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ANTIOXIDANT, ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF EXTRACT OF GIANT FOREST ANT *Dinomyrmex gigas* (Latreille, 1802)

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Abstract. *Giant forest ant Dinomyrmex gigas is one of the largest ants species in the world, native to the rain forests of Southeast Asia. It is known that ants have glands that produce chemical compounds that inhibit the growth of microbes. Therefore, it is necessary to determine the antioxidant and antibacterial activities as well as identify the chemical compounds of D. gigas extract. D. gigas was extracted successively with n-hexane, ethanol and methanol. The antioxidant activity was evaluated by determination of the half-maximal inhibitory concentration (IC_{50}) values while the antibacterial activities of the extracts were determined by measuring the minimum inhibitory concentration (MIC). The results exhibited that the IC_{50} values of n-hexane, ethanolic and methanolic extracts were 336.18±0.0984, 89.16±0.0219 and 90.72±0.0894 µg/mL respectively. The ethanolic extract exhibited the highest AAI value (0.34) followed by methanolic extract (0.33) and n-hexane extract (0.09). Based on AAI values, the extracts were classified as moderate antioxidants. The best MIC values were 625 µg/mL for both ethanolic and methanolic extracts against S. aureus, while MIC values of all extracts against E. coli were >625 µg/mL. Based on MIC values, all of the extracts presented weak activity against both S. aureus and E.coli. The GC-MS analysis showed that there are up to 30 compounds constructed of the ethanolic extract. Three major compounds are ethyl oleate (29.78%), n-hexadecanoic acid (17.54%) and oleic acid (10.65%).*

Keywords: *ant, antibacterial, antioxidant, Dinomyrmex gigas*

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INTRODUCTION

In recent years, the search for new antibacterial sources becomes very important because the resistance of pathogenic bacteria to antibacterial agents has increased significantly. The search for natural antioxidants also gain a lot of attention, this is due to several advantages that natural antioxidants have compared to synthetic antioxidants, namely easy to obtain and have little or insignificant side effects as well as more economically valuable (Vicas et al., 2012). Studies on the medicinal use derived from animals, especially insects have been neglected when compared to plants (Wilsanand et al., 2007). Insects have been well known to live in humid environments that support the growth of pathogenic micro-

Jurnal Biodjati 4(2):263-277, November 2019

JURNAL BIODJATI

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organisms. They have evolved some defense mechanisms against diseases to protect their colonies. One of the defense mechanisms is by producing antimicrobial compounds (Stow & Beattie, 2008), this makes insects as a promising source of new antibiotics (Mendonca et al., 2009; Zeng et al., 2016).

Ants are one of the genus of insects that are considered to be able to produce potent antimicrobial compounds. Based on previous researches by Bot et al. (2002) and Marin et al. (2006), it indicated that one of the primary sources of antimicrobial compounds in various ant species was the metapleural gland. Besides phenolic compounds and carboxylic acids, fatty acids are the major chemical compounds of metapleural gland secretions (Yek & Mueller, 2011). The metapleural gland in *Crematogaster deformis* contains a mixture of phenol which has antiseptic properties.

The metapleural glands in some ant lineages (Dinomyrmex (Camponotus), Oecophylla and Polyrhachis) have been lost (Johnson et al., 2003), several of these ants lineages have developed the proficiency to generate antimicrobial compounds through other glands. The venom glands in some ant species have been identified as a source of antifungal and antibacterial. Ants of subfamily Formicinae, includes Dinomyrmex produce formic acid in their venom glands which are used for defense against vertebrates and other insects, that could also be used to inhibit the growth of fungi (Falotico et al., 2007; Tragust et al., 2013; Tranter & Hughes, 2015). Solenopsins use venom glands to inhibit the growth of *Streptococcus pneumoniae, Staphylococcus aureus and Enterococcus faecalis* (Sullivan et al., 2009). Stinging ants usually use venom glands contain alkaloids, and peptides that possess antimicrobial properties (Moreau, 2013). The growth of both *E. coli* and *S. aureus* can be inhibited by peptides in *Myrmecia pilosula's* venom glands (Inagaki et al., 2004), while ants that do not have stingers, such as ants of the subfamily Dolichoderinae produce antimicrobial compounds from the pygidial glands.

