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by Mokosuli Yeremia

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Full Length Article

Bioactivity of *Apis dorsata* Nest Extract from a Different Geographical Location in Minahasa, North Sulawesi, Indonesia

Mokosuli Yermia Semuel[1*] and Dina O. Rombot[2]
[1]*Department of Biology, Faculty of Mathematics and Natural Science, State University of Manado, Tondano, Sulawesi Utara, Indonesia*
[2]*Faculty of Medicine, Sam Ratulangi University, Manado, Sulawesi Utara, Indonesia*
r correspondence: yermiamokosuli@unima.ac.id
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Abstract

content of *Apis dorsata* Binghami nesting secondary metabolites differs based on the availability of plant feed sources. The present study was conducted to test for *in vivo* Brine Shrimp Lethality Test (BSLT) and radical scavenging activity of the ethanolic extracts of the nest. Nests were obtained from various natural habitats in the Minahasa area. Cytotoxicity was evaluated in LC50 (lethality concentration 50), while radical scavenging activity was evaluated in IC_{50} (inhibitory concentration). This research was conducted in 2021–2022. Results showed that the ethanolic extracts of nest from North Minahasa had a higher phytochemical content intensity than other nest extracts from Minahasa. The DPPH free radical scavenging activity of all the ethanol extract of the nest from Minahasa was stronger than the positive control of ascorbic acid. The best IC_{50} was shown by the nest ethanol extract from North Minahasa (57,67 mg/mL). The toxicity of nest ethanol extract was below 500 mg/mL, where the highest LC_{50} was shown by North Minahasa nest extract (208.498 mg/mL). However, the extract toxicity based on the BSLT test was moderate. Thus, nest extract has the potential to be developed as a bioactive source of herbal medicine. © 2022 Friends Science Publishers

Keywords: Nest extract; Antioxidant; LC50; Cytotoxicity

Introduction

A honey bee nest consists of a hexagonal-shaped chamber held together by Propolis and beeswax. There are eggs, larvae, pollen and royal jelly (Hadisoesilo 2001). Propolis is one of the components that makes up the nest, which is widely studied today. This study used *Apis dorsata* Binghami nest, Sulawesi endemic honey bees (Hadisoesilo 2001; Raffiudin 2002; Semuel *et al*. 2013). The nest of *A. dorsata* consists of only one comb. It is only found in the natural forests of Sulawesi and nearby small islands because it cannot be domesticated (Raffiudin 2002). As a giant honey bee, *A. dorsata* variety and types of food sources are greater than other honey bees (Repi and Semuel 2016; Semuel *et al*. 2013, 2019).

Propolis is a mixture of natural resins collected by bees from various plant species. Bee propolis is the product of plant resin processing with bee saliva, which contains various enzymes and peptides to produce new resins (Przybyłek and Karpinski 2019). Honey bees collected plant resins from plant parts, shoots and exudates (Wagh 2013; Kurek-Gorecka *et al*. 2014; Abdullah *et al*. 2020). Propolis also contains high micronutrients such as vitamins (A, B, C), minerals (Ca, Cu, Fe, Mg, Mn, Na, Fe and Zn) and the enzyme succinate dehydrogenase (Salatino *et al*. 2011). The most secondary metabolites in Propolis are polyphenols (phenolic acids, flavonoids and their esters), steroids, terpenoids and amino acids (Sforcin and Bankova 2011; Kurek-Gorecka *et al*. 2014). Propolis has a colour and bioactive content that varies depending on the plant source (Sforcin and Bankova 2011; Salatino *et al*. 2011; Abdullah *et al*. 2020). The natural resin substance in Propolis provides a sticky aroma and texture at the temperature of the newly formed nest. The health benefits of Propolis include accelerating cell regeneration, repairing leakage of blood vessels, curing diabetes mellitus, immune system stimulator, anticancer, anti prostaglandin, antistress, antioxidant, anti-inflammatory, antibacterial and antihyperlipidemic (Hegazi 1998; Bankova *et al*. 2000; Simone-Finstrom and Spivak 2010; Kubiliene *et al*. 2015). In addition, Propolis in the nest is very potential as an antiviral agent for SARS COV2 (Berretta *et al*. 2020).

