 1995). This is a confirmation that bees are able to find plant sources that provide a good defense against infections in any ecosystem they inhabit, as it was shown for Brazilian propolis (Kujumgiev et al., 1999).

These results confirm that European propolis is definitely of poplar origin and this could be a key for its standardization based on quantification of
Table II. Chemical composition (%)\textsuperscript{a} of ethanol extracts of propolis.

<table>
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<th>Substance</th>
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<th>GR</th>
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<th>BU-8</th>
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<td>–</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.4</td>
<td>0.6</td>
<td>0.6</td>
<td>2.8</td>
<td>0.5</td>
<td>5.6</td>
<td>7.2</td>
<td>0.6</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>Vanillin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.2</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mallic acid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.2</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The ion current generated dependet depends on the characteristics of the compound concerned and it is not a true quantitation.
Table III. Antibacterial and antifungal activity of propolis samples (extracts with 70% ethanol).

<table>
<thead>
<tr>
<th>Sample</th>
<th>S. aureus (diameter of the inhibitory zone ± stand. deviation, mm)</th>
<th>E. coli (diameter of the inhibitory zone ± stand. deviation, mm)</th>
<th>C. albicans (diameter of the inhibitory zone ± stand. deviation, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOV</td>
<td>20 ± 1</td>
<td>0</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>BUR</td>
<td>20 ± 1</td>
<td>0</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td>GR</td>
<td>18.7 ± 0.6</td>
<td>12 ± 0</td>
<td>0</td>
</tr>
<tr>
<td>BU-7</td>
<td>19 ± 2</td>
<td>12 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>BU-8</td>
<td>20 ± 2</td>
<td>12 ± 0</td>
<td>16 ± 1</td>
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<tr>
<td>MU</td>
<td>21 ± 2</td>
<td>13.3 ± 0.6</td>
<td>17 ± 2</td>
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<tr>
<td>IZ</td>
<td>19 ± 2</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>AN</td>
<td>21.7 ± 0.6</td>
<td>14 ± 0</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>AL-1</td>
<td>19 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AL-2</td>
<td>18.7 ± 0.6</td>
<td>0</td>
<td>12 ± 0</td>
</tr>
</tbody>
</table>

*a Mean of three measurements.

the main “poplar” phenolics. They confirm also that in border areas, such as Algeria, where poplars are not always available for propolis collection, other plant sources are used but this does not affect the antibacterial properties of bee glue.

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