JOURNAL OF TROPICAL LIFE SCIENCE
2021, Vol. 11, No. 2, 187 – 192
http://dx.doi.org/10.11594/jtls.11.02.08

Research Article

Potential and Performance of Accelerated Solvent Extraction (ASE) in Obtaining Bioactive Compounds from Bee Propolis: Comparison with Soaking, Ultrasonication, and Microwave-Assisted Methods

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Article history:
Submission December 2020
Revised December 2020
Accepted January 2021

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ABSTRACT

Propolis is a natural resinous substance collected by honeybees from buds and exudates of trees. The material has attracted much attention in recent years as a functional food component since it possesses various biological properties, including antimicrobial, antioxidative, and anti-ulcer properties. In this study, the performance of accelerated solvent extraction (ASE) was assessed and compared with varying methods of extraction: soaking (maceration), ultrasonication, and microwave-assisted methods. Gas chromatography-mass spectrometry (GC-MS) and other spectroscopic techniques, such as absorbance and fluorescence, were employed to assess the efficiency in the extraction of natural products. The antioxidant activity and phenolic content of the different extracts were also determined. Results showed samples obtained from the microwave method showed the highest yield in the extraction of bioactive compounds. Although microwave showed the best method in this study, some issues and recommendations on ASE application for extracting natural products from bee propolis were discussed. Given the ease in controlling extraction temperature with ASE, this technique has a great potential to be a better method for extraction of heat-labile natural products from propolis should optimization of conditions for extraction were further performed.

Keywords: Antioxidant activity, Accelerated solvent extraction, Bee products, Extraction methods, Microwave, Propolis, Ultrasonication

Introduction

Propolis is a natural resinous substance collected by bees from various tree species that the insect uses to protect their beehives against invaders and microorganisms. The substance has a history of being used in medicine, cosmetics, and as an ingredient in food and beverages. Several studies have attributed bee propolis to a wide range of biological properties, such as antioxidant, antifungal, anti-inflammatory, and antibacterial [1]. These natural properties are due to the active components present in propolis extract, mainly phenolics and flavonoids. The chemical composition of propolis samples varies by geographic region, bee species, and even collection time [2-5].

The traditional extraction method for propolis is maceration, followed by soaking in a suitable solvent. However, these methods are deemed cumbersome and time-consuming, requiring 1-10 days.

More recent approaches in the chemical extraction from propolis include ultrasonication [6, 7], supercritical extraction [8], and microwave-assisted extraction [7,9]. These newer methods are considered more efficient and faster in extracting the chemical components from bee propolis because they use less solvent than traditional

How to cite:
Charland JM, Deocaris CC, Micor JRL, Mojica ERE (2021) Potential and Performance of Accelerated Solvent Extraction (ASE) in Obtaining Bioactive Compounds from Bee Propolis: Comparison with Soaking, Ultrasonication, and Microwave-Assisted Methods. Journal of Tropical Life Science 11 (2): 187 – 192. doi: 10.11594/jtls.11.02.08.

approaches. An extraction method that has not been widely explored for bee propolis is accelerated solvent extraction (ASE). ASE is an automated extraction technology that uses high temperature and pressure and has the advantages such as rapid analysis, low organic solvent use, and batch sample processing [10]. It is an efficient and fast approach like the other modern methods and environment-friendly since it uses less solvent volume. By far, there are only a few studies that have been reported to use this method in processing bee propolis [11-13]. This study aims to investigate the potential of ASE and compare its performance with other known extraction methods, such as ultrasonication, microwave-assisted, and soaking or maceration. A comparison of the extraction's effectiveness was made by examining the extracts using GC-MS and by absorbance and fluorescence spectroscopy and measuring the phenolic content and antioxidant properties. ASE can be a very good method since one can modify the extraction temperature to room temperature, which is not achievable for sonication and microwave methods given the longer extraction time with propolis.

Material and Methods

Extraction using different methods

The propolis samples were obtained from a bee farm in Sorsogon, Philippines. After freezing, the samples were pulverized using a mortar and pestle and then allowed to pass thru an 80-mesh sieve to obtain uniform particle size.

