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SHORT COMMUNICATION

Propolis oil extract: quality analysis and evaluation of its antimicrobial activity

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Designing propolis products for external use involves determining the optimal form of propolis for the introduction into dermatological pharmaceuticals and cosmetic preparations. As a potent ingredient, propolis oil extract from raw material harvested in Lithuania was analysed. The rheological characteristics, content of phenolic compounds, major compounds and antimicrobial activity of the propolis oil extract are investigated here for the first time. The propolis oil extract was produced by maceration using different solvents, raw material was collected in Lithuania. Solvent mixture with 96% ethanol increased the rheological stability and extracted amount of phenolic compound. High-performance liquid chromatography identified the potent quality markers for Lithuanian propolis, phenylpropanoid vanillin, coumaric acid and ferulic acid. Antimicrobial activity of propolis oil extract was evaluated in experimental studies in vitro, and the minimal concentration of phenolic compounds that inhibited respective microorganisms was determined. The results demonstrate that phenolic compounds have effective antimicrobial activity in propolis oil extract; thus, it can be compatible with the semisolid preparation.

Keywords: propolis oil extract; phenolic compounds; antimicrobial activity

1. Introduction

Propolis may be used in various forms, including semisolid extract, liquid ethanol extract or aqueous extract (Bankova, Popova, Bogdanov, & Sabatini, 2002; Hu et al., 2005). A surprising fact is that propolis, even though having vastly different chemical composition, has similar antifungal, antibacterial and various other activities (Bankova, 2005; Khalil, 2006; Marcucci et al., 2001; Uzel et al., 2005). Designing propolis products for external use involves determining the optimal form of propolis for introduction into dermatological pharmaceuticals and cosmetic preparations. For this reason, it was relevant to produce propolis oil extract from raw material harvested in Lithuania, and to evaluate the quality of this oil extract through investigating its rheological characteristics, chemical composition and antimicrobial activity. In the production of propolis oil extract, olive oil was chosen.

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as a solvent due to its positive characteristics, i.e. it is well miscible with solid substances, animal fat, wax and paraffins. The oil is able to extract bioactive compounds from propolis (Buriol et al., 2009). Olive oil is used both as an excipient in the production of pharmaceutical and cosmetic preparations (Paiva-Martins, Gordon, & Gameiro, 2003) and as the active substance (Diaz et al., 2006). Literature provides data about the antimicrobial activity of propolis harvested in Lithuania (Pavilonis et al., 2008; Ramanauskiené et al., 2008); yet, it remains important that we determine the antimicrobial activity of propolis preparations such as propolis oil extract; the results then may serve as the basis for selecting suitable indications for the usage of the preparation.

The aim of our study was to investigate the quality of propolis oil extract by determining its rheological properties, chemical composition and antimicrobial activity.

2. Results and discussion

Propolis (lot no. 030608E/2008, produced by close corporation ‘Bitutė’, Vilniaus 7, Kaunas, Lithuania) was macerated with 96% ethanol; the raw material–extractant ratio was 1:2. Ethanol was subsequently evaporated, and the raw material was extracted using olive oil (lot no. 9341.1. 2008, Carl Roth GmbH, Schoemperlenstr. 3–5, Karlsruhe, Germany) (composition no. 1). The temperature of olive oil during extraction was 50–60°C. The extract was filtrated through filter paper under vacuum and cooled down to 20°C. The propolis oil extract was stored at 4°C. Using the second option of production, propolis was extracted using only olive oil (composition no. 2). Compositions of propolis oil extract: no. 1. propolis 30.0, olive oil 70.0, ethanol 96% 60 mL; and no. 2. propolis 30.0, olive oil 70.0.

We selected the analysis technique for the detection of phenolic compounds using spectrophotometry, and to examine the effect of the solvent on the release of phenolic compounds from the studied propolis oil extract. A mixture of pinocembrin and galangin (2:1, w/w) was used for calibration; 0.3 g of propolis oil extract was mixed with 50 mL of 50%, 60%, 70%, 80% and 96% ethanol. The samples were kept for 21 h at the temperature of –12°C and filtrated through membrane filter (pores diameter 0.45 μm). Briefly, 1 mL of the studied solution was transferred into a 50 mL volumetric flask containing 15 mL of distilled water and 4 mL of Folin–Ciocalteu reagent. Subsequently, 6 mL of 20% sodium carbonate was added. The resulting solution was complemented with water up to the 50 mL mark. The solution was then left to stand for 2 h. Absorption was measured at 760 nm wavelength.

The propolis oil extract was developed for introduction into semisolid products as well as for external use as the final product; for this reason, it was expedient to investigate the rheological (rheometer Carri-med CSL100 (TA Instruments, Germany), by applying the plate-and-cone geometry system (cone diameter –40 mm, angle –2° and sample thickness –150 μm), at the temperature of 20°C) characteristics of the produced propolis oil extract to determine its stability under temperature changes.

