PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF PROPOLIS TRIGONA SPP. FROM TWO REGIONS IN LAMPUNG PROVINCE OF INDONESIA

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Abstract: This research was designed to determine the phytochemical components and characteristics of the ethanol extract of propolis Trigona spp. collected from two different areas from Lampung provinces in Indonesia using GC-MS. GC-MS analysis showed the presence of twenty different compounds in ethanol extract of propolis Trigona spp. of West Lampung region. The main phytochemical compounds that are identified from propolis origin West Lampung region is dimethyl amine hydrochloride C2H8ClN as much as 9.71% and twenty compounds identified only twelve compounds were reported to have biological activity. The ethanol extract of propolis Trigona spp. of South Lampung region showed the presence of twelve different bioactive compounds with the highest peak area of 5.92% for methane, tetranitro- (CN4O8), of twelve compounds were identified only eight compounds that are reported to have biological activity. This study showed that propolis origin Indonesia Lampung province is a potential source of natural bioactive compounds for biological and pharmacological applications and has the presence of bioactive compounds that differ from each territory.

Keywords: GC-MS analysis; ethanol extract; phytochemical profile; propolis Trigona spp.

1. Introduction

Propolis is referred to as bee glue, a resin substance, brownish made resin bees collect the sap from trees and then mix it with nectar and wax candles forming substance in the nest. Honeybees use of propolis to seal cracks in the hive and protect their hive from bacterial and fungal infections [1]. At the time of the ancient Egyptians, Greeks and Romans, propolis is used as a remedy for several diseases [2]. Propolis also contain aromatic substances, fragrance substances, and various minerals. Propolis component chemical compounds show a variety of biological effects and pharmacological activity, therefore the researchers are interested in studying the chemical content and biological properties [2,3]. The diversity of chemical composition and biological activity of propolis is associated with the geographical location, the source plant and gathering season [2]. Flavonoids, aromatic acids, acids diterpenoid, triterpenoids, and phenolic compounds are the major components of propolis [4,5]. Some of these compounds are responsible for the biological activity [4,6,7]. There are three sources of organic compounds from propolis: plant origin, compound derived from the metabolism of honey bees, and materials used for the formation of propolis [8].

Indonesia has many kinds of local bees, one of which is known as a producer of propolis is Trigona spp. Trigona spp. bees previously not popular because it produces little honey and propolis difficult extracted but generated more than other bees [9]. This Trigona spp. bees has advantages such as 1) more easily cultivated, 2) more resistant to disease than the Apis mellifera bee, 3) produce a more diverse phytochemical, and 4) propolis higher productivity [10]. Although many bioactive compounds contained in propolis, so far the research on the relationship of bioactive compounds of propolis Trigona spp. Lampung province of has not been yet reported. Therefore, the main objective of this study was to determine the chemical composition, characteristics and relative concentrations of organic compounds in the extract organic material from propolis samples was collected from two different areas in the Indonesian province of Lampung.

2. Materials And Methods

2.1 Preparation of extracts

The research material consisted of raw propolis bee Trigona spp. originating from two regions in Lampung province of Indonesia, which is obtained from local bee-keepers in May 2016.

2.2 Extraction of Propolis

The extraction of propolis from beehive Trigona spp. nests was carried out the Hasan method [9]. A total of 150 grams of honeycomb Trigona spp., macerated with 650 ml of ethanol 70% (soaked while shaken out by using a shaker) for 7 days in a 1000 ml erlenmeyer flask. After 7 days, the filtrate is decanted and then the residue was macerated again with a 50 ml of 70% ethanol new. This process is repeated every day for seven days, until solvent ethanol in the residue seemed clear. Thus, the total solvent (ethanol) used was 1000 ml, and the total time of maceration for 14 days. The filtrate obtained, united in a dark container, and freeze-dried to form a solid extract was then used for subsequent testing.

