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Chemical compounds of herbal honey

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Abstract

Utilization of honey in various foods and beverages can provide benefits for health, where honey contains antioxidant properties. The antioxidant properties come from enzymatic and non-enzymatic substances. Using of herbal plants as a mixture for making herbal honey, this is known as herbal medicine for the people of Indonesia. This study aims to identify the compounds contained in herbal honey. Herbal honey is made from 90% honey; 1.1% ginger; 0.9% kencur (*Kaempferia galangal*); 1.1% garlic; 0.8% bitter powder; 0.6% cardamom; and 0.6% lemon. The results obtained indicate the presence of antioxidant activity IC_{50} 50 which indicates strong antioxidant properties. GC-MS analysis was able to identify 76 types of compounds with percentage values ranging from 0.370 to 4.917%. The compound with the highest percentage value was thujene (4.917%), 1-dehydrogingerdione (3.080), zingerone (2.722%), zerumbodienone (2,622%), α-phelandrene (2,620%), geranial (2.567%),(+)-trans-piperitenol (2,549%), eugenol (2.369%), Limonene (2,369%), neo-allo-ocimene (2,265%), citral (1,590%), β–pinene (2,152%), sabinene (2,153%), menthol (2,085%), (E)-4-(3,4- dimethoxyphenyl) but-3-en-1-ol (1,868%), α-zingiberene (1,743%), geraniol (1,743%), methyl eugenol (1,740%), trans-β-sesquiphellandrol (1,728%), ajoene (1,692%), thymol (1,577%), p-cymene (1,342%), β-eudesmol (1,340%), juniper camphor (1,259%), β-himachalene (1,258%), α-cadinol (1,257%), p-menth-3-en-1-ol (1,221%), α-ylangene (1,217%), α-cadinene (1,092%), myrtenol (1,087%). The types of compounds identified by GC-MS in herbal honey are classified as volatile compounds and include monoterpenes. Meanwhile, the results of the LC-MS analysis succeeded in identifying 199 chemical compounds with percentages ranging from 0.078-12.502%. The compound with the highest percentage was glucose (12,502%), fructose (7,247%), galactose (4,843%) and several compounds belonging to the amino acid group such as valine, proline.

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Introduction

Utilization of honey in various foods and beverages provides many benefits for health (Israili 2014). Honey is a natural liquid that has a sweet taste and is produced by honey bees from flower extracts or other parts of plants (extra floral nectar) (Alvarez *et al.,* 2010 and Gebremariam, 2014). Honey contains most of the carbohydrate groups such as fructose, glucose, maltose, sucrose, oligosaccharides and polysaccharides (Escuredo *et al.,* 2013).

In general, honey consists of 38.5% fructose and 31% glucose from 82.4% total carbohydrates while 12.9% is maltose, sucrose and other sugars (Khan *et al.,* 2007). Other sugars include isomaltose, nigerose, turanose, maltulose; kojibiose; alpha beta-trehalose, gentiobiose, laminaribiose; maltotriose, 1-ketose, panose, isomaltosyl glucose, erlose, isomaltosyltriose, theanderose, centose, isopanose, isomaltosyltetraose and isomalto sylpentaose (Jeffrey *et al.,* 1996). In addition, honey also contains proteins, enzymes, amino and organic acids, lipids, vitamins, minerals, flavor compounds and flavonoids and phenolic compounds (Alvarez *et al.,* 2010 and Arawwawala *et al.,* 2017).

Some of the compounds in honey and its antioxidant properties are well known. Antioxidant properties come from enzymatic and non-enzymatic substances (Gheldof *et al.,* 2002). The amount and type of antioxidants are highly dependent on the source of flowers or varieties of honey, and many studies have shown that there is a relationship between antioxidant activity and total phenol content (Khalil, 2012). Antioxidants are compounds that can slow down and prevent the lipid oxidation process (Ahmad *et al.,* 2012). This compound has a small molecular weight but can inactivate the oxidation reaction of the formation of radicals, so it can inhibit cell damage in the body where the presence of antioxidants is an important parameter to monitor a person's health (Cai *et al.,* 2004; Winarsi 2007). Previous research show that honey is a source of natural antioxidants that are effective in reducing liver damage, cancer, immune-system decline, cataracts, different inflammatory processes (Gheldof *et al.,* 2002). In general, Indonesian people use honey as a mixture in herbal medicine to cure diseases such as infections of the gastrointestinal tract, respiratory tract, and improve body fitness. In addition, honey has the ability to increase the speed of new tissue growth (Wineri, 2014).

