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Phytochemical Profiles of Propolis Trigona Spp. from Three Regions in Indonesia Using GC-MS

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Abstract
This research was designed to determine the phytochemical components of the ethanol extract of propolis *Trigona* spp. using GC-MS. GC-MS analysis showed the presence of twelve different compounds in ethanol extract of propolis *Trigona* spp. from South Sulawesi region. The main phytochemical compounds identified in propolis from South Sulawesi were octadecane (11.87%) and from twelve compounds identified only six compounds were reported to have biological activity. The ethanol extract of propolis *Trigona* spp. from South Kalimantan region indicated the presence of eight different bioactive compounds with highest peak area of 0.96% for tricosane, from eight compounds identified only three compounds were reported to have biological activity. The ethanol extract of propolis *Trigona* spp. from Banten region showed the presence of eight different bioactive compounds with highest peak area of 4.80% for nonacosane and from the eight compounds identified only five compounds were reported to have biological activity. This research confirmed that the existence of different bioactive compounds from each region of origin of propolis in Indonesia.
Keywords: propolis *Trigona* spp., ethanol extract, phytochemical profile, GC-MS analysis

1. Introduction

Propolis is a product produced by insects (honey bees). Bees produce some products such as honey, royal jelly, pollen and propolis. Propolis is referred to as bee glue, a resin substance, brownish-black made by bees by collecting the resin sap from trees and then mixing it with nectar and forming the substance of wax candles in the nest. As the resinous substances, prepared by the honey bee propolis to seal cracks, smooth walls, and to maintain a stable temperature and humidity in the nest throughout the year. Raw Propolis is usually composed of 50% plant resins, 30% wax, 10% essential oils and aromatic, 5% pollen and 5% for other organic substances.

Chemically propolis contains ingredients that are very complex, more than 500 compounds have been identified in propolis from different countries until 2012. It contains flavonoids, phenylpropanoids, terpenoids, stilbenes, lignans, coumarin and prenylated derivative. However, other common chemical components such as alkaloids, iridoids have not been reported in propolis. The characteristic is often explained through plant source[1]. The main compounds that are often found in samples of propolis are flavonoids, organic acids, phenols and various types of enzymes, vitamins and minerals. The compound gives activity as an anti-bacterial, anti-inflammatory, anti-oxidant, pro-oxidant, immuno-enhancement and anti-tumor. For example, flavonoid, as an antioxidant, prevents oxidative damage of DNA caused by reactive oxygen species[2,3,4,5,6].

One species of bee that potentially produce propolis that Indonesia owned is a local bee species *Trigona* spp. Bee *Trigona* spp. is a wild bees in Indonesia that can already be cultivated. The quantity and quality of the chemical components of propolis are affected by bee species and vegetation a source of resin, which may vary among the provinces in Indonesia which Indonesia is a country with a high botanical diversity where it will be reflected in the composition of the different propolis. Differences in chemical composition greatly affects on its ability of bioligical activity[1].

In this research, the source of the propolis is retrieved from three provinces in Indonesia namely South Sulawesi, South Kalimantan and Banten which are known to have the availability of abundant and have bee species *Trigona* spp. different. This research aims to identify the phytochemical profiles of propolis samples *Trigona* spp. obtained from the three regions in Indonesia.

2. MATERIALS AND METHODS

2.1 Preparation of extract

The research material consisted of raw propolis bee *Trigona* spp. Origin of South Sulawesi, South Kalimantan and Banten region, collection of CV Nutrima Sehatalami Bogor obtained from local beekeepers in January 2016.

2.2 Extraction Of Propolis

The extraction of propolis from beehive Trigona spp. nests was carried out using the Hasan method[7]. A total of 150 grams of honeycomb *Trigona* spp., Macerated with 650 ml of ethanol 70% (soaked while digojog by using a shaker) (soaked while shaken out by using a shaker) for 7 days in an erlenmeyer flask 1000 mL. After 7 days, the filtrate was decanted and then the residue was macerated again with 50 ml of 70% ethanol new. This process was repeated every day for seven days, until solvent ethanol in residue seemed clear. Thus, the total solvent (ethanol) used was 1000 ml, and the total time of maceration was for 14 days. The filtrate obtained, united in a dark container, then freeze - dried to form a solid extract which was then used for subsequent testing.

