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Research Article

Antibacterial Activity and Metabolomics Profiling of Torch Ginger (*Etlingera elatior* Jack) Flower Oil Extracted Using Subcritical Carbon Dioxide (CO_2)

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The aim of this study was to identify the bioactive compound and evaluate the antibacterial activity of torch ginger flower oil extracted using subcritical carbon dioxide. The antibacterial activity was evaluated in agar diffusion assay, while MIC and MBC were determined using the microdilution broth assay. The essential oil was subjected to metabolomics profiling using GC-MS and ^{1}H-NMR techniques. The results demonstrated strong antibacterial activity towards *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*. The MIC values were 0.0625, 0.25, and 0.25 mg/mL, and the MBC values were 0.25, 0.5, and 1 mg/mL towards *S. typhimurium*, *S. aureus*, and *E. coli*, respectively. A total of 33 compounds were identified using GC-MS including 15 compounds (45%) known for their antimicrobial activity. In addition, sixteen metabolites were identified using NMR analysis and 8 out of the sixteen metabolites (50%) have antibacterial activity. The extracted oil demonstrated broad range for antibacterial activity and has high potential for applications in pharmaceutical and food industries. *Practical Applications*. The oil extracted from the torch ginger flower was found very stable and has promising applications as antibacterial agent for food and pharmaceutical industries.

1. Introduction

Oils extracted from the parts of aromatic plant such as barks, flowers, fruits, leaves, and rhizomes are economically important due to their applications in foods and pharmaceuticals [1]. The increasing demand for natural bioactive ingredients with biological functions including antioxidant and antibacterial activity has led the researchers to evaluate several promising plants extracts. Torch ginger (*Etlingera elatior* Jack) is an edible aromatic plant rich in phytochemicals and has well-known pharmacological properties [2]. Several previous studies reported the antimicrobial activity of the torch ginger flower oil that was extracted using several organic solvents including acetone, ethanol, methanol, and hexane [3]. However, the antimicrobial activity of the oil extracted from torch ginger flower was reported to be declined after solvent extraction due to oxidative degradation during solvent removal which requires high temperatures [4]. On the other hand, the subcritical carbon dioxide (CO_2) extraction method can preserve the bioactive compound presence in the oil [5]. The CO_2 extraction technique is friendly to environment, requires very low temperatures,

and has low effects on the bioactive compounds including volatile compounds [6]. Previous studies demonstrated the advantages of CO_2 extraction in comparison to solvent extraction for ajwain (*Carum copticum*) oil [7] and cardamom (*Elettaria cardamomum* Maton) oil [8].

The oil of torch ginger flower has been reported in many studies to have antimicrobial activity towards several pathogenic bacteria and identified the chemical compositions including some significant bioactive compounds with antimicrobial activity such as 1-dodecanol [9]. In the previous study, Susanti et al. [10] analysed the chemical compositions of the oil extracted from torch ginger flower obtained from Malaysia and identified 22 compounds including hydrocarbons, aldehydes, alcohols, ketones, esters, and acids. In a recent study, the oil extracted from the rhizome demonstrated strong antibacterial activity against 13 pathogenic bacteria and yeast including MRSA, and the bioactive compounds were identified by GC-MS as linalool formate and eugenol [11]. The oil has promising applications in foods as natural preservatives to replace and reduce the use of synthetic preservatives [12]. On the other hand, torch ginger flower oil has high potential for pharmaceutical applications as natural antibacterial agent. In the previous study, the oil extracted from different herbs was observed to reduce the microbial load and extend the shelf life of Asian sea bass fish for 33 days at 0 to 2° [13]. However, the biodegradation of the bioactive phytochemicals and the loss of antibacterial activity after long storage are the main challenge for using oils in food applications. The stability of the phytochemicals and the antimicrobials activity are affected by the extraction methods and storage conditions [14]. In previous studies, oil of torch ginger flower was mainly extracted using organic solvents and no study determined the stability of the oil during prolonged storage [15]. To the best of our knowledge, there are no studies that determined the antibacterial activity of oil extracted from torch ginger flower using subcritical carbon dioxide (CO_2) extraction. Therefore, the aim of this study was to determine the effects of the CO_2 extraction technique on the antibacterial activity of the oil of torch ginger flower and carry out the metabolic profiling using GC-MS and ^{1}H-NMR based techniques. Moreover, the effects of storage for 12 months at 8°C on the antimicrobial activity were evaluated to determine the oil stability.