Previous research has shown that *A. dorsata* nest extract from Minahasa can reduce the blood lipid profile of rats treated with hyperlipidemia (Mokosuli *et al*. 2019).

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Analysis of flavonoid content using the HPLC method of Sarang *A. dorsata* extract obtained from North Minahasa in September obtained 23 types of flavonoid derivatives (Semuel *et al.* 2019). The bioactive content of *A. dorsata* nest highly depends on the type of feed available. Thus, it is necessary to research the bioactive content of *A. dorsata* nest from Minahasa obtained from various locations and different flowering seasons. The initial bioassay that can be done to determine the pharmacological potential of bioactive natural ingredients that is widely used is the Brine Shrimp Lethality Test (BSLT). BSLT is a method for determining the cytotoxic spectrum of natural extracts (Hamrun *et al.* 2020). Research on the analysis of phytochemical content, antioxidants, and BSLT nests from various regions and habitats in the Minahasa peninsula has been carried out.

Materials and Methods

Honey nest samples

The nests of *A. dorsata* obtained from four locations on the Minahasa peninsula, namely South Minahasa Regency, Southeast Minahasa Regency, Minahasa Regency and North Minahasa Regency (Fig. 1a, b). The nest was obtained directly from the tree where *A. dorsata* made the nest (Fig. 1c). The method of taking the nest using fumigation techniques is assisted by professional forest honey bee seekers. Nests collected at various locations have the same age. The age of the nest used had three months. The nest sample collection was carried out in September 2021.

Manufacture of simplicia

Fresh samples of *A. dorsata* nest were cleaned and then washed in running water. The nest was then dried at 25°C for two days. Next, the nest was dried using an oven for 30 min at 50°C. At the end, the nest was finely chopped then blended until it becomes a powder. Nest powder is used for extraction.

Extraction

The extraction was carried out using the maceration method (Harborne 1996; Mokosuli *et al.* 2019). The solvent and nest powder ratio is 1: 4 (w/v), namely 50 grams of macerated nest powder with 200 mL of 70% ethanol (chemical-pharmaceutical). Erlenmeyer contains solvent and nest powder, covered with aluminum foil, then placed in an orbital shaker for 2 × 24 h at a speed of 50 rpm and a temperature of 25°C. The macerate is then filtered using Whatman filter paper to obtain the filtrate. Finally, the filtrate obtained is dissolved using a Buchi rotary evaporator at a speed of 55 rpm and a temperature of 50°C. Furthermore, the evaporation results are called the crude extract of the nest of *A. dorsata*.

Phytochemical content analysis

Photochemical content analysis using the Harborne (1996) method with slight modifications was done. The phytochemicals analyzed were alkaloids, flavonoids, saponins, tannins, steroids and triterpenoids.

DPPH method free radical reduction activity test

A. dorsata nest extract was made to distribute concentrations (10, 50, 100, 200 and 250 μg/μL). Each one is put into a test tube. Each test tube added 500 μL of DPPH 1 m*M* solution in methanol. The volume was crushed to 5.0 mL, then incubated at 37°C for 30 min. Using a UV Vis spectrophotometer (Parkin Elmer), the absorbance was measured at 515 nm. As a positive control, ascorbic acid (Sigma Aldric) was used with an adjusted concentration (Kumazawa *et al.* 2004; Semuel 2008). The IC50 values are calculated using the linear regression equation formula:

$$\% \text{ inhibition} = \frac{[\text{Absorbance control} - \text{absorbance of the sample}]}{\text{Absorbance control}} \times 100\%$$

BSLT method cytotoxic test

The A. salina Leach cyst was weighed as much as 20 mg then put into a particular container containing filtered seawater; after being aerated, the cyst was left for 48 h under lamp lighting to hatch completely. The hatched larvae are taken for use in toxicity tests—a total of 10 healthy A. Salina Leach larvae (based on the motility and ability of the larvae to search for light) were put into the test vial containing seawater. The ethanol extract solution was added to each test vial with the concentration of the test solution consisting of 10, 100, 500, and 1000 ppm, while the control extract was not added to the extract solution. Each of the three replications was made. Observations were made after 24 h by counting the number of larvae that died from the total larvae included in the test vial (McLaughlin *et al.* 1998).

