Elemental Analysis of *Tetragonula laeviceps* Propolis by X-Ray Fluorescence Spectroscopy

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Abstract. Propolis, a natural substance produced by stingless bees (*Tetragonula laeviceps*), consists of a blend of resins collected from plant exudates and other botanical sources. Bees utilize propolis for hive maintenance and protection against predators. Propolis is widely used in various therapeutic formulations, including treatments for upper respiratory tract infections, burns, acne, herpes, and neurodermatitis. The propolis samples were analyzed using Olympus DELTA Professional Handheld XRF Analyzer. This study employs X-Ray Fluorescence (XRF) analysis to investigate the elemental composition of propolis. Data results of XRF were presented in percentage with limit detection into ppm (%, ppm). The result showed that the three most dominant elements composition in propolis sample from FB 1 were Ca(77.12%) ; K(11.59%), and SO_3(5.18%). Propolis Sample from KP 1 were Fe(31.73%);Ca (26.42%); and K(14.04%). Propolis Sample from KP 2 were K(28.32%); P_2O5(24.35%); and Ca(18.82%). The conclusion was that the three propolis samples contained 2 constant elements, namely Calcium (Ca) and Potassium (K). Calcium and Potassium were found to be essential functions in all organisms.

1 Introduction

Propolis, a natural resin substance produced by stingless bees (*Tetragonula laeviceps*) through the collection of plant parts, buds, and exudates, is known for its various biological activities, including antioxidant, antiobesity, anticancer, and antibacterial properties [1]. Propolis is primarily composed of resins (50%), wax (30%), essential oils (10%), phenolics and flavonoids (10%), and pollen (5%), which also include vitamins and minerals (5%). These components have found applications in creating functional foods and beverages to improve human health and more [2].

The chemical composition of propolis is highly intricate. In recent decades, significant progress has been made in identifying the organic constituents of bee propolis. However, there is a notable gap in the literature regarding the content of trace elements. The limited scientific literature available on the trace mineral composition of propolis underscores the need for further understanding of the potential presence of pollutants (toxic minerals) in this substance, as these pollutants could substantially impact the nutritional properties of derived products [3].

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Different propolis from bee species can be distinguished by mineral compositions [4]. Propolis, notably rich in mineral elements, is a valuable source of essential minerals, containing low concentrations of heavy metals.. Pattern recognition techniques have been applied to assess food quality based on mineral and trace element composition. Propolis contains essential minerals such as magnesium (Mg), calcium (Ca), iodine (I), potassium (K), sodium (Na), copper (Cu), zinc (Zn), manganese (Mn), and iron (Fe), as well as vitamins like B1, B2, B6, C, and E, along with various fatty acids [5]. X-ray fluorescence (XRF) is an elemental analysis technique that provides quantitative chemical information with a superior detection limit compared to Energy Dispersive X-ray Spectroscopy (EDS), making it suitable for trace element analysis [6]. This study focuses on evaluating the essential mineral elements in raw propolis produced by stingless bees Tetragonula laeviceps, which inhabit the same ecological conditions in Indonesia.

XRF is a chemical analysis technique employed to determine the chemical composition of a sample without causing any damage. This method operates on the principle that when a material is exposed to X-ray radiation, the atoms within the sample will absorb energy from the X-rays and subsequently emit this energy in the form of XRF. This XRF exhibits characteristic energies specific to each chemical element. Thus, by measuring the energy of XRF, it is possible to analyze the elements in the sample and determine their concentrations [6]. When X-ray photon particles possess sufficient energy to liberate electrons from an atom, the photoelectric effect occurs, wherein electrons are released as photoelectrons with kinetic energy equal to the difference between the photon energy and the electron binding energy within the atom. X-ray radiation can also induce electrons to transition to lower orbitals, accompanied by the emission of energy, commonly referred to as XRF emission [7].

This study aimed to characterize propolis of Stingless Bees (*Tetragonula laeviceps*) collected from different regions around Yogyakarta on the north side of Mount Merapi, which has a distinctive topography and to analyse the elements composition of propolis by XRF.

2 Materials and Methods

2.1 Collection of propolis from *T. laeviceps* nests

Raw Propolis samples were obtained from two locations, one sample from bee hive in the Forest of Biology UGM (FB1) Yogyakarta, and two more samples (KP1 and KP2) from village gardens in Salam District, Magelang. The collection involved scraping propolis from various parts of the stingless beeshive, including the walls, frames, entrances, and covers. Subsequently, these collected propolis samples were carefully preserved in sterilized flasks and then transported to the Plant Development Structure Laboratory at the Faculty of Biology UGM. The samples were stored in a refrigerator at 4°C until the physicochemical analysis was performed.

2.2 Material and elemental analysis

The raw propolis sample dried first. After that, five (5) gr of propolis samples were inserted in conicle tube. After completely dry the sample was shot using an Olympus handheld X-Ray Flourescent (XRF) analyzer.

