GC-MS Based Metabolite Profiling and Antibacterial Activity of Torch Ginger (*Etlingera elatior*) Flowers Extract

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Abstract: *Torch ginger (*Etlingera elatior*) flowers are well known for their antibacterial effects against* Staphylococcus aureus*, however, the active compounds are still unknown. The purpose of this study was to conduct GC-MS-based metabolite profiling of torch ginger flower and identify compounds correlated with its* S. aureus *antibacterial activity using Orthogonal Projection to Latent Structure (OPLS). Using the well diffusion method, the antibacterial activity of ethanol extract, hexane, chloroform, and ethyl acetate fractions with a concentration of 80 mg/mL were investigated. The ethyl acetate fraction inhibited* S. aureus *growth the most (diameter of inhibition zone, DIZ 13.00–13.20 mm), while the hexane (DIZ 9.55–10.05 mm) and chloroform (DIZ 10.00–11.00 mm) fractions had moderate inhibitory activity, but the ethanol extract had no antibacterial effect. Using OPLS analysis, the GC-MS metabolite profile of all extracts and fractions was linked with the profile of antibacterial activity. This analysis revealed that Dodecanoic acid, 5-Tetradecene, and* n*-Hexadecanoic acid were identified as the compounds that were significantly connected with antibacterial activity.*

Keywords: *antibacterial activity;* Etlingera elatior*; GC-MS; metabolite profiling;* Staphylococcus aureus

INTRODUCTION

Torch ginger is a native plant of Indonesia. Torch ginger is a member of the Zingiberaceae family, the Etlingera genus, and the *Etlingera elatior* species [1]. It is widely used as a medicinal herb and an ingredient in local products such as soap, shampoo, and perfume [2]. Torch ginger, on the other hand, is not as widely used as other spices such as ginger, turmeric, and aromatic ginger. Torch ginger possesses antioxidant, anticancer, antibacterial, antihyperglycemic, and anti-inflammatory properties [3-4].

Staphylococcus aureus is one of the most common pathogenic bacteria in humans. Invasive *S. aureus* infection is becoming recognized progressively as a major cause of severe sepsis in developing countries, with death rates larger than in developed countries [5]. Furthermore, as resistance cases in *S. aureus* increase, these bacteria are becoming increasingly important major pathogens. As a result, efforts must be made to identify prospective antibiotic candidates, particularly from natural sources. According to some previous research, torch ginger blossom has an antibacterial effect

against *S. aureus* [3,6]. Testing the antibacterial activity of *S. aureus* on local torch ginger flower has been carried out by using the modification well diffusion method [7]. However, the identification of components of the antibacterial compound of *S. aureus* from local torch ginger flower has not yet been conducted. Therefore, this study aimed to conduct GC-MS metabolite profiling of torch ginger flower extract and to identify compounds that correlate with its antibacterial activity against growth using multivariate data analysis.

Metabolomics is a comprehensive analysis of components of an organism at a certain time or condition. It can be applied to study the correlation between bioactivity and metabolite profile which is ultimately used to identify the bioactive component in plants efficiently [8]. This method has been carried out by stratified sample extraction using a combination of solvents at the level of polarity [9]. One of the instruments that can be used for metabolomic analysis is Gas Chromatography-Mass Spectrometry (GC-MS). GC coupled to MS can be used to identify and elucidate the structure of compounds. The development of mass detection methods is one of the most widely used techniques for identifying compound structures. The ability to detect the atomic formula or specific functional group of an analyte has become a strong point for the use of MS in metabolomics [10]. Furthermore, GC-MS techniques have an advantage over LC-MS (Liquid Chromatography-Mass Spectrometry) techniques since GC-MS is considerably more repeatable than LC-MS according to the Electron Ionization (EI) approach that is typically used [11]. Several studies successfully identified bioactive metabolites from complex plant extracts using the GC-MS-based metabolomic method [12-14].

To extract information from the large amount of data generated from metabolomics study requires the use of multivariate data analysis. One multivariate data analysis method that can be used to observe a correlation between the metabolite profile of a complex plant extract with its activity is Orthogonal Projection to Latent Structure (OPLS) analysis. This analysis will group the samples based on their bioactivity in the form of an S-plot. In addition, the Y-related coefficient plot indicates metabolites significantly correlate with its bioactivity. Therefore, we used multivariate data analysis/OPLS to facilitate the identification of compounds correlated with antibacterial activity. The known antibacterial compound of the torch ginger flower can be further investigated for its use as an antibiotic drug and functional food that can prevent disease.

