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Antifungal and Antiamoebic Activities, Cytotoxicity, and Toxicity of Aqueous and Ethanolic Extracts of Propolis Produced by Brunei Stingless Bees

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Abstract

Antifungal activities of aqueous and ethanolic extracts of propolis produced by three different stingless bee species, namely *Heterotrigona itama*, *Geniotrigona thoracica*, and *Tetrigona binghami*, found in Brunei, against *Candida albicans* and *Saccharomyces cerevisiae* strains were evaluated. It was found that aqueous extract of the propolis displayed significant activities against the two fungal strains, whereas the antifungal activity of ethanolic extracts was not observed. *T. binghami* propolis had the highest antifungal activity, followed by *G. thoracica* and *H. itama* propolis. The MIC values also indicated that the aqueous extracts (2500–5000 µg/mL) have stronger antifungal activity than the ethanolic extracts (5000–10000 µg/mL), and all the propolis extracts were fungistatic. The brine shrimp nauplii lethality bioassay indicated that the propolis extracts are nontoxic and the cytotoxicity test suggested that the propolis extracts have low anti-amoebic activity against *Acanthamoeba* sp., much lower than that of chlorhexidine. This study revealed the low antifungal and antiamoebic activities of the aqueous and ethanolic propolis extracts from Brunei stingless bees.

Keywords: Propolis, propolis extract, antifungal, *T. binghami*, cytotoxicity, *Acanthamoeba sp*., toxicity

1. Introduction

Over the last few decades, antibiotic resistance in harmful bacteria, fungi, viruses, and parasites has received great attention (Prestinaci et al., 2015). In particular, fungal infections caused by a variety of fungi in our daily environment have become a significant occurrence in humans and are challenging to treat. *Candida* and *Cerevisiae* are some of the most common fungi that affect human health, and overgrowth of these fungi causes a wide variety of mucosal and dermal infections (Cockerill et al., 2012; Nobile and Johnson, 2015; Pappas et al., 2016; Abid et al., 2022). These fungi can also damage internal organs, including the gastrointestinal, respiratory, and urinary tracts (Abid et al., 2022), resulting in high-risk medical conditions, a weakened immune system, and body-wide systemic infections (Patricio et al., 2019). The emerging threat of drug-resistant fungal strains has increased due to the limited amount and efficacy of medical treatments (Wiederhold, 2017; Scorzoni et al., 2017). Therefore, natural products that can reduce the development of virulence factors have been of great interest for effective antifungal treatments (Roemer and Krysan, 2014; Al-Ghamdi et al., 2020; Bendjedid et al., 2022).

Among natural products, propolis, a natural product composed of lipophilic, solid, and resinous substances gathered by bees from various plants and soil combined with their enzymatic saliva (Marcucci, 1995), has been demonstrated to possess a variety of biological and pharmacological properties, including anticancer, antioxidant, antibacterial, antifungal, anti-inflammatory, and antiseptic activities (Wagh, 2013; Lofty, 2006; Gucwa et al., 2018; Abdullah et al., 2020; Ożarowski et al., 2022; Ibrahim and Alqurashi, 2022; Zullkiflee et al., 2022a). Propolis has been reported to be effective against several fungal strains, including *Candida albicans* and *Saccharomyces cerevisiae*. Propolis originates from different countries such as Brazil, Malaysia, Iran, and Poland, present antifungal properties against several *Candida* and *Saccharomyces strains* (Moghim et al., 2021; Gucwa et al., 2018; Yusoff et al., 2016). In general, the bioactive compounds present in propolis have been attributed to their antifungal activity, including caffeic acid phenethyl esters, caffeic acid, flavanone, pinocembrin, p-coumaric acids, and pinobanksin-3-acetate (Anjum et al., 2019; Rivera-Yañez et al., 2021), which inhibit fungal cell division and DNA replication (Patton et al., 2001). Synergistic effects improve the fungicidal activity of the bioactive compounds present in propolis and reduce the development of resistant strains (Alves et al., 2012; Rivera-Yañez et al., 2021). However, the bioactive compounds of propolis vary depending on the bee species, geographical location, surrounding environment, harvesting season, and botanical species around the bee hive. Therefore, the antifungal activity of propolis

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produced by different bee species and/or found in different geographical origins is still of research interest (Bankova et al., 2014).