The raw propolis sample was placed in the sample holder, and the XRF Analyzer was turned on. The XRF calibrator chip was then prepared, and the analyzer was calibrated. Setting the XRF to Mining Plus mode, it was directed to the propolis samples, and the XRF trigger button was pressed. The system was allowed to run for 1 minute. Afterwards, the XRF moved away, and we waited until the red lamp indicator was turned off. The X-ray testing was conducted at Balai Konservasi Borobudur, Magelang, Yogyakarta, Indonesia.

Fig. 1. Olympus handheld X-Ray Flourescent Analyzer

3 Results and Discussion

3.1 Stingless bee species and nest strucuture

Tetragonula laeviceps is one of the stingless bee species found in Java, Sumatra, and Kalimantan. These stingless bees form eusocial colonies and play a crucial role in ecosystems as pollinators. *T. laeviceps* is classified within the family Apidae, subfamily Apinae, and the tribe Meliponini [8].

Diagnostic characteristics of *T. laeviceps* include a predominantly shiny black body with the posterior mesoscutum covered in yellowish setae. The tegula on the base of the front wings is brown. The antennae consist of 11 flagellomeres and are yellowish-brown. The pedicel separating the thorax and abdomen is brown, and the mandibles are composed of two teeth, with brown to blackish coloring [9].

T. laeviceps constructs oval-shaped nests with compartments that separate eggs and food storage, such as pollen and honey sacs. The nest also contains batumen, a type of propolis made from a mixture of wax and resin [10]. Bees typically use cerumen to coat or seal gaps within the nest, although some species of stingless bees combine it with mud [11].

3.2 Propolis chemical composition

Nutrients that are not made by living things are called minerals [12]. Just like vitamins, minerals do not produce energy, but the body still needs minerals, even in small amounts, to carry out all chemical processes in the body. For example, one of the benefits of potassium (K) for body health is reducing the risk of heart disease, and calcium for forms and strengthens bones and teeth [13].

Based on Table 1. The mineral content in sample FB1 showed the presence of the mineral elements Ca, SiO_2, SO_3, Fe, K, Cl, Mn, Cu, and Eu. The Ca mineral content was the highest content, at 77.12%. Mineral K was the second highest content found in the FB1 sample at 11.59%.

Table 1. Chemical composition of propolis (FB1)

Sample		Parameter (Chemical composition)	Results (%)
Code	Variant		
1	FB1	Ca	77,12
		SiO_2	2,75
		SO_3	5,18
		Fe	2,10
		K	11,59
		Cl	0,43
		Mn	0,50
		Cu	0,15
		Eu	0,18

Table 2. Chemical composition of propolis (KP1)

Sample		Parameter (Chemical composition)	Results (%)
Code	Variant		
2	KP 1	Ca	26,42
		SiO_2	6,87
		SO_3	7,34
		P_2O_5	9,35
		Ti	1,35
		Fe	31,73
		K	14,04
		Cl	1,04
		Mn	1,41
		Zn	0,21
		Sr	0,16

Based on Table 2. The mineral content in sample KP1 showed the presence of the mineral elements were Ca, SiO_2, SO_3, P_2O_5, Ti, Fe, K, Cl, Mn, Zn, and Sr. Fe mineral content was the highest content, at 31.73%. Mineral Ca was the second-highest content found in the KP1 sample at 26.42%

Table 3. Chemical composition of propolis (KP2)

Sample		Parameter (Chemical composition)	Results (%)
Code	Variant		
3	KP2	Ca	18,82
		SiO_2	2,92
		SO_3	14,28
		P_2O_5	24,35
		Ti	0,57
		Fe	8,44
		K	28,32
		Cl	1,25
		Mn	0,66
		Zn	0,26

Based on Table 3, the mineral content in the KP 2 sample showed the presence of the mineral elements were Ca, SiO_2, SO_3, P_2O_5, Ti, Fe, K, Cl, Mn, and Zn. The mineral K content was the highest content, at 28.32%. Mineral P_2O_5 was the second highest content found in the KP2 sample at 24.35%.

