ORIGINAL ARTICLE

Evaluation of the Anti-Inflammatory Activity and Chemical Compound Analysis of Propolis Extract *Geniotrigona thoracica* from Pematang Siantar North Sumatera

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ABSTRACT

Introduction: This study aimed to identify the chemical compounds and examine the in vitro anti-inflammatory activity of *Geniotrigona thoracica* propolis extract in inhibiting nitric oxide (NO), tumor necrosis factor alpha (TNF-α), and inducible nitric oxide synthase (iNOS) expression in RAW 264.7 macrophages. **Methods:** Total phenolic and flavonoid content was determined using spectrophotometric methods. Prior to anti-inflammatory studies, cytotoxicity test was performed to determine the safest propolis concentration for RAW 264.7 cells. Anti-inflammatory activity tests including NO production assay (Griess assay), iNOS production and TNF-α production using Enzyme linked Immunosorbent Assay (ELISA) kit. The chemical components of the propolis extract were identified by Liquid chromatography tandem mass spectrometry (LC-MS/MS) technique. **Results:** The TPC and TFC were 374.20 ± 0.51 mg GAE/g extract and 20.66 ± 4.08mg QE/g extract, respectively. Propolis extract at concentrations of 12.5, 25, and 50 µg/mL was non-toxic, making it suitable for anti-inflammatory testing. The anti-inflammatory test results showed inhibition of TNF-α at 12.5 µg/mL EEP, which was 11.06%, inhibition of NO at 50 µg/mL, which was 22%, and inhibition of iNOS at 12.5 µg/mL, which was 41%. LC-MS/MS analysis revealed that propolis extract includes anti-inflammatory bioactive substances such as β-mangostin, garcinone D, garcinone E, DL-stachydrine, betaine, salvinorin-A, mangostin, and 18-β-glycyrrhetinic acid. **Conclusion:** Propolis extract from *Geniotrigona thoracica*, Pematang Siantar area, North Sumatra, Indonesia, has anti-inflammatory bioactive compounds in the results of the LCMS/MS method, and the results of the anti-inflammatory activity on TNF-α, NO, and iNOS indicate the presence of anti-inflammatory activity in the extract.
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Keywords: *Geniotrigona thoracica*, Cytotoxicity, Propolis, Anti-inflammatory, LC-MS/MS, in vitro.

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INTRODUCTION

Inflammation is the body's response to combat invading pathogens, eliminate irritants, and facilitate tissue repair. Inflammation is a natural defense mechanism triggered

by physical injury, toxic substances, microorganisms, or invading objects. Inflammation is frequently associated with redness, discomfort, and heat (1). During inflammatory responses, pro-inflammatory proteins are produced less, and anti-inflammatory proteins that boost the innate immune system are more active (2). Anti-inflammatory medicines can cause side effects and consequences such as reduced renal function, oedema, hypertension, and gastrointestinal bleeding (3). To avoid the harmful side effects of synthetic pharmaceuticals, it is essential to consider natural compounds like propolis when developing new anti-inflammatory medications (4). Research conducted by Gunawan et al, demonstrated that propolis has a better anti-inflammatory impact that is 4-5 times higher in lowering inflammation (5).

Bees produce propolis from plant exudates and shoots (6). Bees use propolis to coat the walls of their honeycombs and protect themselves from invading insects (7). The physical and chemical properties of propolis, like as color, aroma, and propolis content, are affected by the plant from which it extracted. Propolis comes in various colors, from yellow to dark brown, with some being transparent. Propolis is typically light to dark brown, but it can also be green, red, black, yellow, or white (8). This is determined by the flavonoid concentration. Propolis is a traditional medicine used to cure a variety of ailments. Propolis has antioxidant, antibacterial, antiviral, and anti-inflammatory properties (9). The quality of propolis is strongly influenced by bee species and geographical location (10). Propolis is used as an anti-inflammatory agent because it contains CAPE (Caffeic Acid Phenethyl Ester) and quercetin, both of which inhibit T-cell activation (11). CAPE may suppress Nuclear Transcription Factor Kappa B (NF-kB) while activating Interleukin-2 (IL-2), which promotes the growth of T-lymphocytes (12). Propolis has been extensively promoted in many parts of Indonesia, including the Pematang Siantar district in North Sumatra. Stingless bees of the *Geniotrigona thoracica* species are commonly raised by beekeepers in that area. As previously stated, propolis contains flavonoids and polyphenols, both of which have anti-inflammatory properties. So far, no studies habe been conducted on the anti-inflammatory activity or compound composition of *Geniotrigona thoracica* propolis from Pematang Siantar. This study was conducted with the purpose of investigation the potential of propolis *Geniotrigona thoracica* from Pematang Siantar as a natural anti-inflammatory agent.

