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Review Paper

Propolis as a bioindicator of elemental contamination

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ABSTRACT

Bioindicators are organisms used to predict the level of environmental pollution in a selected geographical area. Some of these live organisms and their products are very sensitive to surrounding changes in the concentrations of heavy metals, whether due to geographical or anthropogenic origins. High agricultural and industrial activity can be sources of chemical and other contaminants. Honeybee products are natural mixtures produced by a honey bee. The honeybee products are rich with biologically active compounds, such as carbohydrates, proteins, amino acids, lipids, vitamins, phenolics, minerals and enzymes. The most popular bee products are honey (unifloral and multifloral), bee venom, propolis, bee pollen, bee bread and royal jelly. Propolis is a honeybee product produced by honeybees using plant resins (more than 50%), beeswax, essential oils, pollen and other organic substances (alcohols, acids and their esters, benzofurans, benzopyrenes, chalcones, flavonoids and their esters, glycerol and its esters, lignans, phenylpropanoids, steroids, terpenes and terpenoids). Propolis is a very popular honeybee products with high biological activities, such as anti-oxidant, antimicrobial, anti-inflammatory, immunomodulatory and anticancer activities. Some studies shown in vitro antibacterial activity of propolis against different types of Gram-positive and Gram-negative bacteria and data of synergism exhibited among the various propolis compounds. The chemical composition of propolis is complex, and depends with geographical area and season. The chemical variability presents several challenges to its standardization and quality control. The present review is aimed at highlighting the presence of heavy metals in propolis, produced by the species Apis mellifera, from different geographical areas.

1. Introduction

Propolis, also known as bee glue, is a resinous substance collected by bees from plant juices. It has been demonstrated to possess a wide range of beneficial pharmacological properties, including antibacterial, antiviral, antioxidant, anti-inflammatory, immunostimulatory, hepatoprotective, and cytotoxic activities. Today, propolis is widely used across the globe and is available in its raw form or in combination with other natural products. The demand for commercial products containing propolis continues to grow steadily. Extraction plays a crucial role in enabling the utilization of the bioactive constituents of propolis (*Matuszewska et al*., 2021).

Over the past decade, numerous studies from various countries have actively investigated the

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concentration of heavy metals in different bee products—including honey (both unifloral and multifloral), bee venom, propolis, bee pollen, bee bread, and royal jelly—using inductively coupled plasma atomic emission spectroscopy (ICP-AES). These studies suggest that quantifying heavy metals in bee products is an effective method for environmental monitoring (*Ćirić et al*., 2020, *Spirić et al*., 2019, *Hodel et al*., 2020). Among bee products, propolis has been identified as one of the bee-derived substances containing the highest concentrations of heavy metals (*Vakhonina et al*., 2021). Furthermore, raw propolis materials sometimes fail to meet safety standards required for the production of pharmaceuticals and cosmetics. Several researchers attribute the elevated levels of heavy metals—particularly lead (Pb) and cadmium (Cd)—in propolis to its high content of lipid-like substances and waxes, which may facilitate the accumulation of such contaminants.

Extensive research has been dedicated to analysing the chemical and biological composition of propolis. To date, over 300 distinct compounds have been identified in propolis collected from various geographic regions. The main chemical components of propolis are presented in Figure 1. Propolis is primarily composed of the following constituents: plant resins (50%), wax (30%), essential oils (10%), pollen (5%), and other organic compounds—including sugars, amino acids, vitamins, and minerals—accounting for the remaining 5%. Additionally, propolis contains a variety of phenolic compounds (e.g., flavonoids, polyphenols, phenolic acids and other phenolic aromatic substances (*Devequi-Nunes et al*., 2018; *Galeotti et al*., 2019). Propolis also contains esters, terpenes and terpenoids, steroids, aromatic acids and esters, aldehydes, alcohols, sugars, sugar alcohols and acids, amino acids, vitamins, fatty acids, hydrocarbons, mineral elements and other alcohols (*Stojanović et al*., 2020). On the other hand, some chemical constituents, such as alkaloids and iridoids, have not been detected in propolis, a feature often attributed to its botanical sources.