In this study, the antifungal activities of aqueous and ethanolic extracts of *Tetrigona binghami*, *Heterotrigona itama*, and *Geniotrigona thoracica* propolis against *Candida albicans* and *Saccharomyces cerevisiae* strains were evaluated. The antifungal properties, including the fungal growth inhibition zone, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) of the propolis extracts, were respectively evaluated using the agar well diffusion, broth microdilution, and inoculation methods. In addition, the cytotoxicity of the aqueous and ethanolic extracts of propolis produced by three different stingless bee species against *Acanthamoeba* sp. cells was investigated, and the toxicity of the propolis extracts was tested using a brine shrimp (*Artemia salina* L.) larvae lethality bioassay.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and regents, including 3-(4,5 dimethylthiazol-2y1)-2,5-diphenyltetrazolium bromide (MTT), Mueller-Hinton broth (MHB) microbiology, Mueller-Hinton agar (MHA), and ethanol (95%) were obtained from Merck (Darmstadt, Germany), and were used as received.

2.2. Propolis collection and preparation of propolis extracts

The raw *T. binghami, H. itama*, and *G. thoracica* propolis in this study were collected in August 2021 from the Tasbee Meliponiculture Farm in Tutong District, Brunei Darussalam. Thus, the propolis of the three stingless bee species used in this study was obtained from the same geographical location, surrounding environment, and botanical species around their hives. The stingless bee farm is situated in a suburban area, which is at least 20 km from active agricultural or industrial sites (Abdullah et al., 2020). The collected propolis was rinsed with distilled water, and dried using a dehumidifier at room temperature for 2–3 weeks. After drying, the propolis was ground into small pieces less than 1 millimeter in size.

The propolis extracts were prepared according to a previously reported procedure (Abdullah et al., 2019). Briefly, small pieces of propolis (5 g) were macerated with 125 mL of distilled aqueous or ethanol and the solutions were placed on a temperature-controlled shaker operating at 150 rpm for 18 h at 37 °C. After vacuum filtration, the filtrate was concentrated using a rotary evaporator, followed by drying under vacuum at 40 °C. The dried propolis extracts were then kept in sample vials and dissolved in the necessary solvent for further experiments, as described below.

2.3. Antifungal assay

Candida albicans (ATCC 10231) and *Saccharomyces cerevisiae* (NCPF 3178) strains were provided by the Department of Biodiversity and Environmental Research, Faculty of Science, Universiti Brunei Darussalam. The strains were regenerated from permanent cultures on a regular basis, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Cockerill, 2012).

The antifungal activities of aqueous and ethanolic extracts of *T. binghami*, *H. itama*, and *G. thoracica* popolis against *C. albicans* and *S. cerevisiae* fungal strains were examined using an agar well diffusion test. Here, the fungal strains were prepared by culturing 100 µL of each fungal strain in 5 mL of sterile MHB, which was made by dissolving 19 g MHB in 0.5 L distilled water, and autoclaved at 121 °C for 2 h.

Subsequently, each culture was diluted with MHB, so that the absorbance of the prepared culture at 530 nm was measured to be within 0.08–0.13 OD, to produce a 0.5 McFarland standard culture which contains approximately 1.5×10^6 CFU/mL. The autoclaved MHA solution was added into petri dishes with a diameter of 9 cm and allowed to cool and solidify. A 200 µL of the standardized fungal culture was then uniformly spread over a thin layer of solidified sterile MHA using a sterile glass spreader and dried for a few minutes.

Four wells, each with a diameter of 6 mm, were prepared in Petri dishes. In each well, 40 µL of 80 mg/mL propolis extract in distilled water was added using a micropipette. The petri dishes were then incubated overnight for 48 h at 37 °C, and the diameter of the fungal growth inhibition zone in each well was measured and recorded.

2.4. Minimum inhibitory and fungicidal concentrations

The MIC value representing the lowest concentration of propolis extract to inhibit the growth of incubated *C. albicans* and *S. cerevisiae* fungal strains was evaluated using the broth microdilution method, according to the CLSI guidelines (Cockerill, 2012). Here, a 100 µL of the inoculated fungal cultures was diluted in a sterile MHB (5 mL), incubated for 24 h at 37 °C, and then adjusted to be 0.5 McFarland standard by dilution with MHB with an absorbance of 0.08–0.13 OD at 530 nm, and 0.5 mL of the standardized fungal suspension was further diluted with 74.5 mL of MHB.

