THE ANTIMICROBIAL ACTIVITY OF HONEY AND PROPOLIS AGAINST YEASTS CANDIDA SPECIES

ACTIVITATEA ANTIMICROBIANĂ A MIERII ȘI PROPOLISULUI FAȚĂ DE SPECIILE DE DROJDII CANDIDA

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The aim of this study was to focus on the evaluation of the antimicrobial activity of honey samples and ethanolic extract of propolis (EEP) against Candida species. The honey concentrations - 50 % and 25 % of honey in distilled water were prepared. These preparations were tested for antimicrobial activity against five different types of yeasts: Candida crusei, Candida albicans, Candida (Torulopsis) glabrata, Candida parapsilosis and Candida tropicalis. The disc diffusion method using filter paper discs was employed. The antimicrobial activity was determined as an equivalent of the inhibition zones diameters (in millimeters) after incubation of the cultures for 48 hours. There were not seen an inhibition zones against the yeasts investigated in the 25 % and 50 % concentration of honey samples. The analysis among the tested yeasts showed that Candida crusei was the most sensitive in 70 % of EEP, and the sensitivity of the yeasts decreased in the order: Candida albicans > Candida parapsilosis > Candida tropicalis > Candida glabrata.

Keywords: honey; antifungal action; Candida species, diffusion method

Introduction

The first study on the antimicrobial effects of honey was reported by Van Ketel in 1892 and is mentioned by Dustmann (1979). Since then, various other studies have been published on this subject (Basson et al., 1994; Molan, 1992; Kňazoviká et al., 2009). A great deal of work has been done in an attempt to identify the antimicrobial agents in honey and the range of organisms susceptible to this antimicrobial action. The word “inhibine” was introduced to describe these antimicrobial agents and this term is still used today. One review article lists 64 different bacterial and 13 fungal species on which antimicrobial action has been tested (Theunissen et al., 2001).

Honey and propolis are bee products that have been used for centuries in folk medicine. Several studies have been conducted to authenticate this ‘folklore’
on medicinal properties of honey and there has been a renaissance in the use of honey and propolis as medicine in more recent times (Muli et al., 2008).

Propolis is a complex resinous mixture collected by bees from plant exudates, and mixed with hypo-pharyngeal secretions, beeswax and pollen. In the hive propolis is used for comb construction and polishing, to maintain aseptic hive environment and for protection and adaptation of bees nests. The chemical composition of propolis varies depending on the diversity of plants and geographic locations from which bees collect it (Bankova et al., 2000). The biological activities of propolis (antibacterial, antiviral, antifungal etc) vary according to its source (Muli et al., 2008).

The majority of human mycoses are caused by opportunistic fungi (Boukraa et al., 2008). Opportunistic pathogens produce illness by taking advantage of debilitated or immuno-compromised hosts (McGinnis et al., 1999). *C. albicans* is a fungus which can grow on warm and moist surfaces and cause superficial diseases such as oral and vaginal thrush and chronic mucocutaneous candidiasis (Molero et al., 1998). In ophthalmology, rubbery concretions occur in the presence of infections due to species of *Candida*. Honey is a natural product that is used for its antifungal activity (Irish et al., 2006). Its antimicrobial properties have been extensively reviewed (Molan, 1992). Honey has a potent antibacterial activity and is very effective in clearing infection in wounds and protecting them from becoming infected (Bouleraa et al., 2008). Unlike honey, no inhibitory action has been allocated to starch. In contrary, it is known that starch could be incorporated into microbial media to stimulate their growth (Zangrand Figueira et al., 2000).

In this work we wish to report the results of our study on the antifungal activity of propolis (ethanol extracts) and honey samples from different locations against *Candida* species.

**Materials and Methods**

**Honey and propolis samples and their preparation**

The honey samples were obtained directly from beekeepers during the harvest in the year 2008, from different locations across Slovak Republic. Sixth multifloral honeys were determined. All samples were prepared aseptically and were handled protected from direct sunlight. Honey samples were stored at 4 °C in the dark until they were analysed. The propolis samples originated from different regions of Slovakia, and were frozen and subsequently milled to powder.

**Antifungal activity**

Honey solutions were prepared in two fractions: 50 and 25 % (by mass per volume). The samples of each honey (10 g) and sterile water were stored at 37 °C for 30 min before mixing, to facilitate homogenization. The 50 % (by mass per volume) solutions thus prepared were diluted to 25 %. The samples were assayed immediately after dilution. Propolis (10g) were extracted with use of 80 cm³ in solvent ethanol (70 %) acidified at pH 2, under reflux at 85 °C during 1
hour. After chilling the mixture was centrifuged and supernatant was dried under reduced pressure at temperatures 45-50 °C. The evaporation residue was dissolved in 160 cm³ of solvent mixture ethyl-acetate in the ratio 1:1 and shaken during 30 min. Organic (ethyl-acetate) phase was separated and evaporated; the evaporation residue was dissolved in 10 cm³ of methanol.

The potential antifungal activity of five selected species of fungi was studied using the agar well diffusion method. Five species of fungi were from: *Candida crusei; Candida albicans; Candida (Torulopsis) glabrata; Candida parapsilosis; and Candida tropicalis* by the agar well diffusion method. All fungal isolates were identified microscopically, and samples of each fungus were deposited in the fungal collection bank at the Department of Microbiology, Slovak Agricultural University, Nitra, Slovakia. Fungal isolates were maintained on Sabouraud dextrose agar (SDA, HiMedia), and the cultures were stored at room temperature.

**Culture media and inoculum.**

The strains of fungi *Candida crusei, Candida albicans, Candida (Torulopsis) glabrata, Candida parapsilosis* and *Candida tropicalis* were maintained on Sabouraud dextrose agar (SDA, HiMedia). The concentration of microbial inoculums was within the range of 10⁶ CFU.ml⁻¹ determined by viable counts serial dilutions.