■ EXPERIMENTAL SECTION

Materials

The material used in this study was fresh torch ginger flower bud from Kabanjahe farmer Sumatera Utara, Indonesia. For future use, the voucher specimen was deposited and authenticated at the Herbarium Medanese, Universitas Sumatera Utara, Medan, Indonesia (voucher specimen: 6488/MEDA). Ethanol, *n*-hexane, chloroform, ethyl acetate, dimethyl sulfoxide (DMSO), chloramphenicol, MSTFA (*N*-Methyl-*N*-(trimethylsilyl)trifluoro-acetamide) (gas chromatography grade), and methoxyamine hydrochloride were purchased from Sigma-Aldrich. Pyridine was supplied by Merck. Methoxyamine HCl was supplied by Fisher Scientific (Acros Organics, Geel, Belgium), and liquid nitrogen was purchased from the MOX Company (Petaling Jaya, Malaysia). Nutrient agar media was supplied from Merck. *S. aureus* bacteria (ATCC 25923) was obtained from the Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara. All chemicals were of analytical grade.

Instrumentation

This study used GC-MS of Agilent 6890 and HP 5973 mass detector (Agilent Technologies, Santa Clara, USA), the centrifuge of Eppendorf 5415D (Eppendorf AG, Hamburg, Germany), and the rotary evaporator of Buchi (BÜCHI Labortechnik, Flawil, Switzerland).

Procedure

Samples preparation

Fresh torch ginger flower is sorted, washed, peeled, dried in an oven dryer at 50 °C for 12 h, then crushed into a powder using a food processor and filtered with a 30 mesh filter. Extraction was carried out on 50 g torch ginger flour with 80% ethanol by sonication for 1 h and

followed by centrifugation at 2000 rpm for 30 min. The extract was filtered and the filtrate was collected and dried using a rotary evaporator at 40 °C. The dried extract was subjected to partition liquid-liquid fractionation with *n*-hexane, chloroform, and ethyl acetate. All fractions were dried using a rotary evaporator at a temperature of 40 °C. The extraction and fractionation processes were carried out in 5 replications; thus, 20 samples were obtained in total.

Antibacterial analysis

The antibacterial activity on extraction result was carried out by a modified well diffusion method [15]. The extract and fraction were dissolved in DMSO with a concentration of 80 mg/mL. Next, 500 mL of sterilized NA media were poured into a 25 ± 2 mL Petri plate. The Petri plate containing the media was then allowed to stand for 1 h until it solidified. In this media, three wells of 6 mm diameter were made. A total of 60 µL extract was poured into the well, and other wells were used for positive control and negative control. DMSO:ethanol (6:2) was used as a negative control, and chloramphenicol was used as a positive control (2.50 mg/mL). *S. aureus* with a concentration culture test of 10^6 CFU/mL has been likened to the Mac Farland turbidity standard solution. It was taken, scratched into the media, and incubated at 37 °C for 48 h. Antibacterial activity was calculated based on DIZ (diameter of inhibition zone). The tests were carried out in 5 replications.

GC-MS analysis

Samples derivatization and GC-MS analysis method were referred to [13] method. The extracts (25 mg) were added by 50 µL pyridine in a 2 mL centrifuge tube and sonicated for 10 min at 30 °C. Next, 100 mL of methoxyamine HCl (20 mg/mL pyridine) was added and vortex. It was then incubated for 2 h at 60 °C. Next, 300 mL of MSTFA was added and re-incubated for 30 min at 60 °C. The sample is filtered, transferred into a tube, closed tightly and covered with aluminum foil, and left overnight at room temperature.

Samples were analyzed using the Agilent GC-MS system. A 1 µL sample was injected into the GC-MS system consisting of Agilent 6890 chromatography gas and HP 5973 mass selective detector. The GC column used was a 5% DB-5MS phenyl methyl siloxane column with an inner diameter (ID) of 250 µm and a thickness of 0.25 µm. The initial temperature was set at 50 °C for 3 min and rose to 315 °C for 10 min at a speed of 10 °C/min. Helium gas was used as a carrier gas with a speed of 1 mL/min. The temperature of the injector and ion source was set at 330 and 250 °C, respectively. The mass spectra were obtained using a full scan and monitoring mode with a mass scanning range of 50 to 550 m/z after 7 min of solvent delay.

The spectra for each chromatogram peak were compared with those in the NIST08 database and the retention time (RT) of the primary and secondary metabolites. Chromatogram and mass spectra were processed using Agilent ChemStation, Automated Mass Spectral Deconvolution and Identification System (AMDIS), and Agilent's Deconvoluted Reporting Software (DRS). The data processing approach was used after GC-MS analysis. The main purpose of data processing is to extract all related information from the raw data and turn it into a data matrix. This procedure involves noise filtering, binning data, automatic peak detection, and chromatographic alignment without the need for internal standards (ISs). The XCMS package in version R 2.15.1 was applied to align the GC-MS chromatogram with the following values: xcms Set (fwhm = 30, step = 0.1, Method = bin) and group (bw = 10) and used to extract all information from the data raw and summarize it in Excel sheet format.