2.3 Identification of Compounds with Gas Chromatography - Mass Spectrophotometry (GC-MS)

Extract samples were subsequently analyzed by Pyrolysis Gas Chromatography-Mass Spectrofotometre (*Py*-GC-MS) brand of Shimadzu GCMS-QP2010 Type to determine the organic compounds contained therein. A total of 20 mg of extract was inserted into the quartz chamber in the pyrolysis unit then heated in an oxygen-free environment at a temperature of 400^{o}C. Temperature injector/injet was 280^{o}C and temperature of the interface 280^{o}C.The column used was a capillary column of type RTX-5MS with a length of 60 m, a diameter of 0.25 mm and 0.25 mmID films, containing 5% Dipenyl and 95% methyl polysiloxane. The temperature of the oven was set at at a temperature of 50^{o}C early for 6 minutes, then increased to a temperature of 280^{o}C with the rate of temperature rise 10^{o}C/min and finally let at a temperature of 280^{o}C for 21 minutes. Helium as a carrier gas/mobile phase was set at a constant rate 20 mL/min. Mass spectrometry was set with *Temperature Ion Source* 200^{o}C, *Energy* 70 ev and *Setting Mass Range* (BM) between 40 up to 600 m/z.

2.4 Data Analysis

Extract chemical components were identified by comparing the retention time of the chromatographic peak with WILEY7 database combined with NIST library ver.2.0. Name, molecular weight, molecular formula, and area under peak of the components of the test material were determined. Prediction of biological activity of the compounds was based on Dr. Dr. Duke‟s Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of Agricultural Research Service Service/USDA[8]. Furthermore, the data presented was data that had percent estimate of similarity structure compound (similarity index) ≥ 95% according to to WILEY7 and NIST library ver. 2.0.

3. Results and Discussion

Analysis of GC-MS chromatograms of ethanol extract of propolis *Trigona* spp. originating from the region of South Sulawesi, South Kalimantan and Banten (Figure 1, 2 and 3) showed a hundred peaks indicating the presence of hundred of phytochemical with retention time and different area percent. From the comparison of the mass spectra of compounds with WILEY7 and NIST library ver.2.0, a hundred phytochemical components were marked and identified by retention time (RT), the molecular weight (MW), the molecular formula, and concentration (peak area%) (Table 1, 2 and 3).

As many as fourteen peaks GC-MS chromatograms of ethanol extract of propolis *Trigona* spp. originating from South Sulawesi region of which have approximate percent similarity structure compound (similarity index) ≥ 95% (Table 1). The peak of fourteen, attended twelve different bioactive compounds include octadecane (11,87%), 9-Octadecen-1-ol, (Z)- (1,79%), pentatriacontane (1.49% and 0.23%), limonene (1,53%), tricosane (0,9%), 1-heptacosanol (0,77%), heptacosane (0,48% and 0,22%), 1-hexadecanol (0,64%), 1-hexadecene (0,37%), dioctyl adipate (0,32%), hexadecane (0,16%) and butanoic acid, 2-methyl- (0,12%). Among these compounds, six compounds were reported to have biological activity including ($C_{10}H_{16}$), hexadecane ($C_{16}H_{34}$), 1-hexadecanol ($C_{16}H_{34}O$), dioctyl adipate ($C_{22}H_{42}O_4$), heptacosane ($C_{27}H_{56}$), dan 1-heptacosanol ($C_{27}H_{56}O$). Various phytochemical compounds propolis *Trigona* spp. origin of South Sulawesi region which provides biological activity shown in Table 4.

The results of GC-MS analysis of the propolis South Kalimantan origin, known as much as nine peaks which had percent of forecast the compounds of structural similarity (similarity index) ≥ 95% (Table 2). The peak of nine, attended eight different bioactive compounds such as tricosane (0.96% dan 0.42%), hexadecanoic acid (0,92%), tetratetracontane (0,87%), acetic acid (0,86%), silane, methyl- (0,77%), 2-propanone, 1-(acetyloxy)- (0,16%), propanoic acid, 2-oxo-, methyl ester (0,15%) dan 2-butanone, 1-(acetyloxy)- (0,09%). Among these compounds, three compounds were reported to have biological activity including acetic acid ($C_2H_4O_2$), hexadecanoic acid ($C_{16}H_{32}O_2$) and tetratetracontane ($C_{44}H_{90}$). Phytochemical compounds propolis

Trigona spp. Of origin South Kalimantan which providing biological activity can be seen in Table 4.