Some examples of the use of herbal plants that are commonly mixed with honey such as garlic (*Allium sativum*), containing antioxidants that support the body's protective mechanisms, can be utilized as a therapeutic (Prasonto *et al.,* 2017). Ginger (*Zingiber officinale*) contains volatile and non-volatile compounds of various flavonoid compounds and polyphenols that have high antioxidant activity in preventing the presence of free radicals (Supriyanti 2015). Lemons (*Citrus limon* L.) also contains bioflavonoids, polyphenols, coumarins that have benefits as natural antioxidants (Nizhar 2012).

The purpose of this study was to determine the compounds contained in a mixture of herbal honey that have antioxidant properties that are beneficial for health.

Materials and methods

Sample preparation

The honey comes from forest honey breeders in Malang, Indonesia and the type of honey produced comes from the flower of kapok wood (Randu). The composition of herbal honey consists of 90% of Randu Honey; 1.1% ginger; 0.9% aromatic ginger; 1.1% garlic; 0.8% bitter powder; 0.6% capulogo and 0.6% lemon. The process of making herbal honey is mixing honey with sliced ginger, sliced aromatic ginger, sliced garlic, bitter powder, cardamom and lemon into a container and closed for 20 hours. After that do the filtering of herbal honey and packed in bottles.

Determination of antioxidant activity

Herbs Honey were analyzed for its antioxidant activity using DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay The extract was weighed 20mg, then dissolved with ethanol until the volume was 40 mL. The concentration of the sample extract solution was obtained at 500ppm. Then the dilution was carried out from a concentration of 500ppm,

then taken sequentially from 100µL, 250µL, 500µL, 750µL, 1000µL to obtain concentrations of 10, 25,50, 75 and 100ppm using a micro pipette and dissolved to 5 mL. Then incubated at 250C for 30 minutes in a dark room then the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer (Khalaf *et al.,* 2008). The percentage of antioxidant activity of the fraction in capturing or dampening DPPH expressed in 5 inhibitions obtained using the formula:

$$\%AA=100-((A_B-A_A))\times 100/A_{KN})$$

IC_{50} values are calculated each using the formula of the linear regression equation y = ax +b, where the x-axis is concentration and the y-axis is the inhibition. With a value of y is 50 and a value of x as IC_{50}. The IC_{50} value is to state the concentration of the sample solution needed to reduce DPPH by 50% (Dewi *et al.,* 2016).

Determination of phytocomponent Gas chromatography and mass spectroscopy (GC-MS)

The volatile compound from Herbal Honey analyzed for phytocomponent using GC-MS QP2010SE-Shimadzu under the following condition: column used were ZB-AAA, 10 m length and inner diameter of 0.25 mm and the initial column temperature was 60°C and final temperature was 220°C (6°C/minute), while the injector temperature was 280° C with split mode injector and split ratio of 127.5 and pressure of 15.0 kPa. The flow rate was 30 ml/minute and the flow within the column was 0.60 ml/minute. The detector temperature was 280°C and using Helium as the gas carrier with EI (Electron Impact); and the samples volume injected was 1µl. Compounds were identified by comparing retention indices/comparing mass spectra of each compound with those of authentic samples and library

Liquid chromatography and mass spectrometry (LC-MS)

The coumpond of Herbal Honey were analyzed for phytocomponent using 8040LC/MS Shimadzu under the following conditions: column used were shim Pack FO-ODS (2mm x 150mm, 3µm) capilary voltage 3,0 kv and the initial column temperature was 35°C, while the sample Injection volum 1 µl. The flow gradient was 0/100 at 0 min, 15/85 at 5 min, 21/79 at 20 min, 90/100 at 24 min. The flow rate 0,5 ml/min, were sampling cone 23,0 V with solvent Ethanol 95% and MS focused ion mode are $[M]^+$, while collison energy 5,0 V and Desolvation gas flow 60 ml/hr. The initial Desolvation temperature 350°C use fragmentation method low energy CID and Ionization ESI for Scanning 0,6 sec/scan (mz: 10-100) with Source temperature 100°C and Run time 50 minute