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

Extract samples were subsequently analysed by Pyrolysis Gas Chromatography-Mass Spectrophotometer (Py-GCMS) brand of Type Shimadzu GCMS-QP 2010 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°C. Temperature injector/inject was 280°C and temperature of the interface 280°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 mm-id films, containing 5% diphenyl and 95% methyl polysiloxane. The temperature of the oven was set at at a temperature of 50°C early for 6 minutes, then increased to a temperature of 280°C with the rate of temperature rise 10°C/Min and finally let at a temperature of 280°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°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 the area under the peak of the components of the test material is determined. Prediction of biological activity of the compounds is based on Dr. Duke"s Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of Agricultural Research Service/USDA [11]. Furthermore, the data presented was data that had percent estimate of similarity structure compound (similarity index) ≥ 90% according to the NIST library WILEY7 and ver.2.0.

3. Results and Discussion

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

A total of twenty-two peak GC-MS chromatograms of ethanol extract of propolis Trigona spp. from West Lampung region which have structural similarity percent forecast compound (similarity index) ≥ 90% (Table 1). From twenty-two of the peaks, there were twenty different bioactive compounds include dimethyl amine hydrochloride (9.71%), heptacosane (3.02%, 2.46% and 0.69%), hexadecanoic acid (1.86%), 4H-Pyrans-4- one, 2,3-dihydro-3,5-dihydroxy-6-methyl (1.56%), isosorbide (1.55%), limonene (1.11%), acetaldehyde (0.98%), 9.12-Octadecadien -1-ol, (Z, Z) - (0.98%), acetic anhydride (0.93%), hexadecanoic acid, ethyl ester (0.85%), methanethiol (0.73%), linoleic acid ethyl ester (0.73%), tricosane (0.45%), acetic acid (0.40%), furfural (0.33%), benzenesulfonic acid, 4-Hydroxy- (0.33%), furan, 2 -methyl- (0.26%), trichloromethane (0.17%), propanoic acid, 2-oxo-, methyl ester (0.16%), and 2-propanone, 1-Hydroxy- (0.14%). Among these compounds, twelve compounds reported to have biological activity including acetic acid ($C_2H_4O_2$), 2-propanone, 1-Hydroxy- ($C_3H_6O_2$), furan, 2-methyl (C_5H_6O), furfural ($C_5H_4O_2$), 1, 4-hexadiene, 3,3,5-trimethyl- (C_9H_{16}), Limonene ($C_{10}H_{16}$), 4H-Pyrans-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl ($C_6H_8O_4$), isosorbide ($C_6H_{10}O_4$), benzenesulfonic acid, 4-Hydroxy- ($C_6H_6O_4S$), hexadecanoic acid ($C_{16}H_{32}O_2$), hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$), linoleic acid ethyl ester ($C_{20}H_{36}O_2$), heptacosane ($C_{27}H_{56}$), and triacontane ($C_{30}H_{62}$). Various phytochemical compounds propolis Trigona spp. from West Lampung region which provides biological activity shown in Table 3.

The results of GC-MS analysis of propolis Trigona spp. from South Lampung region, known as much as twelve percent peak of whom had forecast the compounds of structural similarity (similarity index) ≥ 90% (Table 2). The peak of the twelve, was present eleven different bioactive compounds such as methane,

tetranitro- (5.92%), limonene (3.11%), 1,4-hexadiene, 3,3,5-trimethyl- (1.82%), 3,5-heptadien-2-ol, 2,6-dimethyl- (1.77%), hexadecanoic acid (1.52%), heptacosane (0.78% and 0.61%), phenol, 2 , 6-dimethoxy- (1.21%), triacontane (1.04%), mequinol (0.87%), pentatriacontane (0.78%), and 2-Propanone, 1-Hydroxy- (0.42%). Among these compounds, reported eight compounds have biological activity including, 2-propanone, 1-Hydroxy- ($C_3H_6O_2$), 1,4-hexadiene, 3,3,5-trimethyl- (C_9H_{16}), limonene ($C_{10}H_{16}$), 3,5-heptadien-2-ol, 2,6-dimethyl- ($C_9H_{16}O$), phenol, 2,6-dimethoxy- ($C_8H_{10}O_3$), hexadecanoic acid ($C_{16}H_{32}O_2$), heptacosane ($C_{27}H_{56}$), and triacontane ($C_{30}H_{62}$). Phytochemical compounds of propolis Trigona spp. from South Lampung region which provides biological activity can be seen in Table 3.