According to *Stojanović et al.* (2020), the major group of phenolic compounds present in propolis is the flavonoid group, which includes flavones, flavanols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones and neoflavonoids. These compounds significantly contribute to the biological and pharmacological activities of propolis. In addition to flavonoids, propolis contains various phenolic acids, the most commonly identified being ferulic, cinnamic, caffeic, benzoic, salicylic and p-coumaric acids (*Huang et al*., 2014; *Pasupuleti et al*., 2017). Volatile compounds—particularly terpenes and terpenoids—are also present in propolis, although they make up only about 10% of its composition. Nevertheless, they play a significant role in its biological activity. Several studies (cited in *Sforcin*, 2016) have reported the presence of various terpenoids in propolis, including terpineol, camphor, geraniol, nerol and farnesol. Propolis also contains B-complex vitamins, as well as vitamins C and E (*Stojanović et al.*, 2020). Hydrocarbons such as alkanes, alkenes, monoesters, diesters, aromatic esters, fatty acids and steroids have been identified in various types of propolis. Additionally, several enzymes—such as succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphatase and acid phosphatase—have been reported in propolis.

Numerous studies have identified various macro- and microelements in propolis, including calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), manganese (Mn), iron (Fe), silicon (Si),

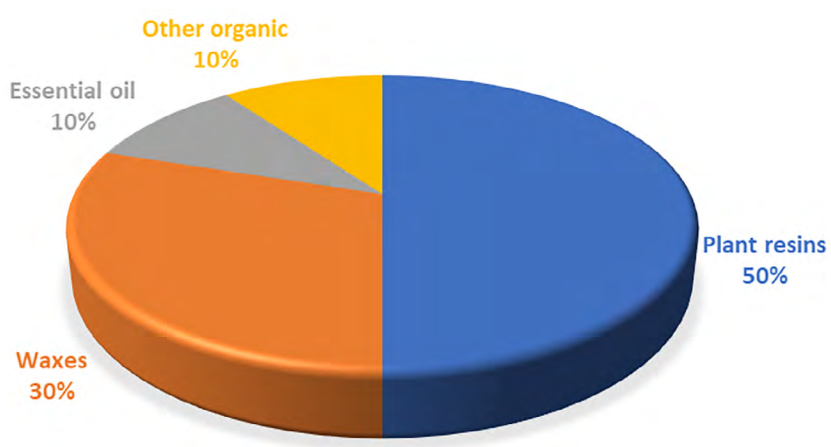

Figure 1. Chemical composition of propolis (adapted from *Stojanović et al*., 2020)

zinc (Zn), selenium (Se), copper (Cu), nickel (Ni), aluminium (Al), boron (B), barium (Ba), chromium (Cr), and strontium (Sr) (*Sforcin*, 2016; *Vakhonina et al*., 2021; *Hodel et al.*, 2020). The trace element profile of propolis can be used as a marker for determining its geographical origin. However, some heavy metals—such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb)—have also been detected in propolis.

The interest in bioindicator-based techniques for the detection and evaluation of environmental contaminants has increased during the past 10 years. It is evident that selection of a suitable organism as a bioindicator is a critical step in the overall biomonitoring activity. As far as the biomonitoring of atmospheric pollution is concerned, the honeybee (*Apis mellifera*) has been the subject of various investigations and may be considered an 'ideal bioindicator' as defined by *Stöcker* (1980). Honeybees are continuously exposed to contaminants present in the (usually widespread) area surrounding the apiary for the duration of their foraging activity (i.e., from spring to autumn). As such, honeybees and their products can supply a suitable amount of biological material to be easily sampled and analysed throughout the year. According to data from ScienceDirect (https://www.sciencedirect.com/), and using the research query "Contaminants in Propolis", the number of published articles has grown from 2003 to 2023. The total number of published articles in the last 20 years ranged from 2003 (four articles) to 2023 (76 articles) (Figure 2).

2. Honeybee products as bioindicators

In Europe, trace elements and heavy metals deposition in soil and plants is one of the most pressing concerns in the science of food safety and food quality (CEC Thematic Strategy for Soil Protection). Different studies (*Jovanović et al*., 2017; *Ćirić et al*., 2020; *Matović et al*., 2018) show the impact of different trace elements and heavy metals on human health as well as the mechanisms of uptake and bioaccumulation of trace elements and heavy metals by plants and other foods for human nutrition. Some of the heavy metals are considered potential carcinogens and are associated with the aetiology of a number of internal diseases. On the other hand, some elements are essential elements for human health (*Jovanović et al*., 2017). Honey bees and their products can be contaminated with heavy metals from different sources. The origin of contamination can be the environment (air, water, plants and soil) and beekeeping practices (*Ćirić et al*., 2020).

