INTRODUCTION

Propolis is a substance derived from plant resins collected by bees and mixed with enzymes found in bee salivary glands and is commonly used to protect the hive [1,2]. Stingless bee propolis has several advantages, such as a higher yield, and the phytochemical composition is more diverse [3].

The geographical location affects the composition of propolis produced [4,5]. The different geographic locations allow there to be different species of bees and plant origin sources. According to Cauchi-Kumul and Segura [6], propolis in different locations can contain different phytochemicals. Araújo et al. and Mahani et al. [7,8] reported that differences in the composition of active compounds or phytochemicals in propolis could affect propolis toxicity. The objective of this study was to determine the phytochemical content and toxicity of propolis.

MATERIALS AND METHODS

Materials

Stingless bee propolis from ten provinces in Indonesia. The selection of provinces is based on the availability of stingless bee cultivators. The parameters tested included the phytochemical content of alkaloids, triterpenoids, total phenolics, total flavonoids, total tannins, and propolis toxicity.

Identification of bee species and plant origin source

The stingless bee species data were obtained using survey and examination using a microscope and camera, then compared with a key book. Then, the plant origin source’s identification base on observations where the bees routinely perched on the plant to collect the resin. Identification carried out on 15 primary plants around the colony.

Alkaloid compound screening

The sample was made alkaline with ammonia added, then added chloroform. The chloroform layer is taken with a pipette, and then adds 2 N hydrochloric acid. The mixture was shaken strong so that there are two layers. The acid layer is taken with a pipette and then divided into three parts. Each tube was added with different reagents, namely, Dragendorff reagent and Mayer reagent. Alkaloid compounds were identified as positive if an orange precipitate was formed after being given Dragendorff’s reagent, a white precipitate with Mayer’s reagent [9].

Triterpenoid compound screening

The sample was added ether. Poured into the vaporizer cup and allow it to evaporate until dry. Furthermore, the reagent is added Liebermann–Burchard. The occurrence of purple color indicates the presence of triterpenoids compounds whereas the blue-green color indicates steroid compounds [9].

Total flavonoid content (TFC)

TFC of each honey sample was determined according to the method was reported by Khalil et al. [10] with minor modification. Briefly, honey 0.20 g was mixed with 4 ml of distilled water. At baseline, 0.3 ml of sodium nitrite (5%, w/v) were added. After 5 min, 0.6 ml aluminum chloride (10% w/v) was added, followed by the addition of 2 ml of sodium hydroxide (1 M) 5 min later. Immediately after that, the volume was increased to 10 ml by the addition of 2.4 ml distilled water. The mixture was vigorously shaken to ensure adequate mixing, and the absorbance was read at 510 nm.

Total phenolic content (TPC)

TPC was measured using the method described by Aloibhi et al. [11]. Briefly, honey about 0.2 g and added 0.5 ml of Folin–Gocalteu reagent and added 7.5 ml aquabidest. Then, the blend was allowed for 10 min at...
RESULTS

Identification of bee species

Based on Table 1, the most widely cultivated bee species is Tetragonula laeviceps. This species found in four different provinces, namely, Banten, West Java, Central Java, and South Kalimantan. South Kalimantan has the most diverse stingless bees than other provinces with three species then followed by North Sumatra and South Sulawesi with two species of stingless bees. Meanwhile, in other provinces, there are only one stingless bee species that cultivate.

Plant origin source

As shown in Table 2, there are 31 types of resin source plants in ten provinces in Indonesia. The most widely distributed plant and used as a source of resin is mango. Mango plants spread across nine provinces, namely, North Sumatra, Banten, West Java, Central Java, South Kalimantan, East Kalimantan, South Sulawesi, West Nusa Tenggara, and Maluku.

Phytochemical propolis

Phytochemical screening tests showed that the propolis with the highest alkaloid and triterpenoid content is Central Java propolis from T. laeviceps species (Table 3). The quantitative phytochemical test shows that TFC of propolis ranges from 0.005% to 0.442%. Then, the TPC in propolis in the range of 0.254-1.897 %, while the TTC of propolis is in the range of 0.023-0.957% (Table 4).

