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THE INFLUENCE OF TECHNOLOGICAL PROCESSES ON EXTRACTION OF CHEMICAL COMPOUNDS OF PROPOLIS

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Key words: *propolis, solvents, extraction, phenolic compounds, flavonoids, high performance liquid chromatography.*

Summary

The main task of this research was to determine and select non-ethanol solvents for raw propolis material, to create technological procedure for extraction and to determine the active compounds in the extracts. Experiments were performed using pure raw propolis material. The analysis of qualitative and quantitative characteristics of propolis solutions was based on propolis samples collected from different climate regions of Lithuania. The aim of the study was to create technological sequence and to obtain optimal concentration of phenolic compounds in the extracts. The optimal composition and specifications of non-ethanol propolis solution were determined in sequence of experiments.

INTRODUCTION

Propolis is attributed to have antioxidative, antimicrobial, anti-inflammatory, antivirus, antifungal, imunomodulatory and other qualities (1–3). Such a wide spectrum of action is determined by complex and diverse composition of chemical compounds found in propolis (4, 5). In recent years, pharmaceutical companies are searching for and developing more and more medications based on natural raw materials, thus making propolis new and important object for investigations.

Propolis is hard, yellowish brown resinous substance. It is highly soluble in ethanol, ether and very poorly soluble in water. Thus the majority of propolis dosage forms (extracts, tinctures etc.) produced by pharmaceutical companies are based on ethanol (6). However, they limit wider use of propolis for treatment of various diseases (in paediatrics, ophthalmology, otolaryngology etc.) (7). Ethanol based propolis preparations increase the risk of adverse effects. What is more, aqueous propolis extracts possess more potent pharmacological effects (8), therefore, according to various authors, development of propolis dosage forms must be oriented to search for effective non-ethanol production technologies.

Another important issue is standardization of propolis active compounds. More than 100 chemical compounds were identified in propolis. However, the composition of substances and their quantities is associated with various factors, such as geographic location, time of the year, plants etc. Therefore, there is a need of reliable criteria for standardization of propolis chemical compounds. Investigators suggest classifying propolis collected in same climatic (temperature) zone by quantities of flavons and flavonols, flavanons and dihydroflavanols and phenols. These parameters correlate significantly with propolis biological activity (9).

MATERIALS AND METHODS

Lithuania is divided into 4 climatic regions: 1 – coastal, 2 – Lower Lithuania, 3 – Middle lowland and 4 – South-eastern highland. Propolis was collected from different climatic regions of Lithuania. The analysis of qualitative and quantitative characteristics of propolis solutions was based on propolis samples in accordance with these climatic regions (this was not an extended epidemiological study). We have determined the major qualitative and quantitative characteristics of propolis solutions from these regions.

Study materials: propolis, solvents: water, glycerol, macrogol 400, phenolic compounds, flavonoids.

Study methods: qualitative methods (chromatic reactions), spectrophotometry, high performance liquid chromatography. High performance liquid chromatography equipment consisted of quaternary gradient pump, degasser, „Hewlett Packard 1100“ (Waldbronn, Germany), autoinjector, „Perkin Elmer 200“ (Norwalh, USA), reversed phase column ODS C-18, d_p=5 μm (250×3 mm) with a precolumn (Knauer, Germany), UV detector „Spectra 200“ (Spectra Physics, USA). Data was registered and analyzed

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using "ChromStar 3.20" software (Bruker, Germany).

Statistical analysis. Experiments were repeated 5 times (n=5), aritmetic mean (x) and 95% confidence intervals (x ±Δ) were calculated.

THE SCHEME AND THE RESULTS OF THE STUDY

Raw propolis for production of aqueous solutions was collected from different climatic regions of Lithuania (1 – coastal, 2 – Lower Lithuania, 3 – Middle lowland, 4 – South-eastern highland. Experiments were performed with raw propolis material satisfying the criteria of pharmacopoeia article (FS 2:2001). We analysed the qualitative and quantitative characteristics of propolis aqueous solutions made from specimens collected according to climatic regions.

One of the most important indicators determining the quality of propolis and its solutions is the concentration of phenolic compounds. For this reason, we evaluated the effectiveness of extraction by quantity of phenolic compounds. It is the primary active substance of propolis and the concentration of phenolic compounds in raw propolis should not be less than 30%.