The beneficial properties of propolis have stimulated growing interest in investigating its chemical composition in relation to its botanical origins.

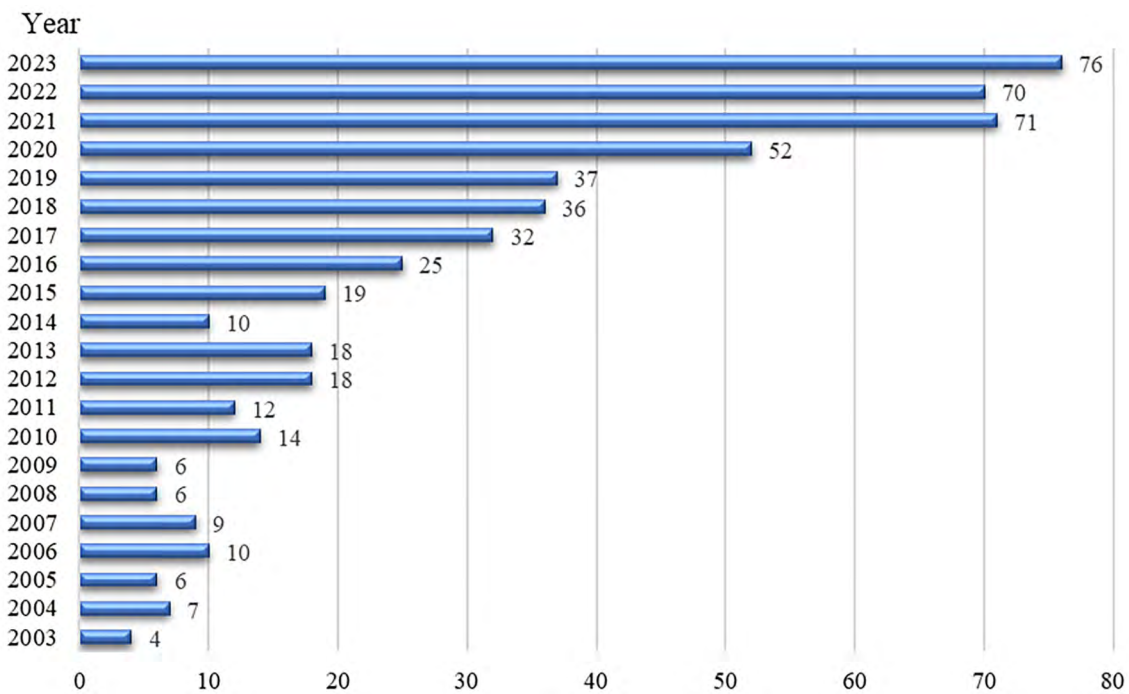

Figure 2. Number of published articles over the past 20 years (data retrieved from the ScienceDirect database using the search query "Contaminants in Propolis").

Propolis produced by *Apis mellifera* contains polyphenolic compounds, with flavonoids—its primary polyphenols—being significantly influenced by the botanical source and the surrounding ecological environment in which the bees reside. The raw materials available to honeybees for the production of propolis primarily consist of plant ingredients secreted in response to injuries, including lipophilic substances from leaves and leaf buds, resins, adhesives, gums and plant ingredients (*Ristivojević et al*., 2015). The botanical composition of these plant-derived substances determines the chemical makeup of propolis, which varies by geographic region. In general, the composition of propolis exhibits high variability due to the diversity of plant species around the hive, from which bees collect the necessary ingredients (*Drescher et al.*, 2019). Furthermore, propolis' chemical profile is influenced by many factors, such as altitude, sunlight exposure, seasonal changes and the characteristics of the bees' foraging areas (*Silva-Carvalho et al*., 2015).

3. Conclusion

Over 500 compounds have been identified in propolis through numerous studies. These include flavonoids, phenylpropanoids, terpenoids, stilbenes, lignans, coumarins and their prenylated derivatives, as reported in various studies. Future research should prioritize the influence of bee species and behaviour on the composition of propolis, which will enhance our understanding of its chemical composition and quality. Comprehensive characterization of propolis from different geographical regions and plant origins is necessary to establish acceptable quantitative standards for various types of propolis. Moreover, the biological activities of propolis need to be correlated with its chemical composition, and standardized products should be used in such studies. Additionally, propolis has potential as a bioindicator for environmental heavy metal deposition. Several studies have confirmed that propolis tends to accumulate heavy metals to a significantly greater extent than honey.

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