CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2026.003

Evaluation of antimicrobial activity from the *Tetragonula* sp. propolis extract

Tran Thanh Toi, Huynh Vy, Do Tan Khang, Nguyen Pham Anh Thi, and Tran Ngoc Quy*

Department of Molecular Biology, Institute of Food and Biotechnology, Can Tho University, Viet Nam

**Corresponding author (tnquy@ctu.edu.vn)*

Article info.

Received 01 Jan 2025
Revised 26 Mar 2025
Accepted 24 Aug 2025

Keywords

Antimicrobial, Cutibacterium acnes, Escherichia coli, Propolis, Staphylococcus aureus, Tetragonula sp.

ABSTRACT

The aim of this study was to investigate the antimicrobial activity against Staphylococcus aureus, Cutibacterium acnes, Escherichia coli, and Candida albicans of the ethanol extract and the n-hexane and ethyl acetate extract of the stingless bee Tetragonula sp. propolis. Additionally, the study also identified the stingless bee species based on morphological characteristics and Cytochrome c oxidase subunit I (COI) gene sequence. The antimicrobial activity of three stingless bee propolis extracts showed resistance against all three bacterial S. aureus, C. acnes, and E. coli. At a concentration of 1000 µg/mL, the ethanol extract exhibited the highest resistance, with C. acnes showing a zone of inhibition of 13.33 mm, while the inhibition zones were 11.33 mm for S. aureus and 10.33 mm for E. coli. The Minimum Inhibitory Concentration (MIC) for the three bacterial S. aureus, C. acnes, and E. coli were found to be 30, 50, and 50 µg/mL, respectively. The study identified the presence of polyphenols, flavonoids, aglycones, phenolics, and ketones quantified the total phenolic and flavonoid content, assessed the antibacterial activity of the propolis extract against the three pathogenic bacteria, and provided the foundation for further research.

1. INTRODUCTION

Currently, compounds extracted from natural sources are being actively researched and applied for the treatment of bacterial and fungal diseases. Propolis is recognized as a leading natural antibiotic. This material is a natural product that includes wax, plant resins, and secretion from the salivary glands of bees. The propolis produced by stingless bees (Meliponini) has been investigated globally and contains chemical components such as flavonoids, terpenes, and phenolic compounds, exhibiting biological effects including antibacterial, antifungal, antioxidant, and anticancer activity (Le et al., 2021). As a result, propolis is widely used in functional foods for disease prevention and treatment. Notably, propolis from stingless bees is particularly effective in harnessing therapeutic properties and is considered safe for humans due to various compounds such as flavonoids, phenolics, terpenes, and others. This type of propolis acts as a reservoir of nutritional and functional compounds. This capability renders propolis a valuable material for the discovery of innovative compounds with notable antibacterial effects (Rocha et al., 2023). Although the propolis of stingless bees has significant biological relevance and antibacterial efficacy, its popularity among the public remains limited. The aim of this research is to evaluate the antibacterial properties obtained from the fractional extracts of stingless bee propolis.

2. MATERIALS AND METHOD

The stingless bee propolis was collected in April 2024 from Soc Trang Province, Viet Nam. The strains of *Staphylococcus aureus*, *Cutibacterium*

acnes, *Escherichia coli*, and *Candida albicans* were provided by the Molecular Biology Laboratory, Institute of Food and Biotechnology, Can Tho University.

2.1. Identification of stingless bee

2.1.1. Morphological Identification

The morphometric characters based on descriptions from published data on *Tetragonula iridipennis* by Sakagami (1978), Vijayakumar and Jeyaraaj (2014), Makkar et al. (2018), and Sharma et al. (2023); *Tetragonula pagdeni* as documented by Vijayakumar and Jeyaraaj (2020); and *Tetragonula travacodica* as reported by Shanas and Faseeh (2019).