On the other hand, 1 mL of each propolis extract (80 mg/mL) was prepared in distilled water, diluted with MHB in the test tube, and vigorously vortexed. By subsequent dilutions with MHB, a series of suspensions of propolis extracts with concentrations of 0.0156, 0.313, 0.625, 1.25, 2.5, 5, 10, 20, and 40 mg/mL were obtained. Finally, 1 mL of the standardized inoculated fungal culture was added into each test tube containing the propolis extract and mixed thoroughly to obtain a final concentration of 5×10^5 CFU/mL. The mixture was then incubated overnight at 37°C.

The MFC value, which is related to the lowest concentration of the propolis extracts showing no visible fungal growth on agar plates, was investigated against *C. albicans* and *S. cerevisiae* fungal strains. The propolis extracts were then swabbed onto the surface of agar plates, using a sterile inoculating loop, and incubated at 37 °C for 48 h. After incubation, the fungal growth on the plates was determined. The concentration of propolis extracts that exhibited no visible fungal growth was considered as the MFC value.

2.5. Cytotoxicity assay

The *in vitro* cytotoxicity of propolis extracts was evaluated against *Acanthamoeba* sp., which was isolated by corneal scraping from patients with keratitis in the

Hospital Kuala Lumpur Isolate, Malaysia. In this sense, protease yeast glucose (PYG) which was prepared by dissolving of protease (3.75 g), yeast extract (3.75 g), and D+ glucose (7.5 g) in 0.5 L of distilled water containing 1.5 mL of page amoeba solution, was used as the culture medium.

The isolated *Acanthamoeba* sp. was then cultured axenically in a T-25 tissue culture flask with 10 mL of PYG media, and was subcultured every four days while being incubated at 30 °C. A 30 µL of propolis extracts dissolved in dimethyl sulfoxide (DMSO) at different concentrations in the range of 35 µg/mL to 4500 µg/mL was mixed with 970 µL of PYG media containing *Acanthamoeba* sp. The mixture was vortexed, and kept in a refrigerator at 4 °C.

Interestingly, various *in vitro* tests have been developed to evaluate the proliferation and viability of *Acanthamoeba sp.* cells. The MTT assay is a common method for colorimetric determination of fungal cell metabolism due to its fast and reliable technique (Hussain et al., 1993). The MTT reagent was utilized to indicate the viability of *Acanthamoeba* cells when treated with the propolis extracts. The MTT assay has several advantages, and has been modified to metabolize the amoeba and other types of cells. Therefore, it is very beneficial to use this method to determine the remained amount of viable *Acanthamoeba sp.* cells following the exposure of cytotoxic agents.

In this study, the cytotoxicity of the propolis extracts was evaluated by determining the 50% inhibitory concentration (IC_{50}) value of *Acanthamoeba* cells based on the MTT assay. The MTT reagent was prepared by dissolving the MTT powder in 1 mL of PBS buffer solution according to the procedure reported by Mosmann (Mosmann, 1983). *Acanthamoeba* cells were first seeded in a 96-well microplate at 1×10^5 cells/well and incubated at 30 °C for 8 h. After incubation, the culture media was removed and replaced with PYG media containing the prepared propolis extracts at various concentrations ranging from 35 µg/mL to 4500 µg/mL. Chlorohexidine was used as a positive control, whereas sterilized PYG media was used as a negative control. The mixtures were then incubated at 30 °C for 24 h. After incubation, 20 µL of the MTT solution was added to each sample and then incubated at 30 °C for 4 h. The purple-blue formazan crystals were then dissolved in 150 µL DMSO, and the absorbance at 570 nm was detected using a MicroElisa reader (Dynatech MR850).

2.6. Toxicity studies

It is essential to assess the toxicity of propolis extracts to determine whether propolis-derived medicines are safe for consumption. In this sense, the brine shrimp nauplii lethality assay was used to evaluate the toxicity and efficacy of phytochemicals found in most natural products. This test allows the determination of the intrinsic toxicity of propolis and the effects of its overdose.