2. Materials and Methods

2.1. Chemicals and Reagents. Ethanol and hexane were purchased from R&M Chemicals (Essex, UK). Mueller–Hinton Agar (MHA), Muller–Hinton broth (MHB), and nutrient agar (NA) were obtained from (Oxoid, Basingstoke, Hampire, England), (Difco, Becton Dickinson, France) and (Merck, Darmstadt, Germany), respectively. Streptomycin was purchased from Oxoid (Hampshire, England).

2.2. Plant Source and Preparation. The fresh torch ginger flowers (50 kg) were purchased from local supplier at Pasar Borong, Selangor. Voucher specimens of torch ginger flowers were identified by a botanist, Dr. Mohd Firdaus Ismail, and deposited at the Phytomedicine Herbarium, Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia, under the voucher number SK 3176/17. The torch ginger flowers were separated from their stalks and stems and washed thoroughly under running water to remove dirt, and their surfaces were cleaned cautiously to remove adhering debris. The excess water was drained, and torch ginger flowers were cut into small pieces using a continuous slicer (thickness: 2 mm). Torch ginger flowers were subjected to oven drying for 16 h in a at 40°C until their moisture content reached $10 \pm 2\%$ using drying oven (Smoke Master Model SMA-112, Tokyo, Japan). The dried torch ginger flowers were grounded using a commercial blender (Blender 8010S, Model HGBTWTS3, Waring Commercial Torrington, USA) and then sieved through a 500 μm mesh size and kept at room temperature for further analyses.

2.3. Subcritical Carbon Dioxide Extraction. The subcritical carbon dioxide (CO_2) extraction was carried out following the method described by Taraj, [16], with modification. The processed torch ginger flowers were soaked in solvent and drained automatically for several times. Carbon dioxide was continuously regenerated by a single stage or flash evaporation in the reboiler. A semicontinuous flow SC-CO_2 extraction system was used in the experiment (FeyeCon Development, Weesp, Netherlands). The extraction conditions were optimized for the temperature 28°C, the pressure 7 MPa, and the time was 12000 min (400 cycle × 3 min). Approximately 150 g of torch ginger flowers was loaded into the extractor unit (1 L capacity). Liquid CO_2 was supplied from the tank to the reboiler unit via the V1 valve and was converted into CO_2 gas. The CO_2 gas was channelled to the condenser unit and condensed into liquid CO_2 again. The liquid CO_2 evaporated while the torch ginger oil was precipitated at the bottom of the reboiler unit. The yield of oil was expressed as the percentage of oil obtained based on the weight of sample used. The torch ginger oil was sealed in the opaque glass bottles and stored at 8°C for further analysis.

2.4. Oil and Microbial Preparations. The oil was dissolved in absolute ethanol at a concentration of 50 mg/mL and filtered using sterilized 0.2 μm syringe filters (Sartorius minisart cellulose, Sartorius Stedim, Göttingen, Germany). The pathogenic bacteria including *Salmonella typhimurium* ATCC14028, *Staphylococcus aureus* ATCC6538, and *Escherichia coli* O157: H7 were obtained from Bioprocessing laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM). The pathogenic bacteria were grown in the nutrient broth (MHB) and adjusted to 10^6 CFU·mL^{-1} approximately using 0.5 Mc Farland (Becton, New Jersey, USA), and the results were reconfirmed at 600 nm wavelength using a microplate reader (PowerWave × 340, BioTek Instruments, Inc, Vermont, USA). The standardized activated bacteria suspensions were used for further analyses.