Result and discussion

Identification chemical compound of Herbal Honey by GC-MS

A typical gas chromatogram of Herbal Honey is shown in Fig. 1 and list of the compounds identified appears in Table 1. Seventy six compounds of Herbal Honey were identified using GC-MS. Seventy six compounds identified percentages ranged from 0.370% - 4.917% and compounds with the highest percentage values were α-thujene (4.917%) followed by 1-dehydrogngerdione (3,080), zingerone (2,722%), geranial (2,567%), eugenol (2,369%). The types of compounds identified in herbal honey can be classified as volatile compounds. Associated with the process of making herbal honey. The entire herbal plant material is reduced in size in the form of a slice then closed and left overnight. The process of making herbal honey was reported by Rahmi *et al.,* 2019 by reducing the size of the material so that volatile compounds can be easily released so that the value of antioxidant activity increases. α-thujene compounds including monoterpenoid compounds are classified as volatile compounds. Reported by Zhou *et al.,* 2002; Bastos and Alves 2003 The main volatile components in honey consist of alcohols, ketones, aldehydes, acids, esters, terpenes.

Identification chemical compound of Herbal Honey by LC-MS

Herbal honey compounds identified by LC-MS are shown in Fig. 2 and details of compound identification are in Table 2. A total of 199 types of compounds were identified using LC-MS and the percentages ranged from 0.078% - 12.502%. The compound with the highest percentage was glucose (12.502%) followed by fructose (7.247%), galactose (4.843%). As well as several amino acid group compounds such as valine, proline.

Table 1. Major pytho-components from Honey Herb using analyzed GC-MS.

Peak	Compound	% RA
1	methyl acetate	1,105
2	ethyl acetate	1,170
3	p-cymene	1,342
4	2,6-dimethyl-1,3,5,7-octatetrane	0,762
5	chavicol	0,953
6	α-phelandrene	2,620
7	β-phellandrene	1,208
8	terpinolene	0,959
9	α-thujene	4,917
10	α-pinene	1,744
11	β -pinene	2,152
12	camphene	0,794
13	β -myrcene	1,302
14	Limonene	2,369
15	(+)-2-carene	1,024
16	neo-allo-ocimene	2,265
17	α-terpinene	1,080
18	γ -terpinene	1,081
19	pentyl propanoate	0,836
20	thymol	1,577
21	p-menth-3-en-1-ol	1,221
22	(+)-pinol	1,077
23	(E)-3,7-dimethyl-3,6-octadienal	0,835
24	citral	1,590
25	myrtenol	1,087
26	β-thujone	0,957
27	geranial	2,567
28	(+)-trans-piperitenol	2,549
29	sabinene	2,153
30	cis-rose oxide	0,557
31	borneol	1,087
32	linalool	0,952
33	citronellal	0,912
34	α-terpineol	0,669
35	geraniol	1,743
36	nerol	0,671
37	menthol	2,085
38	eugenol	2,369
39	methyl eugenol	1,740
40	zingerone	2,722
41	bornyl acetate	1,079
42	calacorene	0,376
43	calamenene	0,454
44	calamene	0,385
45	α-farnesene	0,598
46	β-farnesene	0,825
47	α-cadinene	1,092
48	α-trans-bergamotene	0,597
49	α-cubebene	0,836
50	α-muurolene	0,761
51	β-sesquiphellandrene	0,705
52	α-ylangene	1,217
53	β-bisabolene	0,840
54	β-caryophyllene	0,828
55	α-zingiberene	1,743
56	β-elemene	0,977
57	β-himachalene	1,258
58	δ-elemene	0,595
59	allo aromadendrene	1,079
60	sesquithujene	0,984
61	α-selinene	0,834
62	γ-selinene	0,834
63	δ-cadinene	0,381
64	zerumbodienone	2,622
65	(E)-4-(3,4- dimethoxyphenyl)but-3-en-1- ol	1,868
66	trans-β-sesquiphellandrol	1,728
67	aromadendrene oxide	1,085
68	elemol	0,633
69	farnesol	1,745
70	juniper camphor	1,259
71	α-copaene	0,505
72	β-eudesmol	1,340
73	α-cadinol	1,257
74	α-cedrol	0,857
75	1-dehydrogingerdione	3,080
76	ajoene	1,692

Table 2. Major pytho-components from Honey Herb using analyzed LC-MS.

