Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Propolis Harvesting and Extraction

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Abstract

α-aminophosphonate oxadiazoles (**5a-m**) were prepared in high yields by reacting of 1,3,4-oxadiazole acetohydrazide (**3**) with Propolis is a resin-like material produced by Beehives. Propolis has many vital applications and can be used to make various products. This review paper highlights the methods of propolis harvesting and how to increase its production. Raw propolis has limited uses, so it was essential to understand the methods used to obtain propolis extract. Standard extraction methods are based on the use of a solvent for extraction, and the commonly used solvents are alcohol, water, and glycol, each with its advantages and disadvantages. The solvent extraction method consumes a lot of time, extending for several days, so some modern extraction methods have been used to save time, such as ultrasound-assisted extraction, microwave-assisted extraction, and supercritical carbon dioxide extraction.

Keywords: *Review; Propolis; Propolis harvesting; Propolis extraction; Ultrasound; Microwave; Supercritical carbon dioxide*

Introduction

Beehives produce several useful products such as honey, beeswax, royal jelly, pollen, and propolis. Propolis is a resin-like material of a tan,dark-yellow, orange, or brown color. The propolis composition varies depending on the district, weather, bee species, and types of available plants. Propolis's resin content (flavonoids and related phenolic acids) represents about 50% of its composition. Other constituents comprise beeswax (30%), aromatic material (10%), in addition to pollen, and mechanical admixtures (5% each) [1].

Propolis as a disease-fighting antioxidant has been appreciated for its believed health benefits for thousands of years. Ancient civilizations used propolis to treat infection in wounds, tumors and assist the healing course. Today, propolis is highly required in alternative medicine. Propolis has proven antibiotic, antiseptic, antiviral, and anti-inflammatory properties. Bee propolis can be used to make various products. Propolis could be used in its raw state and obtained in tinctures, tablets, and capsules at health stores [2].

Propolis harvesting

The worker bees gather resins from plants and trees such as poplar, cottonwood, alder, and birch. These sticky resins are normally secreted by these plants during budding, serving the purpose of keeping off bugs, fungus, and diseases. Bees bring the resins back to the hive on their hind legs (pollen baskets). Bee saliva and wax, honey, etc., are mixed with the resins to create the propolis. These sticky resins are taken back to the bee colony for use [3].

The honey bees use propolis to narrow the entrance to the hive, thus making it easier to defend against predators. In addition, propolis is used as a building material and acts as a disinfectant and as an embalming agent to cover surfaces. Propolis is one of nature's most potent antimicrobial substances; it cleans and sterilizes the hive given its natural properties [4].

Harvesting propolis can boost beekeeping returns. The beekeeper may sell raw propolis to supply

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Receive Date: 16 February 2022, Revise Date: 18 April 2022, Accept Date: 26 April 2022 .
DOI: 10.21608/EJCHEM.2022.122043.5469

companies; this adds another valuable product from the hive to the apiary to help offset the costs. Selling propolis is big business due to a very high demand in the industry.

The amount of propolis that can collect from one hive varies. Some beekeepers find they only harvest about 50-150g in a season, but using special procedures and a collection mat can increase significant earnings of 1kg or more [5].

Propolis can be collected by catching the hive scrapings when cleaning out the honey supers during the honey harvest. Scraping is an easier way to get propolis without disturbing the hive. A beehive that has failed and is empty is another source of bee glue. After scraping the wooden surfaces, propolis has to store in a jar or plastic bag in the freezer. Propolis collected in this manner will contain contaminants such as bits of wax, wood scrapings, dead bees, etc. Flavonoids, the major bioactive component of propolis, tend to chelate metals ion as lead, which is one of the primary propolis pollutants. The propolis obtained by the scraped method presented four folds of lead compared with the mesh method of harvest. Hence, beekeepers are advised to use the mesh harvest method to obtain a better quality and safe product [6].

Unpolluted propolis could be collected through a contraption called a propolis trap, which helps increase the amount of propolis gathered and makes the collection very simple. Autumn is the best time of year for propolis production. The bees during this time are working hard to seal any cracks and crevices in their home before winter weather sets in, so autumn is the perfect opportunity to put traps in place [7].

