

Exploring the Anti-Fungal Activity of Malaysian *Heterotrigona itama* Propolis against Candidiasis

1 BACKGROUND

- Heterotrigona itama* (*H. itama*) stands as the most abundant stingless bee species in Malaysia and neighboring regions, with approximately 45 species identified in Malaysia, among which *H. itama* predominates.
- Stingless bee propolis, known as "bee glue," comprises resinous materials from diverse botanical sources and bee secretions, serving crucial roles within the colony such as crack sealing, hive surface coverage, and enhancement of social immunity.
- Ethanol extraction is the primary method for obtaining propolis extracts, yielding a rich array of bioactive compounds including over 300 organic constituents like flavonoids, phenolics, and organic acids.
- Propolis demonstrates remarkable antibacterial and antifungal properties, proving effective against multidrug-resistant strains and *Candida* species implicated in candidiasis.

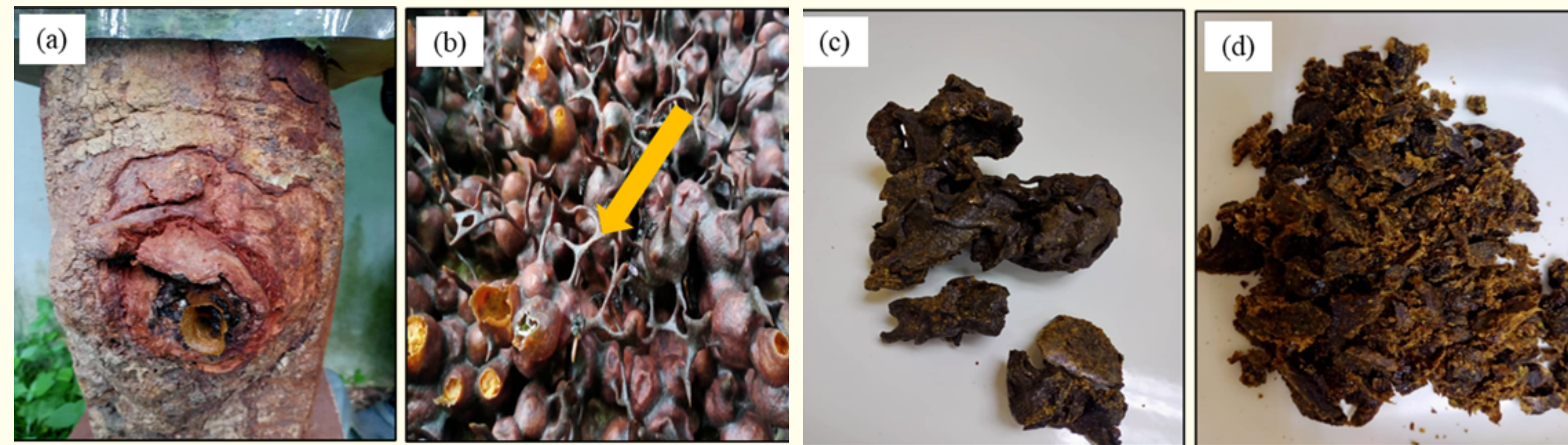


Figure 1 (a) The entrance for the beehive, (b) propolis in the beehive (yellow arrow), (c) the harvested raw propolis, and (d) cut propolis of *H. itama*

2 OBJECTIVE

This study aimed to identify bioactive compounds with anti-fungal activity present in Malaysian *H. itama* propolis against candidiasis.

3 METHODS

H. itama propolis was extracted using the maceration method, and its phytochemical composition was analyzed via Ultraviolet-visible (UV-vis) spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, while anti-fungal properties were evaluated using the agar disk diffusion method.

4 RESULTS AND DISCUSSIONS

Results

Table 1 The phytochemical analysis of ethanolic *H. itama* propolis extract

Extract Type	Appearance	Color	Weight before extraction (g)	Weight after extraction (g)	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg GAE/g)
Aqueous <i>H. itama</i> extract	Semi solid	Yellowish	92.00	35.65	2.17 ± 0.06	6.75 ± 0.85	2.94 ± 1.08
Alkaline hydrolyzed <i>H. itama</i> extract	Solid	Dark brown	60.00	18.28	0.723 ± 0.06	38.36 ± 3.19	4.60 ± 0.40

Table 2 The bioactive groups of ethanolic *H. itama* propolis extract that contributed to anti-fungal activity

Compound	Identified Concentration (%)	Potential Bioactive Properties
Phenol, 2-(3,7-dimethylocta-2,6-dienyl)	95	- Antioxidant properties - Potential antimicrobial properties (including anti-fungal activity)
n-Hexadecanoic acid (Palmitic acid)	99	- Common saturated fatty acid - Energy source - Participates in biological processes - Potential anti-fungal activity
Octadecanoic acid (Stearic acid)	99	- Common saturated fatty acid - Used in soap, cosmetics, and candles - Surfactant and emulsifier - Potential anti-fungal activity
13-Docosenamide, (Z)-83	83	- Long-chain primary fatty amide - Potential applications in slip agents, lubricants, and anti-fogging agents - Potential anti-fungal activity