In several 20 mL vials, we mixed a ratio of 1.0 g of propolis sample with 10 ml ethanol. The bee propolis sample was soaked in the solvent for 24 hours at room temperature for the soaking method. For the ultrasonication method, the propolis samples were sonicated using a laboratory ultrasonicator (Fisher Scientific FS20H). Sonication was performed for two sets of samples: one for 10 min and another for 30 min. For the microwave-assisted extraction (MAE) protocol, the propolis-solvent mixture was placed in a 50 ml beaker and microwaved for a total of 10 seconds (2 x 5 seconds power on and 10 sec off in between) using a standard 700-watt household microwave (Samsung). Finally, for the accelerated solvent extraction (ASE) method, only 0.5 g of propolis per sample run. The following parameters were used for the ASE extraction: 1500 psi, 100°C, 1-minute static, 50% volume wash, and purge time of 60 seconds. After the extraction procedure, each sample was filtered using a 0.45 um filter, and the collected extract (2.0 mL) was aliquoted in Eppendorf tubes and stored in the freezer.

GC-MS

GC-MS analysis was performed with a Hewlett-Packard gas chromatograph 6890 series linked to a Hewlett-Packard 5973 mass selective detector with a 30 µm × 250 µm × 0.28 µm HP5-MS column. The temperature was programmed from 110°C to 280°C at a rate of 10°C/min and a 15-minute hold at 280°C. Helium was used as a carrier gas with a flow rate of 1.5 mL/min. The injector temperature was 110°C with a 2 min hold. The analysis has a full run of 36 minutes, with 5 µL sample extract used or injected in each scan.

Absorbance and fluorescence spectroscopy

The sample was diluted (1/1000) with ethanol for the spectroscopic methods. The extracts' electronic absorbance spectra were measured using a JASCO v-570 spectrophotometer (Easton, MD). Quartz cuvettes (1 mL) were used for all experiments. Pure ethanol was used as a blank. After absorbance measurements, the same samples were analyzed for fluorescence spectra. Emission measurements were made on a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon, Edison, NJ) using 1 nm slits. Each sample's emission spectra were obtained at two different excitation wavelengths: 250 and 330; 350 and 400 nm. The emission spectra of the appropriate blanks were also obtained. All spectroscopic measurements were performed in triplicates (3 sets of 1/1000 dilution).

Assays for antioxidant and total phenolics

The antioxidant activity and total phenolics present in each extract were determined using DPPH assay and Folin-Ciocalteu assay, respectively. Both assays were performed in microplate reader wherein different concentrations of extracts were prepared and used. Each extract (0.1 g/mL) was diluted 10-fold three times to give extract with concentrations ranging from 0.01 to 1×10^{-4} g/ml. Gallic acid (2.5-100.0 ug/mL) was used as a standard. For the DPPH assay, 10 µL of the extract/standard was used and mixed with 200 µL of 0.2 mM DPPH. After 30 minutes, absorbance at 519 nm was obtained.

The antioxidant activity was determined using the given formula below, and IC_{50} was derived to

express the antioxidant activity.

$$\% \text{ Scavenging Activity} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100 \quad (1)$$

Folin-Ciocalteu assay was performed by mixing 10 µL of each sample/standard with 125 µL of Folin-Ciocalteu reagent (0.1N) for 4 minutes and then added with 100 µL of 1.0 M Na2CO3. Absorbance at 745 nm was obtained after 2 hours of incubation. A blank was used that just contained ethanol instead of the extract or standard. All absorbance readings were obtained using Biotek Cytation 5 Image Reader. Both assays were replicated at least three times.

Statistical analysis

Experimental data (absorbance and emission signals in specific wavelengths and assays) were evaluated using student's t-test ($p < 0.05$) to compare their difference.

