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STUDY ON METHODS FOR EXTRACTING PROPOLIS COLLECTED IN MOUNTAIN APIARIES

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ABSTRACT

Propolis (bee glue), a resinous substance that bees harvest from plants, has been used as a popular medicine for hundreds of years and is currently used in natural foods and a variety of pharmaceuticals and cosmetics.
Aim: This study aimed to develop a procedure for obtaining propolis extracts from raw propolis collected from two different regions. The procedure involved a step-by-step approach to ensure maximum extraction efficiency and the measurement of various physical parameters to evaluate the quality of the extracts.
Method: In the period February - April 2023, suitable extracts were obtained from raw propolis of two different botanical origins collected in mountain apiaries in eastern Macedonia. Physical parameters: yields, dry matter, pH, and conductivity have been analyzed in propolis extracts. Two subsequent extractions (by three replicates) were made on the same samples to obtain initial knowledge on the achievement of the highest yield of a certain amount of propolis.
Results: The results lead us to the conclusion that the two-stage extraction with a fresh solvent provides more total extracted dry matter.
Conclusions: The findings emphasize the importance of standardized extraction protocols and further research to better understand the specific bioactive components and their potential health benefits in propolis extracts. Further testing should be performed to analyze the utilization and optimization of unsolved propolis solids.

Keywords: Propolis extracts, two-stage extraction, physical parameters.

INTRODUCTION

Propolis, commonly known as "bee glue," is a resinous substance collected by bees from various plant sources and used for various purposes within the beehive (Bankova et al., 2000). It has gained significant attention in recent years due to its potential therapeutic properties and diverse applications in the pharmaceutical, food, and cosmetic industries (Bankova et al., 2014; Oršolić et al., 2020). The composition of propolis can vary depending on the geographical location, climate, and botanical origin of the collected plant resins, resulting in different bioactive compounds and properties (Marcucci, 1995; Bankova et al., 2014).
While the therapeutic potential of propolis has been extensively studied, less attention has been given to the methods for extracting propolis and optimizing its yield. Efficient extraction methods are crucial for obtaining propolis extracts with high concentrations of bioactive compounds and

ensuring standardized quality for various applications (Daugsch et al., 2008; Khalil et al., 2019). Therefore, a comprehensive study on the methods for extracting propolis, particularly from mountain apiaries, is warranted.

Mountainous regions are known for their diverse flora, which contributes to the unique composition of propolis collected by bees in these areas. Understanding the methods for extracting propolis from mountain apiaries can provide valuable insights into the extraction techniques that optimize the yield and quality of propolis from these specific geographic locations (Khacha-Ananda et al., 2020).

The present study aims to investigate the methods for extracting propolis collected from mountain apiaries to optimize the extraction process and maximize the yield of bioactive compounds. The study will focus on the period from February to April 2023, during which suitable propolis extracts will be obtained from two different botanical sources. The physical parameters of the propolis extracts, including yield, dry matter, pH, and conductivity, will be analyzed to evaluate the effectiveness of different extraction methods.

A two-stage extraction approach will be implemented, involving subsequent extractions using fresh solvents on the same propolis samples. This method will be used to determine the optimal conditions for achieving the highest propolis yield. By comparing the physical parameters of the extracted propolis, valuable insights can be gained regarding the efficiency of the extraction process and the potential for obtaining a higher yield of bioactive compounds.

The results obtained from this study will contribute to the existing knowledge of propolis extraction methods, particularly in the context of mountain apiaries. This research will provide valuable information on the physical parameters of propolis extracts, offering insights into the effectiveness of the extraction process and its potential for optimization. Additionally, the study will lay the foundation for further research on the utilization and potential applications of the remaining propolis solids.

METHODOLOGY

Propolis Collection:

Raw propolis samples were collected from mountain apiaries located in eastern Macedonia between February and April 2023. The collection aimed to obtain samples from two distinct botanical origins, enabling the assessment of potential variations in propolis properties.Extraction Procedure:

The extraction procedure utilized a two-stage method to maximize the yield of bioactive compounds from the propolis samples. Three replicates were conducted for each sample to ensure reliable results. In the first stage, fresh solvent was used for the extraction process. The selection of the solvent was crucial to ensure optimal solubility and preservation of the bioactive constituents present in propolis. The primary objective of this initial extraction stage was to extract as many bioactive compounds as possible.

Following the first stage, the samples underwent a second extraction using the same solvent to extract any remaining propolis constituents that were not extracted during the initial stage. This two-stage extraction approach aimed to maximize extraction efficiency and obtain a higher yield of propolis components.