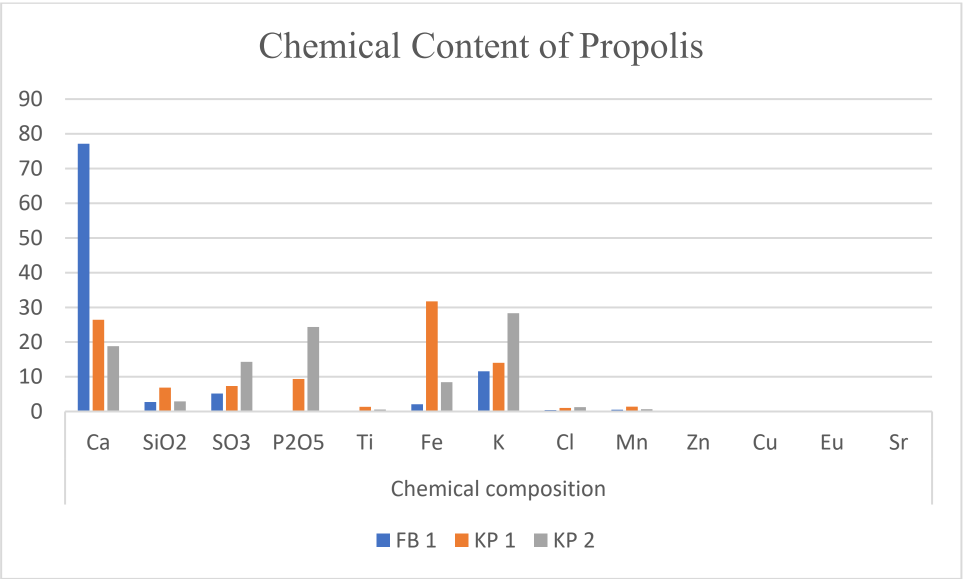

Fig. 2. Propolis composition of FB 1, KP 1, and KP 2 sample

Based on test results, the mineral content of propolis demonstrated that KP1 and KP2 contained minerals such as Ca, SiO_2, SO_3, P_2O_5, Ti, Fe, K, Cl, Mn, and Zn.The mineral content of Fe, Ca, and K in KP1 was higher than KP2 (Figure 2.). Additionally, the mineral content of KP1 was found to be twice as high as that of KP2. Previous research results have shown that propolis contains minerals such as Ca, Mg, Na, Fe, Mn, Cu, and Zn [14,15].

Based on Table 1, the chemical composition of propolis's FB1 sample showed the presence of Cu and Eu elements, but these two elements were not present in samples KP1 and KP2. The content of the Ca element was 3 times greater than in samples of KP1 and KP2. Table 2 shows that the Fe mineral content was higher than in samples FB1 and KP2. The mineral element composition of SiO_2 showed that the amount was twice higher than in the samples of FB1 and KP. In addition, there was not Sr element in samples of FB1 and KP2. Based on table 3. The chemical composition content of sample KP2 showed that the mineral element of K was twice as high as in samples of FB1 and KP1. The SO_3 content was also 2 times higher than samples of FB1 and KP1. In addition, the mineral of K content was 2 times higher than in FB1 and KP1 samples.

The differences in the content of the three samples were attributed to variations in specific geographical locations. FB1 was obtained from the forest of the Faculty of Biology, UGM, located in Sleman Regency, Yogyakarta. Meanwhile, the other sample locations, KP1 and KP2, were collected from the village gardens of Salam District, Magelang, Central Java.The FB1 sample exhibited the highest mineral content, with Ca at 77.12%, followed by K at 11.59%, and SO3 as the third mineral. This is attributed to *T. laeviceps* collecting resin from various plant species in the Forest of the Faculty of Biology, UGM, including mango trees, *Alamanda cathartica* trees, and others. The KP1 sample displayed the highest mineral content, with Fe at 31.73%, followed by Ca at 26.42% and K at 14.04%. Meanwhile, the KP2 sample demonstrated the highest mineral content, with K at 28.32%, followed by P2O5 at 24.35% and Ca at 18.82%.

Based on the analysis, samples of KP1 and KP2 generally showed different results. However, the mineral content of K and Ca showed a dominant and a slightly different result. This phenomenon occurs because in the village gardens of Magelang, there are various types of trees, including durian, eucalyptus, and mango trees. This diversity results in the mineral content of K and Ca showing almost negligible differences in the results. In general, when using an XRF analyzer, propolis is found to contain minerals such as Ca, SiO2, SO3, P2O5, Ti, Fe, K, Cl, Mn, Cu, Eu, Zn, and Sr. Nearly 13 mineral elements have been identified. However, these elements may not be present in all propolis, as their presence depends primarily on the botanical and geographical origin, the species of bee, the harvest season, as well as various environmental factors.

4 Conclusion

The three (3) highest chemical compositions showed that sample FB1 contained a chemical composition of Ca of 77.12%, K was 11.59%, and SO3 was 5.18%. For sample KP1, the Fe was 31.73%, Ca was 26.42%, and K was 14.04%. For sample KP2, the chemical composition of K element was 28.32%, P2O5 was 24.35%, and Ca was 18.82%. The conclusion from the results obtained was that the chemical composition with dominant values in each sample was Ca and K. All mineral elements are not necessarily present in the propolis because their presence depends mainly on the botanical and geographical origin, the species of bee and the harvest season, as well as of various environmental factors.

Further research is still required to explore the potential bioactivity of propolis from the various specific geographical location in various essential function in all organisms.

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