Statistical Analysis

The calculation uses a magnifying glass. The LC_{50} value was determined by probit analysis, with a 95% degree of significance using the IBM SPSS 20.

Results

The nests of *A. dorsata* from Sawangan Minahasa Utara were golden yellow in color. *A. dorsata* was nesting in the stalk of the *Ficus* spp. The nest has a fruity aroma, especially Langsat (*Lansium domesticum* L.) fruit. Nest 1.3 meters wide, hanging from twigs 22 m from the ground. The honey taste still found in the nest is sweet with a slightly sour taste. From observations, around nesting in September, the flowering season of Langsat, durian, mango,

Fig. 1a: Locations for sampling *A. dorsata* nests are marked with an asterisk

Fig. 1b: Location of *A. dorsata* nest in Minahasa, North Sulawesi, Indonesia

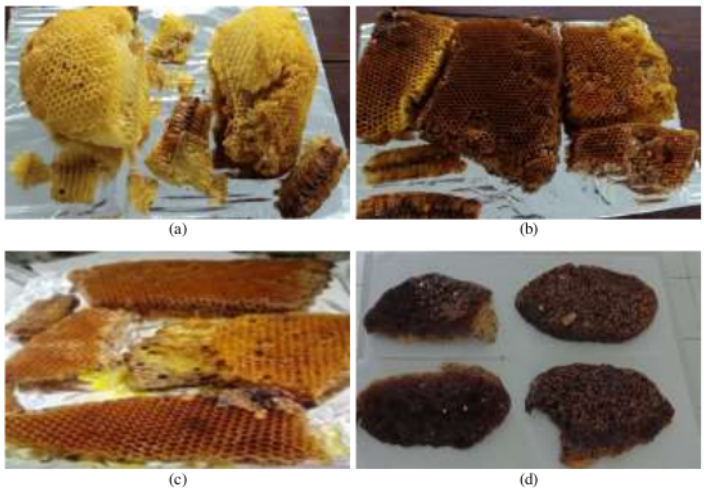

Fig. 1c: Sample nest of *A. dorsata* from (a). Minahasa Utara (b). Minahasa Induk (c). Southeast Minahasa (d). Minahasa Selatan

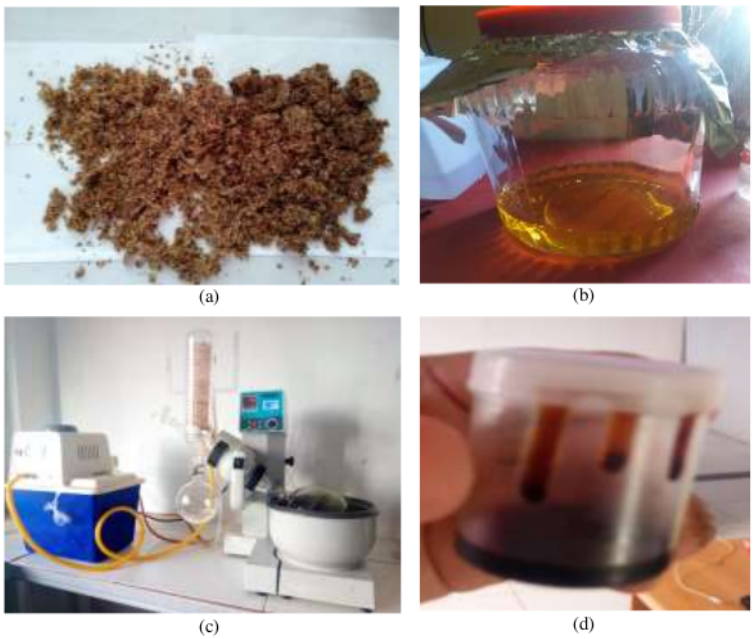

Fig. 2: Nest extract (**a**), Nest powder (**b**), Macerated filtrate (**c**). Solvent evaporation with a rotary evaporator at 450°C, 55 rpm (d). Crude extract