2.1.2. Molecular Identification

DNA of the worker bee was extracted using the PureLink™ Genomic DNA Mini Kit (Invitrogen). Samples with good quality were subjected to PCR for amplification of specific DNA fragments in the 600-700 bp range of the COI gene region using a PCR Thermal Cycler. Gene amplification was carried out in a 30 µL reaction solution, which included 13 µL of BiH2O, 13 µL of master mix (Biohelix, Taiwan), 0.5 µL of each primer at the concentration of 10 pmol/µL (LCO1490: 5′-GGT CAA CAA ATC ATA AAG ATA TTGG-3′ and HCO2198: 5′-TAA ACT TCA GGG TGA CCA AAAAAT CA-3′), as well as 3 µL of genomic DNA. Thermal cycling was carried out in a Mastercycler® nexus X2 - PCR Thermal Cycler, with the protocol commenced with a preheating stage at 94°C for 5 minutes, proceeded by 35 cycles comprising 30 seconds of denaturation at 94°C, 30 seconds of annealing at 50°C, and 1 minute of extension at 72°C. The procedure concluded with a final extension phase at 72°C lasting 10 minutes (Marconi et al., 2022). After running the PCR products on an agarose gel, the results were visualized on a 2% agarose gel to compare the length with the DNA ladder. Clear bands without non-specific products were sequenced. Sequencing results were compared with the NCBI database.

2.2. Propolis extraction

Total Ethanol Extract (EtOH): The collected propolis was ground and soaked in 70% ethanol (4L for 24 hours). The collected extract was combined, passed through filter paper, and concentrated with a rotary evaporator at a temperature of 50°C. The concentrated extract was maintained at a temperature of -8°C for storage and to use in further experiments.

The EtOH was fractionated with n-hexane and Ethyl acetate (EtOAc). These were prepared sequentially based on polarity using liquid-liquid extraction. The collected extracts were concentrated at 50°C and stored at -8°C for downstream assays.

2.3. Qualitative and quantitative analysis of natural compounds

The EtOH, n-hexane, and EtOAc extracts were analyzed for chemical composition through characteristic qualitative chemical reactions as described by Riaz et al. (2018) and Tiwari et al. (2011).

2.3.1. Quantification of phenolic (TPC)

The measurement of total phenolic content was conducted through the application of the Folin-Ciocalteu reagent procedure (Hai et al., 2011, with adjustment). The reaction mixture was formulated by combining 100 µL of the extract with 600 µL of Folin-Ciocalteu reagent (diluted 1:5) and incubating the solution at room temperature for 5 minutes. Subsequently, 800 µL of 10% sodium carbonate solution and 300 µL of distilled water were sequentially introduced, followed by a 30-minute incubation under identical conditions. The absorbance was subsequently quantified at a wavelength of 737 nm. The results are quantified as the amount of gallic acid equivalents (GAE) per gram of the extract (mg GAE/g sample).

2.3.2. Quantification of flavonoids (TFC)

The flavonoid concentration was quantified using a spectrophotometric method as described by Hai et al. (2011), with some adjustments. The reaction mixture included 500 µL of extract, 500 µL of 10% $AlCl_3$, and 500 µL of 10% Na_2CO_3. The absorbance was measured at a wavelength of 437 nm after a 15-minute incubation. The results are quantified as the amount of quercetin equivalents (QE) per gram of the extract (mg QE/g sample).

2.4. Antimicrobial activity by the agar well disk diffusion method

The bacteria were grown in a TSB Broth and maintained at 37°C for a duration of 24 hours to create a microbial suspension with a density of 10^6 CFU/mL. The bacterial population density post-incubation in the TSB medium was quantified through optical density (OD) analysis at a wavelength of 625 nm. The extract was diluted with

10% DMSO. Tetracycline at a concentration of 0.5 mg/mL served as the positive control, and 10% DMSO was used as the negative control.

The bacteria were grown in an SDB Broth and maintained at 37°C for a duration of 24 hours to create a microbial suspension with a density of 10^6 CFU/mL. The turbidity of the fungal suspension was adjusted by the use of a spectrophotometer at 530 nm. The extract was diluted in 10% DMSO. Control solutions included a positive control containing the antifungal Fluconazole at a concentration of 0.5 mg/mL served as the positive control, and 10% DMSO was used as the negative control.

