



## Stingless bees and *Mangifera indica*: A close relationship?

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Propolis is a bee product with various biological activities and phytochemical compositions. This study was aimed to identify the chemical composition and botanical origin of Indonesian stingless bees propolis produced by *Tetragonula laeviceps* in Banten and *Heterotrigona itama* in South Kalimantan, respectively. Propolis extracts were prepared using 70% ethanol and chloroform. The extracts were analysed by GC/MS after silylation. The results showed that the studied stingless bee propolis contained a relatively high amount of sugars, probably due to the harvesting method used. Furthermore, triterpenes were found as the main constituents followed by phenolic lipids. Both samples were *Mangifera indica* type propolis with typical compounds, including cycloartenol, isomangiferolic acid, mangiferolic acid, ambolic acid, alk(en)ylphenols, alk(en)ylresorcinols, and anacardic acids. Therefore, *M. indica* might be a preferred botanical source of propolis from a tropical zone. In addition, the suctioning method in harvesting the stingless bee honey could be recommended to avoid high content of sugar and sugar derivatives.

**Keywords:** Botanical origin, Chemical composition, *Mangifera indica*, Stingless bee propolis.

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### Introduction

Propolis has been used for centuries as a medicinal agent<sup>1</sup>. It is the generic name of honey bee product consisting of resinous material collected from various plants and used by bees to construct their hives and protect the colony. In addition, there is another term to designate propolis produced by stingless bees called geopropolis due to the presence of soil<sup>2,3</sup>. However, sometimes “geopropolis” is used to designate propolis of Meliponini in general, which is not correct, because only some species add soil and clay to the plant resins in their nests<sup>4</sup>.

Propolis has many health benefits because of its biological activities, such as antiviral, antibacterial, antifungal, antitumor, immunomodulatory and antiinflammatory properties<sup>5,6</sup>. Its chemical composition varies greatly between propolis originating from different geographical and climatic regions<sup>2,7</sup>. The location, source of resin and bee species determine the chemical composition of propolis and lead to various biological activities<sup>8</sup>. This fact is a major challenge in propolis research and

application. However, it may be overcome by standardization of propolis based on chemical composition and/or botanical origin<sup>9</sup>.

The botanical origin of propolis has been studied for a long time. It turned out that in some cases honeybees and stingless bees use one and the same resin source, but there are also differences. Indonesia is a tropical country with very abundant biodiversity. Unfortunately, the information about the chemical composition and botanical origin of Indonesian propolis is still limited. Moreover, the published studies are mainly on propolis manufactured by honey bees *Apis mellifera*. Trusheva *et al.*, studied *A. mellifera* propolis from Batu City, East Java and found that alk(en)ylresorcinols, prenylflavanones, and cycloartane-type triterpenes as major components<sup>10</sup>. Wiryowidagdo *et al.*, investigated the chemical composition and cytotoxic activity of *A. mellifera* propolis from West Java, Central Java and East Java<sup>11</sup>. A similar study was also conducted by Halim *et al.*, comparing the bioactive components of *A. mellifera* propolis from Indonesia and Brazil<sup>12</sup>. The only study on stingless bees collected in Indonesia has been recently published investigating propolis of *Tetragonula aff. biroi* and found 4-phenylcoumarins, prenylated flavonoids, and abscisic acid stereoisomers<sup>13</sup>.

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Majority of the previous studies have focused on the chemical composition of stingless bee propolis from Brazil<sup>3,14-16</sup>. In this article, results on the chemical composition of propolis of two stingless bee species *Tetragonula laeviceps* and *Heterotrigona itama* collected in two provinces of Indonesia has been reported.