and other fruit plants. The nests of *A. dorsata* from Kombi Village, Minahasa, are brownish-yellow in colour. The age of the nest is about three months. The taste of honey found in the hive is sweeter than the honey in the North Minahasa nest. However, the hive and honey smelled like cloves. Nests were also found in the *Ficus* spp. tree, with a nest height of 19 meters from the ground. About 300 meters from the nest, there is a clove plantation where the cloves are in the stage of producing flowers and fruit in September. The nests of *A. dorsata* from Ratahan Village, Southeast Minahasa Regency, are brownish-yellow but more yellow than the main minahasa nests. Honey and fruit-scented nest. Nests were found in Aren trees, with 15 meters from the ground. Around the nest is still a natural forest with lots of Aren trees. The nest of *A. dorsata* from Wulurmaatus Village, Modoinding Minahasa Selatan District, was found on a pedu tree (local name), at an altitude of 17 meters from the ground. The nests are dark brown, have a very sweet honey taste and have a fruity aroma (Fig. 2).

A. dorsata honey nest extract

Nest after mashed brownish yellow, scented with cloves. Nest mashed using a blender has been cleaned of larvae. The smell of cloves is caused by the nest location close to a clove plantation, around 700–1000 m. The filtrate resulting from nest maceration with 70% ethanol for 2 × 24 h has a brownish yellow colour (Fig. 2).

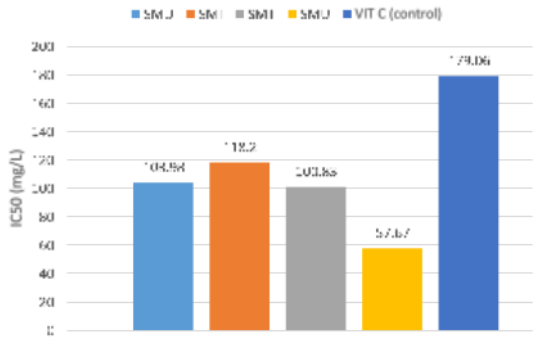

Fig. 3: Free radical scavenging activity of nest extract

After the solvent was evaporated with the Buchi Rotary Evaporator, crude extract of *A. dorsata* nest from South Minahasa was brownish-black with a weight of 50 g. The crude extract had a distinctive nest aroma, honey, cloves and fruit aroma (Fig. 3).

Phytochemical content

Based on the analysis of phytochemical content, all nest samples were identified to contain all phytochemical groups. Nest ethanol extract from all locations in Minahasa contains high intensity flavonoids, saponins and tannins.

Table 1: Percentage of the yield of *A. dorsata* nest extract from various districts in North Sulawesi

Sample	Sample weight (g)	Extract weight (g)	Yield (%)
SMS	50	3.26	6.51
SMI	50	3.21	6.42
SMU	50	3.10	6.20
SMT	50	3.08	6.16

Description:
SMS = nest from South Minahasa : Modoinding
SMI = nest from Minahasa : Kombi
SMU = nest from north Minahasa : Airmadidi
SMT = nest from southeast Minahasa : Ratahan

Table 2: Phytochemical content of the ethanol extract of *A. dorsata* nest from various regions in North Sulawesi

Phytochemical Group	SMS	SMI	SMU	SMT
Alkaloids	+	+	+	+
Flavonoids	++	++	++	++
Saponins	+++	++	+++	++
Tannins	++	++	++	++
Steroids				
- Meyer reagent	+	+	+	+/-
- Wagner reagent	+	+/-	+	+
- Dragendorff reagent	+	++	+/-	+
Triterpenoids				
- Meyer reagent	+	+/-	+	+/-
- Wagner reagent	+/-	+	+/-	+
- Dragendorff reagent	+	+	+	+

Description:
+: there are groups of phytochemical compounds based on the intensity of appearance after being given a reagent.
-: not available based on the intensity of appearance after being given reagent.