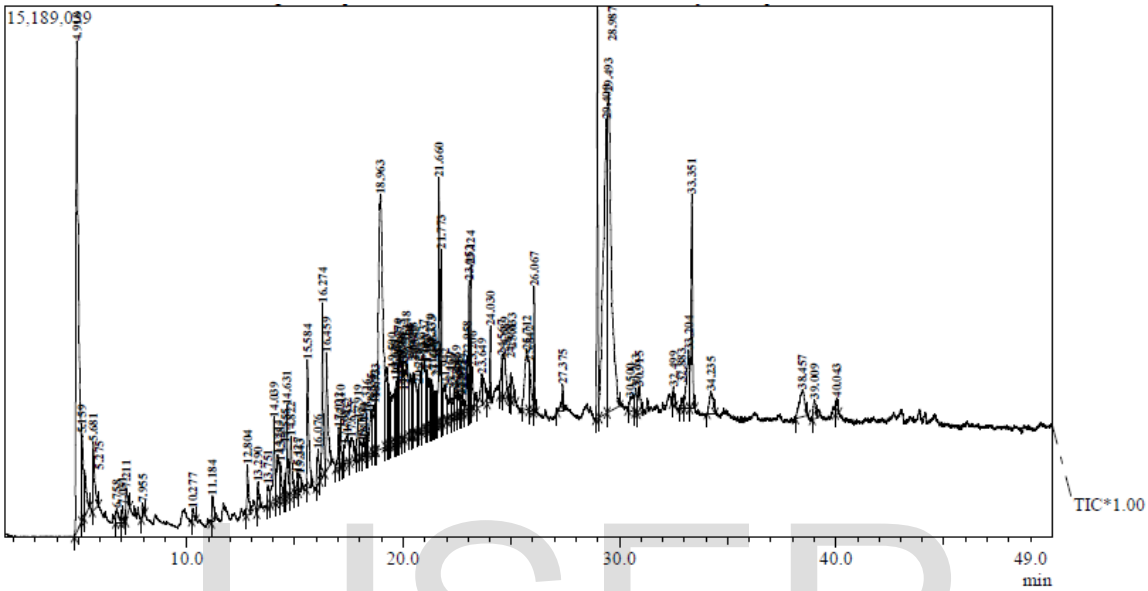

Figure 1. GC-MS chromatograms of the ethanol extract of propolis Trigona spp. of West Lampung region

Table 1. Components phytochemical ethanol extract of propolis Trigona spp. of West Lampung region

No	Peak	Retention Time	Area	Peak Area (%)	Molecular weight	Molecular formula	Name of compound*)
1.	1	4.918	21677008	9.71	81.5450	C2H8ClN	Dimethyl amine hydrochloride
2.	2	5.159	12246634	0.98	44.0526	C_2H_4O	Acetaldehyde
3.	3	5.275	9095640	0.73	48.1070	CH_4S	Methanethiol
4.	4	5.681	11627246	0.93	102.0886	$C_4H_6O_3$	Acetic anhydride
5.	5	6.758	3295349	0.26	82.1005	C_5H_6O	Furan, 2-methyl-
6.	6	7.031	2185445	0.17	119.378	$CHCL_3$	Trichloromethane
7.	7	7.211	5055727	0.40	60,0520	$C_2H_4O_2$	Acetic acid
8.	8	7.955	1801149	0.14	74.0785	$C_3H_6O_2$	2-Propanone, 1-hydroxy-
9.	9	10.277	2012192	0.16	102.0886	$C_4H_6O_3$	Propanoic acid, 2-oxo-, methyl ester
10.	10	11.184	4173614	0.33	96.0841	$C_5H_4O_2$	Furfural
11.	13	13.751	4091531	0.33	174.1740	$C_6H_6O_4S$	Benzenesulfonic acid, 4-hydroxy-
12.	14	14.039	13867623	1.11	136.2340	$C_{10}H_{16}$	Limonene
13.	22	15.584	19566277	1.56	144.1253	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
14.	24	16.274	19428712	1.55	146,1400	$C_6H_{10}O_4$	Isosorbid
15.	63	21.660	23347741	1.86	256.4200	$C_{16}H_{32}O_2$	Hexadecanoic acid

