processes

MDPI

Article

Preparation of Aqueous Propolis Extracts Applying Microwave-Assisted Extraction

Dovaldė Juodeikaitė *, Modestas Žilius and Vitalis Briedis

Department of Clinical Pharmacy, Faculty of Pharmacy, Lithuanian University of Health Sciences, 50162 Kaunas, Lithuania; modestas.zilius@lsmuni.lt (M.Ž.); vitalis.briedis@lsmuni.lt (V.B.)
* Correspondence: dovaldejuodeikaite@gmail.com

Abstract: Water-based propolis extracts usually contain up to 10-fold lower quantities of active ingredients due to poor solubility in water of propolis bioactive compounds when compared with ethanol-based extracts. Since ethanol-based extracts are of limited use, water-based extracts are preferred nowadays. The application of alternative extraction techniques should be evaluated to improve extraction efficiency. Aqueous propolis extracts were prepared using purified water and propylene glycol, 2-hydroxypropyl-beta-cyclodextrin and sodium bicarbonate aqueous solutions. A microwave-assisted extraction method was applied in cycles. The total concentration of hydroxycinnamic acids in aqueous propolis extract produced by four extraction cycles was determined to be 1502.1 ± 130.1 µg/mL and 20% propylene glycol, 10% 2-hydroxypropyl-beta-cyclodextrin and 5% sodium bicarbonate aqueous solutions, increasing the total concentration of hydroxycinnamic acids by 1.6, 1.7 and 1.9-fold, respectively. An application of microwave-assisted extraction method and the procedure of repeating extraction cycles reliably increased the quantity of hydroxycinnamic acids in aqueous propolis extracts. Similarly, the presence of propylene glycol, 2-hydroxypropyl-β-cyclodextrin and sodium bicarbonate increased the concentration of the hydroxycinnamic acids in propolis extracts.

Keywords: propolis; extraction; microwaves

Citation: Juodeikaitė, D.; Žilius, M.; Briedis, V. Preparation of Aqueous Propolis Extracts Applying Microwave-Assisted Extraction. *Processes* **2022**, *10*, 1330. https://doi.org/10.3390/pr10071330

Academic Editors: Antony Kam, Shining Loo and Simon Ming-Yuen Lee

Received: 8 June 2022
Accepted: 1 July 2022
Published: 7 July 2022

1. Introduction

Since the tendency of using natural remedies has been growing, propolis is becoming a popular alternative and complementary medicine product [1]. Propolis is a resinous substance produced by honeybees and it has over 300 bioactive compounds. Most of those are phenolic acids and their esters, flavonoids, chalcones and terpenoids [2]. Due to the variety of compounds in its chemical composition, propolis and its products demonstrate anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant and antiproliferative effects [3]. The traditional way to prepare propolis extracts is by using ethanol as a solvent as most of the propolis bioactive compounds are lipophilic [4].

A traditional extraction method (maceration) is used in the production of ethanol-based propolis extracts [5]. However, ethanol-based propolis extracts have disadvantages: residual taste, smell, and are of limited use in a variety of fields, such as pediatrics, ophthalmology, otorhinolaryngology or even for patients who have alcohol intolerance [6,7]. Developing an alternative way to prepare high-concentrated propolis extracts without the use of ethanol in its manufacturing process would expand the application of propolis extracts into new areas.

A safe, non-toxic alternative solvent for propolis extracts is water, but the production of such extracts is very complex. According to scientific research data, aqueous propolis extracts are approximately 10-fold lower in phenolic compound concentration than ethanol-based extracts, mainly because of poor solubility in water of propolis bioactive compounds [7]. A potential alternative to overcome this issue is choosing an efficient extraction method, as well as applying solubility enhancement techniques.

The microwave-assisted extraction method shortens the extraction duration and increases extraction yield in comparison with traditional extraction methods [8]. Microwaves interact with a polar solvent, rapidly heating it. The moisture in cells begins to evaporate, creating high pressure on cell walls, which leads to cell rupture. Once the cells rupture, the bioactive compounds are released, resulting in an increase in extraction yield [9]. In 2012, Margeretha I. et al. determined that microwaves not only enhance the yield and shorten extraction time, but also show higher selectivity if compared to maceration and reflux extraction methods in extracting flavonoids from propolis [10]. Such results add even more advantages regarding the microwave-assisted extraction method. Applying any of the following solubility enhancement techniques, such as pH adjustment, co-solvency, micellar solubilization, complexation or hydrotrophy, could enhance the solubility of desirable compounds [11] and further result in higher extraction yield. For example, Rodiahwati W., with colleagues, attempted to enhance the solubility of propolis bioactive compounds by adding propylene glycol solution and applying microwave-assisted extraction. They demonstrated that using a 20% propylene glycol solution and adding microwave-assisted extraction had the biggest effect on the extraction yield [12].

Response surface methodology (RSM) is common to use nowadays. This methodology contains a lot of statistical and mathematical techniques, which lead to a more efficient development of any process. RSM shortens the amount of experiments, helps researchers to better identify most affecting variables to their particular model and to determine the optimal point. It is a great tool to optimize different variables, for example, extraction conditions [13]. This is crucial since extraction conditions affect extraction yield significantly. A similar study was conducted whilst using RSM for the experiments of extraction of polyphenols and the optimal extraction conditions were determined successfully [14]. Cavalaro R.I. et al. used the RSM methodology in order to optimize the extraction process from propolis and they determined the optimal point of extraction effectively [15]. Manga E. et al. also used RSM methodology in order to find the optimal extraction conditions of phenolic compounds from *Aframomum citratum* and *Tetrapleura tetraptera* fruits and the optimization process was successful [16].

This study aimed to develop and evaluate aqueous propolis extracts produced by microwave-assisted extraction and to apply the extraction in cycles in order to reach the target concentration. This is significant since extracting bioactive compounds from propolis with water is challenging and the achieved desired product would expand propolis use in different fields.