Table 3: Percentage of nest extract inhibition

Samples	Test concentration (mg/mL)				
	10	50	100	200	250
SMU	16.65	30.22	52.28	58.07	68.06
SMI	11.93	39.5	44.31	52.89	67.23
SMT	11.56	39.65	47.91	59.74	67.4
SMU	11.56	39.65	47.91	59.74	67.4
VIT C (control)	7.67	29.23	40.51	49.76	58.07

However, the content of steroids and triterpenoids is of little intensity. All nest extract samples identified contained alkaloids (Table 1–3).

Antioxidant test

The percentage of inhibition of the extract against DPPH free radicals varied based on the origin of the nest in Minahasa. The highest mean percentage of free radical reduction of all samples at the test concentration of 250 mg/mL. However, there was no significant difference in *A. dorsata* nest extract concentration from various districts in Minahasa. However, the inhibition percentage of extracts at the test concentration of 250 mg/L was higher than the control used, namely vitamin C (Table 3).

The highest IC_{50} of the extract against DPPH free radicals was shown by the nest extract from North Minahasa (SMU), namely 57.67 mg/mL (R = 0.94). Furthermore, SMT nest extract is 100.83 mg/mL (R = 0.99), SMU nest extract is 103.98 mg/mL (R = 0.94) and SMI nest extract is 118.2 mg/mL (R = 0.95). Compared with the control vitamin C which had an IC_{50} of 179.06 mg/mL (R = 0.98), the IC_{50} of nest extract from all locations in Minahasa was still better (Fig. 3).

Bioassay Toxicity Brine Shrimp Lethality Test (BSLT) method

The highest average mortality in all nest samples was shown in the extract treatment with a concentration of 1000 ppm. In contrast, the lowest average mortality was shown in the 10 ppm extract concentration treatment in all nest extract samples. The percentage of mortality of *Artemia salina* Leach larvae increases with increasing test concentration (Fig. 4). From the probit analysis, the graph of the extract mortality plots is in linear regression (Fig. 5).

Based on the probit analysis of the mortality of *Artemia salina* Leach after application of nest extract, the highest LC50 was shown by SMU extract (208.498 mg/mL). In comparison, the extract with the lowest LC50 was SMS extract (395.186 mg/mL). Based on the LC50 values obtained by the SMS, SMI and SMU extracts were less than 500 mg/mL (Fig. 6).

Discussion

The *A. dorsata* nests in Minahasa have different colours, honey taste and aroma characteristics. However, the average length of the nest was relatively the same at the age of 2–3 months, namely 1–1.75 meters and is semicircular (Semuel *et al*. 2019) The differences in taste, aroma and colour are influenced by the forage plants, which are the source of nectar, pollen, plant resins around the nest. The *A. dorsata* bee has a greater variety of forage plants than *A. mellifera*, with a flight distance of about 600–900 meters from the nest (Semuel *et al*. 2013). Nests from North Minahasa are in areas adjacent to plantations and mountainous areas, while nests from Minahasa are in areas adjacent to the sea. Compared to the nests from South Minahasa, which are in the highlands with cooler temperatures and nests from Southeast Minahasa, which are found in mountainous areas and far from people's plantations. Thus, the characteristics of *A. dorsata* hives and honey are also influenced by geographic location (Semuel *et al*. 2013; Semuel and Repi 2015; Semuel *et al*. 2019).