2.5. Determination of Minimum Inhibitory Concentration (MIC) by Resazurin assay

The MIC was evaluated through the colorimetric change of the resazurin dye, in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (2010). The extract solution was diluted in 10% DMSO to create various concentrations. The positive control was Tetracycline 0.5 mg/mL, and the negative control was 10% DMSO. A 100 µL suspension of bacteria (cell density of 10^6 CFU/mL), along with the positive and negative controls, was added to each well containing 100 µL of extract at different concentrations. The wells were maintained at 37°C for 24 hours, followed by the addition of 20 µL of 0.01% resazurin solution to each well.

2.6. Statistical analysis

Data was collected and statistically analyzed through ANOVA analysis using Minitab16 software to test for differences between treatments; Data are calculated, presented as mean±standard deviation and presented in graph form from Microsoft Excel 2013.

3. RESULTS AND DISCUSSION

3.1. Identification of Stingless Bee Species

The COI sequence was compared on the NCBI database using the nucleotide BLAST program, and the results are shown in Figure 1.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Tetragonula pagdeni voucher TpegCOITHAI01 cytochrome oxidase subunit I gene, partial cds; mitochondrial	Tetragonula pag...	774	774	97%	0.0	89.30%	627	KU934145.1
Tetragonula travancorica voucher MFE 123 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochon...	Tetragonula trav...	706	706	100%	0.0	86.87%	685	OP984054.1
Tetragonula sp. 1 MB-2019 voucher GTB261DN1851SASRDW cytochrome oxidase subunit I (COI) gene, partia...	Tetragonula sp. ...	689	689	99%	0.0	86.58%	677	MH347237.1
Tetragonula travancorica voucher VGE1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Tetragonula trav...	684	684	100%	0.0	86.26%	650	OP962445.1
Tetragonula pagdeni isolate Terakanambi, Chamarajanagar cytochrome c oxidase subunit I (COX1) gene, partia...	Tetragonula pag...	662	662	100%	0.0	85.62%	718	OR976105.1
Tetragonula pagdeni isolate Hunsur, Karnataka cytochrome c oxidase subunit I (COX1) gene, partial cds; mitoc...	Tetragonula pag...	662	662	100%	0.0	85.62%	734	OR976104.1
Tetragonula travancorica voucher FER1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Tetragonula trav...	662	662	100%	0.0	85.65%	692	OM293512.1
Tetragonula iridipennis isolate Batlagundu cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Tetragonula iridi...	654	654	97%	0.0	85.78%	657	OR536598.1
Tetragonula iridipennis isolate sirumalai cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Tetragonula iridi...	652	652	98%	0.0	85.67%	657	OR536564.1
Tetragonula iridipennis isolate kodai cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Tetragonula iridi...	649	649	97%	0.0	85.62%	657	OR536596.1
Tetragonula iridipennis isolate natham cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Tetragonula iridi...	636	636	98%	1e-177	85.19%	657	OR536550.1

Figure 1. The genetic identity was compared with data on Blast NCBI

The results illustrated that the COI gene segment extracted and amplified by the research group is similar to three species: *Tetragonula pagdeni*, *Tetragonula travancorica*, and *Tetragonula iridipennis*, with the genetic identity of 89.3%, 86.87%, and 85.58%, respectively.

Based on these results, morphological identification was carried out by comparing the characteristics of the collected stingless bee samples with those reported in published studies.

The morphological measurements of 10 worker bees are presented in Table 1. The total body length ranged from approximately 4.0 to 4.1 mm, with head length and width measuring about 0.97±0.07 mm and 1.71±0.09mm, respectively. The average mesosoma length was recorded as 1.82±0.14 mm, while the average metasoma length was 1.98±0.04 mm. Forewing lengths varied between 3.8 and 4.0 mm, and the hind leg length was approximately 3.8 mm. The head and thorax exhibit a black coloration. The front of the head features subtle distinct markings, with two yellow compound eyes and three ocelli that are red and black. The dorsal abdomen gradually transitions to a darker brown, with segments 1–3 forming alternating yellow and brown rings. The ventral abdomen also displays alternating yellow and brown coloration. The terminal abdominal segments are covered in white setae. The anterior thoracic dorsum contains numerous short white hairs, while the posterior thoracic dorsum and ventral thoracic regions are characterized by dense, long white hairs. The trochanter and posterior edge of the tibial segment are densely covered with hairs.