MATERIALS AND METHODS

Sample

Stingless bee propolis *Geniotrigona thoracica* from Pematang Siantar area, North Sumatera Province, Indonesia. The propolis was harvested in May of 2023, and has been determined at the Biota collection room, Laboratory of Zoologicum University of Indonesia (UIMZ), Department of Biology, FMIPA Universitas Indonesia, Depok with number 172/UN2.F3.11/PDP.02.00/2023.

Chemical and reagent

Phosphate-Buffered Saline/PBS (Biowest, Eropa), DMSO (Merck, Jerman), Thiazolyl blue tetrazolium bromide (Sigma Aldrich, Amerika), Methanol (Merck, Jerman), MTT reagen (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Aldrich, Amerika), Bradford reagen (Sigma Aldrich), Griess reagent (Sigma Aldrich, Amerika), Biotynilated detection ab (Elabscience, Amerika), HRP conjugate (Elabscience, Amerika), Wash buffer solution (Elabscience, Amerika), Substrate reagent (Elabscience, Amerika), Stop solution (Elabscience, Amerika), Standard solution (Elabscience, Amerika), Sulphate Solution (Elabscience, Amerika), Alkali Reagent (Elabscience, Amerika), Chromogenic Agent A (Elabscience, Amerika), Chromogenic Agent B (Elabscience, Amerika).

The extraction of propolis *Geniotrigona thoracica*

The extraction process was conducted by the maceration method with a 96% ethanol solvent, as described by Pratami et al. 2018 (9). The liquid extract was concentrated using a rotary evaporator, yielding a propolis-free wax. This viscous extract was thereafter stored in the refrigerator.

Determination of total phenolic and flavonoid content

Quantitative analysis of total phenolic (TPC) and flavonoid content (TFC) was based on a previous study with some modifications (13). The total phenolic content (TPC) was assessed using the 96-well microplate method, employing a gallic acid standard at concentrations ranging from 12.5 to 250 ppm. Following the addition of 25 µL of either EEP or standard, 100 µL of 25% Folin-Ciocalteu reagent was added and left to incubate for 4 minutes. Subsequently, 75 µL of 1 M Na_2CO_3 solution was added and incubated for 60 seconds, followed by 2-hour incubation at room temperature. Absorbance was measured at a wavelength of 765 nm. The TFC measurements were performed using a standard quercetin solution with concentrations between 12.5 and 250 ppm. A 20 µL aliquot of EEP or quercetin standard at different concentrations was pipetted and mixed with 20 µL of 10% $AlCl_3$, 20 µL of 1M CH_3COOK, and 140 µL of water, followed by agitation for 60 seconds. The mixture was permitted to incubate for 30 minutes at room temperature. The absorbance of the EEP was quantified at a wavelength of 415 nm. The quantification of TPC and TFC was conducted using a microplate reader spectrophotometer (Thermo Scientific Multiskan GO, USA). The absorbance of the standard solution was measured at each concentration, resulting in the calibration curve equation $y = a + bx$. The measurements were performed in triplicate. The total phenolic content (TPC) was measured in mg gallic acid equivalents (GAE) per gram of extract, whereas the total

flavonoid content (TFC) was expressed in mg quercetin equivalents (QE) per gram of extract.