OPLS analysis

OPLS analysis was conducted using SIMCA v.13.01. Two plots were used to interpret the OPLS data; S-plot and a Y-related coefficient plot. A significant correlation is indicated by the value of the Y-related coefficient that is greater than 0.50. The scaling method used the Pareto method because it gives each variant variable numerically the same as the standard deviation [16].

■ RESULTS AND DISCUSSION

Antibacterial Activity

The result of the antibacterial activity test of the extracts and fractions of torch ginger flower showed that

the fraction with the highest activity was ethyl acetate fraction (EF) (DIZ 13.00–13.20 mm), while ethanol extract (EE) showed no inhibitory activity (Fig. 1). The results were in agreement with those of Anzian et al. [17], who reported that oil extracts of torch ginger (*Etlingera elatior* Jack) flower using subcritical carbon dioxide (CO_2) exhibited very strong antibacterial activity against *S. aureus* (14.50 ± 2.21 mm). Research by Susanti et al. [18] showed that the 10 µL concentration of torch ginger flower essential oil could provide 6 mm inhibition in *S. aureus* bacteria. According to Ghasemzadeh et al. [3], Kelantan torch ginger flower extract at a concentration of 10 mg/mL gave 8.40 mm inhibitory power, while Johor torch ginger flower extract gave 4.00 mm inhibitory power, and Pahang torch flower ginger extract gave 9.20 mm inhibitory power. A previous report showed that the chloroform extract had an inhibitory value of 3.78 mm at a concentration of 500 mg/mL [7]. Differences in torch ginger flower source, extraction method, and solvent used can affect the concentration of secondary metabolites in the plants, consequently, influencing their antibacterial activity [3,19].

Metabolites Profiles

A metabolite profile was obtained from chromatograms resulting from GC-MS analysis of 602 metabolite profiles from the retention time of ethanol extract, hexane, chloroform, and ethyl acetate fractions. The compounds were detected by comparing their fragmentation pattern and mass spectra to those in the National Institute of Standards and Technology's mass spectral database (NIST14.L). The most abundant compounds in hexane fractions were 1-Dodecanol, Dodecanoic acid, and Cyclododecane. Meanwhile, the main compounds in chloroform fractions were 1-Dodecanol, Hexadecanoic acid, and Malonic acid. Dodecanoic acid, and (E)-5-Tetradecene were the major compounds of ethyl acetate fractions (Fig. 2). The EFs were the fraction that had the highest antibacterial inhibitory activity. The EFs of torch ginger contained 19 compounds, with Dodecanoic acid (18.60%) being the most abundant (Table 1). The (E)-5-Tetradecene, 1-Tetradecyl acetate, Cyclotetradecane, Tetradecanoic acid, Cyclotetradecane, *n*-Hexadecanoic acid, Tricosane, Dodecanoic acid, and Dodecyl ester were similar to the