Results and Discussions

Four extraction methods were used and compared by analyzing the components and bioactivities of the propolis extracts. The total ion chromatograms (Figure 1) using gas chromatography showed similar profiles for all the extracts obtained using the different methods, with the highest peak observed around 31 min. The microwave extraction method exhibited the highest relative abundance, with the chromatograms showing a relative abundance of approximately 6.6 x 10^6. Ultrasonic extraction showed the highest peak with a relative abundance of 5.4×10^6 at 30 minutes and 5.6×10^6 at 10 minutes. The soaking method exhibited a relative abundance of around 4.7×10^6, while extracts from ASE had the highest relative abundance at approximately 4.1×10^6. Inspection of the peaks only identified the presence of amyrin. It is a pentacyclic triterpene commonly found in propolis [13]. This compound was also reported from the same set of samples used in this study [14]. However, the other peaks present were not identified as MS spectra analysis with the installed library software showed match results lower than 50 percent.

For the absorbance spectroscopy, three distinct peak regions can be observed: 210, 290, and 330 nm (Figure 2). Ultrasonication at 10 min showed the highest absorbance among all extracts. It was followed by microwave extraction, ultrasonication at 30 minutes, soaking, and ASE. Extract from microwave extraction consistently

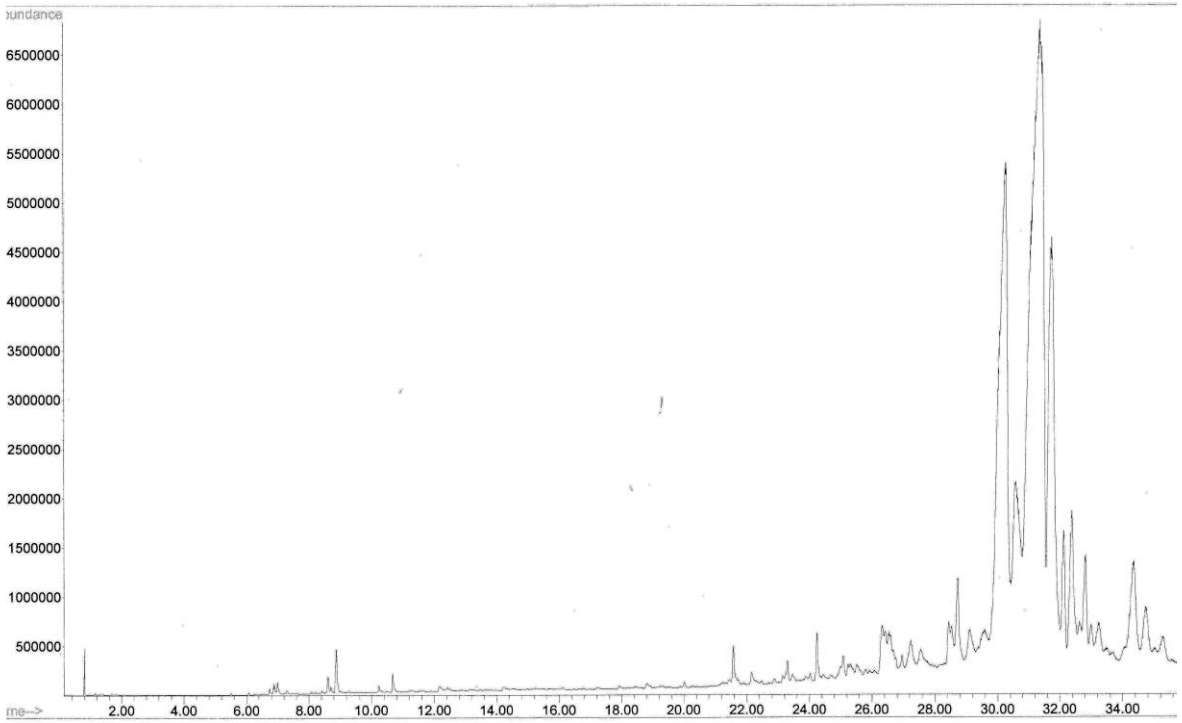


Figure 1. Total ion chromatogram (GC-MS) of propolis extract obtained through microwave extraction. All other chromatograms have the similar profiles but with different intensities

showed the highest intensity excited at 290 nm and 330 nm in fluorescence emission. This trend was followed by ultrasonication at 10 minutes and 30 minutes and soaking. ASE consistently showed the lowest absorbance and fluorescence. Lastly, except for soaking and ultrasonication (30 min) in absorbance and fluorescence excited at 290 nm, all spectra are significantly different.