Propolis trap, for harvesting propolis, is an inexpensive and re-usable object made from a plastic grid that resembles a queen excluder with lots of smaller holes. It is used in place of an inner cover, just below the telescoping surface, to introduce huge numbers of small gaps needing to be filled. A small opening in the outer cover is required, just enough to let light into the hive. The bees get annoyed by the small gaps, which are too small for bee passage. Hence they are forced to fill up these small openings on the trap using the plant secretions referred to as propolis. The bees may take a few days or even months to fill up the propolis on the trap depending on the area or geographical location, the plant species available and the prevailing weather conditions [8].

Once the trap is full, or before winter cold arrives, the trap is removed and placed in a bag inside the freezer for a few hours. After frozen, the trap is twisted and whacked a few times until bits of propolis pop out, and then it is quickly collected before they get warm. It is known that the consistency of propolis varies dramatically with temperature from sticky when warm to hard and brittle when cold. After collecting, the material was promptly stored in a lidded container for transport.

Extraction of propolis

Regardless of its origin, raw propolis is not suitable for direct use in food technology, pharmaceutical, or cosmetic industry applications due to its poor water solubility and high content of impurities. Propolis samples had solid contaminants (mainly waxes, resin, and hazardous substances, asphalt from roads), typical vegetable odor, and a very distinctive, intense scent necessary to be removed [9]. Therefore, propolis must be purified before oral consumption or before use in other applications [10]. Propolis extraction using organic solvents is necessary to extract its bioactive constituents. The process is expected to eliminate inert materials while preserving the polyphenolic fractions [11].

Propolis and propolis extracts have a peculiar odor that is to be overcome. This drawback restricts their use to a few types of food products. An odorless propolis extract could be achieved by treating propolis extract with an anion-exchange resin that eliminates the odorizing ingredients of propolis and maintains its inherent biological activities. This propolis extract can be used in various biologically active compositions [12].

Propolis extracts for use in food production are usually obtained using ethanolic solutions or water. Extraction with ethanol is particularly suitable for obtaining dewaxed extracts rich in polyphenolic components. On the other hand, extraction with pure water is ideal for getting extracts containing water-soluble phenolic acids [13]. Propolis extract contains the primary polyphenols such as flavonoids, phenolic acids, and their esters and has various biological effects, including antimicrobial and antioxidant properties [14].

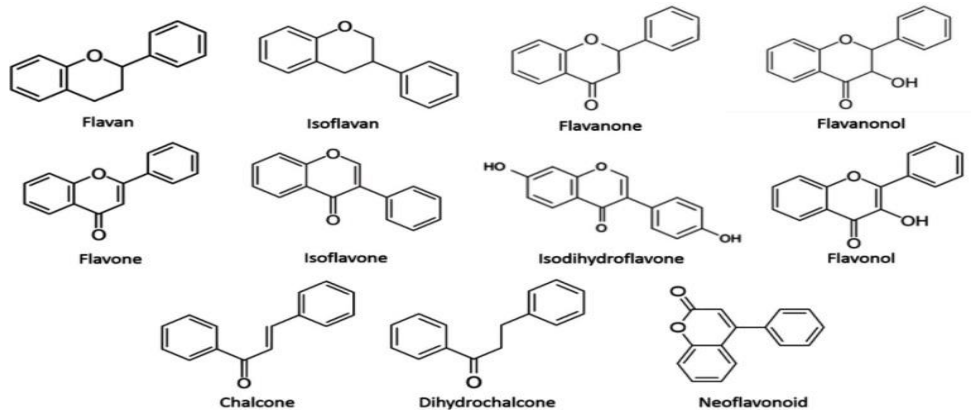

Figure 1: Flavonoid isolated from different propolis and propolis extract samples.

The most common method to obtain a propolis extract is solvent extraction. However, this procedure is being increasingly replaced by ultrasound extraction, whose efficiency for such vegetal compounds as polyphenols has been reported. So, it would be exciting to review which extraction procedure yields the best values for potential functional properties of propolis [15].

Extraction methods and techniques

Concerning the techniques for extracting raw propolis, room temperature maceration and hot reflux extraction were widely used in the past [13]. Propolis extracts are obtained by soaking crumbled propolis in ethanol, glycerol, and/or water. The commercial Poly(vinylidene fluoride) (PVDF) membranes could be used to decrease the solvent in propolis extract with minimum loss of bioactive compounds [10].

