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Original Article

DETERMINATION OF INDONESIAN NATIVE STINGLESS BEE PROPOLIS AS COMPLEMENTARY NUTRACEUTICAL CANDIDATE OF ANTI-TUBERCULOSIS DRUG

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ABSTRACT

Objective: This study aimed to determine Indonesian native stingless bee propolis from ten provinces of Indonesia as complementary nutraceutical candidate of anti-tuberculosis drug (ATD).

Methods: Propolis samples were collected from stingless bee cultivated in ten provinces of Indonesia. The antioxidant capacity test was performed using *2,2-diphenyl-1-picrylhydrazyl* and toxicity test was done using *Brine Shrimp Lethality Test*. The inhibition test of *Mycobacterium tuberculosis* (*Mtb*) was performed using *Lowenstein-Jensen* medium and bacterial colonies were estimated using *Most Probable Number*.

Results: The highest antioxidant capacity was found in *Geniotrigona incisa (G. incisa)* propolis from South Sulawesi Province with an IC_{50} of 100.05 ppm, while the lowest antioxidant capacity was found in *Tetragonula minangkabau* propolis from North Sumatera Province with an IC_{50} of 1378.90 ppm. The lowest propolis toxicity was found in *Geniotrigona thorasica* propolis from South Kalimantan Province with an LC_{50} of >1000.00, while the highest propolis toxicity was found in *Tetragonula laeviceps* (*T. laeviceps*) propolis from Banten Province with an LC_{50} of<50.00. *T. laeviceps* propolis from Banten Province had the lowest *Mtb* inhibition, with the inhibition value of 1.59%. On the other hand, the highest inhibition was shown by *Tetragonula biroi* propolis from South Sulawesi Province and *Tetragonula fuscobalteata* propolis from West Nusa Tenggara Province with 100% inhibition value (equivalent to *rifampicin*).

Conclusion: Based on all determinant parameters, *G. incisa* propolis from South Sulawesi Province has the highest score, and it is defined as complementary nutraceutical candidate of ATD.

Keywords: Indonesian, Nutraceutical, Propolis, Stingless bee, Tuberculosis

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INTRODUCTION

Tuberculosis (TB) is a global infectious disease problem and the second leading cause of death after HIV/AIDS infection. Indonesia ranks 2nd out of 30 countries in the world in terms of TB epidemic, and it is categorized as a high-burden country [1]. One of the problems encountered in the use of the anti-tuberculosis drug (ATD) is the hepatotoxic effect [2, 3]. The hepatotoxic effects of ATD may cause decreased appetite, nausea, dizziness, insomnia, fever and weight loss [4, 5], thereby decreasing nutritional status of the patients, whereas good nutritional status strongly supports the healing process [6].

Hepatotoxicity mechanism of *rifampicin* is mediated by oxidative damage. The antioxidant mechanism in reducing reactive oxygen species (ROS) is strongly suspected as a hepatoprotective mechanism of liver toxicity [7, 8]. Therefore, the provision of antioxidants is expected to reduce the hepatotoxic effects of ATD. Numerous studies have shown that propolis is an antioxidant and able to protect the liver from toxic effects of drugs and ATD [9-13]. Propolis also provides a great protection from the hematologic toxicity of *rifampicin* and *isoniazid* [14].

Stingless bee propolis has strong antibacterial activity [15-16], and it strongly inhibits *Streptococcus sanguinis* [17] and *Streptococcus mutans* [18]. Propolis also has the ability to fight TB infection [19]. Numerous studies have revealed that propolis synergizes with *ampicillin, gentamycin,* and *streptomycin* to kill *Mycobacterium tuberculosis (Mtb)* [20]; synergizes with *streptomycin, rifampicin, isoniazid,* and *ethambutol* [21]; and synergizes with *streptomycin* and *cloxacillin* [22]. Therefore, propolis is an ideal material as an ATD complementary nutraceutical for pulmonary TB healing. Administration of propolis-equipped ATD is expected to reduce the hepatotoxic effects in TB patients and strengthen the ability to fight TB infection.