Propolis toxicity

The propolis with the highest toxicity was Bantren propolis, which came from the T. laeviceps species (Table 5). This propolis has an LC50 value of 50 and gets into a highly toxic category. Another propolis included in the highly toxic category is North Sumatra propolis originating from the Sundatrigona moorei species with an LC50 value of 55.09. The smaller the LC50 value, the more toxic the propolis will be.

Correlation between phytochemical content (TFC, TPC, and TTC) and propolis toxicity

Based on the Spearman correlation test, the significance value between TFC and propolis toxicity was 0.692. This value indicates that the two variables do not have a significant correlation. Then, the significance value obtained between the TPC and propolis toxicity is 0.350. This value indicates that the two variables do not have a significant correlation. Then, the significance value between the TTC and propolis toxicity is 0.692. This value indicates that the two variables have a significant correlation. Besides, it's also known that the correlation coefficient value of the two variables is 0.667. This means that the two variables have a strong correlation (Table 6). Then, a positive value on the correlation coefficient means that these two variables have a unidirectional correlation. Thus, the higher the TFC of propolis, the higher the LC50 value of propolis. In other words, the toxicity is getting lower.

DISCUSSION

The diversity of bee species in various provinces can affect the characteristics of the propolis produced. The diversity of propolis in Indonesia is influenced by differences in resin source plant and bee species [3]. Even so, there is no evidence that enzymes in bees can carry out chemical changes in compounds extracted from plants and used for propolis production [14]. Therefore, it can be said that the bee species affect the characteristics of the propolis produced but indirectly because different bee species will tend to take resin sources for propolis from different plants. This statement is supported by Rasmussen and Cameron [15] who revealed that each species of bee has unique behavior and is not the same as other bee species. Furthermore, stingless bees choose plants that they use as a source of resin based on the distance between flowers and nests, aroma, and flower color [16]. According to Fawzy and Al-Deeb [17], different types of resin plants can cause different phytochemical content of propolis and affect the biological activity of the propolis produced. So from that, it can be said that if the bee species has an indirect effect, while the type of resin source plant directly affects the characteristics of the propolis produced.

Table: Stingless bee species in various provinces in Indonesia

| Province          | Stingless bee species                   |
|------------------|----------------------------------------|
| South Kalimantan | Heterotrigona itama                    |
|                  | Tetragonula laeviceps                  |
|                  | Geniotrigona thoracica                 |
| North Sumatra    | Tetragonula minakabau                 |
|                  | Sundatrigona moorei                   |
| South Sulawesi   | Tetragonula biroi                      |
|                  | Geniotrigona incisa                   |
| Banten           | Tetragonula laeviceps                  |
| West Java        | Tetragonula laeviceps                  |
| Central Java     | Tetragonula laeviceps                  |
| West Kalimantan  | Heterotrigona itama                   |
| East Kalimantan  | Heterotrigona itama                   |
| West Nusa Tenggara | Tetragonula fuscobalteata              |
| Maluku           | Tetragonula fuscobalteata              |
Table 2: Plants origin in various provinces in Indonesia