Besides producing compounds that inhibit microbial growth, some of the ants may also have insecticidal activity (Orivel et al., 2001), antitumor against breast cancer cells (Badr et al., 2012), antioxidant (Ebaid et al., 2014), hypolipidemic effects and anti-inflammatory (Ebaid et al., 2012). Therefore, the bioactivity of ants extracts as antioxidant and antimicrobial needs to be examined, since antibacterial and antioxidant compounds have a fundamental role in preserving and intensifying the quality of human life.

Giant forest ant *Dinomyrmex gigas* (Latreille, 1802), (Figure 1) is one of the largest ants species in the world, native to the rain forests of Southeast Asia. The smallest *D. gigas* has a head width of 3.56 mm with a weight of 135 mg, and the largest has a head width of 6.9 mm and weighs 372 mg (Pfeiffer & Linsenmair, 2007). To the best of our knowledge, *D. gigas* extract has not been investigated to examine the antioxidant and antibacterial activities as well as the identification of their chemical compounds. Based on that reason, it is crucial to investigate their properties.

Figure 1. Giant Forest Ant *Dinomyrmex gigas* (Latreille, 1802)

MATERIALS AND METHODS

Sample Collection

Colonies of *D. gigas* were collected from Bangkirai Hill, East Kalimantan in September 2018. The major workers approximately 28-30 mm in length. Identification was carried out at the Museum Zoologicum Bogoriense, Research Center for Biology-Indonesian Institute of Sciences.

Sample Extraction

One hundred grams of *D. gigas* were crushed and extracted with n-hexane, ethanol and methanol (3 x 24 hours) respectively. The filtrates of each solvent were collected and dried utilizing a rotary evaporator at 35°C to gain a crude extract, and the yield was determined.

Chemical Compounds Analysis by Thin Layer Chromatography (TLC)

The analysis of chemical compounds of *D. gigas* extracts was carried out by TLC. Ten microliters of n-hexane extract with a concentration of 10 mg/mL were transferred to the TLC silica plate (Merck F254) and developed with an eluent system of hexane:ethyl acetate (9:1). Separated chemical compounds were observed under UV light (254 nm), and followed by spraying with color reagent (1% vanillin-sulphuric acid and 1% cerium (IV) sulfate). The ethanolic and methanolic extracts were developed with an eluent system of chloroform:methanol:water (6:4:1).

Antioxidant Activity Assay

The antioxidant activity assay was carried out by the TLC-bioautography method (Wang et al., 2012). Ten microliters of the extract with a concentration of 10 mg/mL were transferred to the TLC silica plate and sprayed with a solution of 0.2% 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. (+)-Catechin (Sigma-Aldrich) was used as a positive control. The yellow spot against the purple background after spraying with DPPH reagent indicated the antioxidant activity.

Determination of IC_{50} Value and Antioxidant Activity Index (AAI)

The IC_{50} value of extracts was carried out by serial microdilution using a 96-well microplate (Takao et al., 2015). The wells of column A was filled with 180 µL of methanol p.a. and 20 µL of extract (10240 µg/mL in DMSO) and mixed thoroughly. Then, 100 µL of methanol p.a was filled in the wells of column B through H. The serial dilution was undertaken with an appropriate final concentration of 512 µg/mL. After finish diluting, each well was added with 100 µL of DPPH solution with a concentration of 61.50 µg/mL. The assay was performed in triplicate. The plates were incubated for 90 minutes under dark conditions at room temperature and the absorbances of the extracts were measured at 517 nm (Varioscan flash, Thermo scientific). The following equation was used to calculate the AAI (Scherer & Godoy, 2009):

$$AAI = \frac{\text{The final concentration of DPPH in the reaction } (\mu g/ml)}{IC_{50}\ (\mu g/ml)}$$

The inhibitory concentration (IC) was calculated using the following equation:

$$IC\ (\%) = \frac{(A_o) - (A_s)}{(A_o)} \times 100\ \%$$

where, Ao and As are the absorbances of the negative control and the sample at different concentrations, respectively.