The IC_{50} for the DPPH assay was determined for the extracts and shown in Table 1. The lower the value of the IC_{50}, the higher is the antioxidant activity. Based on results, the extract from microwave extraction gave the lowest IC_{50} value, followed by ultrasonication (10 min), soaking, and ASE. The antioxidant activity of the extract using the microwave method was consistent with the total phenolic levels. Among the four methods, the soaking technique yielded the least amounts of phenolic compounds. Statistical analysis showed a significant difference in ASE from the other methods (no difference among the other three methods) in the antioxidant assay. However, total phenolic content showed a significant difference between each method except for ASE and ultrasonication (10 min).

The extraction of bioactive compounds from bee propolis is the first step in its utilization as component in dietary supplements, food ingredients, pharmaceuticals as well as cosmetic products. Solvent extraction is the most common approach to extract bioactives from the bee materials due to its ease of use, efficiency, and broad applicability. Albeit, the yield and quality of the extraction depend on the type of solvents, extraction time, method of extraction, and the samples' chemical composition and physical characteristics.

In this study, four methods were used and compared to extract bioactive components from bee propolis. Microwave extraction appeared to the most efficient among the extraction methods explored. The performance of microwave extraction was validated by the results of the instrumental analysis, except for absorbance where it was ranked second, and antioxidant and total phenol measurements. In a study comparing three extraction methods (microwave, ultrasonication, and soaking method), the microwave and ultrasonication methods result in high extraction yield, requiring short timeframes and less labor input [7]. Ultrasonication-based extracts, on the other hand, showed lower phenolic content and antioxidant activity. Extending ultrasonication may still improve extraction efficiency based on previous work [7].

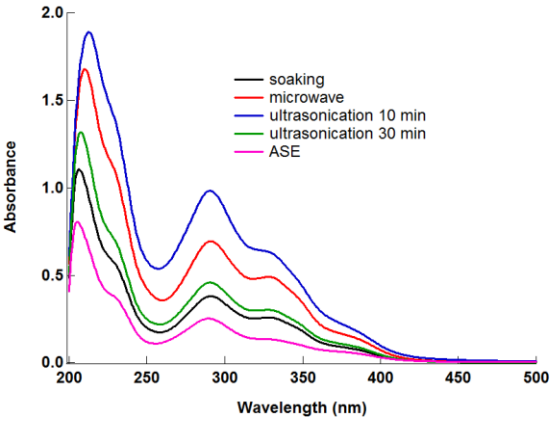
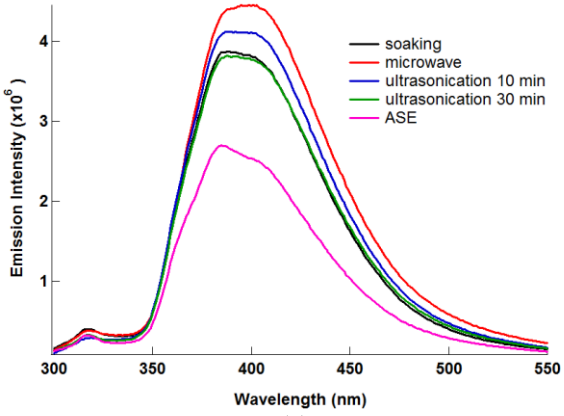


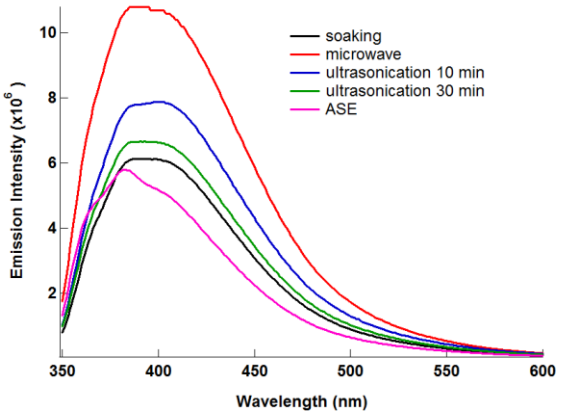
Figure 2. Absorbance of propolis extracts obtained using different extraction methods