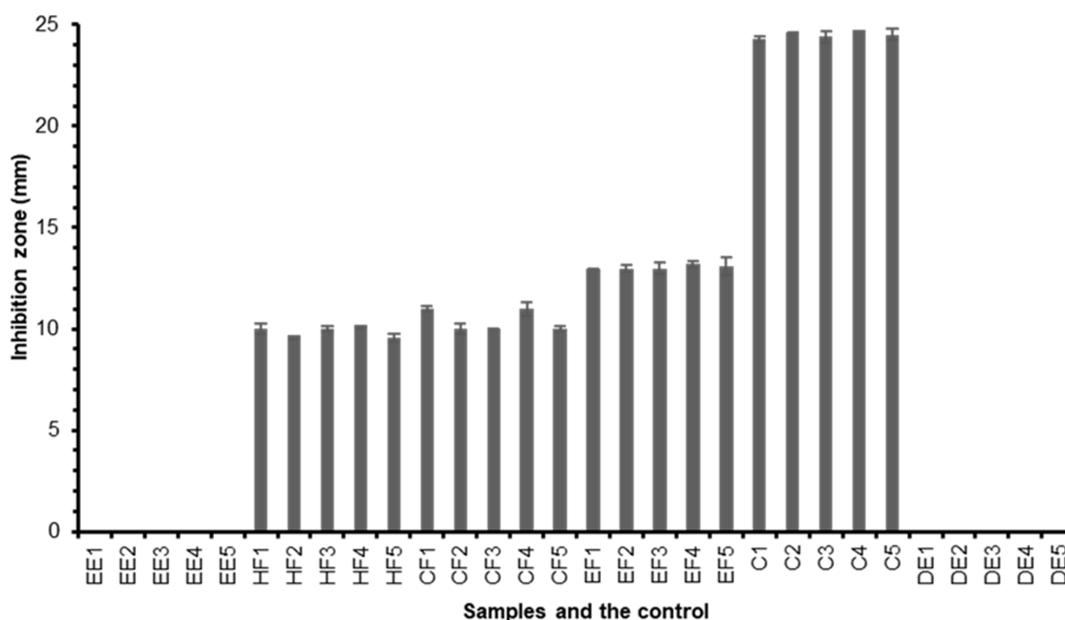

Fig 1. Antibacterial profile of torch ginger extract and fractions against *S. aureus* at 80 mg/mL concentration, 2.50 mg/mL of chloramphenicol (positive control), and DMSO:ethanol (6:2) as a negative control. EE = Ethanol Extract, HF = Hexane Fraction, CF = Chloroform Fraction, EF = Ethyl acetate Fraction, C = Chloramphenicol, and DE = DMSO:ethanol. The DIZ value was the average from two replication with a standard deviation

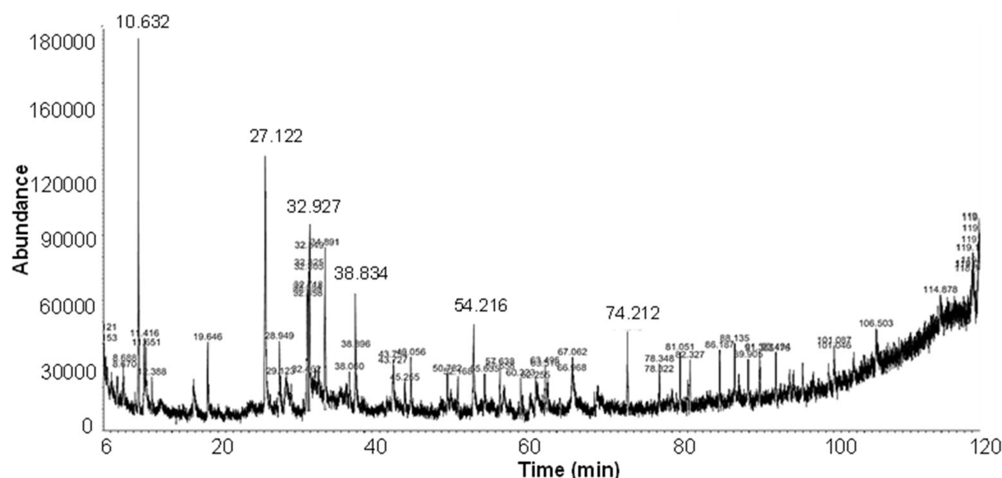

Fig 2. Chromatogram of ethyl acetate fraction from torch ginger

Table 1. Chemical composition of ethyl acetate fraction from torch ginger analyzed by GC-MS analysis