**Materials and Method**

**Sample preparation**

Propolis samples were collected from beehives of two stingless bee species in two provinces of Indonesia: *Tetragonula laeviceps* (Banten province) and *Heterotrigona itama* (South Kalimantan province). Each propolis sample was grounded and extracted using 70% ethanol and chloroform at room temperature (2 x 24 h). The resulted extracts were then filtrated and evaporated to dryness. A total of four extracts were obtained, including 70% ethanol extracts of propolis from Banten (BE) and South Kalimantan (KE), and chloroform extracts of propolis from Banten (BC) and South Kalimantan (KC), respectively. The chloroform extracts were additionally subjected to the procedures for removal of the wax, according to Woisky & Salatino<sup>17</sup>.

**GC/MS analysis**

All four extracts were subjected to GC/MS analysis. About 5 mg of dry propolis extracts were derivatized by mixing with 50 µL of dry pyridine and 75 µL of N,O-bis(trimethylsilyl)-trifluoroacetamide (BTSFA). The mixtures were then heated at 80 °C for 20 min. The GC/MS analysis was performed with an

Agilent gas chromatograph 7890B linked to a MS 5977A mass spectrometer system equipped with a 30 m long, 0.25 mm i.d., and 0.25 µm film thickness HP-5MS capillary column. The temperature was programmed from 75 to 300 °C at a rate of 5 °C/min, and a 20 min hold at 300 °C. Helium was used as a carrier gas at a flow rate of 1 mL/min. The split ratio was 50:1. The injector temperature was set at 280 °C and the interface temperature at 300 °C. The ionization voltage was 70 eV. The identification of the compounds was done using the NIST98 data library and literature data comparison.

**Results**

Remarkable quantitative differences were observed between GC/MS profiles of the extracts obtained by 70% ethanol and chloroform. Samples BE and KE contained sugars with relative abundance over 80 and 20% of TIC, respectively (Fig. 1). In our study on the chloroform extracts (BC and KC), 41 compounds were identified in the samples by GC/MS analysis (Table 1). The results showed that both the samples had similar chemical composition with triterpenes, mainly of cycloartane-type, as major constituents followed by phenolic lipids (alk(en)yl phenols, alk(en)ylresorcinols and anacardic acids) (Fig. 2). These are typical compounds of *M. indica* type propolis.

**Discussion**

A relatively high amount of sugars contained in propolis could be due to the harvesting methods used, as the sugars probably come from honey<sup>8</sup>. The way to

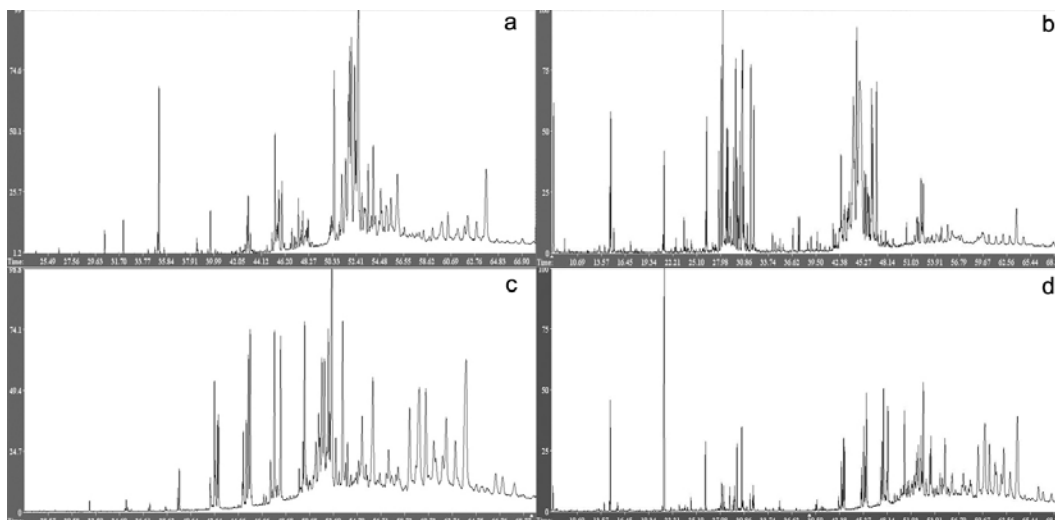


Fig. 1 — GC/MS spectra of tested samples. Ethanol extracts had a higher amount of sugars than chloroform extracts.