Analysis of Physical Parameters:

The propolis extracts obtained from the extraction procedure were analyzed for several physical parameters. These parameters included yield, which measured the quantity of propolis extracted

from the samples. The dry matter content was determined to assess the concentration of bioactive compounds present in the extracts. pH, a measure of acidity or alkalinity, and conductivity, an indicator of ion concentration, were also analyzed to provide insights into the overall properties of the propolis extracts. The analysis of these physical parameters allowed for a comprehensive evaluation of the propolis extracts, providing information on the quantity and quality of the extracted propolis components. These parameters served as indicators of extraction efficiency and potential variations in the properties of propolis from different botanical origins.

Procedure for obtaining Propolis extracts

Firstly, 90 g of raw propolis from each region was weighed using an analytical scale. Then, 300 ml of ethanol 96% was measured for the extraction process. The propolis was crushed using a micronizing mill and immersed in the measured ethanol. The mixture was placed on a magnetic stirrer and stirred twice a day for 10 days in a closed container. After the 10-day extraction period, the extract was filtered using vacuum filtration, and all relevant physical parameters were measured. This step allowed for the assessment of parameters such as yields, dry matter content, pH, and conductivity, providing insights into the characteristics of the propolis extracts.

To further enhance the extraction efficiency, an additional 130 ml of ethanol 96% was added to the filtered extract, which already contained a dry residue. The mixture was stirred on a magnetic stirrer for the next five days to dissolve as much of the soluble dry matter in ethanol as possible. On the fifth day, the mixture was filtered again, and all physical parameters were checked to evaluate the final extract. These parameters provided valuable information regarding the quantity and quality of the extracted propolis. Overall, the developed procedure involved the precise measurement of raw propolis and ethanol, followed by a two-stage extraction process and the measurement of physical parameters. This method allowed for the optimization of propolis extraction and the evaluation of the resulting extracts' characteristics.

RESULTS AND DISCUSSION

This study aimed to develop a procedure for obtaining propolis extracts from raw propolis collected from two different regions. The procedure involved a step-by-step approach to ensure maximum extraction efficiency and the measurement of various physical parameters to evaluate the quality of the extracts.

Table 1. Results of various parameters, including dry matter content (DM), pH value, conductivity (Micro S/cm), and quantities of propolis and 96% ethanol used in the extraction process

Test No.	Propolis origin	DM	pH	Micro S/cm	ppm	m(g)	V(ml)	Mass (g)	96% Ethanol (ml)	96% Ethanol (g)	DR (I) (g)	TM (I)	RE (I) (ml)	TE (II) (ml)	REDM (II)
1.	T1	10.49 %	4.90	38	19	146.1	160	90	300	237	74.67	327.33	135	265	21.3
2.	T2	4.02 %	5.08	34	17	118.19	146		130	103	69.92				
3.	R1	11.69 %	5.22	48	24	117.68	139	90	300	237	76.24	327.33	169	299	68.7
4.	R 2	8.94 %	5.21	50	25	100.28	119		130	103	67.28				

T1,2 – Trsino; R1,2 – Rankovce; DM –dry matter; DR (I) – dry residues of I extraction; TM (I) – total mass in the I extraction; RE (I) – remaining ethanol from the first extraction; TE (II) – total ethanol 96% in the second extraction; REDM (II) – remaining ethanol from II extraction bound to dry matter that can't be filtered

The presented table provides valuable insights into propolis extraction methods I and II, highlighting key parameters such as dry matter content, pH value, conductivity, and the quantities of propolis and 96% ethanol used in the extraction process. These results contribute to our understanding of the efficiency and characteristics of the obtained propolis extracts.

The dry matter content of the propolis extracts obtained through different extraction methods varied significantly. Trsino I extraction exhibited a higher dry matter content of 10.49%, whereas Trsino II extraction resulted in a lower dry matter content of 4.02%. Similarly, Rankovce I extraction showed a higher dry matter content of 11.69%, while Rankovce II extraction yielded a value of 8.94%. These differences suggest that the specific extraction method employed significantly affects the amount of dry matter extracted from propolis.

The pH values of the propolis extracts ranged from 4.90 to 5.22, indicating a slightly acidic nature. This acidity is consistent with the natural characteristics of propolis, which contains organic acids. The slightly acidic pH range is in line with previous studies on propolis extracts, highlighting its inherent properties.

Conductivity measurements provide insights into the presence and concentration of dissolved substances in the extracts. The conductivity values observed in the table ranged from 34 to 50 µS/cm. These variations indicate differences in the dissolved substances and their concentrations in the propolis extracts obtained through different extraction methods. Further analysis is required to identify and characterize these specific dissolved substances.