Figure 2. Morphology of the stingless bee worker

(A. Top-down view, B. Bottom-up view, C. Side view, D. Forewing and Hindwing of the stingless bee, and E. pairs of legs of the stingless bee.

Table 1. Measurements of workers of *Tetragonula sp.*

Character	Measurements (mm)
Body length	4.03±0.05
Antennae	1.91±0.09
Head length	0.97±0.07
Head width	1.71±0.09
Thorax length	1.82±0.14
Thorax width	1.33±0.25
Abdomen length	1.98±0.04
Abdomen width	1.03±0.07
Forewing length	3.97±0.07
Forewing width	1.23±0.07
Hindwing length	2.06±0.08
Hindwing width	0.55±0.09
Foreleg length	2.81±0.09
Middle leg length	3.02±0.04
Hind leg length	3.95±0.07

The analysis revealed that the morphological characteristics of the studied specimens exhibited the highest similarity to *T. iridipennis*. However, Sharma et al. (2023) observed that the morphological traits of *T. iridipennis* worker bees showed slight discrepancies compared to previous studies, likely due to geographic variation. Therefore, the findings suggest that the studied bee species is most likely *T. iridipennis*, but the possibility of it being an undescribed or novel species remains plausible.

3.2. Qualitative and Quantitative analysis of natural compounds

3.2.1. Qualitative results

Preliminary investigations revealed that all three types of extracts contained groups of compounds such as phenolics, tannins, flavonoids, alkaloids, coumarins, quinones, and terpenoids. Propolis contains approximately 150 compounds, including polyphenols, flavonoids, aglycones, phenolics, and ketones (Rozman et al., 2022). Among these, flavonoid molecules, phenolic acids, and their esters exhibit high pharmacological activity in terms of antibacterial properties (Castaldo & Capasso, 2002). The study results are presented in Table 2.

Table 2. Qualitative analysis of natural compounds present in the extracts

Compounds	EtOH	n-hexane	EtOAc
Phenolic	+	+	+
Tannin	+	+	+
Flavonoid	+	+	+
Alkaloid	+	+	+
Coumarin	+	+	+
Quinone	+	+	+
Terpenoid	+	+	+

Note: (+) Present, (-) Absent.

3.2.2. Quantitative result

The total polyphenol content (TPC) and total flavonoid content (TFC) were determined utilizing the standard curve equations established from gallic acid ($y = 0.005x - 0.0206$) with a reliability of ($R^2 = 0.998$) and quercetin ($y = 0.0157x - 0.0191$) with a reliability of ($R^2 = 0.9996$).

The total phenolic content of the extracts was quantified and expressed in terms of milligrams of gallic acid equivalents (mg GAE) per gram of sample. A higher mg GAE/g sample value indicates a greater phenolic content in the extract and vice versa.

In the EtOH extract, the total polyphenol content (TPC) was 102.65 ± 5.21 mg GAE/g extract, while the total flavonoid content (TFC) reached 69.18 ± 0.46 mg QE/g extract. According to the study by Kieu et al. (2019), these results indicate that the TPC in the extract of stingless bee propolis is twice to three times, and the TFC is six times to seven times as high as the TPC and TFC in the propolis extract from honey bees (*Apis mellifera*) sourced from tropical forests in Viet Nam. Therefore, the antimicrobial and antioxidant activities of stingless bee propolis were less potent. However, the TPC in this study was approximately 10 times, and the TFC was nearly twice as high as propolis extracts from several species of stingless bees in Malaysia (Salleh et al., 2021).

Table 3. The presence of TPC and TFC from the fractions of propolis

Extract	TPC (mg GAE/g sample)	TFC (mg QE/g sample)
EtOH	102.65^{B}±9.03	69.18^{A}±0.46
n-hexane	95.65^{B}±1.2	68.16^{A}±0.19
EtOAc	211.25^{A}±19.29	57.97^{B}±0.31

Note: Means+_SD that do not have the same uppercase character in the same column are significantly different (Tukey test, P<0.05). SD= standard diviation

3.3. Results of antimicrobial activity assessment

Based on the recorded results (Table 4), it was observed that the antibacterial activity of the stingless bee propolis extract diluted with DMSO exhibited antibacterial activity against all three bacterial strains: *C. acnes*, *S. aureus*, and *E. coli*. The inhibition zones increased gradually across all three bacteria at concentrations of 50, 100, 200, 600, and 1000 µg/mL. At the maximum concentration of 1000 µg/mL and the minimum concentration of 50 µg/mL for the ethanol and n-hexane extracts, no statistically significant differences were observed across all three bacteria.