No	RT	Compound	Area %	MW	Structure	Precursor ion	$[M-H]^{+/-}$ product ion (m/z)	Ref. (NIST14.L Library)	Ref.
1	12.39	7-Oxooctanoic acid	0.78	158.19	$C_8H_{14}O_3$	159.00	117.10; 75.10	92728	-
2	27.12	(E)-5-Tetradecene	12.80	196.37	$C_{14}H_{28}$	196.00	55.10; 69.00; 83.10	61866	[20]
3	28.95	2,4-Di-tert-butylphenol	2.34	206.32	$C_{14}H_{22}O$	206.30	191.30; 57.30	70632	-
4	32.93	Dodecanoic acid	18.60	200.32	$C_{12}H_{24}O_2$	200.00	55.10; 60.10; 73.20; 128.90	64983	[2,17,20]
5	34.89	1-Tetradecyl acetate	6.71	256.42	$C_{16}H_{32}O_2$	252.40	55.10; 83.00; 97.30	117423	[2]
6	38.84	Cyclotetradecane	3.20	196.38	$C_{14}H_{28}$	196.30	55.10; 83.10;	61850	[20,21]
7	43.73	Tetradecanoic acid	0.14	228.37	$C_{14}H_{28}O_2$	228.00	55.10; 73.00;	91416	[2,18,20]
8	46.06	Cyclotetradecane	1.61	196.38	$C_{14}H_{28}$	196.50	55.10; 83.20	61849	[20,21]
9	52.17	Methyl hexadecanoate	1.19	270.50	$C_{17}H_{34}O_2$	271.40	87.30; 74.00; 87.30; 55.30; 227.30	130818	-
10	54.22	n-Hexadecanoic acid	3.60	256.42	$C_{16}H_{32}O_2$	256.10	73.10; 60.00	117418	[18,20]
11	55.63	Ethyl hexadecanoate	0.37	284.50	$C_{18}H_{36}O_2$	284.00	69.00; 55.00; 88.10; 100.90	144307	-
12	60.33	Myo-Inositol	0.01	180.16	$C_6H_{12}O_6$	193.10	73.20	273399	-
13	63.49	Ethyl linolate	1.11	308.50	$C_{20}H_{36}O_2$	308.00	67.20; 95.00	167365	-
14	67.06	Cyclobutenylbenzene	0.55	130.19	$C_{10}H_{10}$	131.00	131.00; 55.10; 117.00	41663	-
15	78.35	Tricosane	1.06	324.60	$C_{23}H_{48}$	324.20	57.10; 71.00; 85.00	155900	[20]
16	81.05	Dodecyl dodecanoate	1.91	340.60	$C_{22}H_{44}O_2$	334.20	201.20; 57.10; 71.20	182653	[18]
17	82.33	Hexacosane	1.69	366.70	$C_{26}H_{54}$	366.50	57.10; 71.20; 85.10	219427	-
18	86.19	8-Hexylpentadecane	0.96	296.57	$C_{21}H_{44}$	295.60	57.10; 71.20; 85.10	155894	-
19	89.90	Octacosane	1.70	394.76	$C_{28}H_{58}$	394.40	57.10; 71.20; 85.10	235613	-

previous reports [2,17-18,20-21].

We identified several compounds in extracts and fractions of torch ginger, have also been previously reported such as Dodecanoic acid [2,17,20], *n*-Hexadecanoic acid [18,20], 1-Dodecanol [2,17,20], Tetradecene [20], 1-Decanol [17-18], Tetradecanoic acid [2,18,20], 1-Tetradecyl acetate [2], Acetic acid [22], Dodecene [22], 1,3-Propanediol [22], Cyclododecane [20], 9-Tetradecen-1-ol [18,20], Propanedioic acid [20], 13-Octadecenoic acid [20], 9,12-Octadecadienoic acid [20], Tricosane [20], Dodecane [20], and 1-Tetradecanol [18]. Hexane, chloroform, and ethyl acetate fractions containing some of these compounds have inhibitory activity against *S. aureus*. These compounds have been reported to have antibacterial activities, such as Dodecanoic acid (dilution factor of 1:10) against *S. aureus* (15 ± 1.41 mm) and *Streptococcus pneumoniae* (15 ± 0.00 mm), *Mycobacterium tuberculosis* spp. (14 ± 1,41 mm) [23]. Moreover, *n*-Hexadecanoic acid was suspected as an antibacterial compound in

Pentanisia prunelloides against Gram-positive bacteria (*Bacillus subtilis*, *S. aureus*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*) [24]. 1-Dodecanol and 1-Decanol were also reported to have activity against *S. aureus* (MIC 6.25 µg/mL, 50 µg/mL, respectively) and *P. acnes* (MIC 3.13 µg/mL, 25 µg/mL, respectively) [25]. In addition, Tetradecanoic acid-solid lipid nanoparticles (TAS) were also known to have an antibacterial activity where the viable colonies of *Salmonella* CVCC541 bacteria in cells were reduced by 95.83% after TAS treatment [26].

Furthermore, Acetic acid had good activity against clinical and type strains of *P. aeruginosa* at concentrations as low as 0.17%, while 0.31% inhibits MSSA and *A. baumannii* [27]. Kim et al. [28] reported the antibacterial activity of 1,3-Propanediol against *E. coli* (MIC 16%) and *S. aureus* (MIC 16%) bacteria. Moreover, Tricosane was active against Gram-positive: *B. subtilis* CICC 20034; *Flavobacterium SHL45* CICC 51823, Gram-negative: *E. coli* CICC 23845; *P. fluorescens* (*SHL5* CICC 20066, *SHL7* CICC 21620), and Yeast: *S. cerevisiae* (*Ja64* CICC 1346; *Tokay* CICC 1388; *Y-8* CICC 1390) with inhibition zones ranging from 8.03 to 15.97 mm at a concentration of 20 mg/mL [29]. Kubo et al. [30] have reported that 1-Tetradecanol was able to inhibit the growth of *Propionibacterium acnes*, *Brevibacterium ammoniagenes*, and *Streptococcus mutans*.