Peak	Compound	%RA
1	dimethyl sulfide	0,191
2	allyl mercaptan	0,272
3	glycine	0,560
4	alanine	0,369
5	allyl methyl sulfide	0,175
6	2-methyl butanal	0,078
7	isobutyric acid	0,206
8	dimethyl disulfide	0,209
9	serine	0,485
10	(E)-2-hexenal	0,116
11	furfural	0,215
12	hexanal	0,078
13	proline	0,309
14	valine	0,567
15	threonine	0,402
16	heptanal	0,169
17	diallyl sulfide	0,154
18	3-hexenol	0,058
19	2-ethylfuran	0,173
20	2-methylbutanoic acid	0,167
21	cystein	0,302
22	methyl propyl disulfide	0,074
23	2-acetylfuran	0,173
24	5-methylfurfural	0,169
25	niacin	0,402
26	malic acid	0,579
27	α-terpinene	0,176
28	α-pinene	0,302
29	β-pinene	0,248
30	sabinene	0,158
31	dimethyl trisulfide	0,192
32	terpinolene	0,189
33	β-phellandrene	0,207
34	camphene	0,077
35	α-thujene	0,183
36	myrcene	0,293
37	γ-terpinene	0,173
38	α-phellandrene	0,250
39	diallyl disulfide	0,144
40	pentyl propanoate	0,124
41	(+)-2-carene	0,254
42	(E)-anethole	0,078
43	cinnamic acid	1,085
44	(E)-3,7-dimethyl-3,6-octadienal	0,127
45	(Z)-3,7-dimethyl-3,6-octadienal	0,096
46	chrysanthenone	0,661
47	arabinose	2,071
48	thymol	0,077
49	citral	0,085
50	geraniol	0,301
51	borneol	0,187
52	linalool	0,147
53	dipropyl disulfide	0,174
54	α-terpineol	0,130
55	3,4-dihydroxybenzoic acid	0,213
56	allicin	0,602
57	menthol	0,477
58	(-) S-allyl-L-cysteine	0,212
59	trans-S-(1-propenyl)-L-cysteine	0,189
60	isoleucine	0,814
61	leucine	1,085
62	asparagine	0,350
63	myrtenol	0,128
64	aspartic acid	0,360
65	p-coumaric acid	0,910
66	rhamnose	1,718
67	glutamine	0,300
68	lysine	1,085
69	glutamic acid	1,253

Peak	Compound	%RA
70	methionine	0,707
71	xylose	1,673
72	(+)-trans-piperitenol	0,150
73	histidine	0,579
74	phenylalanine	0,779
75	eugenol	0,367
76	vanillic acid	0,953
77	pyridoxine	0,373
78	linalool oxide	0,184
79	gallic acid	1,545
80	1,2-dihydro-2,5,8-trimethylnaphthalene	0,074
81	arginine	0,502
82	ascorbic acid	0,706
83	methyl eugenol	0,365
84	alliin	0,737
85	cycloalliin	0,497
86	isoalliin	0,418
87	esculetin	0,408
88	diallyl trisulfide	0,077
89	allyl trisulfide	0,207
90	caffeic acid	1,089
91	fructose	7,247
92	glucose	12,502
93	galactose	4,843
94	myo inositol	0,600
95	tyrosine	0,508
96	methylselenocysteine	0,147
97	dimethyl diselenide	0,189
98	citric acid	0,497
99	galacturonic acid	0,556
100	ferulic acid	1,183
101	bornyl acetate	0,125
102	calacorene	0,078
103	calamene	0,192
104	ar-curcumene	0,497
105	tryptophan	0,403
106	α-copaene	0,148
107	β-caryophyllene	0,149
108	δ-cadinene	0,124
109	β-farnesene	0,097
110	α-selinene	0,096
111	β-sesquiphellandrene	0,157
112	β-bisabolene	0,484
113	β-elemene	0,192
114	γ-selinene	0,097
115	α-zingiberene	0,673
116	diallyl tetrasulfide	0,097
117	(E)-4-(3,4- dimethoxyphenyl)but-3-en-1- ol	0,049
118	jasmonic acid	0,215
119	ar-curcumen-15-al	0,444
120	morindacin	0,497
121	borreriagenin	0,126
122	zerumbone	0,556
123	pantothenic acid	0,307
124	aromadendrene oxide	0,156
125	spathulenol	0,271
126	α-cadinol	0,050
127	juniper camphor	0,078
128	(E)-4-(2,4,5- trimethoxyphenyl)but-3-en-1- ol	0,559
129	(E)-4-(3,4- dimethoxyphenyl)but-3-en-1- yl acetate	0,056
130	sinapic acid	0,077
131	ajoene	0,110
132	1-dehydrogingerdione	0,169
133	chrysandiol	0,603
134	allyl pentasulfide	0,078
135	thiamin	0,365
136	morindone	0,508
137	apigenin	0,601
138	naringenin	0,450
139	[6]-dehydroshogaol	0,302
140	diallyl hexasulfide	0,154