The antibacterial activity of the EtOAc extract was significantly weaker compared to the EtOH and n-hexane extracts. This could be attributed to the majority of antibacterial compounds being preferentially dissolved in the n-hexane phase, leaving only a small portion in the EtOAc fraction. These findings align with those reported by Yusop et al. (2019), who evaluated the antibacterial effects of n-hexane, ethyl acetate, and ethanol extracts of *Trigona itama* propolis against *S. aureus* and *E. coli*. Their study also demonstrated that the antibacterial efficacy of the ethyl acetate extract was inferior to that of both n-hexane and ethanol extracts.

Table 4. Zone of inhibition (mm) of tested bacteria produced by the propolis fractions.

Sample	Concentrations	*C. acnes*	*S. aureus*	*E. coli*
EtOH	50	2.00^{D}±0.00	1.67^{D}±0.58	5.00^{E}±0.00
	100	8.00^{C}±0.00	4.67^{C}±0.58	6.33^{D}±0.58
	200	11.00^{B}±0.00	7.33^{B}±0.58	8.00^{C}±0.00
	600	11.67^{B}±0.58	10.33^{A}±0.58	9.00^{B}±0.00
	1000	13.33^{A}±0.58	11.67^{A}±0.58	10.33^{A}±0.58
(+) Tetracycline 0.5 mg/mL		16	17	15
(-) DMSO 10%		-	-	-
n-hexan	50	2.00^{E}±0.00	2.00^{E}±0.00	4.33^{E}±0.58
	100	5.00^{D}±0.00	5.00^{D}±0.00	6.00^{D}±0.00
	200	7.00^{C}±0.00	7.33^{C}±0.00	7.00^{C}±0.00
	600	9.00^{B}±0.00	9.67^{B}±0.00	9.00^{B}±0.58
	1000	13.33^{A}±0.58	11.33^{A}±0.00	10.33^{A}±0.58
(+) Tetracycline 0.5 mg/mL		15	15	15
(-) DMSO 10%		-	-	-

Note: Means+_SD that do not have the same uppercase character in the same column are significantly different (Tukey test, P<0.05). SD = standard deviation.

Table 5. Results of diameter antibacterial zones for EtOAc (mm)

Concentrations (mg/mL)	EtOAc		
	C. acnes	*S. aureus*	*E. coli*
1	2.00^{D}±0.00	1.68^{D}±0.58	4.00^{E}±0.00
2	4.00^{C}±0.00	4.67^{C}±0.58	6.00^{D}±0.00
3	6.00^{B}±0.00	7.33^{B}±0.58	7.33^{C}±0.58
4	6.67^{B}±0.58	10.33^{A}±0.58	9.00^{B}±0.00
5	9.67^{A}±0.58	11.33^{A}±0.58	10.33^{A}±0.58
(+) Tetracycline 0.5 mg/mL	15	15	15
(-) DMSO 10%	-	-	-

Note: Means+_SD that do not have same uppercase character in the same column are significantly different (Tukey test, P<0.05). SD = standard deviation.

In the study by Abdullah et al. (2020), the ethanol extract of propolis exhibited antibacterial activity against *S. aureus* and *E. coli*. The antimicrobial activity was more pronounced against Gram-positive bacteria (*S. aureus*) than against Gram-negative bacteria (*E. coli*). This bactericidal action is related to the disruption of ion-channel metabolism through phosphorylation /dephosphorylation reactions. The antimicrobial properties are also associated with the presence of phenolic acids (Popova et al., 2010). There is evidence that propolis disrupts the cell membrane of microorganisms (Torres A.R., 2018).