Jurnal Biodjati 4(2):263-277, November 2019

JURNAL BIODJATI

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Antibacterial Activity Assay

The antibacterial activity assay was carried out by the TLC-bioautography method (Dewanjee et al., 2015). Reference bacterial strains were *Staphylococcus aureus* Ina-CC B4 and *Escherichia coli* Ina-CC B5. Ten microliters of the extract with a concentration of 10 mg/mL were transferred to the TLC silica plate, then it was dipped into bacterial suspension in Mueller-Hinton Broth. The TLC plate was incubated under the humid condition at 37°C for 18 h and sprayed with a solution of p-iodonitrotetrazolium (INT) 4 mg/mL. The inhibition of bacterial growth was indicated by the formation of clear white zones around the extract. Chloramphenicol was used as a positive control.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC value of extracts was carried out by serial microdilution using a 96-well microplate (Pessini et al., 2003). The wells of column A was filled with double-strength of MHB (100 µL), 90 µL of distilled water and 10 µL of extract (25000 µg/mL in DMSO), and mixed thoroughly. Then, a total of 100 µL of MHB was filled in columns B through H. The serial dilution was undertaken with an appropriate final concentration of 625 µg/mL. After finish diluting, each well was added with 100 µL of bacterial suspension (10^5 CFU/mL). The assay was performed in triplicate. After 18 hours of incubation at 37°C, 10 µL of INT with a concentration of 4 mg/mL was added to each well. The color change to red indicates the presence of bacterial growth after added with the INT solution.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was performed using a GC-MS QP 2010 Ultra (Shimadzu, Japan). An exact quantity of six microliters of the extract with a concentration of 1 mg/mL was injected. The chromatographic separation was performed on an Rtx-5-MS column (30 m x 0.25 mm x 0.25 µm, RESTEK, USA) with a flow rate of Helium at 2 mL/min and initial pressure was 121.1 kPa. The injector temperature was 230°C, and then applying for the column temperature program as follows: 50°C for 5 min, 150°C at the rate of 10°C/min and then 250°C for 15 min. The chemical compounds of extract were identified by comparing the mass spectra with the database of NIST 11 (National Institute Standard and Technology, US) and WILEY 8.

RESULTS AND DISCUSSION

The results of this study indicated that the solvent of extraction influence the yield of extracts. The maceration method was used due to its simple treatment and avoiding damage to the active compounds of *D. gigas* that are not resistant to high temperatures during the separation process. The percentage yield of the methanolic extract was found to be the highest (1.948 %), followed by ethanolic extract (0.225 %) and n-hexane extract (0.0129 %).

Analysis of the Chemical Compounds of the Extracts by TLC

The chromatogram profile of *D. gigas* extract with different solvents provided a different chromatogram pattern (Figure 2). Most aromatic molecules or molecules that have conjugated double bonds appear as dark stain spots against the bright-green background on short-waved UV light (254 nm). The formation of different colored stain spots after the TLC plate was sprayed with vanillin sulphuric acid and cerium (IV) sulfate as a color reagent indicates the presence of various chemical compounds in each ex-

tract. Different retention factors on several stain spots indicate that the crude extract of *D. gigas* contained several chemical compounds. The compounds that have the same physical properties as the mobile phase will be retained longer in the mobile phase. The more soluble compounds will be carried by the mobile phase the further up the plate, whereas the less soluble compounds, the higher affinity for the stationary phase will be left behind on the TLC plate.