Analysis of GC-MS chromatograms of ethanol extract of propolis *Trigona* spp. from Banten known to have eight peaks, among of them percent estimate of similarity structure compound (similarity index) ≥ 95% (Table 3). From the eight bioactive compounds, compounds which were indicated (4.80%), tetratetracontane (2.78%), bis(2-ethylhexyl) phthalate (1.79%), hexadecanoic acid (1.06%), tricosane (0.78%), limonene (0.51%), pentadecane (0.41%), and phenol, 2,6-dimethyl- (0.26%). Among these compounds, five compounds were reported to have biological activity including limonene ($C_{10}H_{16}$), hexadecanoic acid ($C_{16}H_{32}O_2$), bis(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$), nonacosane ($C_{29}H_{60}$) dan tetratetracontane ($C_{44}H_{90}$). Phytochemical compounds propolis *Trigona* spp. origin of Banten region that provide biological activity shown in Table 4.

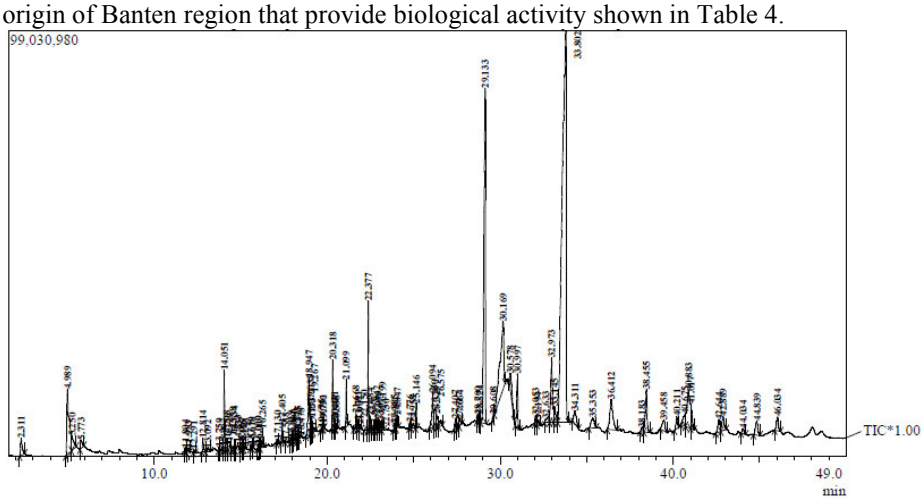

Figure 1. GC-MS chromatograms of the ethanol extract of propolis *Trigona* spp. of South Sulawesi region

Table 1. Components phytochemical ethanol extract of propolis *Trigona* spp. of South Sulawesi region

No.	Peak	Retention Time	Peak Area (%)	Molecular formula	Molecular weight	Name of compound*)
1.	6	11.983	0.12	$C_5H_{10}O_2$	102.1317	Butanoic acid, 2-methyl-
2.	12	14.051	1.53	$C_{10}H_{16}$	136.2340	Limonene
3.	36	19.033	0.37	$C_{16}H_{32}$	224.4253	1-Hexadecene
4.	37	19.073	0.16	$C_{16}H_{34}$	226.4412	Hexadecane
5.	47	21.099	0.64	$C_{16}H_{34}O$	242.4400	1-Hexadecanol
6.	52	22.377	1.79	$C_{18}H_{36}O$	268.4778	9-Octadecen-1-ol, (Z)-
7.	62	24.047	0.23	$C_{35}H_{72}$	492.9462	Pentatriacontane
8.	65	25.146	0.32	$C_{22}H_{42}O_4$	370.5700	Dioctyl adipate
9.	66	26.094	0.90	$C_{23}H_{48}$	324.6272	Tricosane
10.	70	27.407	0.22	$C_{27}H_{56}$	380.7335	Heptacosane
11.	75	29.133	11.87	$C_{18}H_{38}$	254.4943	Octadecane
12.	79	30.997	1.49	$C_{35}H_{72}$	492.9462	Pentatriacontane
13.	92	40.211	0.48	$C_{27}H_{56}$	380.7335	Heptacosane
14.	99	44.839	0.77	$C_{27}H_{56}O$	396.7300	1-Heptacosanol

*) data has percent estimate of similarity structure compound (similarity index) ≥ 95%