Indonesia has a large diversity of propolis sources, due to the diversity of bee species and vegetation of resin sources. It is therefore very interesting to examine biological activities (antioxidant activity, toxicity, and inhibition of *Mtb*) of propolis from various provinces in order to discover the propolis that is able to resist ATD toxic effects, having low toxicity and is able to inhibit *Mtb* infection.

This study aimed to determine Indonesian native stingless bee propolis from ten provinces in Indonesia as complementary nutraceutical candidate of ATD.

MATERIALS AND METHODS

Propolis samples and chemicals

Fourteen different stingless bee propolis samples were collected from stingless bee farm in ten provinces in Indonesia, namely *Tetragonula minangkabau* (*T. minangkabau*) and *Tetragonula moorei* (*T. moorei*) from North Sumatera Province, *Tetragonula laeviceps* (*T. laeviceps)* from Banten Province, *T. laeviceps* from West Java Province, *T. laeviceps* from Central Java Province, *Heterotrigona itama* (*H. itama*) from West Kalimantan Province, *H. itama* from East Kalimantan Province, *H. itama*, *Geniotrigona thorasica* (*G. thorasica*) and *T. laeviceps* from South Kalimantan Province, *Geniotrigona incisa* (*G. incisa*) and *Tetragonula biroi* (*T. biroi*) from South Sulawesi Province, *Tetragonula fuscobalteata* (*T. fuscobalteata*) from West Nusa Tenggara Province, and *T. fuscobalteata* from North Maluku Province. The chemicals used were 70% hydroethanolic extract, *2,2-diphenyl-1-picrylhydrazyl* (DPPH), methanol, dimethyl sulfoxide (DMSO), Trolox, and tween 80% that were obtained from Sigma/Aldrich Co. through distributor company in Jakarta, Indonesia. *Rifampicin* was obtained from a drugstore in Bogor, Indonesia.

Extraction of propolis

All of the propolis samples were chopped into small pieces and macerated with 70% hydroethanolic extract (ratio of sample and ethanol = 1:3) by using water bath shaker at room temperature for 2 d. The filtrate was collected and the residue was macerated two times under the same condition. After filtration, the filtrate was evaporated by a rotary evaporator (3 rpm at 60 °C) and then the pure propolis extract was collected.

Free radical scavenging activity test-2,2-diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging activity was measured using DPPH [23]. Briefly, various propolis extract solutions (2 mg/ml) were added to 2 ml of DPPH, dissolved in methanolic solution (0.1192 mmol/l), and maintained in the dark for 30 min at room temperature. The absorbance was then measured at 517 nm. Methanol was used instead of propolis extract solutions as a control and Trolox was used as a positive control. The results were expressed with IC_{50} value (50% inhibitory concentration), which determined the extract concentration (µg/ml) that provided 50% inhibition. The lower of the value is the greater of the capacity of the antioxidant. The scavenging capacity of the DPPH radical was calculated with the equation below (percent inhibition of the DPPH radical).

$$\% \text{ Inhibition of DPPH} = \frac{(\text{Abs DPPH} - \text{Abs sample1})}{\text{Abs DPPH}} \text{x } 100$$

The extract concentration value was plotted against the percent inhibition of DPPH· and the IC_{50} value was obtained by linear regression. All treatments were run in triplicate.

Toxicity test-brine shrimp lethality test (BSLT)

We used preliminary toxicity of BSLT by using *Artemia salina* Leach [24]. Artemia cysts were prepared by Biopharmaceutical Research Center (BRC), Bogor Agricultural University, Indonesia. The cysts were hatched in the laboratory using artificial seawater at 27–30 °C medium. Appropriate light, pH of 7.5-8.5, alkaline water with a salinity of 3% and temperature of 27-30 °C were regulated during the test. The larvae were collected from hatcheries with a plastic pipette for LC_{50} study.