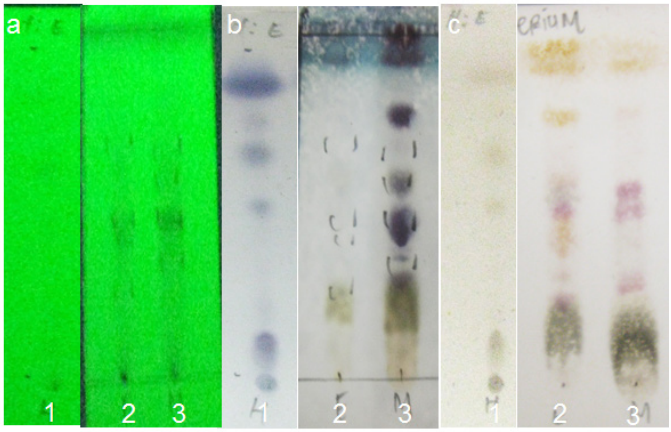

Figure 2. Chromatograms of *D. gigas* extracts: (1) n-hexane extract developed with an eluent system of hexane:ethyl acetate (9:1), (2) ethanolic and (3) methanolic extracts developed with an eluent system of chloroform:methanol:water (6:4:1), (a) viewed under 254 nm, (b) sprayed with 1% vanillin-sulphuric acid, (c) sprayed with 1% cerium (IV) sulfate).

Antioxidant Activity

The qualitative analysis of antioxidant was carried out by the TLC-bioautography on DPPH radical scavenging activity. The TLC-chromatogram (Figure 3) showed the yellow spot on the purple background.

DPPH free radical is most extensively used to evaluate antioxidant activity because it is a stable free radical. The yellow spot on the purple background indicated the presence of the antioxidant activity of the extract (Wang et al., 2012). Based on the result, it indicated that all of the *D. gigas* extracts had antioxidant activity. The antioxidant capacity was indicated by the intensity of the yellow color. The extracts were furthermore analyzed by a quantitative method to determine their potential as an antioxidant. The parameter used in antioxidant activity assay was IC_{50}, defined as the amount of extract concentration required to produce a 50% reduction of DPPH free radical (Molyneux, 2004). The extract concentration and the IC values giving the linear regression equation was used to calculate the IC_{50} value. A calibration curve in the linear by plotting the extract concentration vs the IC value gave a positive correlation coefficient (Figure 4), indicating that the more antioxidant compounds in the extract, the more the ability to reduce free radical activity.

The results showed that the ethanolic extract of *D. gigas* exhibited the highest antioxidant activity with an IC_{50} value of (89.16±0.0219 µg/mL) followed by methanolic extract (90.72±0.0894 µg/mL) and n-hexane extract (336.18±0.0984 µg/mL). (+)-Catechin as a positive control has an IC_{50} value of 2.03±0.1115 (Table 1).

Jurnal Biodjati 4(2):263-277, November 2019

JURNAL BIODJATI

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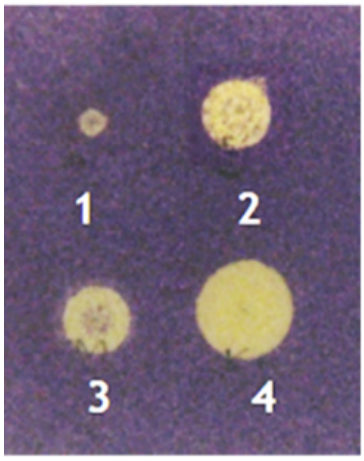

Figure 3. Bioautography of antioxidant activity of *D. gigas*: (1) n-hexane extract, (2) ethanolic extract, (3) methanolic extract and (4) (+)-catechin.

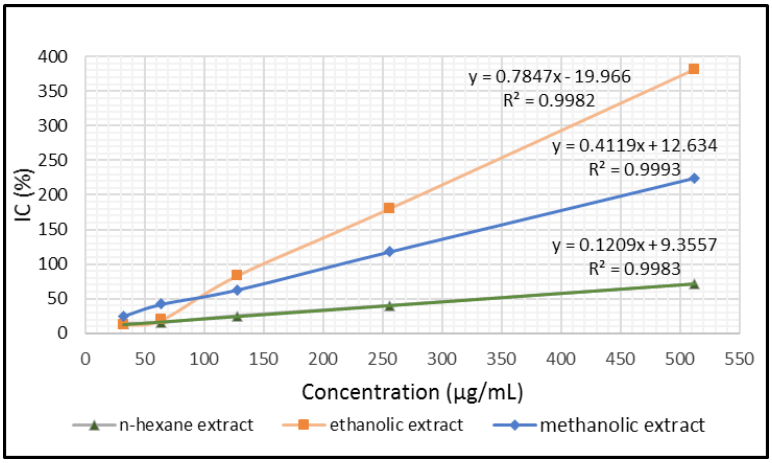

Figure 4. Linear correlation between the extract concentration of *D. gigas* and the IC(%).