It can also be concluded that propolis (*Tetragonula iridipennis*) exhibits antibacterial effects due to active compounds that inhibit bacterial RNA polymerase and cause degradation of the bacterial cytoplasmic membrane. There are currently no reports proving that propolis's antibacterial properties are caused by a single ingredient or group of compounds; rather, they are the result of the synergistic impact of all the compounds that make up the composition (Boisard et al., 2015). Propolis affects the cytoplasm, inhibiting bacterial activity and enzyme function, thus potentially suppressing the growth and proliferation of various bacteria, and may act as a bactericidal agent at high concentrations (Mirzoeva et al., 1997).

3.4. Results of inhibition against *Candida albicans*

Although numerous studies worldwide have demonstrated the antifungal activity of propolis extracts from various stingless bee species against *Candida albicans* (Campos et al., 2015; Santos et al., 2017; Kasote et al., 2019), our study found no inhibition of *Candida albicans* when testing extracts from *Tetragonula sp.* propolis at different concentrations using EtOH, n-hexane, and EtOAc. These findings align with those of Georgieva et al. (2019), who also reported that EtOH extracts of propolis from *Lisotrigona cacciae* were inactive against *Candida albicans*. Since the chemical composition and biological properties of propolis are significantly influenced by the bee species and the plant resins they collect (Popova et al., 2019), further analysis is needed to identify the specific chemical components responsible for these differences.

3.5. MIC based on Resazurin assay

The MIC of the ethanol extract of stingless bee propolis was determined to be 30 µg/mL for *S. aureus*, which is lower compared to 50 µg/mL for *C. acnes and E. coli*. This indicates greater resistance to S. aureus than to the other two bacteria, aligning with studies showing greater inhibition of Gram-positive than Gram-negative bacteria, with *S. aureus* being more sensitive than other bacterial strains (Kasote et al., 2019). According to Campos et al. (2015), the inhibition of EtOH of *T. fiebrigi* propolis was observed against the evaluated followed by *S. aureus*, which was more sensitive than *S. epidermidis, E. faecalis, P. mirabilis, K. pneumoniae,* and *P. aeruginosa*, with the MIC for *S. aureus* at approximately 0.55 ± 0.05 mg/mL. Similar to the study by Santos et al. (2017), the inhibition of microorganisms was observed S. aureus more sensitive than *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. neoformans*, and *C. albicans*, with the MIC for *S. aureus* at approximately 6.13 ± 0.10 mg/mL.

4. CONCLUSION

This study successfully identified the species of stingless bee collected in Soc Trang through morphological and molecular biological methods. Qualitative analysis using chemical methods on the extract of stingless bee propolis (*Tetragonula* sp.) confirmed the presence of several bioactive compounds, including phenolics, tannins, flavonoids, alkaloids, coumarins, quinones, and terpenoids.

The extracts exhibited antibacterial activity against all three bacterial strains: *S. aureus*, *C. acnes*, and *E. coli*. At the highest tested concentration of 1000 µg/mL using the agar well diffusion method, the EtOH and n-hexane extracts showed the greatest inhibitory effect against *C. acnes*, with inhibition zones of 13.33±0.58 mm. At both the highest concentration of 1000 µg/mL and the lowest concentration of 50 µg/mL for EtOH and n-hexane, no statistically significant differences were observed among the three bacterial strains. The MICs for *S. aureus*, *C. acnes*, and *E. coli* were found to be 30, 50, and 50 µg/mL, respectively.

ACKNOWLEDGMENT

This research was funded by Can Tho University, Project Code: TSV2024-222.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Abdullah, N. A., Zullkiflee, N., Zaini, S. N. Z., Taha, H., Hashim, F., & Usman, A. (2020). Phytochemicals, mineral contents, antioxidants, and antimicrobial activities of propolis produced by Brunei stingless bees *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona binghami*. *Saudi Journal of Biological Sciences*, *27*(11), 2902-2911. https://doi.org/10.1016/j.sjbs.2020.09.014

Boisard, S., Ray, A. L., Landreau, A., Kempf, M., Cassisa, V., Flurin, C., & Richomme, P. (2015). Antifungal and Antibacterial Metabolites from a French Poplar Type Propolis. *Evidence-based Complementary and Alternative Medicine*, *2015*, 1–10. https://doi.org/10.1155/2015/319240