LC_{50} was estimated in five dilutions (250, 500, 750, 1000 and 1500 µg/ml) of propolis extracts for 24 h. In each plate, 0.5 ml of propolis extract with different concentrations was added to 4.5 ml of the brine shrimp solution. Ten brine shrimp larvae that had been grown for 48 h were added to each plate. For each propolis concentration, one DMSO with 4.5 ml of brine shrimp solution without propolis extract was used as a control. The plates were sealed with their lids in the darkness at room temperature for 24 h. During the test, feeding and aeration were not allowed. After 24 h, the number of dead and surviving larvae was counted on the plates, and cytotoxicity of the samples was then determined. Each experiment was performed in triplicate. During each experiment, if mortality of the control group was more than 10% of the experiment group, the procedure must be repeated. The mortality was calculated using the following formula:

$$\text{Mortality} = \frac{\text{Accumulation of death larvae}}{\text{Accumulation of death + surviving larvae}} \text{x}100\%$$

The graph was constructed with log concentration as the x-axis and mortality as the y-axis. A substance was confirmed as toxic when LC_{50} of the extract was less than 1000 ppm and LC_{50} of the pure compound was less than 30 ppm.

***Mycobacterium tuberculosis* inhibition test-proportion method**

Mycobacterium tuberculosis inhibition test was based on 100% inhibitory concentration by ATD (i.e. *rifampicin*). At 0.02% *rifampicin* concentration, all tests consistently showed no *Mtb growth* (100% inhibition). Hence, the concentration was used as the standard sample concentration. Therefore, sample concentration also used the same concentration.

Suspension preparation

Sterile screw-cap tubes were filled with 10 glass beads and 1 drop of 0.1% tween 80%. The tubes were then weighed and recorded, for example, M1. *Mtb H37Rv* colony aged 3-6 w was taken with inoculating loop, inserted into the tube, then weighed again, for instance, M2. Weight of *Mtb H37Rv* = M2-M1 = M3. The tube contained *Mtb H37Rv* was vortexed into homogeneous solution and set aside for 10 min to let aerosol down. Sterile distilled water in M3 mg (1 ml ~ 1 mg) was added and homogenized using vortex for 2 min. Finally, it was set aside until the precipitate of coarse particles was formed and the supernatant seemed clear.

Inoculation

A 100 µl supernatant was taken by automatic pipette and yellow tip and inserted into a threaded tube containing *Lowenstein Jensen* (LJ) medium. There were three kinds of LJ medium, namely a) LJ medium without *rifampicin* and propolis (negative control), b) LJ medium with *rifampicin* (positive control), and c) LJ medium with propolis sample (consisting of 14 propolis samples). Two replications of each inoculation were made. The supernatant (inoculation fluid) was flattened to cover the entire surface of the medium.

Colony counting

The colonies growing on the media surface in screw-cap tubes were counted manually with a magnifying glass. The number of colonies was recorded as the *Most Probable Number* (MPN).

Interpretation

The MPN on negative control was considered as 100% growth. Furthermore, inhibition of *Mtb* in propolis sample could be calculated by the following calculation:

$$\text{Mtb inhibition activity of the sample} = 100 - \% \text{ growth of Mtb}$$

Determination of the best candidate

The best candidate was determined based on the total score of each propolis sample obtained from three parameters; i.e. antioxidant capacity, toxicity, and *Mtb* inhibition activity. Antioxidant scores were obtained by sequencing IC_{50} values of propolis samples. The smaller the value of IC_{50}, the more powerful the biological activity and the greater the score. The lowest score was 1 and the highest score was 14 (according to the number of propolis samples). If there were more than one sample having the same IC_{50} score, the same score was given so that the highest score was less than 14. Inhibition score of *Mtb* was obtained by sequencing the inhibition value (%). The greater the inhibition percentage (%), the greater the score. If more than one sample had the same inhibition percentage (%), the same score was given so that the highest score was less than 14. The toxicity score was obtained from LC_{50} value. The smaller the LC_{50} value, the greater the toxicity. The desired propolis was the one with low toxicity. The greater the toxicity, the smaller the score. The method to sort the scores was similar to the determination of other parameter scores.