Table 3 The functional groups of *H. itama* propolis extract for F1 and F2

Functional Group	Wavelength (cm ⁻¹)
f1	
O-H (alcohol/carboxylic acid)	3352.099, 3332.717
C-H (alkanes/alkyl groups)	2924.021, 1447.998, 1340.013, 1011.407, 961.630
C=O (ketones/aldehydes)	1636.685
C=C (alkenes/aromatic compounds)	1600.786, 1502.050
C-O (ethers/alcohols)	1268.917, 1183.037, 1084.644
f2	
O-H (alcohol/carboxylic acid)	3332.717
C-H (alkanes/alkyl groups)	2924.666, 1443.473, 1373.063, 1266.390
C=C (alkenes/aromatic compounds)	1586.399
C-O (alcohol/ether)	1159.946
C-C (alkanes/alkyl groups)	1065.626
C-H out-of-plane bending (substituted benzene rings)	827.719

Table 4 The antioxidant activity of ethanolic *H. itama* propolis extract

Volume fraction of ethanol to water	IC ₅₀ (mg L ⁻¹)
0.0	1905.0
0.2	1480.3
0.4	855.1
0.5	669.5
0.6	183.3
0.8	141.3
1.0	76.5

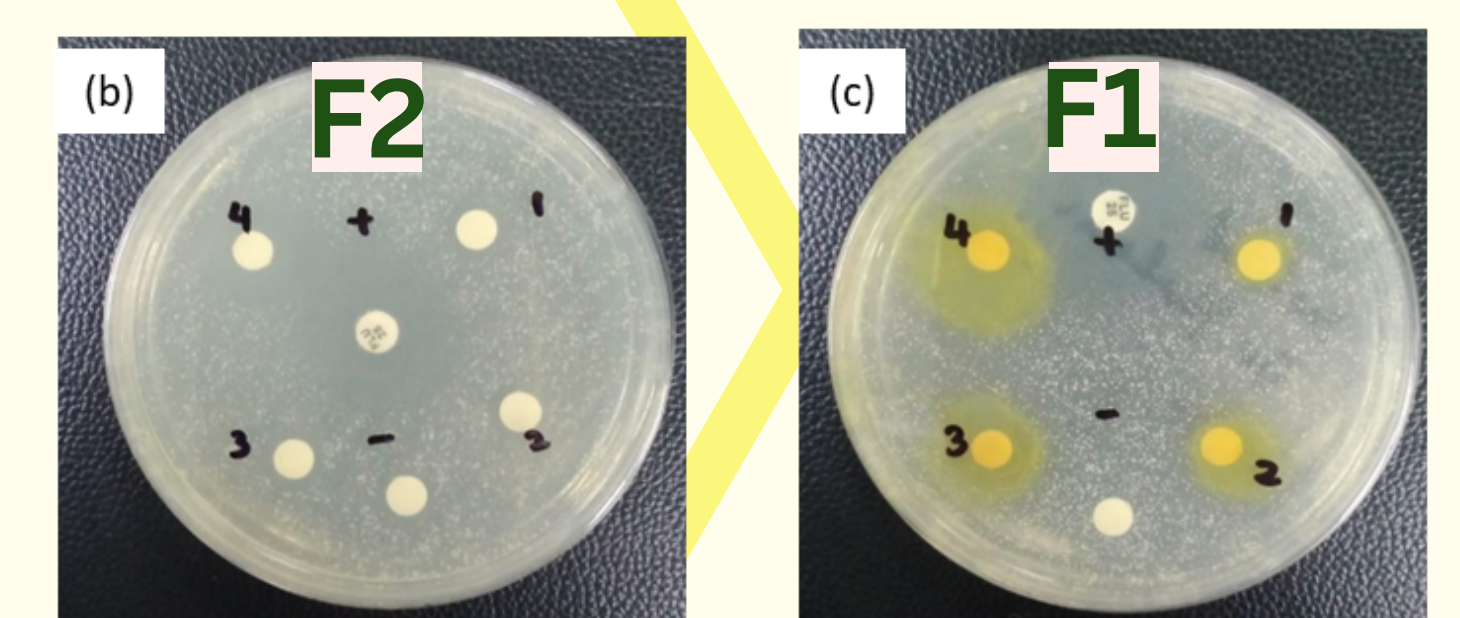
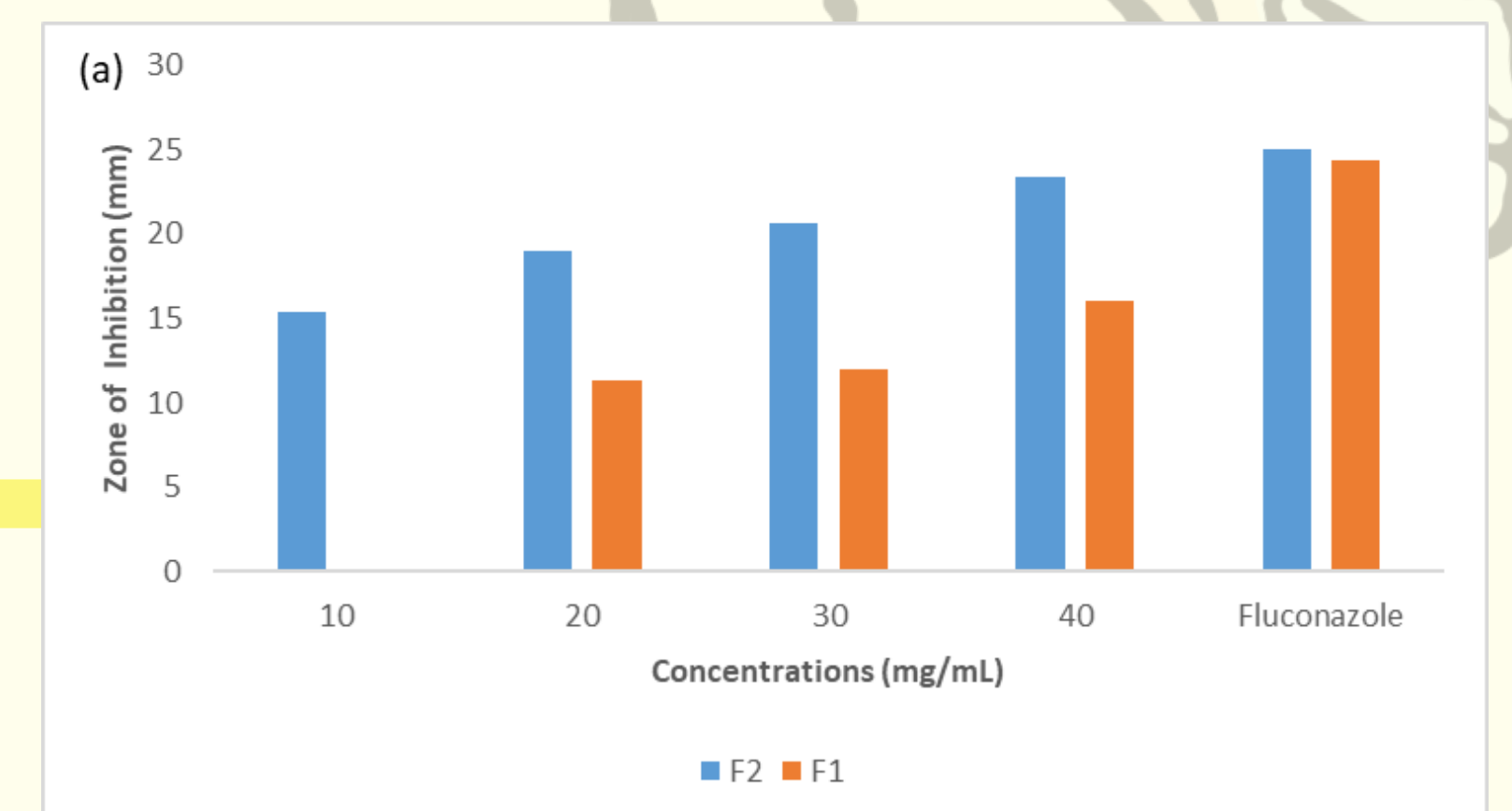


Figure 2 The (a) anti-fungal activity of (b) alkaline hydrolyzed and (c) aqueous of *H. itama* propolis extract with *Candida albicans*

Discussions

- The *H. itama* propolis extract, particularly the alkaline hydrolyzed variant, demonstrated a notably higher TPC and TFC compared to the aqueous extract, indicating its potential richness in bioactive compounds essential for anti-fungal activity benefits.^{1,2,3}
- This study highlighted the potential anti-fungal properties of certain compounds identified in the sample, particularly n-Hexadecanoic acid (Palmitic acid) and Octadecanoic acid (Stearic acid).^{4,5,6}
- FTIR analyses confirmed the presence of bioactive compounds with antioxidant and anti-fungal properties for *H. itama* propolis extract.
- The *H. itama* propolis extract demonstrated significant anti-fungal activity against *Candida* species.^{7,8}

5 CONCLUSION

The findings suggested that *H. itama* propolis extracts hold promise as valuable ingredients in pharmaceutical formulations, nutraceuticals, cosmetics, and functional foods.

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