Cell culture and cytotoxicity test

RAW 264.7 cells (ECACC; 91062702) were retrieved from a -80 ºC freezer vial and cultured as previously described (14). The RAW 264.7 cells were cultured in DMEM (Gibco, Thermo Fisher Scientific, USA) with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, USA) and 1% penicillin-streptomycin solution (Gibco, Thermo Fisher Scientific, USA) at 37°C in a 5% CO_2 incubator. Before they were used in the next test, the cells were cultured in separate cultures and plated until they reached 70% confluence. A modified MTT technique was used for the cytotoxicity test (15). Approximately 1 x 10_5 cells/mL, RAW 264.7 cells were plated onto 96-well plates at and incubated for 24 hours at 37ºC in 5% CO_2 incubator. The cells were again exposed to different concentration of propolis extracts the following day, and they were once more incubated for 24 hours at 37ºC in 5% CO_2 incubator. After the incubation period was over, MTT solution was added to each well and the mixture was once again incubated for 4 hours in the dark at 37ºC in 5% CO_2 incubator. The formed formazan crystals were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA). The viability test was evaluated by measuring the absorbance at 570 nm using microplate reader.

Total protein content using Bradford assay

The protein was evaluated using Bradford reagents as described by Raouhullah (2015) (16). Conditioned medium samples of RAW264.7 cells in the inflammation model were supplemented with approximately 1.5 ml of propolis extract and BSA (bovine serum albumin). (Sigma, A9576). A series of BSA reference standards was prepared with concentrations of 100; 50; 25; 12.5; 6.25; 3.125; 0 (µg/mL). A total of 20 µL of standard solution and samples were added to each well. Then, 200 µL of Quick Start Dye Reagent (Biorad, 500-0205) was added once to each well, and it was reconstituted until thoroughly mixed. Incubate for 5 minutes at room temperature. The color change to blue indicates the presence of protein in the standard or sample, with absorbance measured at a wavelength of 595 nm using a spectrophotometer. The measurement was conducted in duplicate

NO griess, TNF- α, and iNOS assay

Before the NO test response, RAW 264.7 cells were pre-treated with ethanolic extract propolis (EEP) at concentrations of 120, 30, and 7.5 µg/mL for 1 hour. Subsequently, 1 µg/mL lipopolysaccharide (LPS; Sigma Aldrich, USA) was added, and the cells were incubated for 20 hours. Next, 100 µL of supernatant was combined with Griess solution (1% Sulfanilamide and 0.1% N-[naphthyl]ethylendiamine dihydrochloride in 2.5% H_3PO_4) at room temperature for 10 minutes. The assay findings were obtained by measuring at a wavelength of 540 nm using a microplate reader (17).

In addition, the supernatant that was produced from the LPS induction was utilized for the TNF-α and iNOS assay by utilizing a mouse TNF-α and iNOS ELISA kit (Elabscience, China). An equal volume of 100 µL of the standard solution of INOs and TNF-α was put into the well, duplicate. Next, the well was sealed, and then it was incubated at 37°C for 90 minutes. After discarding the solution, a biotinylated detection antibody solution was added to each well at a volume of 100 µL. The wells were then sealed and held in an incubator at 37°C for a duration of one hour. In the following step, the solution was rinsed with wash buffer for 1-2 minutes before being discarded. Three repeated sessions of the treatment were carried out. The plate was covered and incubated at 37°C for 30 minutes after the addition of 100 mL of HRP conjugate each well. A total of five washes were performed on the plate. There was a 90-µL/well substrate that was applied, and it was incubated for 15 minutes at 37°C until the color changed. If the color did not change, the incubation time was added, but not until 30 minutes had passed. After adding 50 µL of stop solution per well, the stop solution was added to each well. Optical density (OD) was determined by utilizing a microplate reader to test the sample at 450 nm (14).

LC-MS/MS analysis

This LC-MS/MS method uses a Vanquish Tandem Q Exactive Plus Orbitrap HRMS UHPLC column instrument (Thermoscientific) equipped with an ACCURE C18 column with an inner diameter of 100x2.1 mm, 1.5 µm, column temperature set at 40 °C, and a flow rate of 0.20 mL/min. The test used a 5000-ppm sample that was diluted in 1 mL of methanol and filtered through a 0.2 µm PTFE membrane with a 2 µL injection volume. LC-MS/MS analysis using mobile phases, namely 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), with a flow rate of 0.2 mL/min, running for 30 minutes with run times of 0–1 minute (5% B), 1–25 minutes (95% B), 25–28 minutes (95% B), and 28–30 minutes (5% B). Mass spectrometry (MS) analysis was performed in positive mode with a mass range of 100–1500 m/z. The data were interpreted using MzCloud and PubChem (18).