Table 1. Antioxidant Activity Index (AAI) and the IC_{50} value of *D. gigas* extracts

No	Sample	IC_{50} (µg/mL)	AAI
1	n-Hexane extract	336.18±0.0984	0.09
2	Ethanolic extract	89.16±0.0219	0.34
3	Methanolic extract	90.72±0.0894	0.33
4	(+)-Catechin	2.03±0.1115	15.18

The IC_{50} values were represented as the means ± standard deviation

The classification of the antioxidant activity of extracts is presented in Table 2. Based on AAI values, those of three extracts were classified as moderate antioxidants, while (+)-catechin showed very strong antioxidant properties. This indicates that the lower the value of IC_{50}, the higher the antioxidant activity index (Molyneux, 2004). The AAI values for the ethanolic and methanolic extracts were higher than that of the n-hexane extract. The difference in antioxidant activity is caused by the efficiency of solvents in extracting antioxidant compounds. Polar solvents such as ethanol and methanol can attract more chemical compounds that might have antioxidant activity than non-polar solvents such as n-hexane.

The extracts showed lower antioxidant activity than the positive control (+)-Catechin. This can be explained that the positive control used is in purified form, while the extracts are the mixtures of chemical compounds. The mixture of chemical compounds in the extracts may neutralize, inhibit, or produce additive or synergistic effects by helping enhance the potential of the active compounds (Dhankhar et al., 2012).

Table 2. The Classification of Antioxidant Activity for Extracts (Scherer & Godoy, 2009)

Antioxidant Activity Index (AAI)	Antioxidant Activity
< 0.05	Poor
0.05-1.00	Moderate
1.00-2.00	Strong
>2.00	Very strong

Antibacterial Activity

The qualitative analysis of antibacterial activity against *S. aureus* and *E. coli* were carried out by the TLC-bioautography method. The results showed that the ethanolic and methanolic extracts could only inhibit the growth *S. aureus* with various inhibition zone diameters, and chloramphenicol as a positive control was active against both *S. aureus* and *E. coli* (Figure 5).

The inhibition of bacterial growth was indicated by the formation of clear white zones around the extract (Das et al., 2010). The purple color on the TLC plate after being sprayed with iodonitrotetrazolium salt (INT) was caused by the reaction of dehydrogenase enzyme in living microorganisms that converts INT to purple formazan (Silva et al., 2005). Chloramphenicol was used as a positive control in the antibacterial activity assay, due to its broad-spectrum activity with MIC range of 1-90 µg/mL (Adetutu et al., 2011).

The antibacterial activity of the extracts was further analyzed quantitatively by determining the MIC value against *S. aureus* and *E. coli*. The lowest extract concentration that can inhibit the growth of bacteria is defined as MIC. The MIC values were >625, 625 and 625 µg/mL for n-hexane, ethanolic and methanolic extracts respectively against *S. aureus*, while MIC values against *E.coli* were >625 µg/mL for all extracts. Chloramphenicol has a MIC value of 4 µg/mL against both reference bacteria (Table 3). Based on the classification of antibacterial activity in Table 4, it can be concluded that all of the extracts presented weak activity against *S. aureus* and *E.coli*, while chloramphenicol exhibit significant antibacterial activity against all tested bacteria.