Peak	Compound	%RA
141	[6]-shogaol	0,485
142	chrysartemin A	0,780
143	chrysartemin B	0,589
144	[6]-paradol	0,372
145	luteolin	0,365
146	kaempferol	0,989
147	fisetin	0,497
148	6-dehydrogingerdione	0,189
149	[6]-gingerdione	0,318
150	γ-glutamyl-S-trans-1- propenyl-cysteine	0,250
151	glutathione	0.148
152	[6]-gingerol	0,509
153	[6]-gingerdiol	0,377
154	morenone-1	0,291
155	[8]-dehydroshogaol	0,099
156	morin	0,554
157	quercetin	0,978
158	[8]-shogaol	0,577
159	bis-demethoxycurcumin	0,477
160	diallyl heptasulfide	0,366
161	myricetin	0,405
162	5,15-dimethylmorindol	0,189
163	[8]-gingerol	0,271
164	1,5-bis(4-hydroxy-3- methoxyphenyl)-penta- (1E,4E)-1,4-dien-3-one	0,097
165	gingerenone C	0,189
166	citrusin C	0,156
167	Homoglutat,093hione	0,324
168	Chlorochrymorin	0,209
169	Morintrifolin A	0,378
170	[10]-dehydroshogaol	0,238
171	4- hydroxycinnamoyl(feruloyl)me thane	0,267
172	chrysanin	0,371
173	[10]-gingerol	0,477
174	1-caffeoylquinic acid	0,707
175	Chlorogenic acid	1,673
176	allithiamine	0,209
177	[1,7-bis(4-hydroxy-3- methoxyphenyl)hepten-3-one]	0,127
178	gingerenone A	0,346
179	[6]-gingesulphonic acid	0,286
180	4-gingesulphonic acid	0,214
181	morintrifolin B	0,343
182	curcumin	0,495
183	hexahydrocurcumin	0,408
184	riboflavin	0,191
185	gingerenone B	0,366
186	roseoside	0,074
187	S(2-carboxypropyl)glutathione	0,319
188	squalene	0,189
189	cyanidin-3-arabinoside	0,367
190	β-amyrin	0,191
191	gitogenin	0,241
192	astragalin	0,344
193	cyanidin-3-O-glucoside	0,448
194	agigenin	0,232
195	chrysanthemin	0,497
196	isoquercitrin	0,961
197	delphinidin-3-glucoside	0,365
198	raffinose	2,147
199	cyanidin-3-(6'- malonylglucoside	0,291

It was reported by Bobis *et al.,* 2018 that honey contains more than 200 components of compounds including fructose, glucose and water as main substances. And Hermosin *et al.,* 2003 have reported that honey also contains several types of abundant amino acids such as proline which constitues 50-85% there are 26 amino acids such as glutamic acid, aspartic acid, glutamine, histidine, glycine, threonine, β-alanine, arginine, α-alanine, aminobutyric acid, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine, tryptophan, phenylalanine, ornithine, lysine, serine, asparagine and alanine. Phenolic compounds (gallic acid, gingerol, shogaol), flavonoids (quercetin, kaempferol) and terpenes (α-

thujene, cinnamic acid) even though the percentage is small but can be classified as compounds that increase antioxidant activity (Kumazawa 2012).