Referring to hepatotoxicity problem in the use of ATD in TB treatment, the desired nutraceutical propolis was the one which has a strong hepatoprotective activity to reduce the hepatotoxic effects of ATD. *Rifampicin* hepatotoxicity mechanism is mediated by oxidative damage [7], and antioxidant mechanism in reducing ROS is strongly suspected as a hepatoprotective mechanism of liver toxicity [8,25]. Therefore, the antioxidant capacity of the sample became the main parameter and had the greatest weight. Additionally, the determination of propolis as the complementary of ATD also considered patient safety (low toxicity) and *Mtb* inhibition ability that could strengthen ATD to fight infection. The weight distribution of determinant parameters was presented in the following table:

Table 1: Determinant parameters and their weighting

Parameter	Weighting (%)
Antioxidant capacity	50
Mtb inhibition activity	25
Toxicity	25
Total	100

Propolis sample that obtained the highest total score was defined as a complementary nutraceutical candidate of ATD.

RESULTS AND DISCUSSION

Antioxidant capacity

Antioxidant capacity was indicated by IC_{50} value. The greater the IC_{50} value, the weaker the antioxidant activity. Conversely, the lower the IC_{50} value, the more powerful the antioxidant activity. This study showed that *T. minangkabau* propolis from North Sumatera Province had the weakest antioxidant capacity (IC_{50} of 1378.95 ppm), whereas *G. incisa* propolis from South Sulawesi Province had the strongest antioxidant capacity (IC_{50} of 100.05 ppm).

The difference in antioxidant capacity of propolis might be due to the difference in the composition of the active compounds in it. The more the active compounds that have antioxidant properties and the stronger the activity, the stronger the antioxidant capacity of a propolis. This assumption is strengthened by the results of the previous study which has suggested that propolis with different phytochemical composition produces different antioxidant capacity [23].

The strength of propolis's antioxidant capacity is influenced by two factors. The first one is the bee species differences; i.e. different bee species have different tastes on plant resins. In this study, *G. incisa* propolis and *T. biroi* propolis came from the same farm with the availability of the same resin source plant. However, both propolis had different antioxidant capacities. These findings strengthen the opinion that each bee species has a unique and different behaviour from other bee species [26]. The second one is the difference in resin source plant. Each plant resin has a unique and distinctive active compound composition between plant species. The difference is very influential on the composition of the active compounds contained in the plant and on its antioxidant capacity. *Silymarin* is a phytochemical that has an antioxidant activity and is an excellent hepatoprotector [27, 28], *propolin G* has a strong anticancer activity [29], *glycosides* have strong anti *Mtb* [30], and *quercetin* has strong antioxidant and antitumor activities [31].

The strength of the antioxidant capacity of *G. incisa* bee propolis is associated with the number of medicinal plants that become its resin sources. The plants are *kaju landong* (*Paraserianthes falcataria*), *palili* (*Lithocarpus celebica Rehder*), *annaja* (*Saurauia costata*), *poringan* (*Baccaurea sp*), *uru* (*Arenga pinnata*), and *sempur* (*Dillenia indica*). These plants are known as medicinal plants by local people. *G. incisa* bees also take resin from food plants, namely mango (*Mangifera indica*) and durian (*Durio ziberthinus*).

Related to antioxidant function, hepatotoxicity mechanism of *rifampicin* is mediated by oxidative damage [7, 32]. Isoniazid also causes liver damage and it can be reduced by strong antioxidant compounds [25]. The *silymarin* (a plant phytochemical compound with strong antioxidant activity) has been proven to protect the liver from toxic effects of *isoniazid, rifampicin,* and *pyrazinamide* and has low toxicity [27].

Table 2: Antioxidant capacity of stingless bee propolis samples from different provinces

Province origin	Bee Species	IC_{50} Antioxidant capacity (ppm)[a]	Score order	Weighting score (50%)
North Sumatera	*T. minangkabau*	1378.95±16.67	1	0.5
North Sumatera	*S. moorei*	208.92±9.57	12	6.0
Banten	*T. laeviceps*	150.83±0.21	13	6.5
West Java	*T. laeviceps*	574.85±4.49	6	3.0
Central Java	*T. laeviceps*	283.05±20.44	9	4.5
West Kalimantan	*H. itama*	227.54±9.32	10	5.0
East Kalimantan	*H. itama*	939.98±6.51	2	1.0
South Kalimantan	*H. itama*	636.61±11.28	4	2.0
South Kalimantan	*T. laeviceps*	580.40±8.61	5	2.5
South Kalimantan	*G. thorasica*	905.06±7.96	3	1.5
South Sulawesi	*G. incisa*	100.05±13.02	14	7.0
South Sulawesi	*T. biroi*	467.93±20.58	8	4.0
West Nusa Tenggara	*T. fuscobalteata*	477.88±7.62	7	3.5
North Maluku	*T. fuscobalteata*	218.65±3.00	11	5.5

[a]mean±SD, $n = 3$.