The toxicity of aqueous and ethanolic propolis extracts was determined using the shrimp lethality bioassay in 12-well plate, according to the procedure reported by Kamyab et al. (Kamyab et al., 2020). Prior to this toxicity test, artificial seawater which was used as a medium in the culture was simulated by preparing a solution of commercial salt at a concentration of 34 g/L in distilled water. The seawater was then poured into a shallow rectangular container which was divided into two compartments using Styrofoam with several 5 mm holes. The saltwater temperature was maintained at 30 °C. Approximately 3 g of brine shrimp eggs were soaked in a Clorox bleach solution for 5 minutes. The eggs were filtered and rinsed with distilled water for a few minutes, ground using filter paper, and then sprinkled into the larger compartment of the container covered with aluminum foil to protect it from light, while the smaller compartment was left under light. After 24 h, the brine shrimp started to hatch, and the larvae were left to mature for an additional 6 h. Once matured, the brine shrimp larvae (*Artemia nauplii* L.) were collected using a Pasteur pipette and kept in a container before being subjected to the toxicity tests.

Approximately, 0.5 mL of propolis extracts in distilled water and 2 mL of the prepared artificial seawater were mixed in 12-well plates, so that the concentrations of the propolis extracts were 100, 10, 1 and 0.1 mg/mL. In this test, instead of propolis extracts, 0.5 mL of distilled water was used as the negative control. Ten nauplii were transferred to each well of the 12-well plates, and artificial water was added to the wells to make a volume of 5 mL. The final concentrations of the propolis extracts were 10, 1, 0.1 and 0.01 mg/mL. The 12-well plates were then placed under a light source at 25–30 °C. After mixing with the propolis extracts for 24 h, the number of surviving and dead shrimp larvae was counted using a magnifying glass. Finally, the toxicity was represented by the LC_{50} value which is the lethal concentration that resulted in the death of 50% of the brine shrimp larvae population, and the value of LC_{50} of more than 1000 ppm suggested that the propolis extract was nontoxic.

2.7. Statistical analysis

All the antifungal, MIC, MFC, antiamoebic activity, cytotoxicity, and toxicity assays of the propolis extracts were performed at least in triplicate or quadruplicate. All collected data were included in the analyses. Statistical analysis, especially the unpaired t-test, was used to compare the significant differences between two means at a significance level of $p < 0.05$. The mean values and standard deviation errors are obtained from the statistical analysis.

3. Results

3.1. Antifungal activities of all propolis extracts

The inhibition zones associated with the antifungal activity of the aqueous and ethanolic extracts of stingless bees *T. binghami, H. itama*, and *G. thoracica* propolis against *C. albicans* and *S. cerevisiae* strains are displayed in Table 1. The results suggested that all aqueous extracts of the propolis have large inhibition zones. On the contrary, those of ethanolic extracts of the propolis were not observed. The aqueous extract of *T. binghami* propolis exhibited the strongest antifungal activity against both *C. albicans* and *S. cerevisiae* strains with inhibition zones of 33.0 mm and 29.7 mm, respectively. This was followed by those of *G. thoracica* and *H. itama* propolis with slightly smaller inhibition zones. The diameters of fungal growth inhibition of aqueous extract of *G. thoracica* propolis were 29.0 mm and 23.0 mm tested against *C. albicans* and *S. cerevisiae,* respectively, while the results for H. *itama* were 27.2 mm and 24.7 mm, respectively.

The MIC values of aqueous and ethanolic extracts of *T. binghami, H. itama*, and *G. thoracica* propolis are gathered in Table 1. Upon tested against *S. cerevisiae*, all aqueous propolis extracts have an MIC value of 5000 µg/mL, except for that of *G. thoracica* propolis (2500 µg/mL). This finding suggested that the aqueous extracts of propolis tend to be lower compared to ethanolic extracts. These MIC values might suggest that the aqueous extracts of propolis are better antifungal agents, supporting the observation of the fungal growth inhibition zones. However, high MIC values generally indicate that *T. binghami, H. itama*, and *G. thoracica* propolis found in Brunei have low antifungal activity.

Table 1. The diameters of inhibition zones of all aqueous and ethanolic propolis extracts tested against *C. albicans* and *S. cerevisiae* fungal strains after incubation for 48 h at 37 °C.