16.	64	21.773	10691055	0.85	284.4772	$C_{18}H_{36}O_2$	Hexadecanoic acid, ethyl ester
17.	74	23.052	12297895	0.98	266.4620	$C_{18}H_{34}O$	9,12-Octadecadien-1-ol, (Z,Z)-
18.	75	23.124	9101690	0.73	308.4986	$C_{20}H_{36}O_2$	Linoleic acid ethyl ester
19.	78	24.030	5684320	0.45	324.6272	$C_{23}H_{48}$	Tricosane
20.	85	26.067	8648029	0.69	380.7335	$C_{27}H_{56}$	Heptacosane
21.	87	28.987	37831915	3.02	380.7335	$C_{27}H_{56}$	Heptacosane
22.	96	33.351	30819585	2.46	380.7335	$C_{27}H_{56}$	Heptacosane

*) data has percent estimate of similarity structure compound (similarity index) ≥ 90%

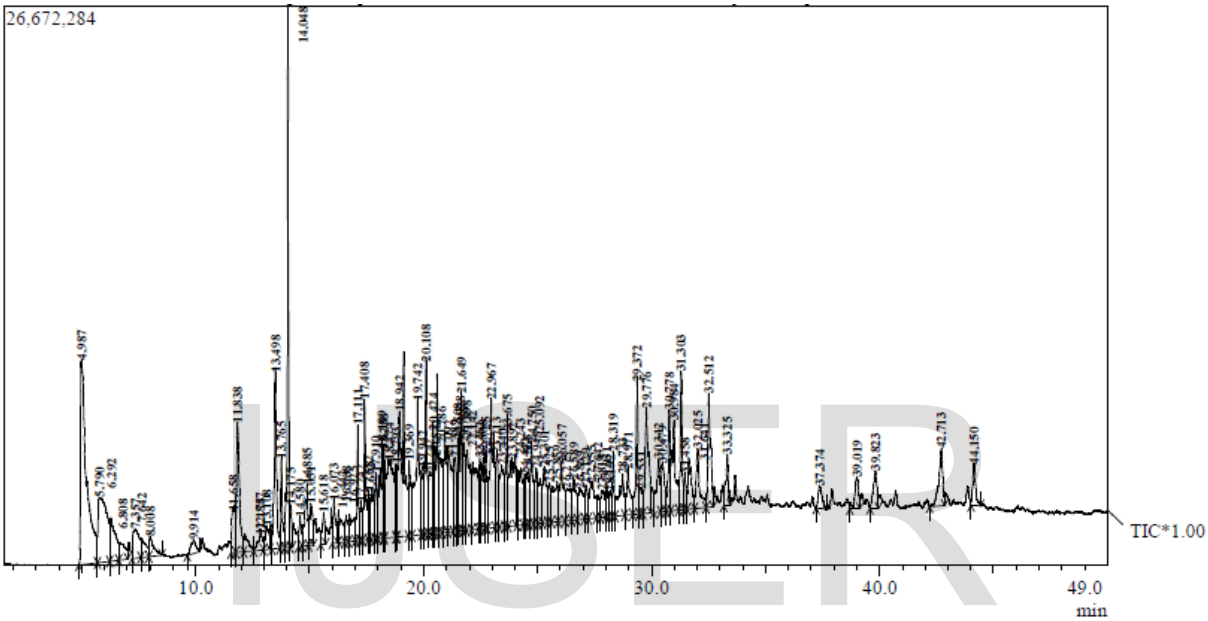

Figure 2. GC-MS chromatograms of the ethanol extract of propolis Trigona spp. of South Lampung region