Free radical scavening activity

The antioxidant activity is expressed by the IC_{50} parameter, that the concentration of sample required to inhibit 50% of free radicals is DPPH. The lower the IC_{50} value, the smaller the concentration of the sample used to ward off 50% of free radicals, meaning that the sample is stronger in counteracting free radicals (Pontis *et al.,*. 2014). The IC_{50} value of herbal honey is 48.35, which means that the active compounds in herbal honey have very strong antioxidant activity. The results of the IC_{50} value produced in this study have the same value as reported by Rahmi *et al.,* 2020 of 50 by referring to the antioxidant properties of IC_{50} which have been reported by Molyneux 2004 that IC_{50} values ranging from 50 -100ppm are quite strong. The antioxidant activity of the material is influenced by the content of phytochemical compounds that act as antioxidants, such as phenolic acids, flavonoids, enzymes (glucose oxidase and catalase), ascorbic acid, carotenoids, organic acids, amino acids and proteins. (Khalil, M.I. *et al.,* 2010.

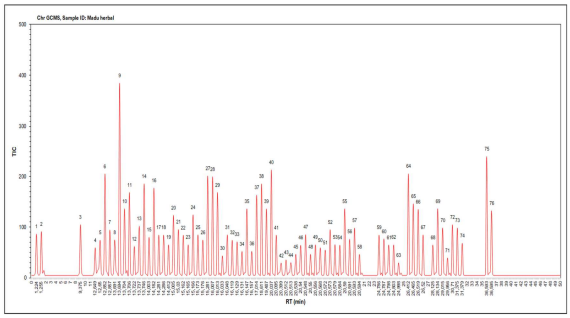

Fig. 1. GC-MS chromatogram of Herbal Honey.
Source: Results of laboratory analysis (Triswaningsih, 2021)

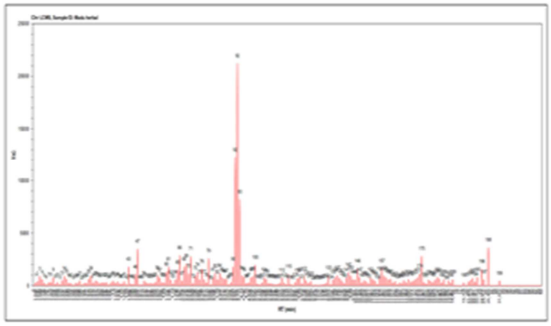

Fig. 2. LC-MS chromatogram of Honey Herb.
Source: Results of laboratory analysis (Triswaningsih, 2021)

Conclusion

Compounds of herbal honey can be identified using GC-MS and LC-MS analysis, where the compounds that have been identified are phenolic compounds, polyphenols, and flavonoids. The IC_{50} value of 50, provides information that it has strong antioxidant properties.

References

Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M. 2010. "Contribution of Honey in Nutrition and Human Health: A Review." Mediterranean J. Nutr. Metab **3,** 15-23.

Ahmad R, Munim A, dan Elya B. 2012. Study of Antioxidant Activity with Reduction of Free Radical DPPH and Xanthine Oxidase Inhibitor of the Extract *Ruellia tuberosa* Linn Leaf. International Research Journal of Pharmacy **3(11).**

Arawwawala LDAM, Hewageegana HGSP. 2017. "Health Benefits and Traditional Uses of Honey: A Review." J. Apither **2,** 9-14.

Bastos C, Alves R. 2003. Compostos voláteis emméis florais. Quim. Nova **26,** 90-96.

Bobiş O, Dezmirean DS, Moise AR. 2018. "Honey and Diabets: The Importance of Natural Simple Sugars in Diet for Preventing and Treating Different Type of Diabets." Hindawi, Oxid. Med. Cell Longev. https://doi.org/10.1155/2018/4757893.

Cai Y, Luo Q, Sun M, Corke H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science **74,** 2157-2184.