Numerous studies have indicated that antioxidant mechanisms in suppressing radical oxygen species (ROS) are strongly suspected to be a hepatoprotective mechanism of liver toxicity [8, 33, 34]. Propolis extract also has a hepatoprotective effect against oxidative stress [35, 36]. Furthermore, murine β TC-6 cell lines incubated with toxicants have increased thiobarbituric acid reactive substances (TBARs), decreased glutathione (GSH) concentrations and cell viability, and increased cell apoptosis. On the other hand, the lowered TBARs increased GSH concentrations, increased cell viability and reduced cell apoptosis were found in cells incubated with propolis extract.

Various research results have shown that propolis is able to protect the liver from mercury exposure [37] and aluminium toxicity exposure [38] through an antioxidant mechanism. It also protects the liver from the toxic effects of *atorvastatin* [38], ethylene glycol [39], and cypermethrin [40]. Propolis extract (150 mg/kg) has been proven to have the good hepatoprotective ability in isoniazid-induced hepatotoxicity in male albino mice [41].

However, the previous study confirmed that the results of plasma antioxidant measurements as a parameter of *in vivo* oxidative damage in mini pig were not aligned with the results found in rats [42]. Therefore, it was highly probable that *in vitro*, *in vivo* and clinical measurements of antioxidants were not always aligned. However, various results of the above studies can be the basis for us to establish that antioxidant capacity is an important criterion and is associated with its potential as a hepatoprotector.

Based on antioxidant capacity, *G. Incisa* propolis from South Sulawesi Province has the strongest potential to protect the liver from toxic effects of ATD.

Toxicity

One of ATD and anti-TB regimen requirements was safe for patients [43]. Similarly, propolis should be safe as a complementary of ATD. The safety was indicated by its toxicity. Toxicity potential was indicated by LC_{50} value. The higher the LC_{50} value, the safer the propolis. Conversely, the lower the LC_{50} value, the stronger the propolis toxicity.

In this examination, the test sample was liquid propolis with propylene glycol as a liquid filler. Therefore, propylene glycol was analyzed as a control. According to table 3, propylene glycol as the liquid filler had toxicity with an LC_{50} value of 652.49 ppm. Thus, the toxicity exhibited by propolis sample was partly derived from propylene glycol contribution. According to the propolis toxicity, there was an interesting fact that the toxicity was widespread (<50.00 to>1000.00 ppm). It means that some propolis have stronger toxicity than the control (652.49 ppm), while some of them have lower toxicity.

These data suggested that there was an interaction possibility between propolis component and propylene glycol, thereby affecting its toxicity level. This phenomenon was very interesting to be learned further, in order to identify which propolis components that were synergetic, neutral or antagonist with propylene glycol.

Table 3: Toxicity of stingless bee propolis samples from different provinces

Province origin	Bee species	Toxicity LC_{50} (ppm) [a]	Score order	Weighting score (25%)
North Sumatera	*T. minangkabau*	621.49±45.40	7	1.75
North Sumatera	*S. moorei*	55.09±12.20	2	0.50
Banten	*T. laeviceps*	<50.00±13.55	1	0.25
West Java	*T. laeviceps*	521.74±18.22	5	1.25
Central Java	*T. laeviceps*	615.84±21.33	6	1.50
West Kalimantan	*H. itama*	802.26±32.55	10	2.50
East Kalimantan	*H. itama*	451.32±25.35	4	1.00
South Kalimantan	*H. itama*	270.60±19.27	3	0.75
South Kalimantan	*T. laeviceps*	838.05±16.22	11	2.75
South Kalimantan	*G. thorasica*	>1000.00±21.76	14	3.50
South Sulawesi	*G. incisa*	854.75±23.82	12	3.00
South Sulawesi	*L. terminata*	656.41±21.10	9	2.25
West Nusa Tenggara	*T. fuscobalteata*	624.34±12.98	8	2.00
North Maluku	*T. fuscobalteata*	932.63±10.88	13	3.25

[a]mean±SD, *n* = 3.