2. Materials and Methods

The materials used in this study: propolis ("Medicata Filia", Vilnius, Lithuania), purified water (produced in LUHS laboratory), ethanol 96% ("Vilniaus degtinė", Vilnius, Lithuania), propylene glycol (Carl Roth GmbH+Co, Karlsruhe, Germany), 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich® Wacker Chemie AG, Burghausen, Germany), sodium bicarbonate (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany), acetonitrile (Chromasolv) and acetic acid (glacial) $\geq$ 99.8% (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany), as well as ultrapure water (produced by filtering purified water through the Millipore Simplicity HPLC-grade water preparation cartridge (Bedford, MA, USA)).

2.1. Preparation of Propolis Material

Prior to extraction, raw propolis was kept at room temperature and protected from direct light. Then, propolis was ground to powder and kept at +4 °C temperature protected from direct light.

2.2. Preparation of Ethanolic Propolis Extracts

To determine phenolic compounds from ground propolis, they were extracted using 70% ethanol at a sample-to-solvent ratio of 1:100 (w/v), stirring on a hotplate magnetic stirrer IKA® C-MAG HS 7, (Staufen, Germany) for 5 h at stirring speed of 200 rpm and at

25 °C temperature (n = 4, where n is the number of experiments performed). Each sample was taken after 1, 2, 3, 4 and 5 h of stirring. After extraction, extracts of propolis were filtered using nylon membrane filter (Macherey-Nagel, CHROMAFIL®Xtra, PA, 13 mm, 0.20 µm) and analyzed by HPLC.

To prepare a concentrated propolis extract, phenolic compounds from ground propolis were extracted using 70% ethanol at a sample-to-solvent ratio of 1:10 (*w*/*v*), stirring on a hotplate magnetic stirrer IKA® C-MAG HS 7 (Staufen, Germany) for 1 h at stirring speed of 200 rpm and at 25 °C temperature (n = 4). After extraction, extracts of propolis were filtered using Frisenette qualitative filter paper, grade 201, 8–12 µm and then using nylon membrane filter (Macherey-Nagel, CHROMAFIL®Xtra, PA, 13 mm, 0.20 µm) and analyzed by HPLC [7].

2.3. Optimization of Microwave-Assisted Extraction

A response surface central composite (α = 1) experimental design methodology was used to optimize the microwave-assisted extraction conditions. The variables chosen were extraction duration (min) and microwave power (W). The microwave power varied from 474 to 800 W and the extraction duration varied from 1 to 5 min. Extraction variables are presented in Table 1.

Table 1. Extraction variables and their levels.

Independent Variable	Symbol	Levels		
		−1	**0**	**1**
Extraction duration (min)	X	1	3	5
Microwave power (W)	Y	472	636	800

The extraction duration was chosen to be 5 min as a maximum duration due to rapid solvent evaporation after 5 min (it reaches up to 65%). In total, 13 experiments were performed (n = 3) and their extraction conditions are presented in Table 2.

Table 2. Extraction conditions during optimization process.

Run	X = Extraction Duration (min)		Y = Microwave Power (W)	
	Level	**Value**	**Level**	**Value**
1	1	5	0	636
2	0	3	0	636
3	0	3	0	636
4	1	5	1	800
5	−1	1	1	800
6	0	3	0	636
7	1	5	−1	472
8	−1	1	−1	472
9	0	3	0	636
10	0	3	−1	472
11	−1	1	0	636
12	0	3	0	636
13	0	3	1	800

Extraction condition efficiency was evaluated determining the concentration of propolis hydroxycinnamic acids. Equation was generated:

$$z = 43.68891 - 38.66370x - 0.080782y + 0.17667xy, \quad (1)$$

where x—extraction duration (min); y—microwave power (W); z—total hydroxycinnamic acid concentration in aqueous propolis extract (µg/mL).

The criterion chosen for the optimization of extraction conditions was a total concentration of hydroxycinnamic acids. According to this criterion, optimized extraction conditions were 5 min of extraction duration and 800 W of microwave power. The fulfillment of criteria was 100%.

2.4. Preparation of Aqueous Propolis Extracts

Hydroxycinnamic acids from ground propolis were extracted using purified water and aqueous solutions of selected solubilizers at a sample-to-solvent ratio of 1:20 (*w*/*v*). The solubilizers were selected according to published scientific research data in order to improve hydroxycinnamic acid solubility: propylene glycol [12], 2-hydroxypropyl-β-cyclodextrin [17] and sodium bicarbonate [18]. Produced aqueous propolis extracts were filtered using polyvinylidene difluoride (PVDF) membrane filter Frisenette ApS, Q-Max, PVDF, 25 mm, 0.22 µm.

Extraction was performed applying a microwave-assisted extraction method using microwave oven Daewoo KOR-61A5 (DAEWOO Electronics UK Ltd., Winnersh, UK) at optimal extraction conditions: 5 min of extraction duration and at nominal 800 W microwave power (n = 4). The extraction cycle was performed 4 times by adding a new portion of ground propolis in order to prepare saturated aqueous propolis extracts. Due to solvent evaporation a fresh portion of purified water was added between each extraction cycle.

Extraction with selected solubilizers was performed in the same way as it was described previously on the saturation procedure of aqueous propolis extracts. Solubilizers were selected according to scientific data and their aqueous solutions of selected solubilizers were: 5%, 10% and 20% of propylene glycol [12], 5%, 10% and 20% of 2-hydroxypropyl-β-cyclodextrin [17] and 1% and 5% of sodium bicarbonate [18]. Due to sodium bicarbonate reactiveness (quick temperature rise and rapid CO_2 release) during the extraction process, the extraction duration was reduced to 2 min.