Table 1 — Chemical composition of Indonesian stingless bee propolis (silylated chloroform extracts)

RT	Compounds	% TIC	
		BC	KC
32.07	Palmitic acid	0.3	0.1
35.19	Oleic acid	2.2	0.1
39.55	Pentadecenyl phenol	0.1	0.2
39.68	Pentadecyl phenol	0.1	0.4
42.29	Heptadecadienyl phenol	0.1	tr.
42.64	Heptadecenyl phenol	0.1	1.3
42.73	Heptadecyl phenol	0.1	0.3
42.89	Pentadecenylresorcinol	0.4	0.8
42.99	Pentadecylresorcinol	0.6	0.9
45.01	Anacardic acid nonadecatrienyl	0	0.2
45.08	Anacardic acid nonadecadienyl	0.2	0.8
45.33	Heptadecadienyl resorcinol	1.8	1.1
45.49	Heptadecenyl resorcinol	0.5	tr.
45.51	Nonadecenyl phenol	tr.	2.0
45.56	Heptadecenyl resorcinol (isomer)	0.3	
45.65	Heptadecenyl resorcinol (isomer)	1.3	2.6
45.94	Heptadecyl resorcinol	1.0	
47.38	Anacardic acid eicosatrienyl	0.8	0.7
47.61	Anacardic acid eicosatrienyl (isomer)	0.4	
47.75	Anacardic acid eicosadienyl	0.7	2.6
48.03	Nonadecenyl resorcinol	0.8	
48.15	Nonadecenyl resorcinol (isomer)	0.3	
48.23	Nonadecenyl resorcinol (isomer)	0.5	2.5
50.19	Anacardic acid eicosatrienyl	1.1	3.0
51.16	Tirucalol	2.3	
51.50	Lanosterol (3-alpha)	3.3	1.5
51.74	$\beta$ -Amyrine	3.3	3.1
51.86	Lanosterol (3-beta)	4.2	
52.01	Triterpenic ketone	5.8	2.7
52.29	$\alpha$ -amyrine	3.3	3.0
52.40	Lupeol	1.8	1.2
52.62	Cycloartenol	9.3	5.8
53.12	Lanosterol acetate	1.0	tr.
53.21	$\beta$ -Amyrine acetate	1.5	tr.
53.47	24-methylene cycloartenol	1.8	3.8
53.91	$\alpha$ -Amyrine acetate	2.3	1.2
56.03	Triterpene diol	3.8	3.6
59.11	Ambolic/isoambolic acid	0.9	2.4
60.44	Mangiferolic/isomangiferolic acid	1.2	2.9
62.16	Ambolic/isoambolic acid	1.6	2.4
63.79	Mangiferolic/isomangiferolic acid	3.3	6.3

RT : Retention time (minute); TIC : Total ion current, The ion current generated depends on the characteristics of the compound and is not a true quantitation; BC : Chloroform extract of propolis from Banten; KC : Chloroform extract of propolis from South Kalimantan; tr : trace amounts

harvest stingless bee honey determines the sugar content of propolis. Honey harvesting in South Kalimantan was done by suctioning with a special hose, thus honey can be removed optimally in this way. On the other hand, harvesting in Banten was done manually by squeezing the honey pots. The latter caused more honey residue in propolis than the