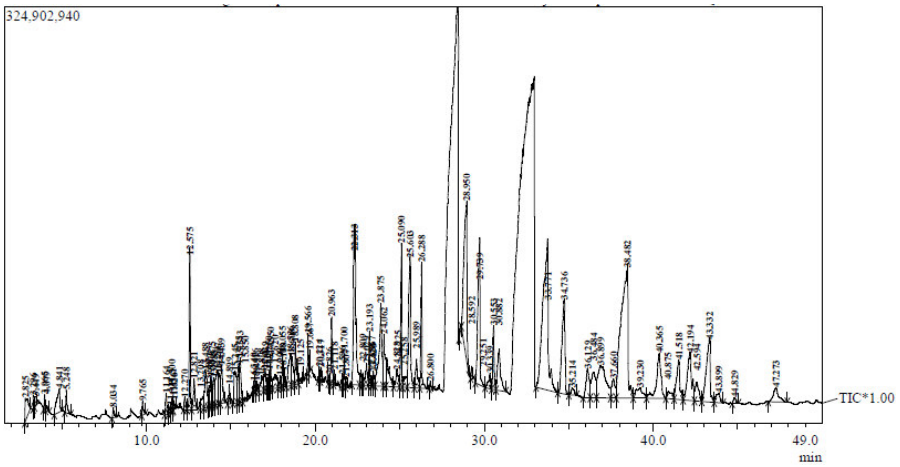

Figure 2. GC-MS Chromatograms of the Ethanol Extract Of Propolis *Trigona* spp. of South Kalimantan

Table 2. Components of the ethanol extract of propolis phytochemical Trigona spp. of South Kalimantan

No.	Peak	Retention Time	Peak Area %	Molecular formula	Molecular weight	Name of compound*)
1.	1	2.825	0.77	CH_6SI_2	46.1438	Silane, methyl-
2.	6	4.841	0.86	$C_2H_4O_2$	60.0520	Acetic acid
3.	8	8.034	0.15	$C_4H_6O_3$	102.0886	Propanoic acid, 2-oxo-, methyl ester
4.	9	9.765	0.16	$C_5H_8O_3$	116.1152	2-Propanone, 1-(acetyloxy)-
5.	13	11.561	0.09	$C_6H_{10}O_3$	130.1418	2-Butanone, 1-(acetyloxy)-
6.	54	20.963	0.92	$C_{16}H_{32}O_2$	256.4200	Hexadecanoic acid
7.	63	23.193	0.42	$C_{23}H_{48}$	324.6272	Tricosane
8.	67	24.062	0.96	$C_{23}H_{48}$	324.6272	Tricosane
9.	100	47.273	0.87	$C_{44}H_{90}$	619.1854	Tetratetracontane

*) data has percent estimate of similarity structure compound (similarity index) ≥ 95%

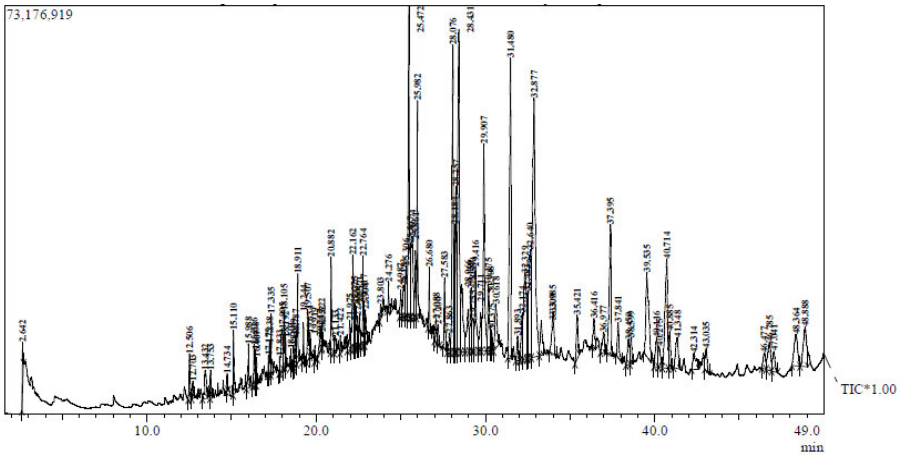

Figure 3. GC-MS chromatograms of the ethanol extract of propolis *Trigona* spp. from Banten region

Table 3. Components phytochemical ethanol extract of propolis *Trigna* spp. from Banten region

No.	Peak	Retention Time	Peak Area %	Molecular formula	Molecular weight	Name of compound*)
1.	2	12.506	0.51	$C_{10}H_{16}$	136.2340	Limonene
2.	5	13.753	0.26	$C_8H_{10}O$	122.1644	Phenol, 2,6-dimethyl-
3.	14	17.335	0.41	$C_{15}H_{32}$	212.4146	Pentadecane
4.	30	20.882	1.06	$C_{16}H_{32}O_2$	256.4200	Hexadecanoic acid
5.	51	25.982	1.79	$C_{24}H_{38}O_4$	390.5561	Bis(2-ethylhexyl) phthalate
6.	55	27.583	0.78	$C_{23}H_{48}$	324.6272	Tricosane
7.	72	31.480	4.80	$C_{29}H_{60}$	408.7867	Nonacosane
8.	84	37.395	2.78	$C_{44}H_{90}$	619.1854	Tetratetracontane