Orthogonal Projection to the Least Square (OPLS) Analysis of Metabolites Profile and Antibacterial Activity

In OPLS analysis, the X data matrix was the metabolite profile in each extract, and fractions correlated with its antibacterial activity as the Y data matrix. The metabolite profile data used the retention time generated from the GC-MS chromatogram of all extracts and fractions, while the antibacterial activity data used the inhibition zone diameter data of extracts and fractions against *S. aureus* bacteria. Using OPLS analysis, these X and Y data were used to determine the correlation of metabolite profiles and antibacterial activity of extracts and fractions.

To interpret the results of the OPLS analysis, S-plot and Y-related coefficient plot were used. The S-plot was used to classify the retention time of extracts and fractions based on the characteristics given by the Y data matrix. The separation of all retention times between those with low and high activity made it easier to identify retention times for further bioactive components using Y-related coefficients. Fig. 3 shows the S-plot of active and inactive retention times. The more positive the X value, the higher activity can be predicted. In Fig. 3, the retention time of 32.93 min showed the greatest X value, so the most active retention time can be expected. The compound at that retention time was Dodecanoic acid.

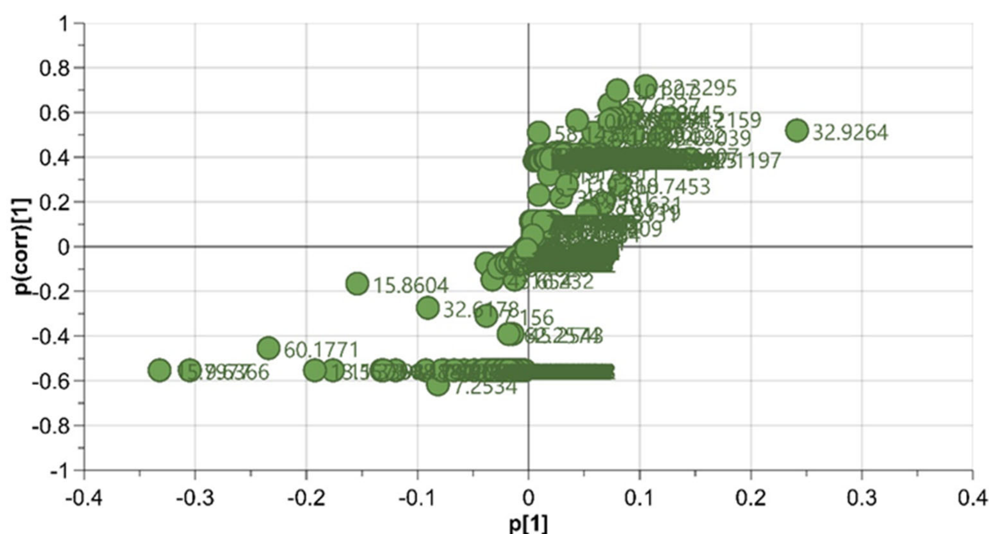

Fig 3. S-plot of extracts and fractions. The more positive the X value, it can be predicted the higher activity

This compound was found in high concentrations in ethyl acetate and hexane fractions. In contrast, the retention times of 15.80 and 60.18 min were the smallest and most predictable as compounds with no antibacterial activity. These compounds predominated in the ethanol extract, which was composed of Glycerol and Myo-Inositol. Glycerol has been reported in torch ginger as well [31]. Additionally, Myo-Inositol has also been found in *Orthosiphon aristatus* (Blume) Miq. (Lamiaceae) [32]. Glycerol and Myo-Inositol are sources of nutrients essential for microbial growth [33].

Furthermore, to determine the high positive correlation value of Y data matrix data with the peak of all retention time, Y-related coefficients were used. A significant correlation is indicated by the Y-related coefficient, which is more than 0.50 [16]. Fig. 4 shows the Y-related coefficient plot, with the highest value found at 32.93 min retention time of 0.81. In addition, there are two retention times with the Y-related coefficient near 0.50. The retention times are 27.12 and 54.22 min, respectively.