The characteristics of the nest also positively correlate with the characteristics of the phytochemical content. The results of the nest phytochemical group analysis found variations in the intensity of the content of each phytochemical class of *A. dorsata* hives from various regions in Minahasa. The highest percentage of DPPH and IC_{50} free radical inhibition was the nest samples from North Minahasa. In contrast, the DPPH and IC_{50} free radical inhibition of the nest samples from Southeast Minahasa and South Minahasa were not significantly different. Based on

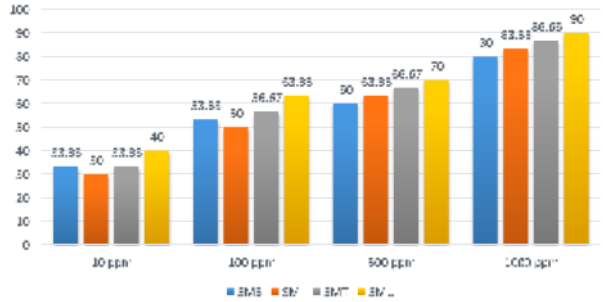

Fig. 4: Comparison of the percentage of mortality of *Artemia salina* Leach larvae based on the origin of the nest sample

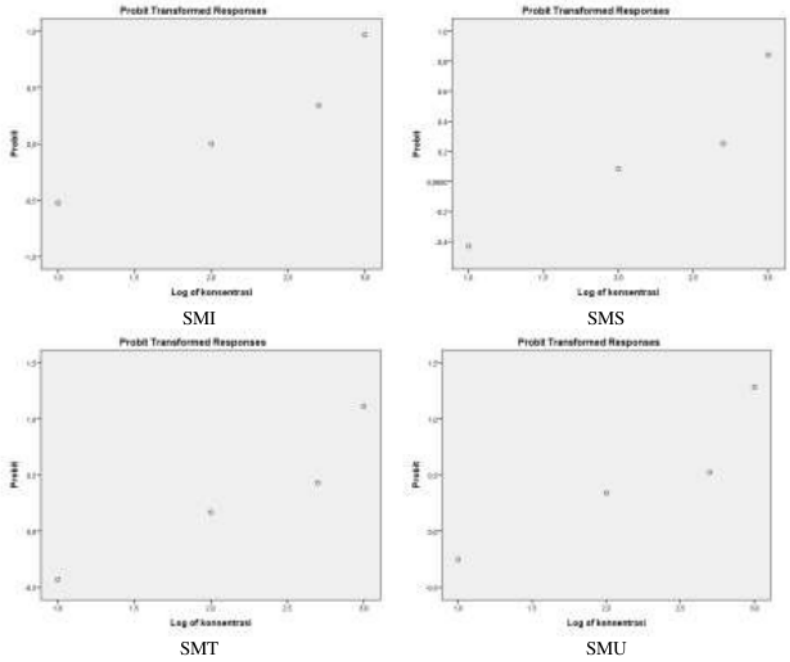

Fig. 5: Graph of LC_{50} probit analysis of *A. dorsata* nest extract

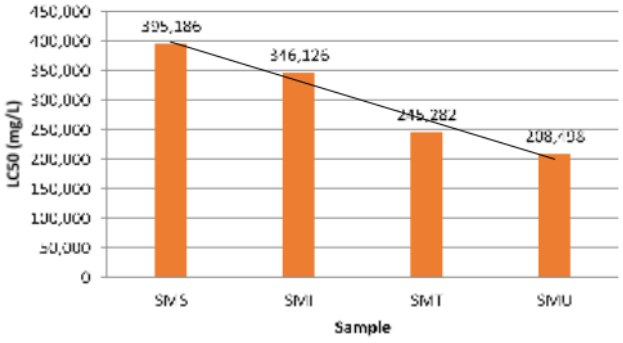

Fig. 6: LC_{50} of nest extract based on sample origin from several districts in Minahasa

the analysis of the phytochemical content of the *A. dorsata* nests from North Minahasa, had a higher intensity than the nest samples from other areas in Minahasa (Semuel *et al*. 2019). However, the IC_{50} of the nest extract was below 150 ppm or better than the positive control for ascorbic acid. Thus, the nest extract's DPPH free radical scavenging activity was different based on the origin of the nest samples in Minahasa.