Ethanol extraction

Different solvents, such as ethanol, water, methanol, ethyl acetate, and others, were used for propolis extraction. Ethanol extracts show the highest activity for most of them. Propolis extraction can be easily achieved by maceration and Soxhlet extraction using ethanol [10]. Ethanol extraction is beneficial to attain propolis extracts with a low wax content and rich in bioactive compounds [11, 14]. Furthermore, ethanol is non-toxic and can be easily removed after extraction if propolis extracts are used as food ingredients [15]. However, alcoholic extraction poses disadvantages, such as strong residual flavor and refusal of some consumers to alcohol [11].

Different techniques for ethanolic extraction of propolis were applied. Generally, 1g crude grounded propolis was extracted using 10 ml of (30-96%, mostly 75%) aqueous ethanol solution. Extraction was carried out at the temperature range from room temperature to 50 ºC with periodical shaking for 1-7 days. The solution was then filtered through a Whatman No. 1 filter paper. The extract solution was concentrated using a rotary evaporator under reduced pressure at 40 °C or lyophilized to attain the propolis ethanolic extract. The extract was stored at 4ºC in the dark until analysis or use. Table 1 summarizes some detailed conditions for ethanolic extraction of propolis.

H_2O extraction

Data on the production of aqueous propolis solutions are limited; however, their indisputable advantages are the low production cost and absence of ethanol in its chemical composition. On the other hand, a disadvantage of water extraction processes is propolis's intense flavor and aroma and an approximately 10-fold lower content of phenolic compounds compared with ethanol extraction due to their lower solubility in water. Nevertheless, some authors found that both water and water/alcohol extraction exhibit similar concentrations of phenolic compounds, thereby producing an inexpensive product with suitable functional properties [11].

Water extraction of propolis was prepared by suspension of 50 g frozen propolis and extracted with 50 mL of distilled water. Extraction was done at 26 ºC for 48h on a shaker at 150 rpm. The extract was centrifuged for 30 min, and the supernatant was collected and evaporated at 25 ºC for 3 days; then, the residual resin was gathered to use in successive tests [26]. Another author used extract filtration seven

times using Whatman No 2 filter paper instead of centrifugation [19].

Siqueira et al. prepared aqueous solutions of ground propolis samples by suspension of 1g propolis with 10 ml of purified water. This suspension was then heated at 40 ºC on a hot plate till complete dissolution. After cooling, the solution was filtered through Whatman filter paper no. 6. Then the filtrate was evaporated at 40 ºC in an oven, and the remaining filtrate solution was kept in the dark at 4 ºC until tested [27].

Phenolic compounds in various plant materials could be extracted with an aqueous cyclodextrin (CD) solution as an eco-friendly solvent instead of conventional organic solvents. Through the extraction of propolis phenolic compounds, the addition of CD to water reduces the extraction time and improves the extraction efficiency. Moreover, the attained phenolic extract shows superior antioxidant activity [28]. Typical extraction was carried out by adding 11.1% (w/w) aqueous solution of CD to 500 g of pulverized propolis at 25 °C in the dark. The extract was filtered using Whatman filter paper No. 1 to remove the waxes and other insoluble components and stored at 4 °C [29].

Table 1

Some detailed conditions for ethanolic extraction of propolis.

Propolis (g)	Ethanol (ml)	Ethanol conc.	Temp. ºC	Time day	Ref
3	100	30	50	1	[16]
20	250	80	RT	7	[17]
--	--	80	--	7	[18]
1	10	70	25	2	[14]
100	200	95	--		[19]
1	10	80	25	2	[20]
10	100	≥99	--	1	[21]
10	100	96	20	7	[22]
30	100	≥99 or 60	RT	7	[9]
1	10	70	37	14	[23]
10	200	96	25	15	[24]
5	45	96	25	7	[24]
10	500	80	40	0.125*	[15]
250	600	80	RT	2	[25]

* Using mechanical agitation for 3 h at 40 °C with an agitation speed of 300 rpm.

Glycerol/ Glycol extraction

It was proven that the employed solvent extraction could influence the effectiveness of the extract's antimicrobial activity [13]. Four propolis extracts were used in industries; ethanol, glycerin, propylene glycol, and oil solutions. It was found that all of the used solvent extracts at the used concentration (ethanol 60%, glycerin, propylene glycol, and edible oil) have comparable antimicrobial properties. The glycerin extract slightly inhibits Gram-positive bacteria, while the oil extract has a wide range of antimicrobial activity. The ethanol and propylene glycol extracts showed a superior activity against yeasts [30].