Investigation of the retention times of 32.93, 27.12, and 54.22 min resulted in the identification of the compounds of Dodecanoic acid, (E)-5-Tetradecene, and *n*-Hexadecanoic acid, respectively (Fig. 5). This identification was made by comparing the fragmentation pattern and molecular weight to mass spectra available in the NIST14.L and the literature [34-35]. Dodecanoic acid showed peak (intensity %): m/z 55.10 (100%), m/z 60.10 (98.41%), m/z 73.20 (92.89%),

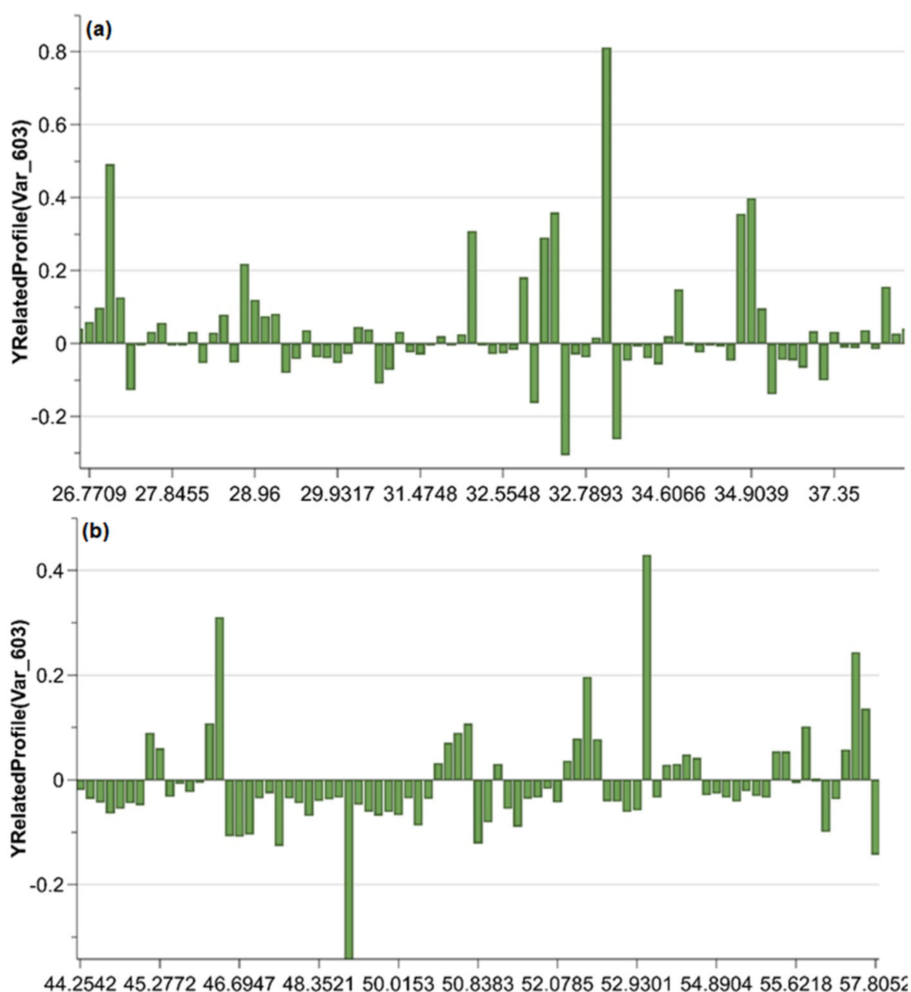

Fig 4. The values of the OPLS Y-related coefficient plot, (a) The plot runs from 26.77 to 37.35 min; (b) The plot runs from 44.25 to 57.80 min