To prepare aqueous propolis extracts on a magnetic stirrer for comparison of extraction method efficiency, a hotplate magnetic stirrer IKA® C-MAG HS 7 (Staufen, Germany) was used. The extraction was performed using 70 °C purified water and stirring for 30 min at a stirring speed of 750 rpm (n = 4). The extraction cycle was performed 4 times in a row following the same saturating procedure principle as described previously.

2.5. Propolis Sample Analysis by High-Performance Liquid Chromatography

Phenolic compounds (*p*-coumaric, ferulic, caffeic, vanillic acids and vanillin) were quantified in propolis extracts using Agilent 1260 Infinity capillary LC (Agilent Technologies, Inc., Santa Clara, CA, USA) with Agilent diode array detector (DAD) and applying validated HPLC method: C18 column (150 × 0.5 mm, 5 µm particle size); the linear elution gradient from 1 to 21% of solvent A (acetonitrile) in B (0.5% (*v*/*v*) acetic acid in ultrapure water) 25 min; the injection volume was 0.2 µL, the flow rate was 20 µL/min and the column temperature was 25 °C. The integration of phenolic compound peaks was performed at 290 nm [19].

2.6. Statistical Analysis

Statistical analysis of experimental data was performed using IBM SPSS Statistics software (version 22.0) and Microsoft Excel 2013. One-way ANOVA (Least Significant Difference (LSD) criteria) and Mann–Whitney U test were used for data analysis. Correlation analysis was performed applying Spearman's rank coefficient. The differences were regarded as significant at $p < 0.05$.

3. Results

3.1. Quantity of Phenolic Compounds in Propolis Material and Ethanolic Extracts

In the extraction of phenolic compounds (*p*-coumaric, ferulic, caffeic, vanillic acids and vanillin) from the propolis material, 70% ethanol was used due to its ability to extract high

quantities of phenolic compounds [20]. The total concentration of phenolic compounds was determined to be 28.4 ± 1.3 mg/g (as in propolis material) after 1 h of extraction. Further extraction (it was performed up to 5 h) did not have a statistically significant ($p > 0.05$) effect, meaning the propolis material was extracted after a 1 h extraction procedure. The profile of phenolic compounds in propolis material is presented in Table 3.

Table 3. Profile of phenolic compounds in propolis raw material.

Compound	Concentration (mg/g)	Percentage of Total Phenolic Compounds (%)
p-Coumaric acid	10.5 ± 0.4	37.1 ± 0.5
Ferulic acid	7.6 ± 0.4	26.8 ± 0.3
Vanillin	6.0 ± 0.4	21.2 ± 0.6
Vanillic acid	3.7 ± 0.2	12.9 ± 0.7
Caffeic acid	0.6 ± 0.1	2.0 ± 0.3

p-Coumaric acid was extracted in the largest amounts and the caffeic acid was extracted in the lowest amounts from propolis material. These findings confirmed that the dominating compounds in Lithuanian propolis are *p*-coumaric and ferulic acids [21–23], as well as phenolic aldehyde vanillin [21,22].

After preparing ethanolic propolis extract, the total concentration of phenolic compounds was determined to be 2407.8 ± 61.5 μg/mL. The profile of phenolic compounds is presented in Table 4.

Table 4. Profile of phenolic compounds in ethanolic propolis extract.

Compound	Concentration (μg/mL)	Percentage of Total Phenolic Compounds (%)
p-Coumaric acid	857.8 ± 24.3	35.6 ± 0.3
Ferulic acid	618.7 ± 14.8	25.7 ± 0.1
Vanillin	543.8 ± 11.0	22.6 ± 0.1
Vanillic acid	341.7 ± 11.5	14.2 ± 0.3
Caffeic acid	45.8 ± 3.7	1.9 ± 0.1

The distribution of vanillin and vanillic acid percentage-wise in the concentrated ethanolic propolis extract was statistically significantly higher ($p < 0.05$) than in propolis material, and *p*-coumaric and ferulic acids were statistically significantly lower ($p < 0.05$), except for the caffeic acid—there was no statistically significant ($p > 0.05$) difference. Aforementioned values were chosen as the values of control in the further analysis.

3.2. Determination of Optimal Microwave-Assisted Extraction Conditions

The experimental design methodology was used to optimize microwave-assisted extraction conditions and a response surface central composite design model was applied because it is considered to be suitable and commonly used in the optimization of extraction conditions of phenolic compounds [24,25]. During the preparation of aqueous propolis extracts, according to generated microwave-assisted extraction conditions, vanillin and vanillic acid concentrations were fluctuating in the same batches and samples, and the differences were statistically significant ($p < 0.05$). For example, vanillin concentration varied from 4.7 μg/mL to 105.6 μg/mL, vanillic acid concentration varied from 47.3 μg/mL to 122.4 μg/mL. According to published scientific research data, vanillin is capable of undergoing oxidation into vanillic acid whilst exposed to environment oxygen [26,27]. Mourtzinos I. et al. tested vanillin by exposing its solutions to high temperature (100–180 °C) and they noted that the higher temperature and the longer heating time, the more vanillin was converted into vanillic acid [28].

Due to such instability in vanillin and vanillic acid concentrations in aqueous propolis extracts, their values were eliminated from further analysis. Further aqueous propolis extract concentrations were evaluated according to hydroxycinnamic acids—*p*-coumaric,

ferulic and caffeic acids. The optimization of the extraction condition results is presented in Figure 1.