| Plant                  | NS | B   | Wj | EJ | WK | EK | SK | SS | WNT | M  |
|-----------------------|----|-----|----|----|----|----|----|----|-----|----|
| Acacia (Acacia mangium) |    |     |    |    |    |    |    |    |     |    |
| Avocado (Persea americana) |    |     |    |    |    |    |    |    |     |    |
| Cempaka (Magnolia champaca) |    |     |    |    |    |    |    |    |     |    |
| Cempedak (Artocarpus integer) |    |     |    |    |    |    |    |    |     |    |
| Dadap (Erythrina variegata) |    |     |    |    |    |    |    |    |     |    |
| Damar (Agathis dammarana) |    |     |    |    |    |    |    |    |     |    |
| Durian (Durio zibethinus) |    |     |    |    |    |    |    |    |     |    |
| Castor plant (Ricinus communis) |    |     |    |    |    |    |    |    |     |    |
| jengkol (Archidendron pauciflorum) |    |     |    |    |    |    |    |    |     |    |
| Pomelo (Citrus maxima) |    |     |    |    |    |    |    |    |     |    |
| Lemon (Citrus limon) |    |     |    |    |    |    |    |    |     |    |
| Kaju London (Diospyros celebica) |    |     |    |    |    |    |    |    |     |    |
| Cacao (Theobroma cacao) |    |     |    |    |    |    |    |    |     |    |
| Rubber (Hevea brasiliensis) |    |     |    |    |    |    |    |    |     |    |
| Kuwani (Mangifera odorata) |    |     |    |    |    |    |    |    |     |    |
| Mahogany (Swietenia mahagoni) |    |     |    |    |    |    |    |    |     |    |
| Mango (Mangifera indica) |    |     |    |    |    |    |    |    |     |    |
| Mangosteen (Garcinia mangostana) |    |     |    |    |    |    |    |    |     |    |
| Jackfruit (Artocarpus heterophyllus) |    |     |    |    |    |    |    |    |     |    |
| Pali (Lithocarpus sundasicus) |    |     |    |    |    |    |    |    |     |    |
| Petai (Parkia speciosa) |    |     |    |    |    |    |    |    |     |    |
| Pine (Pinus merkusii) |    |     |    |    |    |    |    |    |     |    |
| Banana (Musa acuminata) |    |     |    |    |    |    |    |    |     |    |
| Poringan (Codiaeum variegatum) |    |     |    |    |    |    |    |    |     |    |
| Randu (Ceiba pentandra) |    |     |    |    |    |    |    |    |     |    |
| Rengas (Gluta rengas) |    |     |    |    |    |    |    |    |     |    |
| Resak Air (Vatica pauciflora) |    |     |    |    |    |    |    |    |     |    |
| Sempur (Dillenia indica) |    |     |    |    |    |    |    |    |     |    |
| Rubber cassava (Manihot Glaziiovii) |    |     |    |    |    |    |    |    |     |    |
| Soursop (Annona muricata) |    |     |    |    |    |    |    |    |     |    |

NS: North Sumatra, B: Banten WJ: West Java, CJ: Central Java, WK: West Kalimantan, EK: East Kalimantan, SK: South Kalimantan, SS: South Sulawesi, WNT: West Nusa

One of the characteristics of propolis being tested is its phytochemical content. Propolis, which contains the highest alkaloid compounds, is found in Central Java propolis from the bee T. Laeviceps. The plant source of the resin influences the high alkaloid content in propolis. Central Java Province has seven types of resin source plants, six of which contain alkaloid compounds, including acacia, mahogany, mango, jackfruit, banana, and soursop. Triterpenoid compounds are known to have antibacterial, anticancer, and analgesic [18]. The propolis with the highest triterpenoid content found in Central Java propolis comes from the bee T. Laeviceps. Central Java has seven types of resin source plants, five of which are plants containing triterpenoids. These plants include acacia, durian, jackfruit, banana, and soursop. Triterpenoid compounds are known to have anti-tuberculosis activity [19].

The propolis with the highest TFC was South Sulawesi propolis, which came from Tetrangula biroi. The TFC in propolis is 0.442 %. TFC of propolis is influenced by the type of plant that is used as a source of propolis resin by bees [20]. There are nine types of resin source plants in South Sulawesi that contain flavonoids, including durian, mango,
The toxicity of propolis can be influenced, one of which is the plant source of resin. According to Mensah et al. [26], some traditional medicine plants can be potentially toxic. It is also supported by Mahani et al. [27] who states that resins from medicinal plants tend to produce propolis with high biological activity and toxicity. Furthermore, from the research, it can be seen that some of the medicinal plants studied had LC_{50} values of less than 100 ppm [28].

The propolis with the highest TPC was South Sulawesi propolis from the G. incisa species with a content of 0.96%. The type of resin source plant also influences propolis tannin content. South Sulawesi Province is known to have seven types of resin source plants that contain tannin compounds, including durian, cempaka, cocoa, pine, randu, resak, and rubber cassava. Tannins have various kinds of biological activities such as antibacterial and antioxidant [25].