2.5. Antibacterial Assay. The antibacterial activity of torch ginger flower oil was evaluated to determine the potential applications in foods and pharmaceutical industries. Agar

disc-diffusion assay was carried out to determine the antibacterial activity according to the method described in the Clinical and Laboratory Standard Institute [17]. Briefly, Muller–Hinton Agar (MHA) plates were inoculated with the pathogenic bacteria using a sterile swab. A total of 20 μL of the oil extracts (50 mg/mL) was placed on blank paper discs (6 mm) and left for drying before the experiment in MHA plates. Absolute ethanol (100%) served as a negative control, while streptomycin (25 μg/disc, Oxoid, Hampshire, England) served as the positive control. The plates were incubated at 37°C for 24 h, and the antibacterial activity was determined by measuring the diameter of the clear zones of inhibition. The assay was done in triplicate for each bacterium.

2.6. Determination of Minimal Inhibitory Concentration and Bactericidal Concentration. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined following the microdilution broth method [17]. The oil was dissolved in DMSO (10%), and eight different concentrations were prepared (80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/mL). A total of 180 μL of MHB containing 10^6 CFU·mL^{-1} from each pathogenic bacterium was added to the wells, and 20 μL of the different oil concentrations was placed in the wells. As the oil (20 μL) diluted in the broth (180 μL), the final concentrations of the oil in the wells were 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL. The positive control was streptomycin prepared following the same oil concentrations, and the negative control was 10% DMSO. The plates were incubated aerobically at 37°C for 24 h. The MBC was determined by inoculating 5 μL from each well from the 96-well microtitter plate on MHA plates and incubation at 37°C for 24 h. The complete growth inhibition represented the MBC. The MIC was determined as the lowest concentration of oil that fully inhibits the bacterial growth. All the experiments were carried out in triplicate.

2.7. GC-MS Metabolic Profiling. The torch ginger flower oil bioactive compounds were identified using QP2010 ultra gas chromatography-mass spectrometer (Shimadzu Corporation, Kyota, Japan) following the method described by Wei et al. [18]. The oil was diluted in the ratio of 1 : 10 with ethanol, and 1 μL of the extract was injected into BPX5 capillary column (30.0 m × 0.25 mm × 0.25 μm, composed of 5% phenyl/95% methylpolysilphenylene/siloxane) (Trajan Scientific, Victoria, Australia). Helium was the carrier gas, and a split ratio of 1 : 10 was used. The oven temperature was kept at 50°C and then gradually increased at a rate of 3°C/min to 300°C at a linear velocity 32.4 cm/sec and held for about 10 min. The temperature at the injection port and detector temperature was 280°C. Mass spectra were taken at 70 eV (a scan interval of 0.1 s and scan range from 40 to 700 m/z). The metabolites were identified by matching their mass spectra with those of stored standard compounds in the database using the Shimadzu National Institute of Standards and Technology Mass Spectral database (Shimadzu NIST-MS [18]). The name, molecular weight, and structure of the components of the test extracts were ascertained.

2.8. ^{1}H-NMR Metabolic Profiling. The metabolomics profiling was carried out for the torch ginger flower oil to identify the metabolites that demonstrated antibacterial activity. Metabolomics profiling was performed using ^{1}H-NMR following the method as described by Mediani et al. [19]. Briefly, 10 mg of the oil sample was mixed with 0.375 mL of CH_3OH-d_4 and 0.375 mL of KH_2PO_4 buffer in D_2O (pH 6 adjusted with NaOH) containing 0.1% TSP. The mixture was subjected to vortex for 1 minute and then then ultrasonicated for 15 minutes at 30°C. The mixture was centrifuged at 13,000 rpm for 10 minutes, and the supernatant (600 μL) was transferred to the NMR tube for ^{1}H-NMR analysis using a 500 MHz spectrometer (Varian INOVA model Inc., California, USA). The NMR spectra were analysed using Chenomx NMR Suite version 8.1 (Alberta, Canada) to identify the metabolites and confirmed with Human Metabolome Database (HMDB) [20].