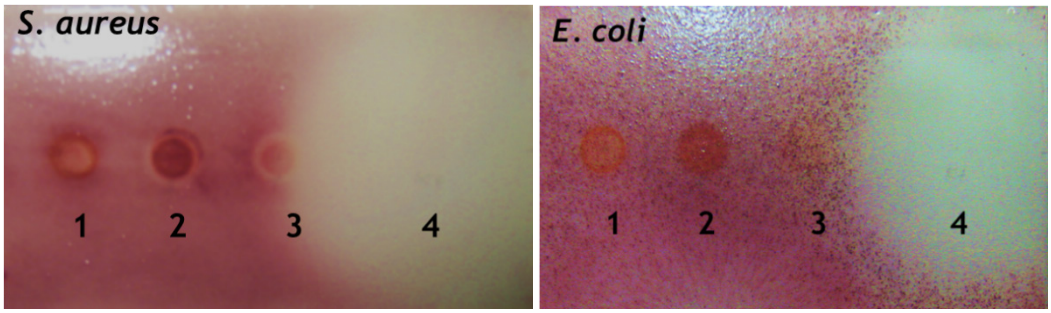

Figure 5. Bioautography of antibacterial activity of *D. gigas* against *S. aureus* (left) and *E. coli* (right): (1) n-hexane extract, (2) ethanolic extract, (3) methanolic extract, and (4) chloramphenicol.

Jurnal Biodjati 4(2):263-277, November 2019

JURNAL BIODJATI

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Table 3. The Minimum Inhibitory Concentrations (MIC) of *D. gigas* extracts

No	Sample	MIC (µg/mL)	
		S. aureus	*E. coli*
1	n-Hexane extract	>625	>625
2	Ethanolic extract	625	>625
3	Methanolic extract	625	>625
4	Chloramphenicol	4	4

Table 4. The Classification of Antibacterial Activity for Extracts (Pessini et al., 2003)

MIC (µg/ml)	Antibacterial Activity
< 100	Strong
100-500	Moderate
500-1000	Weak
> 1000	Inactive

In the present study, the extracts were more effective against *S.aureus* than *E.coli*. The difference in sensitivity between two types of Gram bacteria may be associated with the differences in the morphology and molecular components of membranes. The presence of the additional protection provided by the outer membrane layer contains lipopolysaccharide in Gram-negative bacteria that make this bacterium tend to be less permeable to antibacterial substances than Gram-positive bacteria (Epand et al., 2016).

GC-MS Profiling of *D. gigas* Extract

The identification of chemical compounds of *D. gigas* extract was carried out using GC-MS (Figure 6).

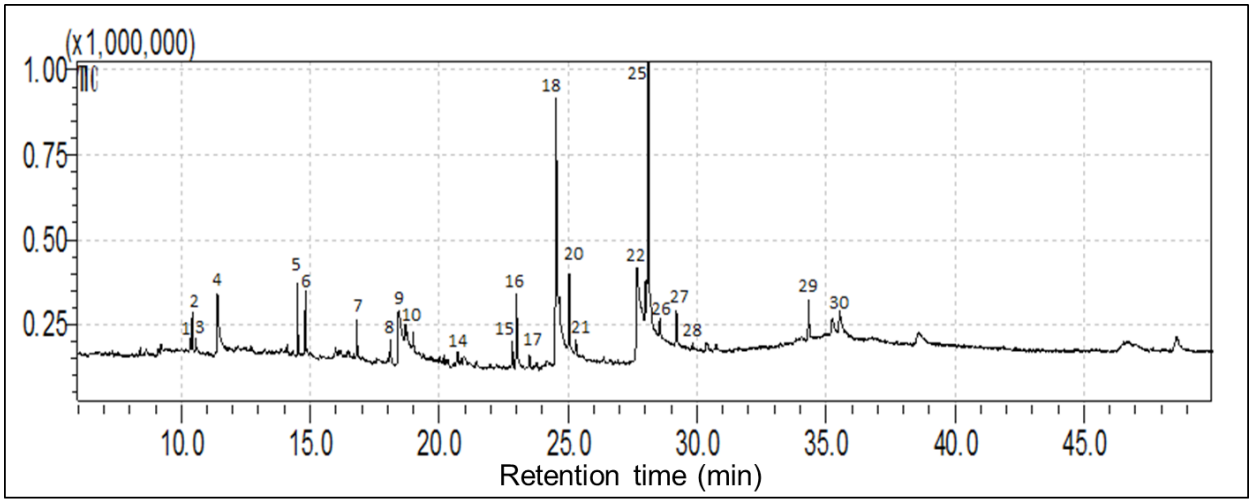

Figure 6. GC-MS chromatogram of the ethanolic extract of *D. gigas*. The peak numbers correspond to the numbers in Table 5.