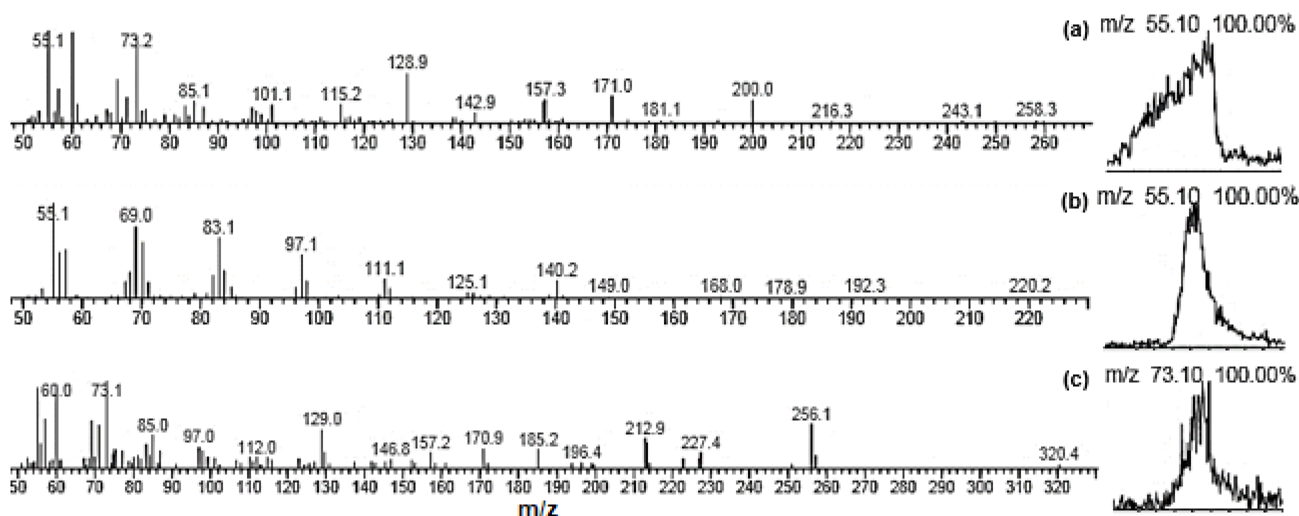

Fig 5. MS fragmentation pattern for retention times of (a) 32.93 min, (b) 27.12 min, and (c) 54.22 min

m/z 128.90 (53.86%), m/z 69.20 (46.78%), while in the literature it had peak (intensity %): m/z 55.10 (100%), m/z 43.00 (92%), m/z 73.00 (90%), m/z 60.00 (85%), m/z 129.00 (30%), m/z 85.00 (30%) [35]. (E)-5-Tetradecene showed peak (intensity %): m/z 55.10 (100%), m/z 69.00 (75.72%), m/z 83.10 (63.86%), m/z 70.20 (59.14%), m/z 57.20 (51.41%), while in the literature for 1-Tetradecene had peak: m/z 55, m/z 41, m/z 43, m/z 83, m/z 57, m/z 69, m/z 97, m/z 168 [34]. *n*-Hexadecanoic acid showed peak (intensity %): m/z 73.10 (100%), m/z 60.00 (93.86%), m/z 55.20 (92.04%), m/z 57.00 (56.69%), m/z 69.20 (55.05%), while in the literature it had peak (intensity %): m/z 73 (100%), m/z 129 (30%), m/z 97 (12%), m/z 256 (12%), m/z 213 (11%) [36].

E. elatior plant extract with *n*-hexane has been reported to have antibacterial activity of *C. gloeosporiodes,* which is identified as Dodecanoic acid, β-Sitosterol, and Stigmasterol [37]. In addition, essential oil components of the Etlingera genus such as Dodecanoic (lauric) acid, Decanoic (capric) acid, aldehydes of Dodecanal, monoterpenes of α-Pinene, β-Pinene, and sesquiterpene of (E)-Caryophyllene have been reported to have antibacterial, antifungal, and antiviral activities [38]. There have been no reports of Tetradecene's antibacterial activity, whereas *n*-Hexadecanoic acid has been extensively described as having antimicrobial activities [39]. In a previous study [40], *Plectranthus amboinicus* leaves extract showed inhibitory activity on *Bacillus subtilis*, Methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* which was correlated with the occurrence of more *n*-Hexadecanoic acid bioactive compounds. Dodecanoic acid and *n*-Hexadecanoic acid are free fatty acids (FFA) whose main antibacterial action is cell membranes, where they interfere with oxidative phosphorylation and the electron transport chain [38]. FFAs are reported to have a non-specific action mode and broad activity spectrum that can be applied in various medicine, food preservation, and agriculture, making them attractive as antibacterial agents where the use of conventional antibiotics is prohibited [38].

■ CONCLUSION

Antibacterial activity tests on the growth of *Staphylococcus aureus* showed the greatest inhibition (DIZ) in the ethyl acetate fraction (13.00–13.20 mm) and followed by chloroform (DIZ 10.00–11.00 mm) and hexane (DIZ 9.55–10.05 mm) fractions of torch ginger (*Etlingera elatior*) flower with a concentration of 80 mg/mL. The result of the OPLS analysis showed that the component at a retention time of 32.93 min correlated significantly with the growth of *S. aureus* bacteria (Y related coefficient value of 0.81). The compound was identified as Dodecanoic acid. Furthermore, (E)-5-Tetradecene and *n*-Hexadecanoic acid compounds were also discovered to be strongly connected to their activity

(Y related coefficient value near to 0.50). Dodecanoic acid and *n*-Hexadecanoic acid compounds have also been reported to have antimicrobial activity, but not (E)-5-Tetradecene. Purification of the (E)-5-Tetradecene compound can be done in further research to confirm its antibacterial activity.

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AUTHOR CONTRIBUTIONS

WHM participated in doing research, AP participated in writing the script, NDY contributed to the analysis of OPLS and revised the manuscript, LML contributed to the extraction of the sample, and AK conducted GCMS analysis. All authors agreed to the final version of this manuscript.

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