Absorbance spectrometry is a widely used method for analyzing bee products because of its non-destructive nature [15]. Since the propolis samples came from one source, the absorbance profiles obtained are similar; despite difference in extraction method was used (Figure 3). Even though ultrasonication at 10 minutes gave the highest absorbance values, on one end, the extracts derived from the microwave method displayed the most increased fluorescence. We also noticed that the intensity at the excitation wavelength of 330 nm (Figure 3a) is higher than 290 nm (Figure 3b). This indicates that our propolis extracts from the microwave method were more fluorescent. The sample from the ASE method consistently had the lowest absorbance and lowest emission intensity. Its fluorescence spectrum was also different compared to the other methods. Notably, the extract from ASE had higher phenol content than that of the extract from soaking process but was closer in amounts to the extracts obtained using the ultrasonication method.

Among the four methods, ASE had the lowest antioxidant property. Initially, the same mass of the sample was used for ASE based on the methodology reported by the group of Oliveira Dembogurski [12]. However, we observed some technical issue when our instrument indicated an erroneous run at higher pressure, $\geq$1500 psi. Interestingly, our propolis samples adhered on the inner surface of the sample cylinder forming a large chunk at higher temperatures, $\geq$100°C. These observations were noted with other propolis samples from different geographic sources (data not shown). Some of our samples also formed sticky



(a)



(b)

Figure 3. Emission intensity of propolis extracts obtained using different extraction methods. Emission spectra were excited at 290 nm (a) and 330 nm (b)

Table 1. Antioxidant activity and phenol content of extracts.

Extraction Method	Antioxidant IC_{50} (mg/mL)	Phenol content (mg/mL)
Soaking	2.72 ± 0.61	54.38 ± 0.63
US (10 min)	2.04 ± 0.57	201.62 ± 0.84
US (30 min)	2.29 ± 0.64	222.66 + 0.63
Microwave	1.77 ± 0.58	428.52± 1.16
ASE	7.37 ± 1.32	205.07 ± 0.63

*US = ultrasonication

To resolve these issues, we modified some of the parameters. First, the amount of solvent washing volume was reduced from 150% to 50%, and the purge time was limited to 60 sec and exceeding 100 sec. Second, the mass of the original sample before extraction was also reduced to half. With these new conditions, we successfully obtained and analyzed the propolis extracts from ASE. It is also worth noting that although extraction temperature may be increased to enhance solubility and mass transfer rate of the analyte [11], this may prove to be problematic as well. Some propolis compounds are easily hydrolyzed or oxidized, thus prolonging the extraction times, and decreasing the extraction yield. Intuitively, this may be the reason only a few studies have been published on the application of ASE for propolis [11-12].

There is a need to do additional studies on the use of ASE for propolis samples. One crucial area not covered by this study is the optimization of other ASE parameters, such as temperature and pressure, which can affect extraction efficiency [11]. In one study that observed the effect of temperature ranging from 20-80°C, 40°C was the most efficient temperature for extracting phenolic compounds from propolis [11]. In terms of pressure, extraction efficiency increased beyond 1500 psi and reached a threshold at 2000 psi [11].

Conclusion

This study compared the extraction performance of four methodologies. We believe our results can provide future guidance in improving the quality and quantity of bioactive compounds extracted from propolis. Although microwave-assisted extraction gave the best results and based on its initial performance, the potential of ASE cannot be ignored especially if the same amount of materials were used. There are inherent advantages of ASE to realize its full potential in the extraction and analysis of bioactive compounds from propolis. There is a need to further optimize the method by manipulating other variables, such as extraction solvent, time, pressure, and temperature. ASE can be performed on room temperature but high pressure which is more advantageous to sonication and microwave methods where temperature increases at longer period resulting in degeneration of bioactive compounds.