Table 5. Chemical compounds of ethanol extract of *D. gigas*

Peak no.	Retention time (min)	Area	Area Percentage (%)	Chemical Compounds	Activity
1	10.338	93120	0.51	Hexamethylcyclotrisiloxane	
2	10.428	205998	1.13	Undecane	Alarm pheromone (Mizunami et al., 2010)
3	10.555	78734	0.43	L-Leucine, ethyl ester	
4	11.402	541613	2.96	2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-Pyran-4-one	Antioxidant (Kanzler et al., 2016); anti-inflammatory, antiproliferative, antimicrobial (Velayutham & Karthi, 2015); trail pheromone (Cerda et al., 2014)
5	14.517	353827	1.93	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methyl ethyl)propyl ester	Fungicide and bactericide (Haque et al., 2009)
6	14.808	318514	1.74	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethyl pentyl ester	Fungicide and bactericide (Haque et al., 2009)
7	16.808	209939	1.15	Butylated Hydroxytoluene (BHT)	Antioxidant (Bouftira et al., 2007; Ibtissem et al., 2010)
8	18.106	151704	0.83	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	
9	18.433	776299	4.24	Ethyl alpha-d-glucopyranoside	Antituberculous, antioxidant, alpha amylase inhibitor, hypolipidemic, anticonvulsant (Velayutham & Karthi, 2015)
10	18.688	144380	0.79	3-(2,3-epoxypropoxy)propyl] ethoxydimethyl-silane	
11	18.775	110983	0.61	Benzophenone	Anti-HIV, antioxidant, antiviral, antimicrobial, antifungal, and cytotoxic (Wu et al., 2014)
12	18.994	108350	0.59	Tributyl phosphate	
13	20.175	46987	0.26	4-(1,1-dimethylpropyl)-phenol	Antioxidant (Foti, 2007)
14	20.725	82338	0.45	Diallyl phthalate	
15	22.838	215304	1.18	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	
16	22.997	589189	3.22	1-Hexadecanol	Antimycoplasma (Fletcher et al., 1981)

Peak no.	Retention time (min)	Area	Area Percentage (%)	Chemical Compounds	Activity
17	23.501	121700	0.66	Lidocaine	
18	24.532	3210902	17.54	n-Hexadecanoic acid	Antiinflammatory (Aparna et al., 2012), antioxidants, hypocholesterolemic, nematicide, 5 alpha-reductase inhibitors, antiandrogenic, flavor, hemolytic (Kumar et al., 2010; Tyagi & Agarwal, 2017), and larvicidal activity against mosquitoes (Rahuman et al., 2000)
19	24.659	518424	2.83	Ethyl 9-hexadecenoate	
20	25.041	633219	3.46	Hexadecanoic acid, ethyl ester	Antioxidant, hemolytic, hypocholesterolemic, nematicide, antiandrogenic (Tyagi & Agarwal, 2017)
21	25.303	137598	0.75	Butanoic acid, 2-[2,4-bis(1,1-dimethylpropyl) phenoxy]-	
22	27.68	1948564	10.65	Oleic Acid	Cancer preventive, antiandrogenic (Ma'arif et al., 2016), antioxidant (Wei et al., 2016), anti-inflammatory, dermatitigenic, 5 alpha-reductase inhibitors, anemiagenic, hypocholesterolemic (Vadivel & Gopalakrishnan, 2011)
23	27.85	560886	3.06	Bis(2-ethylhexyl) maleate	
24	28.002	661775	3.62	Linoleic acid ethyl ester	Antiarthritic, antiandrogenic, 5 alpha-reductase inhibitor, hypocholesterolemic, antiacne, nematicide (Tyagi & Agarwal, 2017)
25	28.103	5450469	29.78	Ethyl Oleate	Primer pheromone (Castillo et al., 2012), flavor (Duke, 2016)
26	28.56	144783	0.79	Octadecanoic acid, ethyl ester	
27	29.197	329007	1.8	2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester	Antidermatitic, antioxidant, antihepatocarcinogenic, antioxidant, antitumor, flavor (Duke, 2016)
28	29.819	57305	0.31	Tributyl acetylcitrate	
29	34.326	353802	1.93	Bis(2-ethylhexyl) phthalate	
30	35.53	145877	0.8	1-Cyclohexyl-2-methyl-prop-2-en-1-one	