The mass of propolis and the volume of 96% ethanol used in the extractions varied among the samples. These parameters play a crucial role in determining the efficiency and yield of the extraction process. The total mass in the first extraction, including the propolis and ethanol, ranged from 117.68 g to 146.1 g, while the remaining ethanol from the first extraction varied between 135 ml and 169 ml. These results highlight the importance of optimizing the solvent-to-propolis ratio to maximize the extraction efficiency.

In the second extraction, additional ethanol was added to the dry residue from the first extraction. The total volume of 96% ethanol used in the second extraction ranged from 103 ml to 130 ml. The remaining ethanol from the second extraction, bound to the dry matter that cannot be filtered, was observed to be 21.3 g and 68.7 g for Trsino 1 and Rankovce 1, respectively. These findings indicate that some propolis solids may not be fully dissolved or extracted, suggesting the need for further optimization of the extraction process.

The varying levels of dry matter content observed in the propolis extracts are consistent with previous studies that have reported differences in the chemical composition of propolis extracts obtained through different extraction methods. For instance, Bankova (2005) reported variations in the dry matter content of propolis extracts collected from different geographical regions, indicating the influence of botanical sources and extraction techniques on the composition of propolis. Similarly, Burdock (1998) highlighted the impact of the solvent and extraction parameters on the dry matter content and bioactive compound profile of propolis extracts. These studies support the notion that different extraction methods can result in variations in the dry matter content of propolis extracts.

The pH values obtained in the present study fall within the reported range for propolis extracts. Previous research by Sforcin (2007) demonstrated that propolis extracts typically exhibit slightly acidic pH values due to the presence of organic acids, such as caffeic acid and p-coumaric acid.

The observed pH values in the current study align with these findings, reinforcing the natural acidic nature of propolis extracts.
The conductivity measurements provide insights into the presence of dissolved substances in the propolis extracts. While specific information on the nature of these substances is not available in the table, previous studies have reported the presence of various bioactive compounds in propolis, including flavonoids, phenolic acids, terpenoids, and enzymes. Campos et al. (2014) discussed the anti-inflammatory and healing actions of propolis, highlighting the role of these bioactive compounds. The differences in conductivity values observed in the current study may be attributed to variations in the concentration and types of dissolved substances in the propolis extracts obtained through different extraction methods.
The quantities of propolis and 96% ethanol used in the extraction process significantly impact the extraction efficiency and yield. The mass of propolis and volume of ethanol varied among the extraction methods, indicating differences in the extraction conditions. Optimal extraction conditions, including the ratio of propolis to solvent and extraction time, have been investigated in previous studies. Burdock (1998) emphasized the importance of selecting appropriate extraction solvents and optimizing the extraction parameters to maximize the extraction efficiency and obtain propolis extracts with desirable properties.

CONCLUSIONS

In conclusion, the obtained results of propolis extraction methods I and II, providing insights into the dry matter content, pH value, conductivity, and quantities of propolis and ethanol used in the extraction process. The observed variations in the dry matter content among the extraction methods can be attributed to factors such as botanical origin, geographical location, season of collection, and extraction techniques employed. The choice of solvent, in this case, 96% ethanol, also influenced the extraction efficiency and composition of the extracts. The pH values indicate a slightly acidic nature, while the conductivity measurements reflect the presence and concentration of dissolved substances in the extracts.
In summary, the presented research highlights the importance of extraction techniques, solvent choice, and the inherent variability in propolis composition. These findings provide a basis for further studies aimed at harnessing the potential health benefits of propolis extracts.
Contribution to the Field:
- By building upon previous research, this study contributes to the field by expanding upon existing knowledge and identifying potential improvements in Propolis extraction methods.
- The collection of Propolis samples from mountain apiaries allows for a better understanding of how environmental factors influence its composition, contributing to the field's knowledge of regional variations.
- Developing standardized protocols for sample preparation improves the clarity and reproducibility of the study, enhancing the field's ability to compare results and draw accurate conclusions.

RECOMMENDATION

The findings emphasize the importance of standardized extraction protocols and further research to better understand the specific bioactive components and their potential health benefits in propolis extracts. It is crucial to consider the botanical origin and solvent choice when conducting propolis extractions to obtain consistent and reproducible results.

Moreover, studying the variations in chemical profiles and bioactive compounds of propolis collected from different sources can contribute to the development of optimized extraction methods and formulation of propolis-based products with desired properties.

Further investigations should aim to identify and quantify the specific bioactive compounds present in propolis extracts obtained using different extraction methods. Additionally, evaluating the biological activities and potential therapeutic applications of these extracts can provide valuable insights into their pharmacological properties. Standardized and well-characterized propolis extracts have the potential for various applications, such as in pharmaceuticals, functional foods, and natural health products.

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