The aim of the study was to discover non-ethanolic indifferent solvent capable of dissolving raw propolis, to optimize the sequence of extraction and technological conditions to obtain optimal concentration of phenolic compounds. Optimal concentration of phenolic compounds was based on by relevant scientific articles.

Experiments were performed in several stages. We answered the following questions during the experiments:

1. Does the climatic region where raw propolis material was collected influence the concentration of phenolic compounds in propolis aqueous solution?
2. Does the concentration of raw propolis material influence the concentration of phenolic compounds in propolis aqueous solution?
3. Which solvent is the most efficient for extraction of phenolic compounds?
4. What technological conditions are needed for the highest extraction of phenolic compounds?
5. What is the optimal time-span for extraction?

DISCUSSION

Grained and specially processed raw propolis material was used for production of solution. The main problem was that propolis is very poorly soluble in water and its aqueous solutions turn white.

We chose water, aqueous glycerol, aqueous macrogol, aqueous glycerol plus macrogol, aqueous macrogol plus sodium citrate solutions as solvents. Extraction in the experiments was performed by several methods (extraction in room temperature, extraction in 70°C and extraction in 100°C). During the experiments the time-span of extraction was determined. Considering diverse technological modifications used in the experiments, we are presenting only the most relevant data.

The influence of raw propolis on the quantity of phenolic compounds was evaluated by comparison of concentration of phenolic compounds in 10% aqueous solutions of propolis collected from 4 climatic regions of Lithuania.

We have performed a comparison of measures of phenolic compounds in propolis gathered in different regions (the difference of means was evaluated using Scheffe test). We did not find any significant differences of phenolic compounds in different regions. The concentration of phenolic compounds in 10% aqueous solution of propolis was 0.098–0.12%. We have discovered a trend showing that a mean concentration of phenolic compounds in propolis is higher in the third climatic region (0.12±0.009%). As our study shows, Lithuanian climate has a minor influence in quality of propolis, i.e. more phenolic compounds were found in propolis from drier climatic region (3 – Middle lowlands).

The influence of raw propolis concentration o the quantity of phenolic compounds was evaluated by selecting different propolis concentrations (from 10% to 40%).

Technological conditions: solvent water, extraction was performed in room temperature for 5 hours. The results are given in table 1.

In this experiment, we have identified that if the amount of raw propolis is increased, the quantity of phenolic compounds is increased significantly ($p<0.05$) (The average results compared to 10% propolis concentration). Discussion: if the amount of propolis is increased, the price of dosage form also increases.

In the next stage we searched for indifferent solvent allowing better extraction of phenolic compounds. Experiments were made using different modes of temperature for extraction (room tempereature, 70°C and 100°C). As a solvent we chose: I – water; II – aqueous glycerol

Table 1. The impact of raw propolis concentration on concentration of phenolic compounds.

Concentration of raw propolis	10%	20%	30%	40%
Concentration of phenolic compounds	0.104±0.01	0.15±0,006*	0.26±0.009*	0.38±0.01*

**(p< 0.05)*

(20%); III – aqueous macrogol (20%); IV – aqueous glycerol (10%) and macrogol (10%); V –aqueous macrogol (20%) and sodium citrate (1%) solutions.

Technological conditions: raw propolis 10%, one of the solvents, extraction performed in room temperature for 5 hours (mode A) and in 70°C for 15 minutes (mode B).

(Note. The results of extraction in 100°C are not given because there were no significant differences in concentration of phenolic compounds, compared to 70°C mode).

The results are given in table 2

The concentration of phenolic compounds in obtained solutions ranged from 0.11% to 0.78%, pH ranged from 4.2 to 6.8. Solutions were clear, yellow, viscous (with macrogol and glycerol) liquids. Solutions remain stable when stored, i.e. the colour remains unchanged, no precipitate is observed and they do not turn white.

In the first stage of the experiment we have identified that there is a significant difference in amount of phenolic compounds extracted from raw propolis using different solvents ($p<0.05$). The highest concentration of phenolic compounds was achieved by extracting propolis with aqueous macrogol solution as well as aqueous macrogol and sodium citrate solution.