Statistical analysis

The results are expressed as the mean ± standard deviation of the mean (SD). All the data here were thrice replication for each experiment. The statistical analysis and all graphs were performed using GraphPad prism 8.

RESULT

Total phenolic and flavonoid content

The TPC value and TFC of propolis *G. thoracica* were 374.20 ± 0.51 mg GAE/g extract and 20.66 ± 4.08mg QE/g extract, respectively.

Total protein content using Bradford assay

The results of measuring total protein levels with the

Bradford method show that propolis extracts with different concentrations have average protein levels ranging from 0.793±0.066 - 0.913± 0.076 µg/mL (Fig 1).

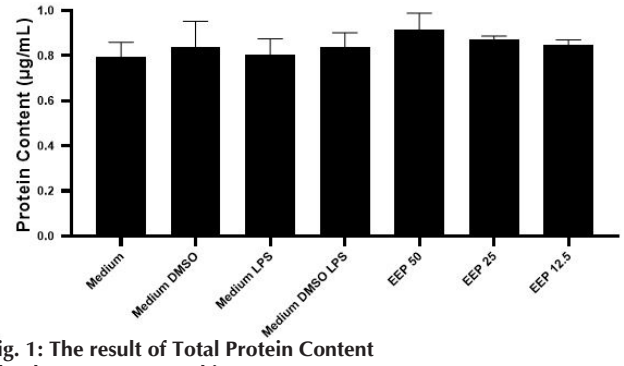

Fig. 1: The result of Total Protein Content
The data was presented in mean ± SD, n=3

Cytotoxicity test using MTT assay

The cytotoxic test showed a percentage of viability ranging from 53.50±2.5 to 126.21±8.4 as seen in Fig. 2.

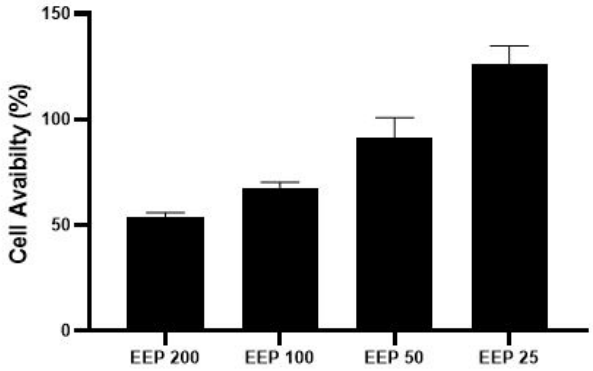

Fig. 2: The result of cytotoxicity test
The data was presented in mean ± SD, n=3

NO Griess assay test

The results of the standard solution concentration series on the NO standard curve read on the microplate reader were entered into the linear regression equation y = 0.0112x + 0.1527, R^2 = 0.9996. It can be seen in the test results shown in Fig. 3 that NO production in the negative control (only medium) was relatively lower because the negative control was not given LPS induction as an inflammatory stimulus. In the test group, it can be seen that the concentration of EEP 50 µg/mL affects the decrease in NO production. The trend was seen in the administration of propolis extract at a concentration of 25 µg/mL, where the results showed that NO production dropped significantly compared to the concentration of 12.5 µg/mL.