According to table 3, the safest propolis is propolis *G. thorasica* from South Kalimantan Province, with an LC_{50} of more than 1000.00 (score order is 14, weighting score is 3.5). In terms of safety, it is the best propolis. Conversely, the most toxic propolis is *T. laeviceps* propolis from Banten Province, with an LC_{50} of less than 50.00 (score order is 1, weighting score is 0.25).

As is the case with antioxidant capacity, the difference in the toxicity of propolis is influenced by bee species and plant species of resin sources. A study result suggested that the difference in the toxicity of propolis might be due to the differences in the composition of the active compounds it contained and each active compound had different toxicity levels [44]. The results of this toxicity test were also in line with another study which suggested that the biological activity and phytochemical composition were influenced by the geographical location and the plant origin of the resin [31].

G. thorasica bee propolis (the lowest toxicity) is derived from the resin of jackfruit (*Artocarpus heterophyllus*), mangosteen (*Garcinia mangostana*), durian (*Durio zibethinus*), mango (*Mangifera indica*), and lead tree (*Leucaena leucocephala*). These data regarding resin source plant provide clues that the low toxicity of *G. thorasica* propolis is influenced by plant food resins that are usually non-toxic.

The highest toxicity occurs in the liver, followed by brain and kidneys [45]. Therefore, propolis toxicity becomes one of the considerations in the determination of ATD complementary nutraceutical candidate. Numerous studies have shown that propolis has low liver toxicity. Behavior changes and clinical toxicity were not found in experimental mice receiving an ethanolic extract of propolis up to a dose of 2000 mg/kg body weight (BW) during a 45-d study. Nevertheless, there were hematological and biochemical changes at that dose compared to the control [46]. Another study on Swiss mice also showed no signs of toxicity in the propolis hydroalcoholic extract administration with doses of 1000, 2000 and 4000 mg/kg BW. No death was found in all the treatment groups, and blood biochemical analysis showed decreased levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) indicating that propolis was hepatoprotective [44]. Toxic effects were undetectable in mice receiving 25 µl propolis/administration four times a day [47]. Furthermore, adult female Sprague-Dawley mice receiving 200 mg propolis/d orally were able to resist the toxic effects of oral administration of 200 mg methoxychlor/kg BW twice a week [48]. A review stated that LD_{50} of propolis in various mouse species ranged from 300 to 2050 mg/kg [49].

However, we recognize that BSLT method cannot be a strong basis for the determination of toxicity, but these data are useful for early predictions of propolis toxicity. Further toxicity analysis should be performed on the determined candidates to establish the propolis that is eligible to be declared a complementary nutraceutical of ATD.

Mycobacterium tuberculosis strain *H37Rv* inhibitory activity

Various studies have revealed that propolis has the ability against *Mtb* germ and synergizes with ATD [19, 22]. Therefore, propolis ability in inhibiting *Mtb H37Rv* becomes one of the considerations in our study.

In the present study, *rifampicin* with a concentration of 0.02% (positive control) consistently showed no colony growth of *Mtb H37Rv* or 100% inhibition. In contrast, 100% colony growth or 0% inhibition were found in negative control (without *rifampicin* or propolis).

Table 4: *Mycobacterium tuberculosis* strain H37Rv inhibition activity of stingless bee propolis samples from different provinces