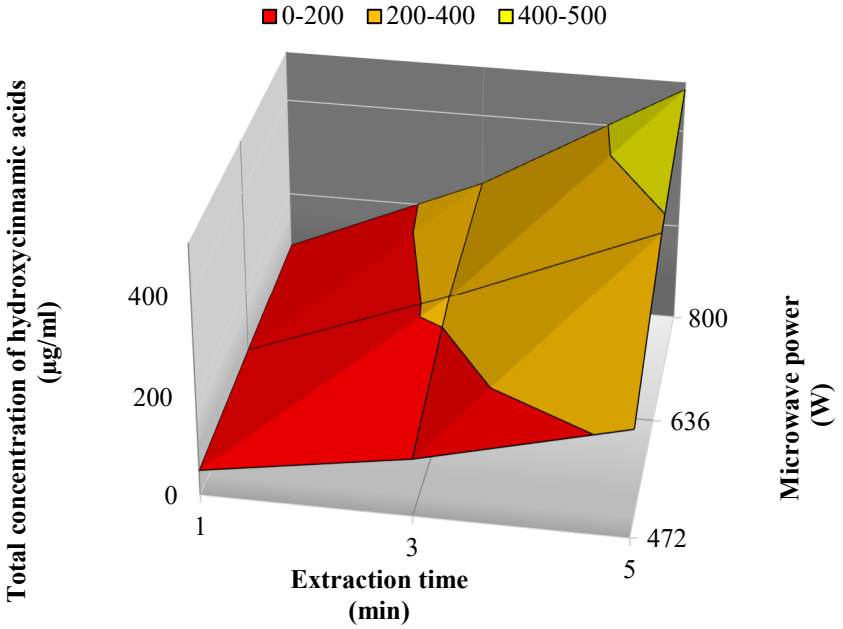


Figure 1. The effect of microwave power and extraction duration on total concentration of hydroxycinnamic acids.

Higher microwave power and longer extraction time resulted in higher concentration of total hydroxycinnamic acids in aqueous propolis extracts. Extraction time had a statistically significant ($p < 0.05$) effect on total hydroxycinnamic acid concentration; a strong correlation was observed (r = 0.843). No statistically significant ($p > 0.05$) correlation was determined between microwave power and total hydroxycinnamic acid concentration.

Since vanillin and vanillic acid were eliminated from further analysis, the hydroxycinnamic acid concentrations in the control sample (concentrated ethanolic propolis extract) were re-calculated percentage wise. Re-calculated values are presented in Table 5.

Table 5. Re-calculated values of hydroxycinnamic acids of concentrated ethanolic propolis extract.

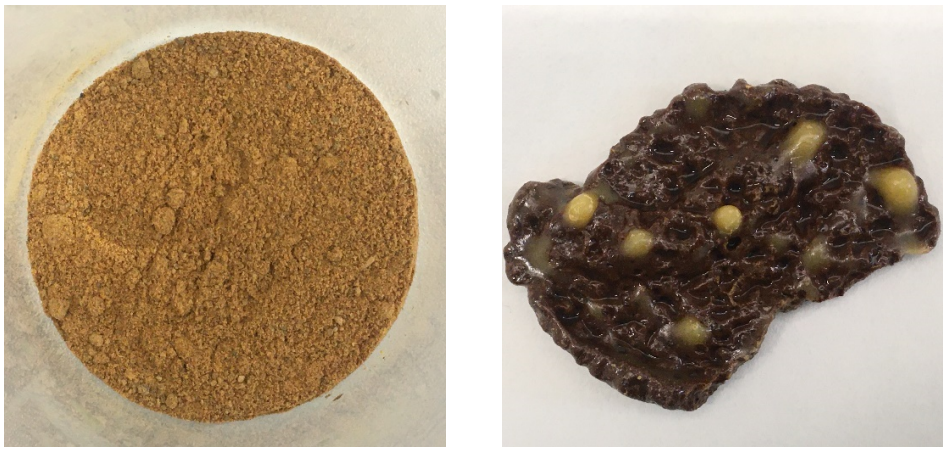
Compound	Concentration (µg/mL)	Percentage of Total Hydroxycinnamic Acids (%)
p-Coumaric acid	857.8 ± 24.3	56.3 ± 0.3
Ferulic acid	618.7 ± 14.8	40.6 ± 0.2
Caffeic acid	45.8 ± 3.7	3.0 ± 0.2

After re-calculation, the total hydroxycinnamic acid concentration in a control sample was obtained to be 1522.3 ± 41.1 µg/mL. Aforementioned values were used in further analysis as comparison values.

The total hydroxycinnamic acid concentration in optimal aqueous propolis extract was approximately 3.1-fold lower if compared to the control sample. In comparison of individual hydroxycinnamic acids, *p*-coumaric and caffeic acids were extracted more using purified water than ethanol, percentage wise. Aqueous propolis extracts were kept for 7 days at +4 °C temperature and the accumulated propolis residue was analyzed—only 3.0 ± 0.3% of total hydrocinnamic acids formed residue and caffeic acid was not detected.

3.3. Saturation of Aqueous Propolis Extracts

During microwave-assisted extraction of propolis compounds, propolis binds itself into a lipophilic mass, decreasing its surface area as well as reducing the extraction efficiency (Figure 2).



(**a**) (**b**)

Figure 2. (**a**) Propolis before microwave-assisted extraction; (**b**) propolis after microwave-assisted extraction.

Due to such changes in propolis material and a decrease in extraction efficiency, the extraction cycle was repeated four times in a row, adding a new portion of propolis material after removing the previous one. A fresh portion of purified water was also added due to solvent evaporation during the extraction process. A similar extraction procedure is commonly used in the re-percolation extraction method, when the first portion of extract is used to soak and extract a fresh portion of used material, then the second portion of extract is used to soak and extract a fresh portion of used material, then the third portion of extract continuing the same principle of extraction [29]. The obtained results, after applying microwave-assisted extraction at optimal conditions and performing four cycles of extraction, are presented in Figure 3.