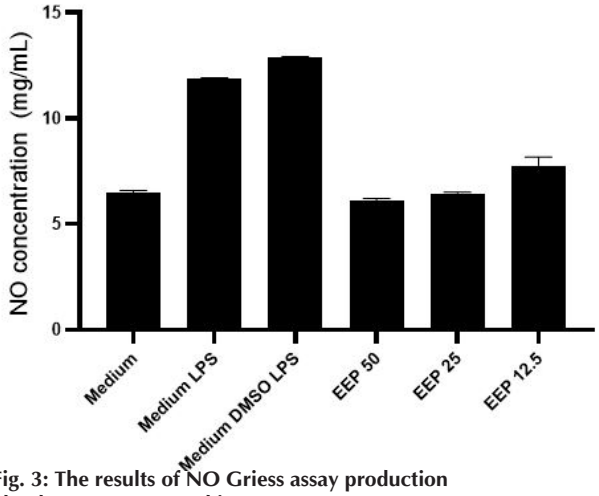

Fig. 3: The results of NO Griess assay production
The data was presented in mean ± SD, n=3

TNF- α ELISA assay test

The concentrations in the TNF-α standard obtained linear regression equation with he following equation: y = 0.0038x + 0.099, R^2 = 0.9918. From the results of these levels, it can be seen that the negative control, namely culture media and RAW 264.7 cells, did not show high levels, because the negative control was not induced with LPS. The TNF-α test results showed that the test group given propolis extract at a concentration of 50 µg/mL produced better results than at concentrations of 25 and 12.5 (µg/mL). It can be said that propolis extract at a concentration of 50 µg/mL can reduce TNF-α levels and inhibit TNF-α cytokine production in RAW 264.7 cells induced with LPS. From these results, it can be compared with the positive control which shows relatively higher results due to the induction of LPS (Fig 4).

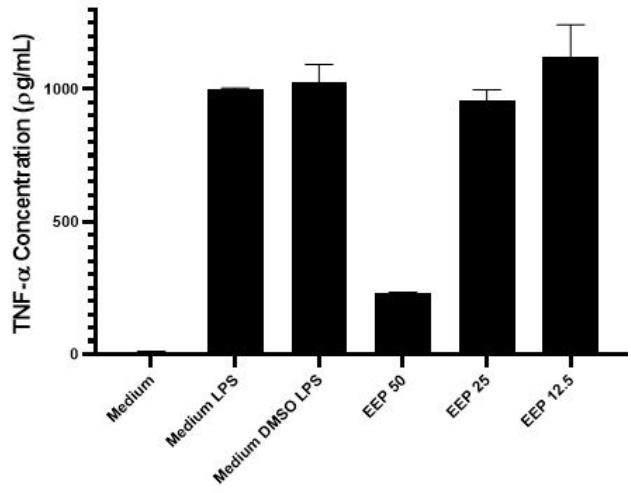

Fig. 4: The results of the TNF-α ELISA assay production
The data was presented in mean ± SD, n=2

iNOS ELISA assay test

The iNOS curve was made using the standard solution contained in the iNOS ELISA Kit. This standard curve determination uses a concentration series of 0 – 20 ng/L. Absorbance data from the results of iNOS standard solution was entered into linear regression and obtained, the result y = 0.0906x + 0.0632, R^2 = 0.995. The test results in Fig. 5 show that the negative control has low levels because only RAW 264.7 cells and culture media are not induced with LPS, while the difference in the positive control has very high results where the positive control is induced with LPS which indicates that the positive control produces high levels of iNOS. The propolis extract concentration of 50 µg/mL resulted in low iNOS production compared to the propolis extract concentrations of 25 and 12.5 (µg/mL). It can be said that the decrease in iNOS production and activity is due to the large concentration of propolis extract given to RAW 264.7 cells that have been induced with LPS.