We have also determined a direct influence between thermal mode extraction of phenolic compounds: the amount of phenolic compounds increases significantly ($p<0,05$) using different thermal modes. This study proves that thermal mode should be used in the production of propolis solutions, since it directly influences the amount of phenolic compounds.

To determine the optimal time-span for extraction, we evaluated the concentration of phenolic compounds (the quantity of phenolic compounds was examined after 24, 48, 72 and 96 hours). We have found one noteworthy trend during this experiment: the concentration of phenolic compounds in propolis solutions obtained in thermal mode did not change later during extraction. This can be explained by changes in propolis structure occuring during thermal extraction (rolls are formed) and extraction of phenolic compounds is taking place only during thermal process. Yet, in solvents that were produced in room temperature (the quantity of phenolic compounds was examined after 24, 48, 72 and 96 hours) we observed a trend of increase of concentration of phenolic compounds later during extraction. However, there were no differences in average amount of pheno-

Table 2. The influence of technological processes on quantitative and qualitative characteristics of propolis aqueous solutions.

Solvent	I	II	III	IV	V
Concentration of phenolic compounds (mode A)	0.11±0.005	0.16±0.002	0.30±0.004	0.17±0.002	0.27 ±0.003
Concentration of phenolic compounds (mode B)	0.25±0.005*	0.21±0.008*	0.73±0.01*	0.21±0.008*	0.78±0.004*
pH	4.2±0.04	4.4±0.06	4.2±0.02	4.0±0.08	6.8±0.1
Identity of phenolic compounds	Match	match	match	match	match
Appearance (Yellow color, no precipitation and opalescence	match	match	match	match	match

(p< 0.05)

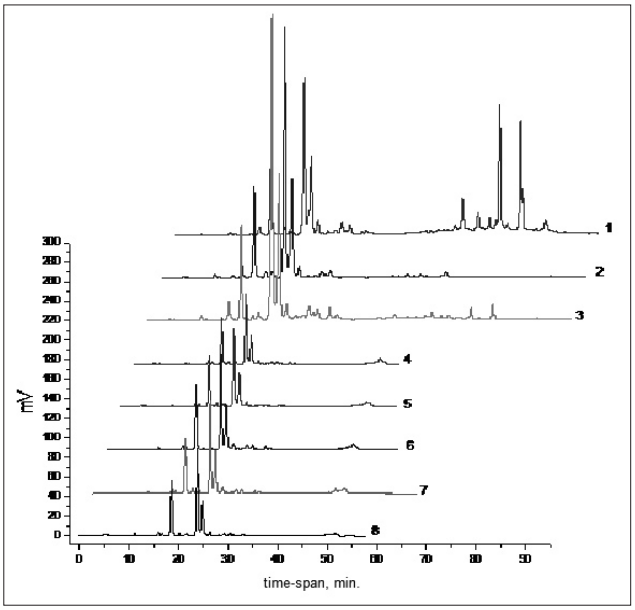

Figure. Chromatograms of propolis extracts.
No. 1 (III) – aqueous 20% macrogol solution (extraction at 70°C temperature);
No. 2 (V) – aqueous macrogol and sodium citrate solution (extraction at room temperature);
No. 3 (V) – aqueous macrogol and sodium citrate solution (extraction at 70°C temperature);
No. 4 (I) – aqueous solution (extraction at room temperature);
No. 5 (II) – aqueous glicerol (extraction at room temperature);
No. 6 (I) – aqueous (extraction at 70°C temperature);
No. 7 (IV) – aqueous glicerol and macrogol (extraction at room temperature);
No. 8 – aqueous (extraction at room temperature). Amount of raw propolis – 20%.

4 µl of extract injected. UV detection at 315 nm wave length. Eluent flow 0.3ml/min. Chromatography performed in gradient mode. Eluent A: water with 0.05% trifluoroacetate, eluent B: methanol with 0.05% trifluoroacetic acid. Chromatograms 4–8 were obtained by programing following gradient: in 35 minutes from 33 to 50% B, 45 minutes – 95% B; chromatograms 1–3 were obtained by programing following gradient: in 35 minutes from 33 to 50% B, 80 minutes – 90% B, 85 minutes – 95% B.

lic compounds in all series during the storage process. To sum up, we have determined that there were no significant changes in amount of phenolic compounds during time spans.