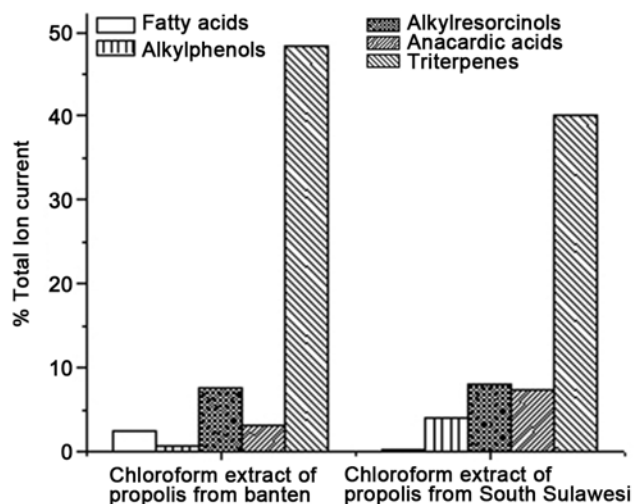


Fig. 2 — Major compound groups contained in the chloroform extract of Indonesian propolis. Triterpenes were the main constituent of stingless bee propolis from Indonesia followed by phenolic lipids.

former procedure, as it was obvious in the chemical profile of BE. The presence of primary metabolites, such as sugar, amino acids, and nucleic acids in high amount could interfere in the identification of secondary metabolites<sup>18</sup>. Thus, further identification was conducted on chloroform extracts.

Till now, propolis of *H. itama* has been studied predominantly for its pharmacological properties and was found to possess antimicrobial<sup>19-22</sup>, antioxidant<sup>19,20,23,24</sup>, cytotoxic<sup>23</sup>, and anti-inflammatory<sup>24</sup> activities. Chemical studies are limited; a few authors have determined total phenolics<sup>22,24,25</sup>. The only detailed study of the chemical composition was performed by UHPLC-Q-TPF/MS and NMR, where phenolic acids, flavones, triterpenes, and phytosterols were identified or tentatively identified<sup>26</sup>. Propolis of *Tetragonula laeviceps* from Thailand demonstrated antibacterial activity and was found to contain prenylated xanthones, coming from the resin of *Garcinia mangostana*<sup>27,28</sup>.

The combination of phenolic lipids (alk(en)yl phenols, alk(en)ylresorcinols and anacardic acids) and triterpenes, mainly of cycloartane-type (cycloartenol, mangiferolic acid, isomangiferolic acid), was found to be typical for the resin of mango fruits (*M. indica*)<sup>29</sup>. This is supported by Sulaeman *et al.*, who conducted observational study in 10 provinces of Indonesia and found mango as the most widespread flora around the bee hives<sup>30</sup>. Previous studies have also revealed *M. indica* resin as the main source of *A. mellifera*

propolis in different continents: Brazil<sup>31</sup>, Myanmar<sup>32</sup>, Indonesia<sup>10</sup>, Oman<sup>33</sup>, Thailand<sup>34</sup>, Fiji Islands<sup>35</sup>, and Cameroon<sup>36</sup>. It was found to be the botanical source of propolis of stingless bee species, too: *Tetragonula sapiens* in Indonesia<sup>37</sup>, *Trigona minor* in Vietnam<sup>38</sup>, and *Trigonaincisa* in Indonesia<sup>39</sup>. Obviously, this resin is attractive to both *A. mellifera* and Meliponini, and *M. indica* is an important source of resin for bees. Based on our results and literature data, we raised a hypothesis that *M. indica* is a preferred botanical source of propolis from the tropical zone.

Our findings compared to the abovementioned published results demonstrate that stingless bees of the same species might use different resin sources in different environments. It could be suggested that the availability of the resin, combined with its olfactory characteristics and its mechanical properties (soft enough to be scraped by the mandibles of the bees) are the features which drive the choice of the stingless bees. This suggestion is supported by the fact that Meliponini of different species choose the same resin source because it has the right properties and is readily available.

### Conclusion

The results of the present study revealed the similar chemistry for propolis produced by two stingless bee species, in two provinces in Indonesia. *M. indica* plants seem to be a preferred source for resin collection in the tropical zone. To avoid high content of sugar and sugar derivatives, chloroform instead of 70% ethanol as the extracting solvent could be recommended when studying stingless bee propolis or to harvest the stingless bee honey by suctioning method.

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### Conflict of interest

We declare no potential conflict of interest

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