The major chemical compounds in the ethanolic extract of *D. gigas* are saturated and unsaturated fatty acids. Three major compounds are ethyl oleate (29.78%), n-hexadecenoic acid (17.54%), and oleic acid (10.65%). Those chemical compounds were also identified in a traditional edible insect species in China, *Polyrhachis vicina* Roger (Edible black ant) (Shen et al., 2006).

The GC-MS analysis revealed that BHT, a lipophilic organic compound (fat-soluble) which is commonly used as a synthetic antioxidant for cosmetics, food additive, and pharmaceuticals occurs naturally in the ethanolic extract of *D. gigas*. This result of the study is corresponding to Bouftira et al. (2007) and Ibtissem et al. (2010), that BHT occurs naturally in *Mesembryanthemum crystallinum* (the purple leaves of the halophyte plant).

This finding also showed that undecane was found in the ethanolic extract of *D. gigas*. Undecane is an alkane hydrocarbon with the chemical formula $CH_3(CH_2)_9CH_3$. Many formicine species utilize this compound as an alarm pheromone (Lenz et al., 2012). Alarm pheromone plays an important role in defense of the colony in ants. A study by Mizunami et al. (2010) reported that formic acid and undecane were alarm pheromone compounds of workers of the carpenter ant *Camponotus obscureipes*.

One pair of isomers, propanoic acid 2-methyl-,2,2-dimethyl-1-(2-hydroxy-1-methyl ethyl)propyl ester and propanoic acid, 2-methyl-,3-hydroxy-2,4,4-trimethyl pentyl ester, reported for this species. Propanoic acid is a weak acid that has been proven to show efficacy in inhibiting the growth of fungi (*Aspergillus flavus, A. niger*, *A. versicolor*, *Chaetomium globosum*, *Penicillium funiculosum, P. expansum*, *P. spinulosum*, *P. roqueforti*), bacteria (*Aerobacter aerogenes, Bacillus subtilis*, *Escherichia coli*, *E. freundii*, *Staphylococcus aureus, Serratia marcescens, Pseudomonas aeroginosa*, *P. fluorescens, Proteus vulgaris*) and yeast (*Candida albicans*, *C. krusel*, *Mansenula anomala*, *Pichia fermentans*, *Oidinum* sp., *Saccharomyces cerevisiae*, *S. vini*) (Haque et al., 2009).

The antioxidant activity of the ethanolic extract of *D. gigas* may be attributed to the presence of flavonoids (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one), phenolic compounds [4-(1,1-dimethyl propyl)phenol], butylated hydroxytoluene (BHT), and fatty acids. BHT is an additive that is often used in foods and cosmetics to prevent oxidative rancidity. BHT is usually intended to prevent the appearance of ketones and aldehydes which can give unpleasant odor products into cosmetics (such as bronzing agents, creams, make-up removers, eyelift products, oils, lotions, and sunscreens) (Capitan-vallvey et al., 2002). Fatty acids are mainly used in cosmetic products as an emulsifier and the production of soap (Kelm & Wickett, 2017). This present study showed that the ethanolic extract of *D. gigas* may contain valuable bioactive compounds exhibited antioxidant and antibacterial activity. This is the first study related to antibacterial and antioxidant activities as well as GC-MS analysis of an extract of giant forest ant *D. gigas*.

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