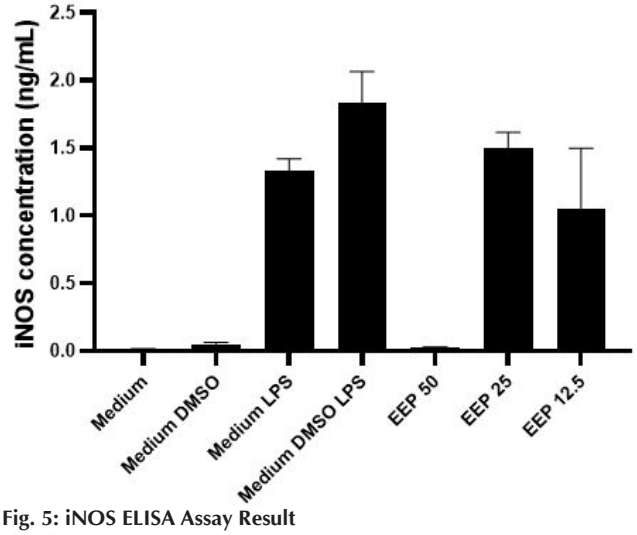

Fig. 5: iNOS ELISA Assay Result
The data was presented in mean ± SD, n=2

LC-MS/MS analysis

The results obtained in the LC-MS/MS analysis instrument test was presented in Fig. 6. The processed raw data has been analysed through the data processing stage first to get the final results. In this study, several compounds were selected that have the potential to be anti-inflammatory bioactive compounds due to their anti-inflammatory effects. The list of compounds in *Geniotrigona thoracica* bee propolis that have potential as bioactive compounds for anti-inflammatory activity can be seen in Table I.

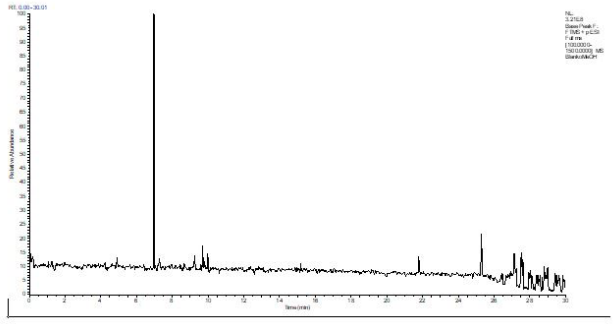

Fig. 5: iNOS ELISA Assay Result
The data was presented in mean ± SD, n=2

Table I: List of potential bioactive anti-inflammatory compounds

Bioactive Compounds	Group	Retention time (minutes)	Experimental Mass m/z	Teoritical Mass m/z	Mass Error	Reference
Betaine	Amino acid	1.08	118.0864	118.08677	0.670	(19,20)
DL-Stachydrine	Amino acid	1.13	144.1017	144.1024	1.050	(21)
Salvinorin-A	Terpenoid	13.89	433.1846	432.1784	2.320	(22,23)
Garcinone D	Xanthon	19.07	429.1893	428.1835	2.830	(24)
β-mangostin	Xanthon	1.08	425.1945	424.1885	1.98	(25)
Mangostin	Xanthon	22.19	411.1789	411.1725	3.00	(26)
18-β-Glycyrrhetinic acid	Triterpenoid glycoside	23.89	471.3467	470.3396	1.28	(27)
Garcinone E	Xanthon	24.21	465.2263	464.2198	1.82	(24)

DISCUSSION

Propolis from *G. thoracica*, a stingless bee species native to Indonesia, is known for its rich bioactive compounds, particularly its total phenolic and flavonoid content. These compounds contribute to its strong antioxidant properties, making it highly valuable in traditional medicine and natural remedies. The phenolic content is critical as it plays a vital role in neutralizing free radicals, reducing oxidative stress, and supporting overall health. Meanwhile, flavonoids enhance the immune response and exhibit anti-inflammatory and antimicrobial effects. Studies on Indonesian *G. thoracica* propolis reveal significant levels of these bioactive compounds, making it a promising source for natural therapeutic applications (13).

Phenolics and flavonoids, known for their strong antioxidant capabilities, help neutralize free radicals and reduce oxidative stress, which is closely linked to

inflammation (28). Studies have shown that the high levels of these bioactives in *G. thoracica* propolis contribute to its ability to inhibit pro-inflammatory mediators, making it a potential natural remedy for inflammatory-related conditions (29). Additionally, the diverse plant sources visited by this bee species in Indonesia's rich biodiversity may further enhance the potency and variation of the phenolic and flavonoid profiles, contributing to its overall health benefits (30).