Dewi T, Alifah I, Bhayangkara T, P dan Jason GJ. 2016. Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung (*Mimusops elengi* L). Prosiding Seminar Nasional Teknik Kimia 1-7

Escuredo O, Míguez M, Fernández-González M, Seijo MC. 2013. "Nutritional Value and Antioxidant Activity of Honeys Produced in a European Atlantic Area." Food Chem **138,** 851-856.

Gheldof N, Wang XH, Engeseth NJ. 2002. Identification and quantification of antioxidant components of honeys from various floral sources. J. Agric. Food Chem **50,** 5870-5877.

Gebremariam T, Brhane G. 2014. Determination Of Quality And Adulteration Effects Of Honey From Adigrat And Its Surrounding Areas. International Journal Of Technology Enhancements And Emerging Engineering Research **2,** 2347-4289.

Hermosin I, Chicón RM, Dolores Cabezudo M. 2003. "Free Amino Acid Composition and Botanical Origin of Honey." Food Chem **83,** 263-268.

Israili ZH. 2014. "Antimicrobial Properties of Honey." Am. J. Therapeutics **21,** 304-323

Jeffrey AE, Echazarreta CM. 1996. "Medical Uses of Honey." Rev. Biomed **7,** 43-49.

Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z, Farah H. Antioxidant Activity of Some Common Plants. Turky Journal Biology **51,** 55. 2008.

Khan F, Abadin ZU, Rauf N. 2007. "Honey: Nutritional and Medicinal Value." Int. J. Clin. Pract **61,** 1705-1707.

Khalil MI, Sulaiman SA, Boukraa L. 2010. Antioxidant Properties of Honey and its Role in Preventing Health Disorder. The Open Nutraceuticals Journal Vol. **3,** 6-16

Khalil MDI. 2012. Content and Antioxidant Properties of Processed Tualang Honey Collected From Different Regions in Malaysia. International Journal of Pharmacy and Pharmaceutical Sciences **4,** 214 - 219.

Kumazawa S. 2012. Antioxidant Activity in Honeys of Various Floral Origins: Isolation and Identification of Antioxidants in Peppermint Honey. Food Science and Technology Research **18(5),** 679 ± 685.

Molyneux P. 2004. The Use of the Stable Free Radical Diphenylpicryl-Hydrazyl (DPPH) for Estimating Antioxidant activity. Songklanakarin Journal of Science Technology **26(2),** 211 - 216.

Nizhar U. 2012. Level Optimum Sari Buah Lemon (*Citrus limon*) Sebagai Bahan Penggumpal Pembuatan Keju Cottage. Skripsi. Fakultas Peternakan, Universitas Hasanuddin. Makassar.

Pontis JA, Costa MA, Silva SJR, Flach A. 2014. Color, Phenolic, Flavonoid Content and Antioxidant Activity of Honey from Roraima, Brazil. Food Science and Technology **34(1),** 69-73

Prasonto D, Riyanti E, dan Gartika M. 2017. Uji Aktivitas Antioksidan Ekstrak Bawang Putih (*Allium sativum*). ODONTO Dental Journal **4(2),** 122-128.

Rahmi S, Hasanuddin Husin. 2020. Analisis Sensori dan Aktivitas Antioksidan Menggunakan Metode DPPH Pada Campuran Bawang Putih, Jahe, Lemon dan Madu Sebagai Suplemen Herbal. Pro Food (Jurnal Ilmu dan Teknologi Pangan) **6(1),** Mei 2020 e-ISSN: 2443-3446. P. 599-608

Supriyanti H. 2015. Untung Besar Budidaya Jahe Merah. Araska. Yogyakarta.

Winarsi H. 2007. Antioksidan Alami dan Radikal Bebas. Yogyakarta: Kanisius.

Wineri E. 2014. Perbandingan Daya Hambat Madu Alami dengan Madu Kemasan secara In Vitro terhadap Streptococcus beta hemoliticus Group A sebagai Penyebab Faringitis. Jurnal Kesehatan Andalas.

Zhou Q, Wintersteen CL, Cadwallader RW. 2002. Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey. J. Agric. Food Chem **50,** 2016-2021.