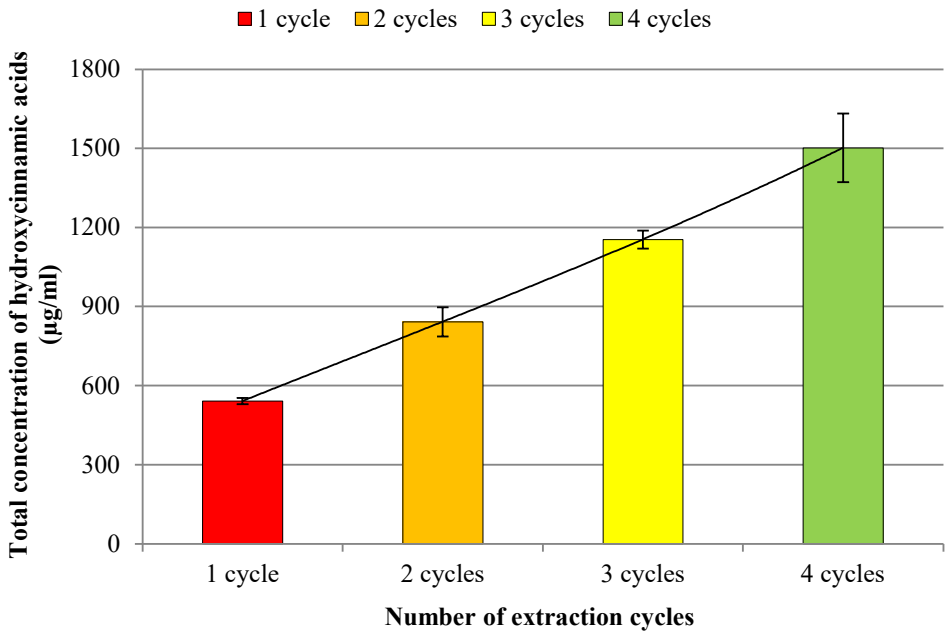


Figure 3. The effect of the number of extraction cycles on total concentration of hydroxycinnamic acids.

The obtained data for the increase in total concentration of hydroxycinnamic acids in aqueous propolis extracts after each extraction cycle was an exact fit for the third order polynomial function (coefficient of determination was $R^2 = 1$). An equation was generated:

$$y = 3.9808 \times 3 - 18.191 \times 2 + 327.54x + 228.27$$

where x—number of extraction cycles; y—total hydroxycinnamic acid concentration (μg/mL).

The total concentration of hydroxycinnamic acids was determined to be 541.6 ± 11.5 μg/mL after the first extraction cycle. After each extraction cycle, the concentration increased by 1.6-fold, 1.4-fold, and 1.2-fold, respectively. The increase in total concentrations of hydroxycinnamic acids was statistically significant ($p < 0.01$). In comparison with concentrations after the first and last extraction cycles, a 2.8-fold higher concentration of hydroxycinnamic acids in aqueous propolis extracts was determined after the last extraction cycle. In terms of percentage, a statistically significant ($p < 0.05$) increase in *p*-coumaric and ferulic acids after the last extraction cycle was observed. The concentration in aqueous propolis extract, obtained after 4 extraction cycles (1502.1 ± 130.1 μg/mL), was compared to the concentration of the control-sample-concentrated ethanolic propolis extract, and there was no statistically significant ($p > 0.05$) difference observed. In terms of percentage, purified water performed more efficiently in the extraction of *p*-coumaric and caffeic acids, and ethanol was more efficient in the extraction of ferulic acid. The mentioned differences were statistically significant ($p < 0.05$).

3.4. Comparison of Extraction Methods

Additional aqueous propolis extracts were produced by applying a traditional stirring method, mainly because it is a well-established method to yield aqueous propolis extracts nowadays [30,31]. The traditional procedure was modified and performed repeating an extraction cycle four times in a row. The total concentration of hydroxycinnamic acids in aqueous propolis extracts prepared by the stirring method was determined to be 257.4 ± 29.4 μg/mL and it was 5.8-fold lower than the concentration in aqueous propolis extracts prepared by microwave-assisted extraction. The obtained data confirm the fact that microwave-assisted extraction shortens the extraction duration and increases the extraction yield in comparison with traditional extraction methods [32–34].

3.5. Determination of Solubilizer Efficiency

To improve the efficiency of the extraction method, solubilizers are used in the extraction process. Three solubilizers that enhance the solubility of lipophilic compounds by different mechanisms were chosen—propylene glycol (PG) as a co-solvent, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) to form complexes and sodium bicarbonate ($NaHCO_3$) to adjust pH levels and possibly form sodium salts with acids. The analysis was completed once to determine the tendency and ability in solubility enhancement of selected solubilizers. The results are presented in Figure 4.

The aqueous propolis extract containing only purified water was chosen as a control sample. Aqueous solutions of 20% of propylene glycol, 10% of 2-hydroxypropyl-β-cyclodextrin and 5% of sodium bicarbonate had the highest effect on the increase in concentration of hydroxycinnamic acids in aqueous propolis extracts and increased concentration of hydroxycinnamic acids by 1.6-fold, 1.7-fold and 1.9-fold, respectively. At this stage, further experiments are needed since the aqueous solutions are very saturated and there is a big possibility that a precipitation process will begin.