Table 2. Components phytochemical ethanol extract of propolis Trigona spp. of South Lampung region

No	Peak	Retention Time	Area	Peak Area (%)	Molecular weight	Molecular formula	Name of compound*)
1.	1	4.987	181027114	5.92	196.0327	CN_4O_8	Methane, tetranitro-
2.	7	8.008	12798532	0.42	74.0785	$C_3H_6O_2$	2-Propanone, 1-hydroxy-
3.	10	11.838	55786019	1.82	124.1252	C_9H_{16}	1,4-hexadiene,3,3,5-trimethyl-
4.	14	13.498	54094762	1.77	140.1201	$C_9H_{16}O$	3,5-Heptadien-2-ol, 2,6-dimethyl-
5.	16	14.048	95021669	3.11	136.2340	$C_{10}H_{16}$	Limonene
6.	19	14.885	26605570	0.87	124.1372	$C_7H_8O_2$	Mequinol
7.	28	17.408	36890375	1.21	154.1632	$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy-
8.	49	21.649	46470042	1.52	256.42	$C_{16}H_{32}O_2$	Hexadecanoic acid
9.	65	24.948	23826698	0.78	492.9462	$C_{35}H_{72}$	Pentatriacontane
10.	70	26.057	31814403	1.04	422.8133	$C_{30}H_{62}$	Triacontane
11.	82	28.971	23865096	0.78	380.7335	$C_{27}H_{56}$	Heptacosane
12.	95	33.325	18632670	0.61	380.7335	$C_{27}H_{56}$	Heptacosane

*) data has percent estimate of similarity structure compound (similarity index) ≥ 90%

Relative total of twenty-seven compounds have been identified by the ethanol extract of propolis from two regions in the Lampung provinces, Indonesia. Among them, compound acetaldehyde, methanethiol, acetic anhydride, acetic acid, dimethyl amine hydrochloride, furan, 2-methyl, furfural, propanoic acid, 2-oxo-, methyl ester, trichloromethane, 4H-Pyrans-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl, isosorbide, benzenesulfonic acid, 4-Hydroxy-, 9,12-octadecadien-1-ol, (Z, Z)-,hexadecanoic acid, ethyl ester, linoleic acid ethyl ester, and

tricosane found present in propolis samples from West Lampung but is not found in propolis from South Lampung, 1,4-hexadiene, 3,3,5-trimethyl-, mequinol, 3,5-heptadien-2-ol , 2,6-dimethyl-, phenol, 2,6-dimethoxy-, methane, tetranitro-, triacontane, and pentatriacontane found present in the South Lampung propolis which does not exist in propolis from West Lampung. Compound 2-propanone, 1-Hydroxy-, limonene, hexadecanoic acid, and heptacosane present in both the samples of propolis from West Lampung and South Lampung (Table 3).

Table 3. Activity components identified in the sample of the ethanol extract of propolis from two regions in Lampung province using GC-MS

No.	Name of Compound	MF	Sample of propolis		Activity*)
			West Lampung	South Lampung	
1.	Acetaldehyde	C_2H_4O	x		perfumery
2.	Methanethiol	CH_4S	x		no activity reported
3.	Acetic anhydride	$C_4H_6O_3$	x		no activity reported
4.	Acetic acid	$C_2H_4O_2$	x		anti-bacterial; anti-otitic; anti-salmonella; anti-vaginitic; expectorant; acidulant; fungicide
5.	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	x	x	preservative
6.	Dimethyl amine hydrochloride	C2H8ClN	x		tanning
7.	Furan, 2-methyl-	C_5H_6O	x		anti-diabetic
8.	Furfural	$C_5H_4O_2$	x		anti-septic, flavor, fungicide, insecticide, irritant, pesticide
9.	Propanoic acid, 2-oxo-, methyl ester	$C_4H_6O_3$	x		flavor, fungicide, irritant, perfumery, pesticide
10.	Trichloromethane	$CHCL_3$	x		no activity reported
11.	1,4-hexadiene,3,3,5-trimethyl-	C_9H_{16}		x	anti-microbial
12.	Mequinol	$C_7H_8O_2$		x	depigmenting (a melanin synthesis inhibitor)
13.	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
14.	3,5-Heptadien-2-ol, 2,6-dimethyl-	$C_9H_{16}O$		x	anti-microbial, anti-viral
15.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	x		anti-microbial, anti-inflammatory, anti-proliferative anti-oxidant, automatic nerve activity
16.	Isosorbid	$C_6H_{10}O_4$	x		diuretic