High performance liquid chromatography was also used to detect propolis phenolic compounds (Figure). It should be mentioned that properties of aqueous propolis solution with macrogol differed from all examined solutions (Figure , chromatogram 1). Chromatogram of this solution was more complex: in addition to the fraction of phenolic acids (group of peaks up to 40 minutes), there was a considerable amount of less-polar compounds (peak group eluted during 40 – 80 minutes). Eluent with lower eluting power was sufficient for chromatography of aqueous solutions (4–8 chromatograms). Since more polar phenolic acids were dominant in the extracts, all these compounds were eluted from the column in 35 minutes using eluent consisting of up to 50% of methanol. For chromatography of aqueous solutions with macrogol and sodium citrate (chromatograms 1–3) the gradient was extended up to 85 minutes (95% methanol eluent) to wash less polar compounds (flavonoids) from the column. Considerably higher concentration of phenolic acids in extract was found in aqueous solution with macrogol and sodium citrate (extraction at 70°C temperature) (chromatogram 3). Furthermore, low concentration of flavanoids was found in this solution. Extraction of the same solution at room temperature (chromatogram 2) resulted in slightly lower concentration phenolic acids; less-polar compounds were not detected after this procedure and in other extracts (chromatograms 4–8).

CONCLUSIONS

1. The investigation revealed that the increase in the concentration of raw propolis material significantly increases the amount of phenolic compounds ($p<0,05$). Discussion: if the amount of propolis is increased, the price of dosage form also increases.

2. If raw propolis material is extracted with different solvents, the amount of phenolic compounds changes significantly ($p<0.05$). The highest concentration of phenolic compounds was achieved by extracting raw propolis material with aqueous macrogol and aqueous macrogol plus sodium citrate solutions.

3. Thermal extraction mode directly influences the extraction of phenolic compounds: extracting with the same solution at higher temperatures causes the increase of concentration of phenolic compounds ($p<0.05$)

4. There are no significant changes in concentration of phenolic compounds during time-spans of extraction.

References

1. Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol 1998;36:347-63.

2. Castaldo S, Capasso F. Propolis, an old remedy used in modern medicine. Fitoterapia 2002;73(suppl 1):S1-6.

3. Farre R, Frasquet I, Sanchez A. Propolis and human health. Ars Pharmaceutica 2004;45:121-43.

4. Mani F, Damasceno HC, Novelli EL, Martins EA, Sforcin JM. Propolis: Effect of different concentrations, extracts and intake period on seric biochemical variables. J Ethnopharmacol 2006;21:105(1-2):95-8. Epub 2005 Nov 15.

5. Popova M, Silica S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine 2005;12(3):221-8.

6. Kosalec I, Bakmaz M, Pepeljnjak S, Vladimir-Knezević S. Flavonoid analysis and antimicrobial activity of commercially available propolis products. Acta Pharm 2004;54(1):65-72.

7. Duran N, Koc A, Oksuz H, Tamer C, Akaydin Y, Kozlu T, et al. The protective role of topical propolis on experimental keratitis via nitric oxide levels in rabbits. Mol Cell Biochem 2006;281(1-2):153-61.

8. Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of propolis. Phytother Res 2001;15:561-71.

9. Bankova V. Chemical diversity of propolis and the problem of standardization. J Ethnopharmacol 2005;100(1-2):114-7.

TECHNOLOGINIŲ PROCESŲ ĮTAKA PROPOLIO CHEMINIŲ JUNGINIŲ EKSTRAKCIJAI

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Raktažodžiai: propolis, tirpikliai, ekstrakcija, fenoliniai junginiai, flavanoidai, efektyvioji skysčių chromatografija.

Santrauka

Pagrindinis šio tyrimo uždavinys – nustatyti ir atrinkti neetanolinius tirpiklius propolio žaliavai, sukurti technologinę ekstrakcijos seką bei nustatyti veikliąsias medžiagas. Eksperimentai atlikti su gryna propolio žaliava. Iš skirtingų Lietuvos klimato regionų atrinktų propolio bandinių pagrindu analizuoti propolio tirpalų kokybiniai ir kiekybiniai rodikliai. Tyrimo tikslas. Sukurti technologinę seką ir gauti optimalią fenolinių junginių koncentraciją. Eksperimentų seka nustatyta optimali propolio neetanolinio tirpalo sudėtis ir technologinės sąlygos.

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