Stingless bee species	**The diameter of inhibition zone (mm)**			
	C. albicans		***S. cerevisiae***	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
T. binghami	33.0 ± 1.0	ND	29.7 ± 2.5	ND
H. itama	27.2 ± 1.4	ND	24.7 ± 2.5	ND
G. thoracica	29.0 ± 1.0	ND	23.0 ± 2.0	ND
	MIC value (µg /mL)			
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
T. binghami	5000	>10000	5000	>10000
H. itama	5000	5000	5000	10000
G. thoracica	5000	>10000	2500	>10000

*ND = not detected

In the MFC tests, aqueous and ethanolic extracts of *T. binghami, H. itama*, and *G. thoracica* propolis showed fungal inhibition on all agar plates. These results indicated that propolis extracts inhibited fungal growth, suggesting that they were fungistatic rather than fungicidal in nature.

3.2. Cytotoxicity results of propolis extracts

The plots of the percentage of *Acanthamoeba* cell viability at different concentrations of aqueous and ethanolic extracts of *T. binghami, H. itama*, and *G. thoracica* propolis, along with chlorhexidine (positive control), are shown in Figure 1. The IC_{50} values of all the propolis extracts were determined from their respective graphs. It was found that among the propolis extracts, only the aqueous extract of *T. binghami* propolis has the value of IC_{50} (3635 µg/mL). This IC_{50} value is much higher than that of chlorhexidine (36.75 µg/mL), suggesting that the efficacy of propolis extracts was very low, i.e. two orders of magnitude lower than that of chlorhexidine.

Table 2. The IC_{50} values of all aqueous and ethanolic propolis extracts using the MTT assay

Stingless bee species	**IC_{50} values (µg/mL)**		
	Aqueous extract	Ethanolic extract	**Chlorhexidine**
G. thoracica	ND	ND	
H. Itama	ND	ND	36.75
T. binghami	3635	ND	