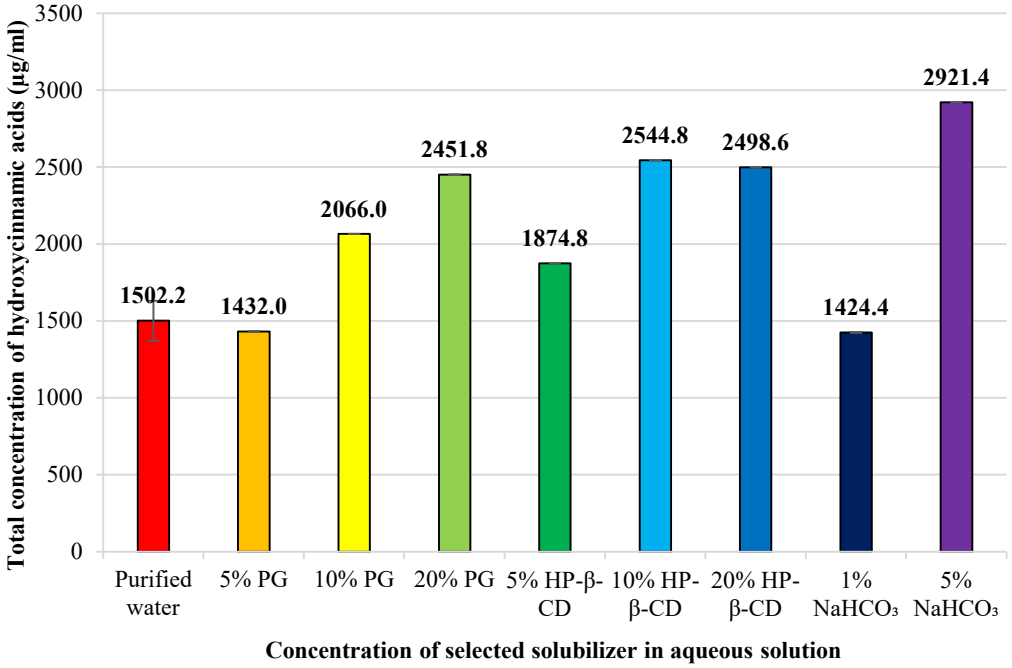


Figure 4. The effect of selected solubilizers on total concentration of hydroxycinnamic acids in aqueous solutions.

4. Discussion

Our study highlights the importance of using a modern extraction method in order to achieve the desired concentration in aqueous propolis extracts. We demonstrate that using microwave-assisted extraction, a better yield of propolis bioactive compounds can be achieved in comparison to traditional extraction methods. What is unique about our study is adding repeated extraction cycles in the microwave-assisted extraction procedure, which led to a target concentration attainment in aqueous propolis extracts. Preparation of such extracts could expand the use of propolis products in the pharmaceutical and/or medical fields.

In early studies, Trusheva B. et al. compared different extraction methods and their effect in propolis bioactive compound (mainly phenolics and flavonoids) yield. They found out that microwave-assisted extraction and ultrasound-assisted extraction both improved extraction yield significantly compared to the traditional maceration process. It has been demonstrated that longer irradiation times result in a decreased amount of extracted bioactive compounds, possibly due to degradation [35]. This finding is similar to our study result, since a significant shift was demonstrated in vanillin and vanillic acid concentrations due to oxidation processes after using the microwave-assisted extraction method. For future reference, a modification in our microwave-assisted extraction method is recommended in order to be able to control the temperature during the process and it would possibly avoid such phenolic compound degradation. Later on, Jang M. et al. confirmed that microwave-assisted extraction shortened extraction time and improved yield by 2.25-fold [36]. These findings are supported by our results, demonstrating that the yield increased two-fold when comparing maceration and the first extraction cycle of microwave-assisted extraction. Yet, adding more extraction cycles to the process of extracting phenolic acids from propolis leads to even better yield, as our study demonstrates. We mentioned Rodiahwati W. et al. and their study earlier by using 20% propylene glycol solution to enhance the extraction yield of propolis bioactive compounds. Our results confirm theirs, since different propylene glycol concentrations (5%, 10% and 20%) were compared, and it was demonstrated that 20% propylene glycol solution enhanced the propolis bioactive compound solubility most effectively. Our experiments using solubilizers show a great perspective to go even further and maximize the extraction yield, confirming

the previous study findings. Adding solubilizers could possibly reduce the amount of extraction cycles for the desired concentration to be achieved, which leads to a faster extraction procedure but this needs to be investigated in further studies. Heidari G. et al. combined microwaves and ultrasound into a sequential process and found out that it produced significantly higher yield if compared to maceration and Soxhlet extraction processes. They state that this method is considered to be low cost, fast and energy saving [37]. Wyan L. et al. compared microwave and ultrasonication extraction methods to traditional extraction methods when extracting propolis bioactive compounds. They confirmed that microwave and ultrasonication extraction methods serve as a rapid and improved extraction method [38]. These findings add a new perspective from combining a few modern extraction methods in order to achieve even better extraction yield.

The findings of this study confirm the results mentioned earlier and add a new perspective for extracting propolis bioactive compounds in cycles, which improves the extraction yield significantly. Microwave-assisted extraction in cycles is a quite unique process and it is a promising technique to extract propolis bioactive compounds in the future. Novel extraction methods are efficient in providing higher extraction yields in comparison to traditional, time-consuming extraction methods. Adding solubility enhancement techniques also improves the extraction yield, which is also a promising technique for future studies. Nevertheless, there is a need to further explore extraction methods and their different variables, such as extraction time, temperature, power and their influence on the bioactive compounds and their degradation processes.

5. Conclusions

The application of a microwave-assisted extraction method and the procedure of repeated extraction cycles efficiently increases the concentration of hydroxycinnamic acids (*p*-coumaric, ferulic and caffeic acids) in aqueous propolis extracts. The concentration determined in aqueous propolis extracts prepared by microwave-assisted extraction does not differ significantly from the concentration determined in ethanolic propolis extracts, which means a target concentration of aqueous extracts could be achieved. Microwave-assisted extraction is shorter in duration and a more efficient method in comparison to traditional extraction methods. Aqueous solutions of propylene glycol, 2-hydroxypropyl-β-cyclodextrin and sodium bicarbonate support the increase in the concentration of the hydroxycinnamic acids in propolis extracts.