Campos, J. F., Santos, U. P. D., Da Rocha, P. D. S., Damião, M. J., Balestieri, J. B. P., Cardoso, C. a. L., Paredes-Gamero, E. J., Estevinho, L. M., De Picoli Souza, K., & Santos, E. L. D. (2015). Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of propolis from the stingless bee *Tetragonisca fiebrigi* (Jataí). *Evidence-based Complementary and Alternative Medicine*, *2015*, 1-11. https://doi.org/10.1155/2015/296186

Castaldo, S., & Capasso, F. (2002). Propolis, an old remedy used in modern medicine. *Fitoterapia*, *73*, S1–S6. https://doi.org/10.1016/s0367-326x(02)00185-5

Georgieva, K., Popova, M., Dimitrova, L., Trusheva, B., Thanh, L. N., Phuong, D. T. L., Lien, N. T. P., Najdenski, H., & Bankova, V. (2019). Phytochemical analysis of Vietnamese propolis produced by the stingless bee *Lisotrigona cacciae*. *PLoS ONE*, *14*(4), e0216074. https://doi.org/10.1371/journal.pone.0216074

Hai, N. X., Nhan, N. T., Mai, N. T. T., (2011). Comparative study on total polyphenols, flavonoids content, and antioxidant activity of propolis from Viet Nam and other countries. *Science & Technology Development, 14*(2), 66-74. https://doi.org/10.32508/stdj.v14i2.1945

Kasote, D. M., Pawar, M. V., Gundu, S. S., Bhatia, R., Nandre, V. S., Jagtap, S. D., Mahajan, S. G., & Kulkarni, M. V. (2019). Chemical profiling, antioxidant, and antimicrobial activities of Indian stingless bees propolis samples. *Journal of Apicultural Research*, *58*(4), 617-625. https://doi.org/10.1080/00218839.2019.1584960

Kieu, T. T. M., Thoa, H. T., & Ngoc, D. T. B. (2019). Isolation, quantification of the active pharmaceutical compounds in propolis in Vietnam's rainforests. *Journal of Science and Technology – NTTU*, *8*, 60-65.

Le, T. N., Thoa, H. T., Oanh, N. T. T., Giap, T. H., Quyen, V. T., Ha, N. T. T., Phuong, D. T. L., Lien, N. T. K., & Hang, N. T. M. (2021). Cycloartane triterpenoids and biological activities from the propolis of the stingless bee *Lisotrigona furva*. *Vietnam Journal of Chemistry*, *59*(4), 426-430. https://doi.org/10.1002/vjch.201900143

Makkar, G. S., Chhuneja, P. K., & Singh, J. (2018). Stingless Bee, *Tetragonula iridipennis* Smith, 1854 (Hymenoptera: Apidae: Meliponini): Molecular and Morphological Characterization. *Proceedings of the National Academy of Sciences India Section B Biological Sciences*, *88*(1), 285–291. https://doi.org/10.1007/s40011-016-0757-4

Marconi, M., Modesti, A., Alvarez, L. P., Ogoña, P. V., Mendoza, A. C., Giove, D. V., Luna, J. O., Di Giulio, A., & Mancini, E. (2022). DNA barcoding of stingless bees (*Hymenoptera: meliponini*) in northern Peruvian forests: A plea for integrative taxonomy. *Diversity*, *14*(8), 632. https://doi.org/10.3390/d14080632

Mirzoeva, O., Grishanin, R., & Calder, P. (1997). Antimicrobial action of propolis and some of its components: the effects on growth, membrane potential and motility of bacteria. *Microbiological Research*, *152*(3), 239-246. https://doi.org/10.1016/s0944-5013(97)80034-1.

Popova, M. P., Graikou, K., Chinou, I., & Bankova, V. S. (2010). GC-MS Profiling of Diterpene Compounds in Mediterranean Propolis from Greece. *Journal of Agricultural and Food Chemistry*, *58*(5), 3167-3176. https://doi.org/10.1021/jf903841k

Popova, M., Trusheva, B., & Bankova, V. (2019). Propolis of stingless bees: A phytochemist's guide through the jungle of tropical biodiversity. *Phytomedicine*, *86*, 153098. https://doi.org/10.1016/j.phymed.2019.153098

Riaz, B., Zahoor, M. K., Zahoor, M. A., Majeed, H. N., Javed, I., Ahmad, A., Jabeen, F., Zulhussnain, M., & Sultana, K. (2018). Toxicity, Phytochemical Composition, and enzyme inhibitory activities of some indigenous weed plant extracts in Fruit Fly, Drosophila melanogaster. *Evidence-based Complementary and Alternative Medicine*, *2018*(1). https://doi.org/10.1155/2018/2325659.