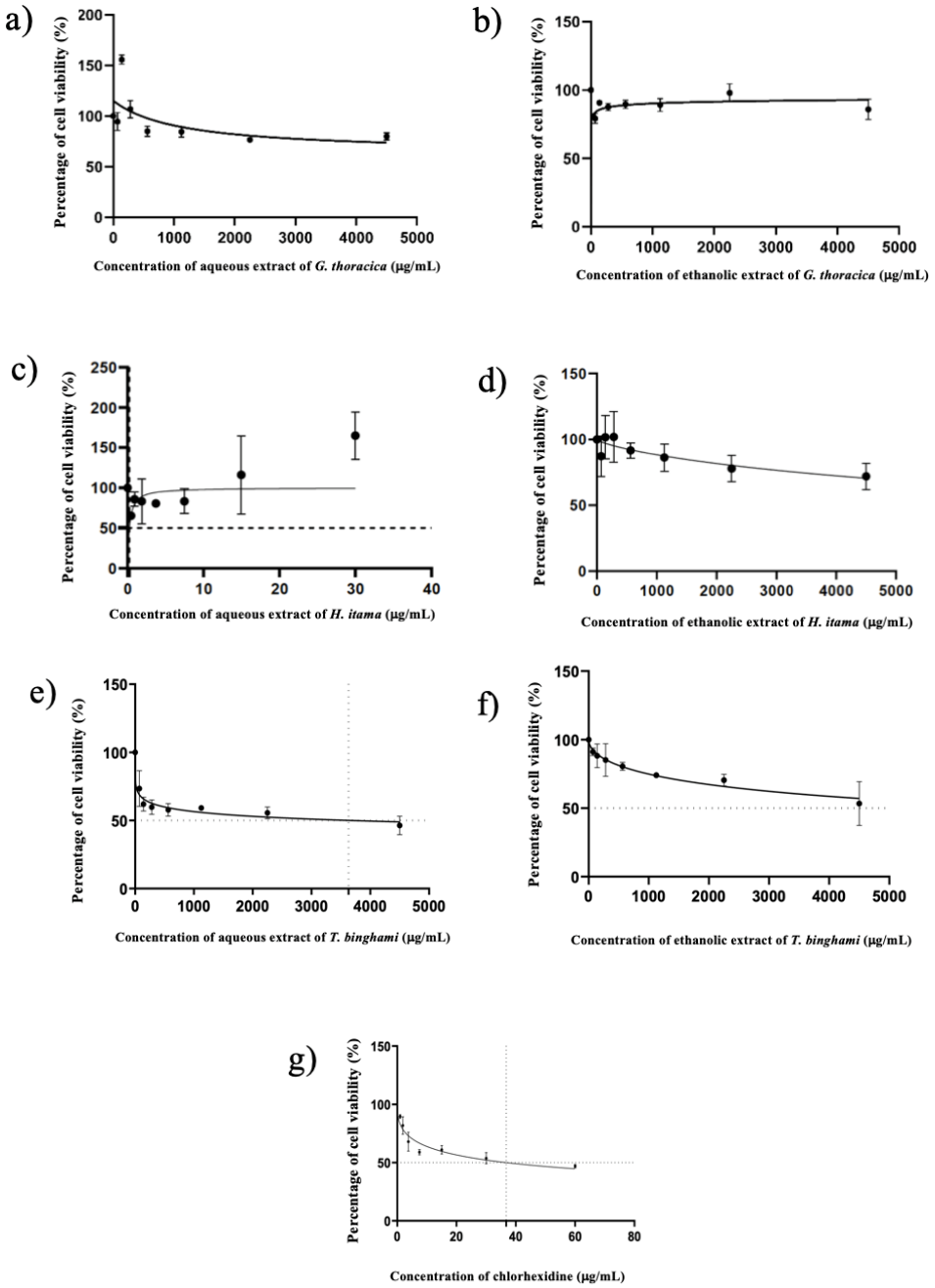

Figure 1. The plots of percentage of *Acanthamoeba* cells viability against the concentration of aqueous ethanolic extracts of stingless bees (**a**,**b**) *G. thoracica*, (**c**,**d**) *H. itama*, and (**e**,**f**) *T. binghami* propolis along with (**g**) chlorhexidine (positive control).

3.3. Toxicity tests

The results of the brine shrimp nauplii lethality tests of the aqueous and ethanolic extracts of *T. binghami*, *H. itama*, and *G. thoracica* propolis are gathered in Table 3. The LC_{50} was determined by plotting the percentage mortality against the logarithmic concentration of the propolis extracts, and was found to be higher than 1000 mg/mL. Therefore, in general, the brine shrimp nauplii lethality tests suggested that all propolis extracts in this study are non-toxic or have low toxicity. Nevertheless, at concentrations as low as 0.1 mg/mL, the aqueous extracts of *T. binghami*, *H. itama*, and *G. thoracica* propolis resulted in 3–13% mortality of the nauplii. In comparison, the ethanolic extract of *H. itama* propolis at the same concentration showed higher mortality (20%), whereas that of *T. binghami* propolis exhibited the lowest percentage of mortality (0%).

Table 3. The LC_{50} of all aqueous and ethanolic propolis extracts which resulted in death of 50% of nauplii at various concentration after 24 h

Bee species	Extract	Concentration (mg/mL)	No. of surviving nauplii (after 24 h)				Mortality (%)	LC_{50} (mg/mL)
			Test 1	Test 2	Test 3	Total		
G. thoracica	Aqueous	0.1	6	10	10	26	13	>1000
		1	10	7	9	26	13	
		10	10	6	9	25	13	
		100	9	10	10	29	3	
	Ethanolic	0.1	9	10	7	26	13	>1000
		1	9	10	10	29	3	
		10	8	9	10	27	10	
		100	10	9	10	29	3	
H. Itama	Aqueous	0.1	9	10	10	29	3	>1000
		1	9	6	8	23	23	
		10	7	3	8	18	40	
		100	7	10	8	25	17	
	Ethanolic	0.1	9	8	7	24	20	>1000
		1	6	7	9	22	27	
		10	7	3	6	16	47	
		100	4	2	10	16	47	
T. binghami	Aqueous	0.1	9	10	10	29	3	>1000
		1	9	10	10	29	3	
		10	3	8	9	20	33	
		100	8	2	7	17	43	
	Ethanolic	0.1	10	10	10	30	0	>1000
		1	9	10	8	27	10	
		10	9	10	10	29	3	
		100	4	3	6	13	57	

4. Discussion

Propolis is rich in bioactive compounds with a variety of therapeutic potential that come from different sources including plants, microorganisms, etc. Propolis has been shown to be an excellent treatment option for a variety of diseases, as summarized in a recent review article (Zullkiflee et al., 2022b). Among the biological properties that have been studied worldwide, antimicrobial effects are the most commonly studied. However, due to the distinct flora in the surrounding area of bee hives being diverse in each region and country in the world, antimicrobial properties of stingless bee propolis depend on the origin from which the propolis samples are harvested. Moreover, the extraction method, osmotic effect, and phytochemical properties of propolis can also influence its antifungal activity.