2.9. Statistical Analysis. All experiments for disc diffusion were performed three times with triplication ($n = 3 \times 3$). The results were interpreted as mean ± standard deviation (SD). Analysis of variance was performed, and the significant differences recorded between mean values were determined by Tukey's pair wise comparison test (level of significance of $P < 0.05$). Statistical analyses were conducted using MINITAB 16 software (Minitab, Inc., State College, Pennsylvania, USA).

3. Results and Discussion

The oil of torch ginger flower demonstrated strong antibacterial activity towards the tested pathogenic bacteria in the agar disc-diffusion assay. The diameter of inhibition zones against *S. aureus* and *E. coli* was significantly ($P < 0.05$) higher than that of the positive control, while the positive control exhibited higher clear zone towards *S. typhimurium* (Table 1). The positive control (streptomycin) inhibition zones ranged from 8.5 ± 0.4951 b to 19.5 ± 0.354 mm against the tested pathogenic bacteria. However, the 10% DMSO (negative control) did not show growth inhibition towards the selected bacteria. The oil extracted using subcritical carbon dioxide (CO_2) demonstrated very strong antibacterial activity against *S. aureus* (14.5 ± 2.211 mm). In a previous study, the antibacterial activity of torch ginger flowers oil extracted using dichloromethane against *Bacillus cereus* was 13 mm [10]. Moreover, Wijekoon et al. [3] reported that the pathogenic bacteria, namely, *B. cereus*, *B. subtilis*, *S. aureus*, and *Listeria monocytogenes*, showed moderated susceptibility to the oil of torch ginger flower that was extracted using solvents. The results of this study demonstrated significantly strong antibacterial activity for torch ginger flower oil extracted using subcritical CO_2 technique against the tested pathogenic bacteria.

The antibacterial activity was further evaluated using 96-well microtitter plate assay to determine the MIC and MBC.

Table 1: Antibacterial activity of torch ginger (*E. elatior* Jack) flower oil (50 mg/mL) extracted using subcritical CO_2.

Microorganism	Inhibition zone (mm)	
	EO (20 mg/disc)	Streptomycin (25 μg/disc)
Salmonella typhimurium	17.5 ± 0.827^b	19.5 ± 0.354^a
Staphylococcus aureus	14.5 ± 2.211^a	12.5 ± 1.424^b
Escherichia coli	14 ± 1.324^a	8.5 ± 0.4951^b

Values are expressed as mean ± standard deviation ($n = 9$). Different letters show the significant differences ($P < 0.05$), and same letters show no significant differences in the row.

Table 2: The MIC and MBC of torch ginger (*E. elatior* Jack) flower oil against pathogenic bacteria in comparison to streptomycin.

Microorganism	EO		Streptomycin	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Salmonella typhimurium	0.625	2.5	2.5	5
Staphylococcus aureus	2.5	5	5	10
Escherichia coli	2.5	10	5	10

The MIC value of torch ginger flower oil was 0.0625 mg/mL for *S. typhimurium*, 0.25 mg/mL for *S. aureus*, and 0.25 mg/mL for *E. coli*. On the other hand, the MBC values were 0.25 mg/mL for *S. typhimurium*, 0.5 mg/mL for *S. aureus*, and 0.25 mg/mL for *E. coli* (Table 2). In a previous study, Abdelwahab et al. [21] studied the antibacterial activity of torch ginger oil extracted with different solvents. The results showed no growth inhibition against 3 of the tested pathogenic bacteria, while the MIC value for *S. aureus* was 10 mg/mL. However, in this study, the MIC for *S. aureus* was 0.25 mg/mL which is significantly ($P < 0.05$) low in comparison to the previous studies. In another study, the MIC values for torch ginger aqueous and ethanolic extracts ranged from 37.5–125 mg/mL to 50–75 mg/mL, respectively, while the MBC values ranged from 50–175 mg/mL and 50 mg/mL for aqueous and ethanolic extracts, respectively [22]. The oil extracted by CO_2 demonstrated significantly higher antibacterial activity and lower values for the MIC and MBC towards the pathogenic bacteria in comparison to the solvent extractions reported in the previous studies.