Rocha, V. M., Portela, R. D., dos Anjos, J. P., de Souza, C. O., & Umsza-Guez, M. A. (2023). Stingless bee propolis: composition, biological activities and its applications in the food industry. *Food Production, Processing and Nutrition*, *5*(1), 1-13. https://doi.org/10.1186/s43014-023-00146-z

Rozman, A. S., Hashim, N., Maringgal, B., & Abdan, K. (2022). A comprehensive review of stingless bee products: phytochemical composition and beneficial properties of honey, propolis, and pollen. *Applied Sciences*, *12*(13), 6370. https://doi.org/10.3390/app12136370

Sakagami, S. F. (1978). Tetragonula stingless bees of the continental Asia and Sri Lanka. *Journal of the*

Faculty of Science, Hokkaido University, ser. VI, Zoology, 21(2), 165-247.

Salleh, S. N. a. S., Hanapiah, N. a. M., Ahmad, H., Johari, W. L. W., Osman, N. H., & Mamat, M. R. (2021). Determination of total phenolics, flavonoids, and antioxidant activity and GC-MS analysis of Malaysian stingless bee propolis water extracts. *Scientifica*, *2021*, 1-11. https://doi.org/10.1155/2021/3789351

Santos, H., Campos, J., Santos, C., Balestieri, J., Silva, D., Carollo, C., De Picoli Souza, K., Estevinho, L., & Santos, E. D. (2017). Chemical Profile and Antioxidant, Anti-Inflammatory, Antimutagenic and Antimicrobial Activities of Geopropolis from the Stingless Bee *Melipona orbignyi*. *International Journal of Molecular Sciences*, *18*(5), 953. https://doi.org/10.3390/ijms18050953

Shanas, S., & Faseeh, P. (2019). A new subgenus and three new species of stingless bees (Hymenoptera: Apidae: Apinae: Meliponini) from India. *ENTOMON, 44*(1), 33-48. https://doi.org/10.33307/entomon.v44i1.424

Sharma, V. G., Jethva, D. M., Wadaskar, P. S., Kachot, A. V., & Davaria P. J. (2023). Morphometric studies of stingless bees (*Tetragonula iridipennis* Smith) in Saurashtra region of Gujarat state. *The Pharma Innovation Journal*, *12*(5), 1582-1585.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H., (2011). Phytochemical screening and Extraction: A Review. *International Pharmaceutical Science, 1*(1), 98-106.

Torres, A., Sandjo, L., Friedemann, M., Tomazzoli, M., Maraschin, M., Mello, C., & Santos, A. (2018). Chemical characterization, antioxidant and antimicrobial activity of propolis obtained from Melipona quadrifasciata quadrifasciata and *Tetragonisca angustula* stingless bees. *Brazilian Journal of Medical and Biological Research*, *51*(6). https://doi.org/10.1590/1414-431x20187118

Vijayakumar, K., & Jeyaraaj, R. (2014). Taxonomic notes on stingless bee *Trigona (Tetragonula) iridipennis* Smith (Hymenoptera: Apidae) from India. *Journal of Threatened Taxa*, *6*(11), 6480-6484. https://doi.org/10.11609/JoTT.o3773.6480-4

Vijayakumar, K., & Jeyaraaj, R. (2020). Taxonomic notes on *Tetragonula pagdeni* Schwarz (Apidae: Meliponini) from India. *International Journal of Advanced Life Sciences*, *13*(1), 22-26.

Yusop, S. a. T. W., Sukairi, A. H., Sabri, W. M. a. W., & Asaruddin, M. R. (2019). Antioxidant, Antimicrobial and Cytotoxicity Activities of Propolis from Beladin, Sarawak Stingless Bees *Trigona itama* Extract. *Materials Today Proceedings*, *19*, 1752–1760. https://doi.org/10.1016/j.matpr.2019.11.213