The antimicrobial effect of the natural honey was tested using the agar well diffusion method. Overnight microbial cultures were used for surface inoculation of Petri dishes containing 15 ml of Sabouraud dextrose agar (SDA). Each Petri dish was spread on with 0.5 ml of strain inoculum streaked thoroughly all over the surface of the MA. Subsequently, four equidistant wells 9 mm in diameter each were punched into the inoculated medium with sterile glass Pasteur pipettes and were filled up with 250 µl of honey using a precise eppendorph. All plates were incubated at 25 °C and inhibition zones were measured after two days. Five different strains of *Candida* species were tested in sets of plates, which were simultaneously processed for each strain. All the experiments were repeated twice, including control with plain 40 % phenol every time. After incubation the zones of inhibition of the growth of the fungi around the disks were measured. The mean values of three trials were calculated.

**Results and Discussion**

Propolis and honey has been found to possess antimicrobial activity and this has been attributed to specific chemicals in the propolis and honey (Kačániová et al., 2008; Kňazovická et al., 2009).

The minimal inhibitory concentration of *Rhododendron smirnovii* leaf extract against *C. glabrata* was 128 µg.ml⁻¹. The leaf extract of *R.smirnovii* was found to have lower activity against yeasts (*C. albicans, C. crusei* and *C. parapsilosis*) at a concentration of 256 µg. ml⁻¹ (Tezgülçakir, 2005).
Antifungal activities of the honey samples with 25% concentration against fungi *Candida crusei, Candida albicans, Candida (Torulopsis) glabrata, Candida parapsilosis* and *Candida tropicalis* strains are presented in Table 1. The antimicrobial activity of honey samples was assessed by the diameter (mm) of the obtained sterile zones around the disks. No inhibition zones were seen against the yeasts investigated in the 25% concentration of honey samples.

The sugar control and the three honey samples stimulated the growth of *C. albicans* and were optimal between 2.5% and 5%. Increased honey concentrations resulted in reduced growth of *C. albicans*; wasbessie honey at a concentration of 25% demonstrated 29.4% inhibition on the growth of *C. albicans*, while the control, bluegum and fynbos honey produced only partial inhibition (Theunissen et al., 2001).

### Table 1

<table>
<thead>
<tr>
<th>Species of yeast</th>
<th>Inhibitory zones (in mm) of 25% honey concentration</th>
<th>Number of honey samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.</td>
<td>2.</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida crusei</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>NI</td>
<td>NI</td>
</tr>
</tbody>
</table>

NI: No inhibition zone

Antifungal activities of the honey samples with 50% concentration against fungi *Candida crusei, Candida albicans, Candida (Torulopsis) glabrata, Candida parapsilosis* and *Candida tropicalis* strains are presented in Table 2. The antimicrobial activity of honey samples was assessed by the diameter (mm) of the obtained sterile zones around the disks. No inhibition zones are seen against the yeasts investigated in the 25% concentration of honey samples.

### Table 2

<table>
<thead>
<tr>
<th>Species of yeast</th>
<th>Inhibitory zones (in mm) of 50% honey concentration</th>
<th>Number of honey samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.</td>
<td>2.</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida crusei</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>NI</td>
<td>NI</td>
</tr>
</tbody>
</table>

NI: No inhibition zone
Undiluted honey showed high inhibitory effect on bacterial growth compared to diluted honey of 75%, 50% and 25% concentrations. Similarly, the 75% and 50% concentrations had significantly higher inhibitory effects compared to the 25% concentration; however, there were no significant differences in the inhibitory effect of the 75% and 50% honey concentrations. Inhibitory effects of the honey were noted on *B. subtilis* and *S. typhi* in some instances. No inhibitory effect was noted on *E. coli, S. aureus, A. niger* and *C. albicans* (Muli et al., 2008).

Antifungal activities of the 70% ethanol extract against fungi *Candida crusei, Candida albicans, Candida (Torulopsis) glabrata, Candida parapsilosis* and *Candida tropicalis* strains are presented in Table 3. The antimicrobial activity of honey samples was assessed by the diameter (mm) of the obtained sterile zones around the disks.

Table 3

<table>
<thead>
<tr>
<th>Species of yeast</th>
<th>Inhibitory zones mean±SD (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>3.75±1.77</td>
</tr>
<tr>
<td><em>Candida crusei</em></td>
<td>6.00±2.83</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>3.50±0.77</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>3.75±1.77</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>3.00±1.41</td>
</tr>
</tbody>
</table>

Analysis among the tested yeasts showed that *Candida crusei* was the most sensitive in 70% of EEP, and the sensitivity of the yeasts decreased in the order: *Candida albicans > Candida parapsilosis > Candida tropicalis > Candida glabrata*.

The EEP had the significantly higher inhibitory effect on the Gram positive bacteria, *Bacillus subtilis* compared to all other bacterial and fungal strains *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Aspergillus niger*. The inhibition on *Bacillus subtilis* however, was not statistically different to that observed for the yeast strain, *C. albicans*. Among the Gram negative bacteria, EEP had higher inhibitory effect on *S. typhi* compared to *P. aeruginosa* and *E. coli*.

The antimicrobial effect of the pollen extracts, propolis extracts and honey samples was tested using the agar well diffusion method in study Khazovická et al. (2009). The inhibition zones varied at pollen and propolis extracts.

**Conclusions**

Our results indicate that ethanolic extract of propolis EEP had higher antimicrobial activity against the tested microbes compared to its honey. There is need for characterization of the active components in the propolis extracts.
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References

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