Plant oils contain a great number of secondary metabolites characterized by strong aromas that are used in food and pharmaceutical industries. The oils have a complex composition containing hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (acids, acetals, alcohols, aldehydes, esters, ethers, ketones, lactones, oxides, and phenols). In this study, subcritical CO_2 extraction yielded in 5.5% pure yellow oil with a strong antibacterial activity. The strong antibacterial activity of torch ginger flower oil might be due to the high content of fatty alcohol and/or fatty acids [23]. Moreover, major chemical compounds present in torch ginger flower oil such as polyphenols, flavonoids, anthocyanins, and tannins can also possess strong antimicrobial activities against pathogenic bacteria [24]. In this study, the GC-MS profiling led to identify 33 compounds in the oil of torch ginger flower including 15 compounds that are well-known for their antibacterial activity (Table 3). The predominant chemical classes of the oil consisted of oxygenated compounds including alcohol (15.53%) followed by aldehydes (7.81%), esters (5.06%) acids (1.27%), and (8.23%) terpene hydrocarbons (monoterpenes and sesquiterpenes). Several previous studies reported the antibacterial activity of fatty acid alcohols against different pathogenic bacteria [24, 38]. Chiang et al. [23] suggested that compounds present at low levels are also having high potential for antibacterial activity that has synergic effects and more than one specific mechanism. However, the strong antibacterial activity of torch ginger flower oil in this study could be due to the extraction method, different sensitivity of the test strain, and the species of the plant. Zoghbi and Andrade [39] identified 15 compounds using GC-MS including 1-dodecanol as major component followed by dodecanal and α-pinene. In another study, the oil extracted from different parts of Malaysian torch ginger were analysed by GC-MS, the flowers and rhizomes contained 1-dodecanediol diacetate (40.4%) and cyclododecane (34.5%), while the leaf contained β-pinene (19.7%), β-caryophyllene (15.4%) and trans-β-farnesene (27.1%), and the stem compounds were 1,1-dodecanediol diacetate (34.3%) and trans-5-dodecene (27.0%) [40]. The results of this study are in agreement with the previous studies, and major compounds identified are well-known for their antibacterial activity.

The bioactive metabolites were further identified using ^{1}H-NMR in combination with the compounds available at Chenomx database. A total of 16 metabolites were identified in the oil of torch ginger extracted by subcritical carbon dioxide including 8 metabolites known for their antimicrobial activity (Table 4). Several acids were identified for the first time in the oil of torch ginger flower such as azelaic acid, butyric acid, citraconic acid, capric acid, caprylic acid, valeric acid, citric acid, syringic acid, chlorogenic acid, and citraconic acid (Figure 1). The results revealed the presence of several saturated fatty acids such as hexacosanoic acid, capric acid, and caprylic acid at high concentrations. Several studies reported the correlation between antibacterial activity and the short fatty acids [43, 47]. NMR analysis is used as the rapid identification method of the main metabolites and their concentrations, especially the metabolites

Table 3: GC-MS metabolomics profiling of torch ginger (*E. elatior* Jack) flower oil.