Province origin	Bee species	MPN [a]	Growth of *mtb* (%)	*Mtb* inhibition activity (%)	Score order	Weighting score (25%)
Positive control (*rifampicin*)	-	0.0±0.0	0.00	100.00	-	-
Negative control	-	157.5±7.5	100.00	0.00	-	-
North Sumatera	*T. minangkabau*)*	-	-	-	-	-
North Sumatera	*S. moorei*	*106.5±1.5*	67.62	32.38	6	1.50
Banten	*T. laeviceps*	*155.0±0.0*	98.41	1.59	1	0.25
West Java	*T. laeviceps*	*150.0±0.0*	95.24	4.76	3	0.75
Central Java	*T. laeviceps*	*137.5±2.5*	87.30	12.70	4	1.00
West Kalimantan	*H. itama*	*152.5±2.5*	96.83	3.17	2	0.50
East Kalimantan	*H. itama*	*120.0±0.0*	76.19	23.81	5	1.25
South Kalimantan	*H. itama*	*150.0±0.0*	95.24	4.76	3	0.75
South Kalimantan	*T. laeviceps*)*	-	-	-	-	-
South Kalimantan	*G. thorasica*	*35.0±5.0*	22.22	77.78	8	2.00
South Sulawesi	*G. incisa*	*79.0±11.0*	50.16	49.84	7	1.75
South Sulawesi	*T. biroi*	*0.0±0.0*	0.00	100.00	9	2.25
West Nusa Tenggara	*T. fuscobalteata*	*0.0±0.0*	0.00	100.00	9	2.25
North Maluku	*T. fuscobalteata*	*152.5±2.5*	96.83	3.17	2	0.50

[a]mean±SD, *n* = 3, **)* the data were eliminated due to the failed test.

It was interesting that different propolis had different *Mtb H37Rv* inhibitory capability. At the same level as *rifampicin*, *T. laeviceps* propolis from Banten Province showed a very low inhibition of 1.59% (score order was 1, weighting score was 0.25). Meanwhile, *T. biroi* propolis from South Sulawesi Province and *T. fuscobalteata* propolis from West Nusa Tenggara Province showed maximum inhibition (100%) with score order of 9 and weighting score of 2.25. Their abilities were equivalent to *rifampicin*.

The investigation results regarding plant origin of the resin showed that *T. fuscobalteata* propolis from West Nusa Tenggara Province collected resin from Ceara rubber tree (*Manihot glaziovii*), mangosteen *(Garcinia mangostana)*, mango *(Mangifera indica),* jackfruit *(Artocarpus heterophyllus)*, *cempedak* (*Artocarpus integer*), durian (*Durio ziberthinus*), pomelo (*Citrus maxima*), banana *(Musa spp.)*, and castor oil plant *(Ricinus communis).* The resin source plants were divided into two groups; i.e. medicinal plants and food plants. The medicinal plants were Ceara rubber tree (*Manihot glaziovii*), mangosteen (*Garcinia mangostana*), pomelo (*Citrus maxima*), and castor oil plant (*Ricinus communis*). Meanwhile, the food plants were mangosteen (*Garcinia mangostana*), mango (*Mangifera indica*), jackfruit (*Artocarpus heterophyllus*), *cempedak (Artocarpus integer)*, durian (*Durio ziberthinus*), pomelo (*Citrus maxima*), and sweet orange (*Citrus sinensis*). The resin source plants of *T. fuscobalteata* from West Nusa Tenggara Province had similarities with the resin source plants of *G. incisa* propolis and *T. biroi* propolis from South Sulawesi that were dominated by medicinal plants, which had an effect on the strong *Mtb* inhibition activity. In this study, it appeared that the more the resins from medicinal plants, the stronger the biological activity and toxicity of the propolis. Conversely, the more the resins from food plants, the weaker the biological activity and toxicity.

This study strengthens the results of various previous studies which have shown that the antimycobacterial activity of propolis varies. The water extract of propolis has a low *Mtb H37Rv* inhibitory ability [50]. In contrast, numerous studies have indicated that ethanol extract of propolis has good *Mtb* inhibitory ability [19-22, 31, 51, 52].

The difference in the inhibitory of *Mtb H37Rv* strengthens the opinion that biological activity of propolis is diverse and influenced by the content of its active compounds [31]. In our study, the differences were allegedly influenced by different species of bees and the origin of plant resins.

Our study showed that *T. biroi* propolis from South Sulawesi and *T. fuscobalteata* propolis from West Nusa Tenggara Province were the best propolis in inhibiting *Mtb H37Rv*. However, this test had limitations. There were two *Mtb H37Rv* inhibitory data not available due to test failure and lack of test samples; i.e. *T. minangkabau* propolis from North Sumatera Province and *T. laeviceps* propolis from South Kalimantan Province. Both of them might have a stronger ability to inhibit *Mtb H37Rv* than other propolis.