Glycerin extract was prepared by mixing 5.0 g of propolis powder with 45 ml of glycerin, and the suspension was warmed at 40°C for 30 min. The extraction was conducted in the dark at room temperature for 7 days, with occasional manual shaking. The volume, after filtration using cotton wool, was adjusted to 50 ml. The extract was stored in the dark at 5°C until use [24].

Unfortunately, most of the biologically active substances in propolis are scarcely soluble in water or oil, which is usually used in the pharmaceutical

industry. In contrast, ethanolic extracts cannot be used to treat certain diseases and have some limitations of application in the pharmaceutical industry. Non-ethanolic solvent and higher extraction temperatures allow efficient extraction of propolis. This method allows obtaining low-wax propolis extracts, which are rich in biologically active compounds [31]. Polyethylene glycol (PEG) is an alternative solvent for propolis, allowing more efficient extraction of various active substances from propolis when compared to water extractions [11].

For efficient extraction of biologically active substances from propolis that is poorly soluble in water, extraction at 70 °C temperature and using PEG and water mixture or PEG, olive oil and water mixture were applied. PEG provides comparable total phenolic compounds in the extract with that found in ethanolic extract. In addition, PEG extracts demonstrate efficient antioxidant and antimicrobial activity [32]. Propolis extract could be diluted to 5%, 10%, and 15% solution with propylene glycol. In addition, propylene glycols can dissolve beeswax in the propolis to produce a sticky oily solution which is applied as a coating material [33].

Advanced techniques in propolis extraction

Some advanced techniques could be used in propolis extraction such as ultrasound-assisted extraction (UAE) [34], microwave-assisted extraction (MAE)[34-36], and supercritical carbon dioxide extraction [37]. These methods provide the advantages of reduced extraction times and lower solvents used in the extraction and higher extraction yields than conventional methods.

Ultrasound-assisted extraction (UAE)

The traditional propolis extraction process is long and time-consuming and requires a considerable amount of ethanol. During extraction of crude propolis, ultrasonication improves the selectivity of removal and recovery of active compounds. As a consequence of decreasing the extraction time (30 min), the extraction unit scale can be considerably reduced. Such a technique is required in industries for viable economic and green extraction procedures [38].

Ultrasonic could assist the typical ethanolic extraction by a method called ultrasound-assisted extraction (UAE). UAE uses ultrasonic energy for extraction with an ultrasonic bath and/or probe. UAE makes cavitation bubbles that collapse and produce higher shear and results in complete extraction [13]. Ultrasound assists contact between the propolis and solvent, increasing the mixing speed of the components and removing the stagnant layer barrier. Also, ultrasound participates in propolis fragmentation and thus enhances its contact with the solvent. It also increases the cell pores, which facilitate solvent penetration. These courses accelerated mass exchange between the material and the solvent, which increased the extraction yields [39].

Crude propolis samples were dried and then crushed well, homogenized with an electric blender, and passed throughout a 2 mm mesh sieve. 2 g of dried ground sample were immersed in 10 ml of 96% ethanol in ultrasonic bath equipment 28 kHz/1100 W at 35 ◦C for 60 min. The ethanolic extracts were concentrated under reduced pressure in a rotary evaporator at 45 ºC to yield a solid residue ready for use [40]. In another trial, 2g of propolis were extracted under dark conditions with 30 ml of 80% ethanol in an ultrasonic bath with a heating frequency of 40 kHz for 20 min. Afterwards, the mixture was filtered (Whatman filter paper No. 4). The solid residue was re-extracted twice using the same conditions to extract the most bioactive compounds from the crude propolis. The propolis extracts were stored in the dark at −20 °C until analyses. A comparison of three extraction methods of propolis; shaking extraction (SE), ultrasound-assisted extraction (UAE), and ultrasound-assisted shaking extraction (SUAE), were carried out using 70% ethanol, extraction times of 1 or 7 days, propolis to ethanol ratios of 1:10 or 1:5. In the (SE) method, samples were shaken (200 rpm) at 28 ºC for 1 or 7 days. In the (UAE) method, samples were subjected sonication process for 10, 20, and 30 min at a power of 210 W and a frequency of 20 kHz. In the (SUAE) method, samples were subjected to both shaking and then to the ultrasound as before. The obtained extracts were filtered through Whatman No. 4 filter paper and subsequently condensed under reduced pressure at 40 ºC. Then, the extracts were freeze-dried and stored in dark containers at 4 ºC. The extraction method affects the extraction yields, antimicrobial properties of extracts, and the contents of phenolic and flavonoid compounds. SUAE process provided a higher yield, superior antimicrobial

activity, and high content of phenols and flavonoids than SE and UEA [13].