Acknowledgement

This material is based upon work supported by the National Science Foundation under grant no. 1828320. We also like to thank Prof. Ruel Desamero of CUNY York College for the use of the spectroscopic instruments.

References

1. Pellati F, Prencipe FP, Bertelli D, Benvenuti S (2013) 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. Journal of Pharmaceutical and Biomedical Analysis. 81-82, 126-132. doi: 10.1016/j.jpba.2013.04.003.
2. Bankova V (2005) Chemical diversity of propolis and the problem of standardization. Journal of Ethnopharmacology 100 (1-2): 114-117. doi: 10.1016/j.jep.2005.05.004.
3. Barbosa da Silva Cunha I, Lucia Tiveron Rodrigues M, Cesar Meurer E et al. (2006) Effect of the maceration time on chemical composition of extracts of Brazilian propolis. Journal of Apicultural Research 45 (3): 137-144. doi: 10.1080/00218839.2006.11101332.
4. Kujumgiev A, Tsvetkova I, Serkedjieva Y et al. (1999) Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology 64 (3): 235-240. doi: 10.1016/s0378-8741(98)00131-7.
5. Tielemans A, Deocaris CC, Mojica ERE Antioxidant activity and phenolic content of bee propolis from selected countries. Submitted to Journal of Tropical Life Sciences.
6. Khacha-Ananda S, Tragoolpua K, Chantawannakul P, Tragoolpua Y (2013) Antioxidant and anti-cancer cell proliferation activity of propolis extracts from two extraction methods. Asian Pacific Journal of Cancer Prevention. 14 (11): 6991-6995. doi: 10.7314/apjcp.2013.14.11.6991.
7. Trusheva B, Trunkova D, Bankova V (2007) Different extraction methods of biologically active components from propolis: a preliminary study. Chemistry Central Journal 1, Article 13. doi: 10.1186/1752-153x-1-13.
8. Idrus NFM, Yian LN, Idham Z et al. (2018) Mini review: Application of supercritical carbon dioxide in extraction of propolis extract. Malaysian Journal of Fundamental and Applied Sciences. 14 (4): 387-396. doi: 10.11113/mjfas.v14n4.1088.
9. Hamzah N, Leo CP (2015) Microwave-assisted extraction of Trigona propolis: The effects of processing parameters. International Journal of Food Engineering. 11 (6): 861-870. doi: 10.1515/ijfe-2015-0106
10. Azwanida N (2015) A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal and Aromatic Plants 4 (196): 2167-0412. doi: 10.4172/2167-0412.1000196.
11. Erdogan S, Ates B, Durmaz G et al. (2011) Pressurized liquid extraction of phenolic compounds from Anatolia propolis and their radical scavenging capacities. Food and Chemical Toxicology 49: 1592-1597. doi: 10.1016/j.fct.2011.04.006.
12. de Oliveira Dembogurski DS, Trentin DS, Boaretto AG et al. (2018) Brown propolis-metabolomic innovative approach to determine compounds capable of killing Staphylococcus aureus biofilm and Trichomonas vaginalis. Food Research International 111: 661-673. doi: 10.1016/j.foodres.2018.05.033.
13. Yam-Puc A, Santana-Hernandez AA, Yah-Nahuat PN et al. (2019) Pentacyclic triterpenes and other constituents in propolis extractfrom Melipona beecheii collected in Yucatan, México. Revista Brasileira de Farmacognosia 29: 358-363. doi: 10.1016/j.bjp.2019.01.006.
14. Ragasa CY, Galian RAF, Ebajo Jr. VD et al. (2015). Propolins and glyasperin A from stingless bee nest. Revista Brasileira de Farmacognosia 25 (2): 177-179. doi: 10.1016/j.bjp.2015.03.006.
15. Maldonado L, Marcinkevicious K, Borelli R et al. (2020) Differentiation of Argentine propolis from different species of bees and geographical origins by UV spectroscopy and chemometric analysis. Journal of the Saudi Society of Agriculutral Sciences 19 (2): 185-191. doi: 10.1016/j.jssas.2018.09.003.