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17.	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$		x	anti-oxidant
18.	Benzenesulfonic acid, 4-hydroxy-	$C_6H_6O_4S$	x		anti-microbial, anti-oxidant
19.	Methane, tetranitro-	CN_4O_8		x	no activity reported
20.	Hexadecanoic acid	$C_{16}H_{32}O_2$	x	x	anti-oxidant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, hypocholesterolemic, nematicide, pesticide, lubricant
21.	9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	x		anti-preventive, flavour, fungicide, pesticide, perfumery
22.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	x		anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, anti-histaminic, anti-eczemic, anti-acne, alpha reductase inhibitor, anti-androgenic, anti-arthritic, anti-coronary
23.	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	x		anti-microbial, anti-plasmodial, hypocholesterolemic
24.	Tricosane	$C_{23}H_{48}$	x		no activity reported
25.	Heptacosane	$C_{27}H_{56}$	x	x	anti-bacterial
26.	Triacontane	$C_{30}H_{62}$		x	anti-bacterial, anti-diabetic, anti-tumor
27.	Pentatriacontane	$C_{35}H_{72}$		x	herbistat

* Source: Dr. Duke's Phytochemical and Ethnobotanical Databases (online database)

In this study, from twenty seven components was identified sixteen compounds have biological activity. Compounds such as acetic acid is used as an antibacterial well against the micro-organism Pseudomonas aeruginosa [12]; 2-propanone, 1-Hydroxy- typically has activity as a preservative [13]; furan, 2-methyl-used as anti-diabetic [14]; furfural is used as an antimicrobial [15]; 1,4-hexadiene, 3,3,5-trimethyl- used as an antimicrobial [16]; limonene an isoprenoid group poly-phenyl compound, a metabolite intermediate in the synthesis of cholesterol have immunomodulatory activity [17]; 3,5-heptadien-2-ol, 2,6-dimethyl- used as an antimicrobial [18]; 4H-Pyrans-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-used as an antimicrobial [19]; isosorbide is used as a diuretic [20]; phenol, 2,6-dimethoxy- used as an antioxidant [21]; benzenesulfonic acid, 4-Hydroxy- used as an antimicrobial [22]; hexadecanoic acid is used as an antioxidant [23]; hexadecanoic acid, ethyl ester used as anti-inflammatory [19, 23]; linoleic acid ethyl ester used as an antimicrobial [23]; heptacosane used as an antibacterial [24]; and triacontane used as an antibacterial [25]. Some of the compounds identified in propolis from Lampung province is also found in propolis originating from three other Indonesian regions such as acetic acid, limonene and heptacosane [26]. The presence of a variety of bioactive compounds confirms the application of propolis for various ailments by herbal practitioners. In addition, the results of GC-MS profiles can be used as a tool for identifying phytochemical bioactive components.

4. CONCLUSION

The results obtained in this study indicate that the twenty-seven phytochemical compounds were identified bioactive compounds where sixteen of which have biological activity. Therefore, the data generated from these experiments provide a basis for using propolis chemical widely as a therapeutic agent that can be used to treat various diseases. This study offers a basis to make propolis as an herbal alternative for various diseases including diabetes, cancer, microbial infection, inflammation etc.

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