Extraction of the active substances from propolis oil extract solution was performed using 96% ethanol as a solvent. After extraction, the propolis extract was
filtered through filter paper. Detection of phenolic acids was performed via high-performance liquid chromatography (HPLC). HPLC analysis with UV/PDA detection was carried out using the Waters 2690 chromatography system model (Waters, Milford, USA), equipped with a Waters 2487 UV/Vis detector and Waters 996 PDA detector (Ivanauskas, Jakštas, Radušienė, Lukošius, & Baranauskas, 2008). For separation, a Hichrom column Hypersil HSODS-150A 150 x 4.6 mm (Hichrom Ltd., Berkshire, UK) and a H5ODS-10C guard-cartridge were used. The data were collected and analysed using a personal computer and the Waters Millennium 2000® chromatographic manager system (Waters Corporation, Milford, USA). The mobile phase of the method consisted of solvent A (methanol) and solvent B (0.5% (v/v) acetic acid in water). The elution profile was: 10% A in B, 0 min; 60% A in B, 28 min; and 10% A in B, 30 min. All gradients were linear. The flow rate was 1 mL min\(^{-1}\), the column temperature was ambient and the injection volume was 10 µL. UV detection was performed at 290 nm. The eluted components were identified on the basis of the retention time by comparison with the retention time of the reference standard. The identity of constituents was also confirmed with PDA detector by comparison with UV spectra of the reference standard in the wavelength range of 190–400 nm.

During the microbiological study, we determined the minimum inhibitory concentration (MIC) of the preparation, i.e. the highest dilution (the lowest concentration) of the preparation that still inhibited the growth of a concrete standard culture of microorganisms. The study was performed in aseptic conditions. The preparation (1 g) was dissolved in 8 mL of solution (4 mL of sterile saline and 4 mL of 96% ethanol) and was emulsified in a water bath kept at 40–45°C, resulting in the main dilution of the studied preparation (test tube 1). Following the preparation of the main solutions of the studied preparations, they were diluted using 10 mL of Mueller–Hinton agar (Mueller–Hinton Agar, Becton, Dickinson and Company), where the inhibitory effect (MIC) of the studied preparations on the growth of standard microorganisms (Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 33499, Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 12459, Bacillus subtilis ATCC 6623, Bacillus cereus ATCC 8035 and Candida albicans ATCC 60193) was evaluated. Standard bacterial cultures were introduced on Petri dishes containing Mueller–Hinton agar of every dilution. Olive oil was used as experiment control. The cultures were incubated for 24 h in a thermostat at the temperature of 37°C, subsequently evaluating the growth of microorganisms (formation of microorganism colonies) in the site of introduction.

Statistical analysis was performed using statistical software package Statistica 5.5. Student’s \(t\)-test was applied, and \(p < 0.05\) was used as the level of significance. All samples were prepared in triplicate.

We selected the analysis technique for the detection of phenolic compounds using spectrophotometry, and to examine the effect of the solvent on the release of phenolic compounds from the studied propolis oil extract (Figure 1).

Studies showed that the greatest amount of phenolic compounds was released from propolis oil extract for sample preparation when 96% ethanol was used as a solvent (Figure 1). For this reason, 96% ethanol was used as a solvent in further quality tests.
The results of rheological studies showed that adding propolis decreases the viscosity of olive oil because the viscosity coefficient ($K$) of propolis is lower than that of olive oil. It is obvious that the method of the production and composition of propolis oil extract affect the changes in viscosity at different temperatures. The temperature-dependent changes ($p < 0.05$) in rheological characteristics suggest that during production the temperature factor may affect the stability of the product. The flow index ($n$) did not change significantly with increasing temperature. This leads to a conclusion that the fluidity of propolis oil extract is not affected by temperature changes ranging between $40^\circ C$ and $60^\circ C$. The results of the study showed that propolis oil extract with composition no. 1 was more resistant to temperature changes, compared to propolis oil extract with composition no. 2.

HPLC was used to identify phenolic acids, phenylpropanoid (vanillin), and to determine their content in propolis oil extract.

Data presented in Figure 2 show that the predominating phenolic acids in propolis oil extract were ferulic acid and coumaric acid. Studies have also shown that vanillin was another predominating substance in propolis oil extract. The results of our study correspond with those published in literature, indicating that the predominant substances in propolis harvested in Lithuania are vanillin, ferulic acid and coumaric acid (Ivanauskas et al., 2008; Ramanauskiene et al., 2008). The results of the studies confirmed that suitable technology for the production of propolis oil extract was selected. The same active substances as in propolis raw material were indentified (Ramanauskienë et al., 2008).

Active substances in propolis have antimicrobial and antibacterial effects. For this reason, the antimicrobial activity of propolis oil extract was evaluated using experimental studies in vitro, and minimal concentration of phenolic compounds that inhibited respective microorganisms was determined.

The results (Figure 3) showed that the Gram-negative bacterium *E. coli* and *K. pneumoniae* were most resistant to the effect of propolis oil extract. Other Gram-negative bacteria were also more resistant to the studied propolis preparation,
compared to Gram-positive bacteria. The most sensitive microorganisms to the studied preparations were those that had eucaryotic cell structure – *C. albicans* and also a spore procaryotic bacterium *B. cereus*. Olive oil did not inhibit the growth of standard microorganism.

3. Conclusion
The results of this study showed that the optimal method for the production of propolis oil extract was maceration using the mixture olive oil/96% ethanol as an extractant. The results of the rheological investigation confirmed that propolis oil extract of such composition was resistant to temperature changes – i.e. it remained stable. The selected production technique ensures high quality of the preparation because propolis oil extract was found to contain the same active substances as
found in raw material of propolis, and tests of antimicrobial activity confirmed the effectiveness of the preparation against respective bacteria.

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References