*) Data has percent estimate of similarity structure compound (similarity index) ≥ 95%

Table 4. Activity components identified in the sample of the ethanol extract of propolis from three regions in Indonesia using GC-MS

No.	Name of Compound	MF	Samples Propolis			Activity*)
			South Sulawesi	South Kalimantan	Banten	
1.	Silane, methyl-	CH_6SI_2		X		no activity reported
2.	Acetic acid	$C_2H_4O_2$		X		anti-bacterial; anti-otitic; anti-salmonella; anti-vaginitic; expectorant; acidulant; fungicide
3.	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$		x		flavoring agents
4.	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	x			flavor and fragrance agents
5.	2-Propanone, 1-(acetyloxy)-	$C_5H_8O_3$		X		flavor and fragrance agents
6.	Phenol, 2,6-dimethyl-	$C_8H_{10}O$			x	flavor and fragrance agents
7.	2-Butanone, 1-(acetyloxy)-	$C_6H_{10}O_3$		X		flavoring agents
8.	Limonene	$C_{10}H_{16}$	x		x	immunomodulator, anti-oxidant, anti-bacterial, anti-inflammatory, anti-adenomic, anti-alzheimeran, anti-asthmatic, anti-cancer, anti-esophagitic, anti-feedant, anti-flu, anti-lithic, anti-lymphomic, anti-metastatic, anti-mutagenic, anti-obesity, anti-septic, anti-spasmodic, anti-tumor, anti-acetylcholinesterase, anti-allergic, anti-angiogenic, anti-atherogenic, anti-carcinomic, anti-nociceptive, anti-allergic, anti-angiogenic, anti-atherogenic, anti-tussive, apoptotic, bronchoprotectant, candidistat, chemopreventive, cholesterolytic, detoxicant, enterocontractant, expectorant, flavor, fungistat, GST-inducer, herbicide, histaminic, insecticide, insectifuge, interleukin-6-inhibitor
9.	Pentadecane	$C_{15}H_{32}$			x	fragrance agents
10.	1-Hexadecene	$C_{16}H_{32}$	x			no activity reported

11.	Hexadecane	$C_{16}H_{34}$	x			anti-oxidant, anti-microbial
12.	1-Hexadecanol	$C_{16}H_{34}O$	x			anti-oxidant
13.	Octadecane	$C_{18}H_{38}$	x			no activity reported
14.	Hexadecanoic acid	$C_{16}H_{32}O_2$		X	x	anti-oxidant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, hypocholesterolemic, nematicide, pesticide, lubricant
15.	9-Octadecen-1-ol, (Z)-	$C_{18}H_{36}O$	x			no activity reported
16.	Tricosane	$C_{23}H_{48}$	x	X	x	no activity reported
17.	Dioctyl adipate	$C_{22}H_{42}O_4$	x			anti-bacterial
18.	Heptacosane	$C_{27}H_{56}$	x			anti-bacterial
19.	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$			x	anti-mutagenic, anti-leukaemic
20.	1-Heptacosanol	$C_{27}H_{56}O$	x			anti-cancer, anti-oxidant, anti-microbial, nematicidal
21.	Nonacosane	$C_{29}H_{60}$			x	anti-mutagenic
22.	Pentatriacontane	$C_{35}H_{72}$	x			Herbistat
23.	Tetratetracontane	$C_{44}H_{90}$		X	x	hypoglycaemic, anti-oxidant

* Source: Dr. Duke's Phytochemical and Ethnobotanical Databases

Compared with the findings of this research, in two samples of propolis ethanol extract Turkey[9] were reported to contain twenty-four and eighteen of chemical compounds while twenty chemical compounds were found in propolis ethanol extract of red Brazil[10]. It should be noted that tricosane ($C_{23}H_{48}$) were found to be present at our three propolis *Trigona* spp. also detected present in propolis Portugal [11, 12] and India [13]. While octadecane ($C_{18}H_{38}$) was a phytochemical compounds highest (11.87%) was found in our propolis also found to be present in propolis Turkey [14] and Iran [15].

4. Conclusion

In this research, the ethanol extract of propolis *Trigona* spp. each originating from South Sulawesi, South Kalimantan and Banten were identified containing twelve, eight and eight phytochemical compounds that easily evaporate. From phytochemical compounds identified were obtained six, three and five compounds which were reported to have biological activity.

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