Author Contributions: Conceptualization, M.Ž. and V.B.; Data curation, D.J. and M.Ž.; Formal analysis, D.J. and M.Ž.; Investigation, D.J. and M.Ž.; Methodology, D.J. and M.Ž.; Resources, V.B.; Supervision, M.Ž. and V.B.; Visualization, D.J.; Writing—Original draft, D.J.; Writing—Review & editing, D.J., M.Ž. and V.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Keskin, M.; Keskin, Ş.; Kolayli, S. Preparation of alcohol free propolis-alginate microcapsules, characterization and release property. *LWT* **2019**, *108*, 89–96. [CrossRef]
2. Ristivojević, P.; Trifković, J.; Andrić, F.; Milojković-Opsenica, D. Poplar-type propolis: Chemical composition, botanical origin and biological activity. *Nat. Prod. Commun.* **2015**, *10*, 1869–1876. [CrossRef]
3. Ahmed, R.; Tanvir, E.M.; Hossen, M.S.; Afroz, R.; Ahmmed, I.; Rumpa, N.E.N.; Paul, S.; Gan, S.H.; Sulaiman, S.A.; Khalil, M.I. Antioxidant properties and cardioprotective mechanism of Malaysian propolis in rats. *Evid. Based Complement. Altern. Med.* **2017**, *2017*, 5370545. [CrossRef] [PubMed]

4. Ecem Bayram, N.; Gerçek, Y.C.; Bayram, S.; Toğar, B. Effects of processing methods and extraction solvents on the chemical content and bioactive properties of propolis. *J. Food Meas. Charact.* **2020**, *14*, 905–916. [CrossRef]
5. Escriche, I.; Juan-Borrás, M. Standardizing the analysis of phenolic profile in propolis. *Food Res. Int.* **2018**, *106*, 834–841. [CrossRef] [PubMed]
6. Kubiliene, L.; Maruska, A.; Gailiunaite, V. The Influence of Technological Processes On Extraction of Chemical Compounds of Propolis. *Biomedicina* **2011**, *21*, 105–108.
7. Kubiliene, L.; Laugaliene, V.; Pavilonis, A.; Maruska, A.; Majiene, D.; Barcauskaite, K.; Kubilius, R.; Kasparaviciene, G.; Savickas, A. Alternative preparation of propolis extracts: Comparison of their composition and biological activities. *BMC Complement. Altern. Med.* **2015**, *15*, 156. [CrossRef] [PubMed]
8. Švarc-Gajić, J.; Stojanović, Z.; Segura Carretero, A.; Arráez Román, D.; Borrás, I.; Vasiljević, I. Development of a microwave-assisted extraction for the analysis of phenolic compounds from Rosmarinus officinalis. *J. Food Eng.* **2013**, *119*, 525–532. [CrossRef]
9. Tatke, P.; Jaiswal, Y. An overview of microwave assisted extraction and its applications in herbal drug research. *Res. J. Med. Plant* **2011**, *5*, 21–31. [CrossRef]
10. Margeretha, I.; Suniarti, D.F.; Herda, E.; Mas'ud, Z.A. Optimization and comparative study of different extraction methods of biologically active components of Indonesian propolis *Trigona* spp. *J. Nat. Prod.* **2012**, *5*, 233–242.
11. Vemula, V.R.; Lagishetty, V.; Lingala, S. Solubility enhancement techniques. *Int. J. Pharm. Sci. Rev. Res.* **2010**, *5*, 41–51.
12. Rodiahwati, W.; Ariskanopitasari, A.; Saleh, I.K. Identification of total bioflavonoid compound of propolis extract from wild honey bee hives apis dorsata in Sumbawa Region, Indonesia. *Appl. Sci. Eng. Prog.* **2019**, *12*, 37–43. [CrossRef]
13. Mohamad Said, K.A.; Mohamed Amin, M.A. Overview on the Response Surface Methodology (RSM) in Extraction Processes. *J. Appl. Sci. Process Eng.* **2016**, *2*, 8–17. [CrossRef]
14. Montenegro-Landívar, M.F.; Tapia-Quirós, P.; Vecino, X.; Reig, M.; Valderrama, C.; Granados, M.; Cortina, J.L.; Saurina, J. Fruit and vegetable processing wastes as natural sources of antioxidant-rich extracts: Evaluation of advanced extraction technologies by surface response methodology. *J. Environ. Chem. Eng.* **2021**, *9*, 105330. [CrossRef]
15. Cavalaro, R.I.; da Cruz, R.G.; Dupont, S.; de Moura, J.M.L.N.; de Souza Vieira, T.M.F. In vitro and in vivo antioxidant properties of bioactive compounds from green propolis obtained by ultrasound-assisted extraction. *Food Chem. X* **2019**, *4*, 100054. [CrossRef]
16. Manga, E.; Brostaux, Y.; Ngondi, J.L.; Sindic, M. Optimisation of phenolic compounds and antioxidant activity extraction conditions of a roasted mix of *Tetrapleura tetraptera* (Schumach & Thonn.) and *Aframomum citratum* (C. Pereira) fruits using response surface methodology (RSM). *Saudi J. Biol. Sci.* **2020**, *27*, 2054–2064. [PubMed]
17. Vasilaki, A.; Hatzikamari, M.; Stagkos-Georgiadis, A.; Goula, A.M.; Mourtzinos, I. A natural approach in food preservation: Propolis extract as sorbate alternative in non-carbonated beverage. *Food Chem.* **2019**, *298*, 125080. [CrossRef]
18. Qin, P.; Wei, A.; Zhao, D.; Yao, Y.; Yang, X.; Dun, B.; Ren, G. Low concentration of sodium bicarbonate improves the bioactive compound levels and antioxidant and α-glucosidase inhibitory activities of tartary buckwheat sprouts. *Food Chem.* **2017**, *224*, 124–130. [CrossRef] [PubMed]
19. Žilius, M.; Ramanauskiene, K.; Briedis, V. Release of propolis phenolic acids from semisolid formulations and their penetration into the human skin in vitro. *Evid. Based Complement. Altern. Med.* **2013**, *2013*, 958717. [CrossRef]
20. Cunha, I.B.S.; Sawaya, A.C.H.F.; Caetano, F.M.; Shimizu, M.T.; Marcucci, M.C.; Drezza, F.T.; Povia, G.S.; Carvalho, P.O. Factors that influence the yield and composition of Brazilian propolis extracts. *J. Braz. Chem. Soc.* **2004**, *15*, 964–970. [CrossRef]
21. Adomavičiute, E.; Pupkevičiute, S.; Juškaite, V.; Žilius, M.; Stanys, S.; Pavilonis, A.; Briedis, V. Formation and investigation of electrospun PLA materials with propolis extracts and silver nanoparticles for biomedical applications. *J. Nanomater.* **2017**, *2017*, 8612819. [CrossRef]
22. Kubiliene, L.; Jekabsone, A.; Zilius, M.; Trumbeckaite, S.; Simanaviciute, D.; Gerbutaviciene, R.; Majiene, D. Comparison of aqueous, polyethylene glycol-aqueous and ethanolic propolis extracts: Antioxidant and mitochondria modulating properties. *BMC Complement. Altern. Med.* **2018**, *18*, 165. [CrossRef] [PubMed]
23. Ramanauskiene, K.; Savickas, A.; Inkeniene, A.; Vitkevičius, K.; Kasparavičiene, G.; Briedis, V.; Amšiejus, A. Analysis of content of phenolic acids in Lithuanian propolis using high-performance liquid chromatography technique. *Medicina* **2009**, *45*, 712. [CrossRef] [PubMed]
24. Mao, H.; Qiu, Z.; Xie, B.; Wang, Z.; Shen, Z.; Hou, W. Development and application of ultra-high temperature drilling fluids in offshore oilfield around bohai sea bay Basin, China. In Proceedings of the Offshore Technology Conference Asia, Kuala Lumpur, Malaysia, 22–25 March 2016; pp. 1201–1222.
25. Pellati, F.; Prencipe, F.P.; Bertelli, D.; Benvenuti, S. An efficient chemical analysis of phenolic acids and flavonoids in raw propolis by microwave-assisted extraction combined with high-performance liquid chromatography using the fused-core technology. *J. Pharm. Biomed. Anal.* **2013**, *81–82*, 126–132. [CrossRef]
26. Englis, D.T.; Manchester, M. Oxidation of Vanillin to Vanillic Acid. *Anal. Chem.* **1949**, *21*, 591–593. [CrossRef]
27. Svard, M.; Gracin, S.; Rasmuson, A.C. Oiling Out or Molten Hydrate—Liquid–Liquid Phase Separation in the System Vanillin–Water. *J. Pharm. Sci.* **2007**, *96*, 2390–2398. [CrossRef]
28. Mourtzinos, I.; Konteles, S.; Kalogeropoulos, N.; Karathanos, V.T. Thermal oxidation of vanillin affects its antioxidant and antimicrobial properties. *Food Chem.* **2009**, *114*, 791–797. [CrossRef]
29. Ramanauskienė, K.; Savickas, A.; Bernatonienė, J. The influence of extraction method on the quality of the liquid extract of St John's wort. *Medicina* **2004**, *40*, 745–749.