Based on the Spearman correlation test results, the higher the TFC, the lower the toxicity. This is slightly contrary to various sources that state that flavonoid compounds have a cytotoxic activity increasing propolis toxicity. Vukovic et al. [33] stated that the flavonoids isolated from propolis showed cytotoxic, proapoptotic, and antioxidant activities. Based on Josipović and Oršolić [34], research regarding the cytotoxic effect of flavonoids on leukemia cells, it was found that the flavonoids quercetin type showed the highest cytotoxic effect, besides caffeic acid, and chrysin also showed high cytotoxic levels. Kaempferol flavonoids are also known to exhibit cytotoxic properties in human pancreatic cancer cells. This kaempferol compound can reduce the viability of pancreatic cancer cells by increasing apoptosis. Quercetin is also known to have potent cytotoxicity because it can induce apoptosis, suppress proliferation, and viability in HepG2 cancer cells [35].

Even so, the resulting correlation data could be due to the flavonoid compounds found in the propolis being tested was dominated by flavonoids with low cytotoxic activity. Therefore, even though the levels of flavonoids are high, the toxicity of these compounds remains low. This statement is supported by Yadegarynia et al. [36], which states that different types of flavonoids have different cytotoxic activities. Tests conducted on breast cancer cells showed that the flavonoids apigenin, luteolin, chrysin, kaempferol, and quercetin had strong cytotoxic activity. In contrast, the flavonoids of the Daidzein, Naringenin, Catechin, and Myricetin types showed weak cytotoxic activity and did not even show the ability to kill cells. Based on research, it is also known that there is not always a correlation between the tested phytochemicals and their level of toxicity [37]. This could be because the compounds that are more toxic to the test organism are other compounds outside the phenolic and tannin groups.

CONCLUSION

Stingless bee propolis from various provinces in Indonesia contains various phytochemicals and toxicities. This is influenced by the types of bees and the source of resin plants in each province. The phytochemical content of flavonoids in propolis also shows a strong correlation with propolis toxicity.

Table 5: The toxicity of Indonesian stingless bee propolis

| Province    | Bee species                  | Toxicity LC_{50} (ppm) | Note       |
|-------------|------------------------------|------------------------|------------|
| Banten      | Tetragonula laeviceps        | <5.00                  | Highly toxic |
| North Sumatra| Sundastrigona moorei         | 5.09                   | Highly toxic |
| South Kalimantan| Heterotrigona itama     | 27.06                  | Moderately toxic |
| East Kalimantan| Heterotrigona itama         | 45.32                  | Moderately toxic |
| West Java   | Tetragonula laeviceps        | 52.17                  | Moderately toxic |
| Central Java| Tetrangula laeviceps         | 61.58                  | Moderately toxic |
| North Sumatra| Tetrangula minangkabau       | 62.14                  | Moderately toxic |
| West Nusa   | Tetrangula fuscobaltea      | 62.43                  | Moderately toxic |
| Tenggara   | Tetrangula biroi             | 65.61                  | Moderately toxic |
| West Kalimantan| Heterotrigona itama      | 80.22                  | Moderately toxic |
| South Kalimantan| Tetrangula laeviceps     | 83.80                  | Moderately toxic |
| South Sulawesi| Genistriographina incisa   | 85.47                  | Moderately toxic |
| Maluku      | Tetrangula fuscobaltea      | 93.26                  | Moderately toxic |
| South Kalimantan| Genistriographina thoracica| >1000.00               | Slightly toxic |

Table 6: Correlation (Spearman correlation test) between phytochemical content (TFC, TPC, TTC) and propolis toxicity

| Toksisitas (LC_{50}) | TFC | TTC | TPC |
|----------------------|-----|-----|-----|
| Correlation Coefficient | 0.667** | 0.116 | 0.270 |
| Sig. (two-tailed)     | 0.009 | 0.692 | 0.350 |

**Correlation is significant at the 0.01 level (two-tailed), TFC: Total flavonoid content, TPC: Total phenolic content, TTC: Total tannin content
AUTHORS CONTRIBUTION
MM, AS, HH, NN designed the experimental study and carried out the analysis. MM, ZZ, JS, AS, HH, NN contributed in preparing the manuscript and revision. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST
The authors declared that they have no conflict of interest.

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