Determination of the best candidate

The best candidate was determined by three determinant parameters; i.e. antioxidant capacity, toxicity, and inhibition of *Mtb*. Antioxidant capacity was the main criterion because the primary mechanism to protect the liver from the toxic effects of ATD was to reduce the oxidant radical of ATD metabolites.

Moreover, additional criteria were safety (low toxicity) and having good ability to inhibit *Mtb H37Rv*. Therefore, antioxidant capacity, toxicity, and *Mtb H37Rv* inhibition were given the weight of 50%, 25%, and 25%, respectively. The selected propolis candidate was the one with the highest total score. With these three criteria, the ATD-complementary propolis was expected to have a major ability as hepatoprotector, as a solution of one of the main problems in the use of ATD. In addition, ATD-complementary propolis was also safe (not toxic) and having good ability to fight *Mtb* infection.

The highest score was achieved by *G. incisa* propolis originating from South Sulawesi Province, with a score of 11.75. The score was mainly obtained from antioxidant activity (7.0), toxicity (3.0) and *Mtb H37Rv* inhibition (1.75). An antioxidant capacity score of this propolis was the highest compared to other propolis samples. Meanwhile, in terms of toxicity, this propolis was not the safest. There were two safer propolis samples; i.e. *G. thorasica* propolis from South Kalimantan Province with a score of 3.50 and *T. fuscobalteata* propolis from North Maluku Province with a score of 3.25. Likewise in the *Mtb H37Rv* inhibition, this propolis was not the strongest. There were three samples of propolis which had stronger *Mtb* inhibition; i.e. *T. biroi* propolis from South Sulawesi Province and *T. fuscobalteata* propolis from West Nusa Tenggara Province that had the same score (2.25), as well as *G. thorasica* propolis from South Kalimantan Province with a score of 2.00.

Tabel 5: Determination of the best candidate

Province origin	Bee species	Antioxidant activity	Toxicity	*Mtb* inhibition activity	Total score
		W. score (50%)	W. score (25%)	W. score (25%)	
North Sumatera	*T. minangkabau*	0.5	1.75	-	2.25
North Sumatera	*S. moorei*	6.0	0.50	1.50	8.00
Banten	*T. laeviceps*	6.5	0.25	0.25	7.00
West Java	*T. laeviceps*	3.0	1.25	0.75	5.00
Central Java	*T. laeviceps*	4.5	1.50	1.00	7.00
West Kalimantan	*H. itama*	5.0	2.50	0.50	8.00
East Kalimantan	*H. itama*	1.0	1.00	1.25	3.25
South Kalimantan	*H. itama*	2.0	0.75	0.75	3.50
South Kalimantan	*T. laeviceps*	2.5	2.75	-	5.25
South Kalimantan	*G. thorasica*	1.5	3.50	2.00	7.00
South Sulawesi	*G. incisa*	7.0	3.00	1.75	11.75
South Sulawesi	*T. biroi*	4.0	2.25	2.25	8.50
West Nusa Tenggara	*T. fuscobalteata*	3.5	2.00	2.25	7.75
North Maluku	*T. fuscobalteata*	5.5	3.25	0.50	9.25

Based on these three test parameters, *G. incisa* propolis from South Sulawesi Province was chosen as the best candidate of complementary nutraceutical of ATD. It had the following characteristics: 1) had the strongest potential to reduce the hepatotoxic effects of ATD, 2) had low toxicity potential, and 3) had strong potential to inhibit *Mtb* infection.

CONCLUSION

Based on the antioxidant capacity test, the highest score was owned by *G. incisa* propolis from South Sulawesi Province. The toxicity test indicated that *G. thorasica* propolis from South Kalimantan Province had the lowest toxicity. Furthermore, *Mtb H37Rv* inhibition test showed that the highest score was achieved by *T. biroi* propolis from South Sulawesi Province and *T. fuscobalteata* propolis from West Nusa Tenggara Province. Based on all test parameters, the highest score was achieved by *G. incisa* propolis from South Sulawesi Province. Thus, it was defined as ATD complementary nutraceutical candidate. Further studies on animal models and clinical studies are needed to establish this propolis as the complementary nutraceutical of ATD.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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