The inflammatory test includes various components, such as quantifying proteins using the Bradford assay. The Bradford microplate protein test is a rapid and appropriate technique. This method could replace the time-consuming method and provide numerous advantages for students and laboratories requiring protein concentration determination via the Bradford assay (16). It is based on the binding of dye reagent to proteins, which causes a shift in the dye's absorbance maximum. This change can be quantitatively measured using a spectrophotometer. The intensity of the blue color produced is proportional to the amount of protein present in the sample. To obtain accurate results, a standard curve is usually prepared using a known protein, such as bovine serum albumin (BSA), for comparison. The total protein content was comparable to Sahlan et al study, which found that varying doses of *T. sapiens* bee propolis extract from South Sulawesi result in variable total protein content. This study's total protein content is lower than that of *T. sapiens* propolis extract, averaging between 203.08 - 208.30 µg/mL (14).

A cytotoxicity test is commonly performed to evaluate the effects of compounds like propolis on the viability of cells, such as RAW 264.7 cells, a murine macrophage cell line. This assay helps determine the potential toxic effects of propolis by measuring how it affects cell survival. Typically, a MTT assay is used, where cells are treated with varying concentrations of propolis. The viability of the cells is assessed based on their ability to metabolize certain substrates, such as MTT, into a colored product. A reduction in color intensity indicates decreased cell viability, suggesting cytotoxic effects at higher concentrations of propolis (14). The cytotoxicity test is crucial for determining the safe and effective concentration of propolis for further anti-inflammatory studies. This test ensures that propolis has therapeutic potential without adversely affecting normal cell function. The safe extract concentrations for RAW 264.7 cells were determined based on the percent viability values. Extract concentrations with a percent viability value above 80% are considered safe for this test. Sahlan et al. demonstrated that propolis from T. sapiens at concentrations of 150 - 200 ppm significantly decreased the viability of RAW 264.7 cells to 88.14% ± 8.43% and 75.99% ± 2.44% respectively compared to the negative control (14). The study confirmed that the concentration of propolis influenced cell viability, either increasing or lowering it, which could help determine the ideal propolis dosage.

Propolis extract has been shown to exhibit anti-inflammatory properties by reducing NO production in RAW 264.7 cells, a commonly used murine macrophage cell line for inflammation studies. When macrophages are activated by inflammatory stimuli, such as LPS, they produce excessive nitric oxide through the enzyme iNOS (29). Elevated levels of NO contribute to inflammation and tissue damage. Propolis extract, rich in bioactive compounds, can inhibit the expression of iNOS, thus lowering NO production. This reduction in NO helps modulate the inflammatory response (31), indicating the potential of propolis extract as a natural anti-inflammatory agent. Flavonoids can block the enzyme-inducible cyclooxygenase synthase and iNOs by attaching to the PPAR-γ receptor on macrophages (32). Previous researched verified that propolis components such as quercetin, chrysin, and CAPE were efficient in reducing LPS-induced inflammation (33). Of the three propolis components, CAPE directly contributed to reducing NO levels (4). Furthermore, combining propolis components has a synergistic impact on the inflammatory response. While CAPE exhibits the highest inhibitory effect on NO generation, quercetin directly suppresses iNOS expression (34). By dampening the production of inflammatory mediators, propolis may prevent excessive inflammation and promote better immune regulation, making it valuable for therapeutic applications in inflammation-related disorders (35). The anti-inflammatory test results of our investigation showed that propolis *T. sapiens* at a concentration of 120 µg/mL exhibited an anti-inflammatory effect, as indicated by a nitric oxide level of 101.09 ± 1.49 µmol/L (14). Iranian propolis suppressed the growth of RAW 264.7 cells with an IC_{50} of 15 ± 3.2 µg/mL and decreased NO generation by 0.15 µg/mL of propolis extract (34). Propolis from the UK with a temperate climate exhibits anti-inflammatory properties and effectively inhibits NO production in macrophage cells with an IC_{50} value of 50 µg/mL (36). The iNOS level of *T. sapiens* propolis extract from South Sulawesi was 5.42 ± 0.82 ng/mL (14).