In this study, the results showed that the aqueous extracts of *T. binghami, H. itama*, and *G. thoracica* propolis produced large inhibition zones, whereas the ethanolic extracts showed no antifungal activity. This was supported by the lower MIC values of *C. albicans* and *S. cerevisiae* in the presence of the aqueous extracts than the ethanolic extracts. This finding indicated that water could extract more antifungal compounds than ethanol. In other words, the antifungal activities responsible for propolis extracts are highly polar organic compounds. The various antifungal activities of the aqueous extracts of *T. binghami, H. itama*, and *G. thoracica* propolis may be associated with their different chemical components. In other words, the different species of stingless bees might collect chemical components from different botanical plants that are available in the area surrounding their meliponi farm, and a large variety of the collected bioactive compounds could be attributed to the different antifungal activities (Sforcin and Bankova, 2011; Montero and Mori, 2012). In particular, in addition to the flavonoid and phenolic compounds present in propolis (Zullkiflee et al., 2022a), aromatic esters and acids present in the resins are also attributed to their antifungal activity (Montero and Mori, 2012). In this sense, the permeability of the cytoplasmic membrane of fungal cells is strongly affected by the phenolic acids present in propolis, causing leakage of intracellular components such as inorganic ions, nucleic acids, and proteins, which resulted in complete cell mortality (Montero and Mori, 2012; Farnesi et al., 2009). In comparison, the efficacy of propolis of the same bee species originated from different geographical locations to inhibit the development of yeasts may also differ, indicating that the botanical origin of propolis has a substantial impact on its antifungal activity.

The MFC tests suggested that propolis extracts are fungistatic, rather than fungicidal. A similar finding has been reported for the antifungal activity of *Trigona thoracica* propolis against *C. albicans* strains (Kačániová et al., 2013). It is worth noting that the fungistatic properties of propolis are of interest for the application of this bee glue in many different medicinal treatments including asthma, diabetes, ulcers, skin infections, and wound healing (Shehu et al., 2015).

In this study, the primary toxicity test using the brine shrimp nauplii lethality assay indicated that the aqueous and ethanolic extracts of *T. binghami, H. itama*, and *G. thoracica* propolis are not toxic. Although there have been numerous reports stating that propolis is relatively nontoxic and safe for consumption, there are still a few studies that state otherwise (Vakhonina et al., 2021). Nevertheless, despite the disparity in reported toxicities of propolis, some propolis extracts, irrespective of their stingless bee species, may have a very low intrinsic toxicity, which is likely caused by the preparation of the extracts or the presence of a small amount of the bioactive compounds in propolis. For example, it was reported that, propolis also contains flavonoids which are active ingredients and known for their relatively low toxicity (Burdock, 1998). Phytochemicals such as toxic metals (arsenic, lead, chromium, mercury, and cadmium) found in propolis can also greatly influence its toxicity (Ahangari et al., 2018; Hodel et al., 2020). These toxic metals are often associated with contamination, especially if the propolis originates around active sites such as industrial areas, mining, and active urbanization, or it is exposure to fertilizers and pesticides (González-Martín et al., 2015; Hodel et al., 2020). Overexposure to high amount of these metals can cause toxicity (Jaishankar et al., 2014).

5. Conclusion

The present study revealed that all aqueous extracts of *T. binghami*, *H. itama*, and *G. thoracica* propolis displayed significant antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae* strains, supported by the lower MIC values of aqueous extracts compared to ethanolic extracts. The MFC values indicated that all the aqueous and ethanolic propolis extracts were fungistatic. The brine shrimp nauplii lethality bioassay indicated that the propolis extracts were non-toxic, and the cytotoxicity test suggested that the propolis extracts have a low anti-amoebic activity against *Acanthamoeba* sp., much lower than that of chlorhexidine. Overall, this study revealed that the aqueous and ethanolic propolis extracts of the three stingless bees found in Brunei showed low antifungal and antiamoebic activities. A deeper understanding of the antifungal and antiamoebic activities of these stingless bees propolis should be provided by comprehensive research to identify specific compounds present in propolis.

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