30. Gülçin, I.; Bursal, E.; Şehitoğlu, M.H.; Bilsel, M.; Gören, A.C. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. *Food Chem. Toxicol.* **2010**, *48*, 2227–2238. [CrossRef]
31. De Moura, S.A.L.; Negri, G.; Salatino, A.; Lima, L.D.D.C.; Dourado, L.P.A.; Mendes, J.B.; Andrade, S.P.; Ferreira, M.A.V.D.; Cara, D.C. Aqueous extract of Brazilian green propolis: Primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. *Evid. Based Complement. Altern. Med.* **2011**, *2011*, 748283. [CrossRef]
32. Kaderides, K.; Papaoikonomou, L.; Serafim, M.; Goula, A.M. Microwave-assisted extraction of phenolics from pomegranate peels: Optimization, kinetics, and comparison with ultrasounds extraction. *Chem. Eng. Process.-Process Intensif.* **2019**, *137*, 1–11. [CrossRef]
33. Rafiee, Z.; Jafari, S.M.; Alami, M.; Khomeiri, M. Microwave-assisted extraction of phenolic compounds from olive leaves; a comparison with maceration. *J. Anim. Plant Sci.* **2011**, *21*, 738–745.
34. Simsek, M.; Sumnu, G.; Sahin, S. Microwave Assisted Extraction of Phenolic Compounds from Sour Cherry Pomace. *Sep. Sci. Technol.* **2012**, *47*, 1248–1254. [CrossRef]
35. Trusheva, B.; Trunkova, D.; Bankova, V. Different extraction methods of biologically active components from propolis; a preliminary study. *Chem. Cent. J.* **2007**, *1*, 13. [CrossRef] [PubMed]
36. Jang, M.; Sheu, S.; Wang, C.; Yeh, Y.; Sung, K. Optimization Analysis of the Experimental Parameters on the Extraction Process of Propolis. *Lect. Notes Eng. Comput. Sci.* **2009**, *2175*, 1295–1299.
37. Heidari, G.; Najafpour, G.D.; Mohammadi, M.; Moghadamnia, A.A. Microwave ultrasound assisted extraction: Determination of quercetin for antibacterial and antioxidant activities of Iranian propolis. *Int. J. Eng. Trans. B Appl.* **2019**, *32*, 1057–1064.
38. Wyan, L.O.; Charland, J.M.; Mojica, E.E. Comparative Study of the Extraction Methods for the Instrumental Analysis of Bee Propolis. *Undergrad. J. Teach. Res.* **2021**, *1*, 51–62.