In another study, propolis was extracted by a sequential sonication using polar solvents (ethanol, ethyl acetate, and hexane). Three cycles of 60 min were carried out for each solvent, and at every cycle, the extract was withdrawn, and the new solvent was added. The solvents were recovered using a rotary evaporator at 40 °C. [39]

Microwave-assisted extraction (MAE)

MAE of bioactive constituents from raw propolis is a fast extraction method compared to maceration and even the UAE. However, lower extraction selectivity with a high amount of unwanted wax in the extracts was a drawback. Also, extended irradiation periods decreased the proportion of extracted active components due to degradation [34].

MAE of raw propolis in the closed vessel was helpful to decrease the extraction time and solvent quantity. The effects of extraction parameters, temperature, time, applied power, and liquor ratio on the extract yield, total phenolic, flavonoids, and antioxidant activity were investigated. At low microwave power, an enhancement in yield and quality of propolis extract was detected by extending the extraction time. At higher microwave power, a considerable decrease in the yield was recorded with the risk of ethanol release due to the increased pressure on the vessel. Precise control of temperature below 125°C, allow propolis extraction in a shorter time (15 min) and lower volume of used ethanol (sample to solvent ratio of 1:5 (w/v)) as well as avoiding extract degradation in contrast to maceration [36]. This finding follows an earlier study that affirmed that the extraction of polyphenols from raw propolis by MAE techniques was improved by a shorter extraction time and lower volume of solvent needed [36].

Various published results demonstrate that both MAE and UAE modern technologies result in a comparable extraction efficiency compared to traditional extraction technologies under milder extraction conditions and shorter processing time [41, 42].

Supercritical carbon dioxide ($scCO_2$) extraction

Recently $scCO_2$ has increased utilization for the extraction of active constituents from natural products [43]. Carbon dioxide is an ultimate solvent for the extraction of lipophilic substances since it is readily available, non-explosive, non-toxic, and easy to remove from the final product. scCO2 is attractive for propolis extraction due to propolis' resinous nature and the presence of lipophilic compounds. During $scCO_2$ extraction of propolis, pressure and time have the most significant effect on the extraction yield and extract composition, while temperature has a minor impact. The sample was soaked in $scCO_2$ for about 30 min and then extracted at room temperature and pressure for different times at 2 L/min (wet gas meter). The extract was obtained by the separator and accurately weighted. At optimum conditions of 317 bar, 45 °C, gas flow 2 L/min, and 6.5 h, the maximum yield of 14.3% extracted material is obtained. $scCO_2$ is usually used to produce certain types of extracts rich in lipophilic constituents; it could also be used as a pre-treatment before ethanolic extractions to facilitate further extraction. The extracts obtained by $scCO_2$ have a different composition compared with those extracted with ethanol. The propolis residues after $scCO_2$ extraction still contain a reasonable amount of flavones and phenolics which can be acquired by traditional extraction methods [37, 44]. Using of $scCO_2$ to obtain antioxidant fractions from propolis extract was established [45, 46].

Conclusion

The propolis amount collected by catching the hive scrapings could be increased up to 10 folds through a propolis trap contraption. However, raw propolis is not suitable for direct use in food technology, pharmaceutical, or cosmetic industry applications, so it should be purified. Propolis extracts are obtained by soaking crumbled propolis in ethanol, glycerol, and/or water. Ethanol extracts show the highest activity towards propolis extraction compared with other solvents; it is beneficial to attain propolis extracts with a low wax content and rich in bioactive compounds. Advantages of aqueous extraction are the low production cost and absence of ethanol in its chemical composition. Glycol extracts demonstrate efficient antioxidant and antimicrobial activity. Ultrasound-assisted, microwave-assisted, and supercritical carbon dioxide extraction provide the advantages of reduced extraction times and lower solvent used in the extraction and provided higher extraction yields than conventional methods.

Conflict of Interests

The authors declare that they have no conflict to interests.

Acknowledgement

The authors acknowledge the financial support of this paper by Science. Technology & Innovation Funding Authority (STDF) under grant (45892).

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