Based on the results of the BSLT test, the toxicity of the nest extracts of SMS, SMI, SMT and SMU were below 500 mg/mL, thus the toxicity of the nest extract was in the moderate category in *A. salina* Leach. The high-intensity content of phytochemicals, namely flavonoids, saponins and tannins, affects the toxicity of the extract. The active compound in nest propolis is soluble in ethanol (Kubiliene *et al*. 2015). These phenolic group compounds are toxic in *A. salina* Leach larvae (Semuel 2008; Ullah *et al*. 2013; Marzuki *et al*. 2019; Rasyid *et al*. 2020). The active compound activity in the extract that enters the larvae and goes to the cells, causing metabolic and cytotoxic disorders. Cytotoxic effects can be observed quickly within 24 h (McLaughlin 1998; Dwijayanti *et al*. 2015; Rachman *et al*. 2020). Phenolic compounds can induce apoptosis in cells (Semuel 2008). Tunisian ethanolic extract of Propolis exhibits strong antioxidant, antibacterial and antiproliferative activity (Béji-Srairi *et al*. 2020). BSLT has been used by many researchers, including initial toxicity testing, including fungal extracts (Carballo *et al*. 2002), langsat stem bark extract (Semuel 2008), Averhoa bilimbi leaf extract (Rachman *et al*. 2020), metal weight and pesticides (Hussain *et al*. 2006), *Arecha catechu* seed extract (Rasyid *et al*. 2020), *Ruellia tuberisa* L. leaf extract (Vitalia *et al*. 2016) and rosella flower extract (Purbowati *et al*. 2015). Shrimp larvae are known to have thin skin to be sensitive to their environment. Foreign substances or compounds that exist in the environment will diffuse into the body so that they affect the cells. If the compound that enters the cell is toxic, it will kill the shrimp larvae. According to Carballo *et al*. (2002), an extract of natural ingredients has potential as medicine if it has an LC50 according to the BSLT test of less than 1000 mg/mL. The toxicity of natural extracts based on LC50 values is very strong (< 10 ppm), strong (10–100 ppm), moderate (100–500 ppm) and weak (500–1000 ppm) (Meyer *et al*. 1982; McLaughlin *et al*. 1998).

The propolis extract from Bangladesh honey bees obtained LC50 57.99 (Tanvir *et al*. 2018), compared with the crude extract of honey bee hives in this study, LC50 honey nest extract of *A. dorsata* has the potential to be developed as a bioactive source that has pharmacological properties. The nest extract of *A. cerana* showed strong toxicity (LC50 67,744 and 86,98 (Yasmin *et al*. 2019); Propolis from Trigona itama from Beladin Serawak showed low toxicity but high free radical scavenging activity (EC50 17,18 mg/mL) (Yusop *et al*. 2019). In previous research, the nest ethanol extract from North Minahasa showed strong antioxidant activity with IC50 6.69 mg/mL, while n-hexane extract had IC50 6.76 mg/mL (Semuel *et al*. 2019). The IC50 obtained was greater in this study but still had a strong free radical scavenging activity > 200 mg/mL Season and diversity of bee food sources (Semuel *et al*. 2013, 2019).

Empirically, the Minahasa people consume bee hives directly because they are believed to have medicinal properties. Bee hives are believed to reduce blood cholesterol, uric acid, treat diabetes and have anti-tumor properties (Kaunang and Mokosuli 2017). The medium toxicity category indicates that the active compounds present in the nest extract from all locations in Minahasa have pharmacological activities. With the LC50 in the moderate category it confirms that nest extract can be consumed directly by humans.

Conclusion

The analysis of the toxicity test of *A. dorsata* nest extract had LC50 54.457. Based on the LC50 value, the *A. dorsata* forest honey nest can be consumed directly by humans due to containing bioactive medicinal potential.

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Author Contributions

Author Contributions MYA designed the experiment, collected and prepared the materials, analyzed the data and drafted the manuscript. DR prepared the nest extracts, collected data and reviewed the manuscript. MYA and DR collected data and revised the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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