Macrophages that have been activated are responsible for the initiation of chronic inflammation by increasing the production of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) (37). Multiple investigations have shown that propolis displays pro-inflammatory properties in an immunosuppressed setting, indicating its potential as an immunomodulator and/or immune-restoring agent (29). In response to stimuli like lipopolysaccharide (LPS), activated macrophages release a cytokine called tumor necrosis factor-alpha (TNF-α), which is a pro-inflammatory cytokine. It is an essential component in the inflammatory cascade. High levels of TNF-α contribute to the progression of inflammation and can lead to tissue damage. Propolis, which is rich in phenolic compounds and flavonoids, has been shown to downregulate the expression and

release of TNF-α by inhibiting key inflammatory pathways, such as NF-κB activation. By reducing TNF-α production, propolis helps to control the inflammatory response, potentially preventing chronic inflammation and related diseases. This makes propolis a promising natural agent for managing conditions characterized by excessive inflammation, such as arthritis or other immune-mediated disorders. The previous study of propolis extract has been conducted to evaluate the production of TNF-α in LPS-treated RAW 267.4 cells. The TNF-αvalues of *T. sapiens* propolis from South Sulawesi were measured at 304.28 ± 30.25 pg/mL (14). The Mayan propolis demonstrated a decrease in the production of pro-inflammatory cytokines TNF-α by up to 210 pg/mL (38). Propolis extract has been shown to inhibit the generation of TNF-α generated by LPS. The reduction in TNF-α levels is directly related to the concentration of propolis extract administered.

At a concentration of 50 ppm of propolis *G. thoracica* extract, there is a reduction in TNF-α levels. But other studies have shown that propolis extract at low doses can lowering the TNF-α production, than it high dose (14). This effect may be attributed to propolis' immunomodulatory properties. When RAW 264.7 macrophage cells are exposed to a low dose for a brief duration, it could enhance the average macrophage phagocytic power index. Conversely, when exposed to high doses over an extended period, propolis may act as an immunosuppressant on the average macrophage phagocytic power index. Propolis exhibits IFN-γ-like properties that stimulate and enhance the function of macrophages and T cells. Macrophage activation releases cytokines such as IL-1, IL-6, IL-12, and TNF-α, and stimulates T cells. T cell activation releases IFN-γ, which hinders the formation of antibodies by B cells. Propolis's IFN-γ-like activity activates cell-mediated immune responses when humoral immune response generation is inhibited (39). Propolis has demonstrated potent anti-inflammatory effects in primarily through the inhibition of key inflammatory pathways, involves the inhibition of cyclooxygenase-2 (COX-2), an enzyme that plays a crucial role in the conversion of arachidonic acid to pro-inflammatory prostaglandins. By suppressing COX-2 expression, propolis effectively reduces the production of these inflammatory mediators, thereby alleviating inflammation. By targeting these critical pathways, propolis not only mitigates the inflammatory cascade but also helps restore homeostasis in the immune response. These mechanisms highlight the potential of propolis as a natural anti-inflammatory agent, offering insights into its therapeutic applications in managing inflammatory diseases (29,40).

The results of the determination of LC-MS/MS showed that higher antioxidants and anti-inflammatory agent, that thought contain from several flavonoid compounds. This study presents a novel investigation into the anti-inflammatory properties of propolis sourced from *G. thoracica* bees in Pematang Siantar, North Sumatra, Indonesia, contributing to the limited body of research focused on this unique stingless bee species. Unlike propolis from other bee species, the chemical composition and bioactivity of *G. thoracica* propolis may vary due to the distinct flora available in its geographical region. Our findings demonstrate that this specific propolis extract significantly reduces pro-inflammatory mediators, such as nitric oxide, INOS, and TNF-α, thereby highlighting its potential as a natural anti-inflammatory agent. This research not only expands the understanding of the therapeutic applications of propolis but also emphasizes the importance of regional biodiversity in developing natural remedies, paving the way for further studies on the pharmacological potential of propolis derived from diverse bee species.

CONCLUSION

Propolis extract from *Geniotrigona thoracica*, Pematang Siantar area, North Sumatra, Indonesia, has anti-inflammatory bioactive compounds in the results of the LCMS/MS method, and the results of the anti-inflammatory activity on TNF-α, NO, and iNOS indicate the presence of anti-inflammatory activity.

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