No.	Compound name	RT[a]	Peak area %	Activity	Reference
1	Cyclohexane	2.60	0.73	—	—
2	Cyclopentanol, 1-methyl-	4.33	0.12	—	—
3	*α*-Pinene	8.16	2.23	Antibacterial	Nissen et al. [25]
4	*cis*-pinen-3-ol	8.95	0.16	—	—
5	Bornylene	12.07	0.17	Antibacterial	Goudjil et al. [26]
6	2-Nonanone	15.02	0.15	Antibacterial	Orlandaand and nascimento, [27]
7	Nonan-2-ol	15.49	0.33	—	—
8	*trans*-verbenol	17.81	0.31	Antibacterial	Utegenova et al. [28]
9	*α*-Terpineol	20.26	0.25	Antibacterial	Li et al. [29]
10	Decanal	20.64	0.65	Antibacterial	Verma et al. [30]
11	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)	20.99	0.26	—	—
12	1-Decanol	23.79	1.46	Antibacterial	Togashi et al. [31]
13	2-Undecanone	24.74	1.55	Antibacterial	Orlandaand and nascimento, [27]
14	Methyl nonyl carbinol	25.11	0.37	—	—
15	Undecanal	25.45	0.18	Antibacterial	Kubo et al. [32]
16	1-Undecanol	28.46	0.30	Antibacterial	Togashi et al. [31]
17	Decanoic acid	29.36	0.30	Antibacterial	Huang et al. [33]
18	(±)-*α*-Terpinyl acetate	29.76	0.19	—	—
19	Dodecanal	30.30	6.50	—	—
20	*trans*-Caryophyllene	30.52	2.09	—	—
21	*α*-Humulene	32.16	1.88	Antibacterial	Azizan et al. [34]
22	Dodec-(8Z)-en-1-ol	32.44	0.17	—	—
23	1-Dodecanol	33.46	11.44	—	—
24	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)	33.60	0.87	—	—
25	2-Tridecanone	33.93	1.09	—	—
26	*α*-Bisabolol	34.31	0.04	Antibacterial	de sousa oliveira et al. [35]
27	Mentha-1(7),8-dien-2-ol, cis-para	34.56	0.23	—	—
28	Methyl dodecanoate	34.96	0.84	—	—
29	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl	36.52	0.35	—	—
30	Dodecanal <dimethyl-> acetal	36.89	0.48	—	—
31	Oleic acid	37.10	0.09	Antibacterial	Dilika et al. [36]
32	Lauryl acetate	38.49	4.03	—	—
33	Lauric acid	38.77	0.88	Antibacterial	Nakatsuji et al. [37]

[a]RT, retention time (min).

Table 4: Chemical shifts and concentrations of the bioactive metabolites identified in the oil of torch ginger flower.

No.	Metabolites	Chemical shift	Concentration mM	Activity	Reference
1	Hexacosanoic acid	*δ* 0.87 (t)	0.3351	Antimicrobial	Singh and Singh, [41]
2	Methylmalonic acid	*δ* 1.23 (d)	1.0311	—	—
3	Azelaic acid	*δ* 1.54 (m)	0.1341	Antimicrobial	Leeming et al. [42]
4	Butyric acid	*δ* 1.55 (tq)	0.316	Antibacterial	Fernández-Rubio et al. [43]
5	Citraconic acid	*δ* 1.93 (s), *δ* 5.598 (d)	0.0925	—	—
6	Capric acid	*δ* 2.16 (br s), *δ* 1.53 (br s), *δ* 1.27 (br s), *δ* 0.85 (br s)	0.3272	Antimicrobial	Bergsson et al. [44]
7	Caprylic acid	*δ* 2.16 (t), *δ* 1.53 (m), *δ* 1.27 (d), *δ* 0.85 (m)	0.2272	Antimicrobial	Nair et al. [45]
8	Valeric acid	*δ* 2.19 (t)	0.3137	Antimicrobial	Sunkara et al. [46]
9	Citric acid	*δ* 2.66 (d), *δ* 2.52 (d)	0.0299	Antimicrobial	Allende et al. [47]
10	Ethanolamine	*δ* 3.13 (d, $J = 19.8$ Hz)	0.0222	—	—
11	Trimethylamine N-oxide	*δ* 3.25 (s)	0.157	—	—
12	1,3-Dimethyluric acid	*δ* 3.29 (s)	0.4813	—	—
13	1,3-Dimethyluric	*δ* 3.428 (s), *δ* 3.298 (s)	0.0257	—	—
14	Sarcosine	*δ* 3.6 (s)	0.0536	—	—
15	Syringic acid	*δ* 3.84 (s)	0.0126	—	—
16	Chlorogenic acid	*δ* 5.33 (m)	0.1234	Antimicrobial	Tajik et al. [48]

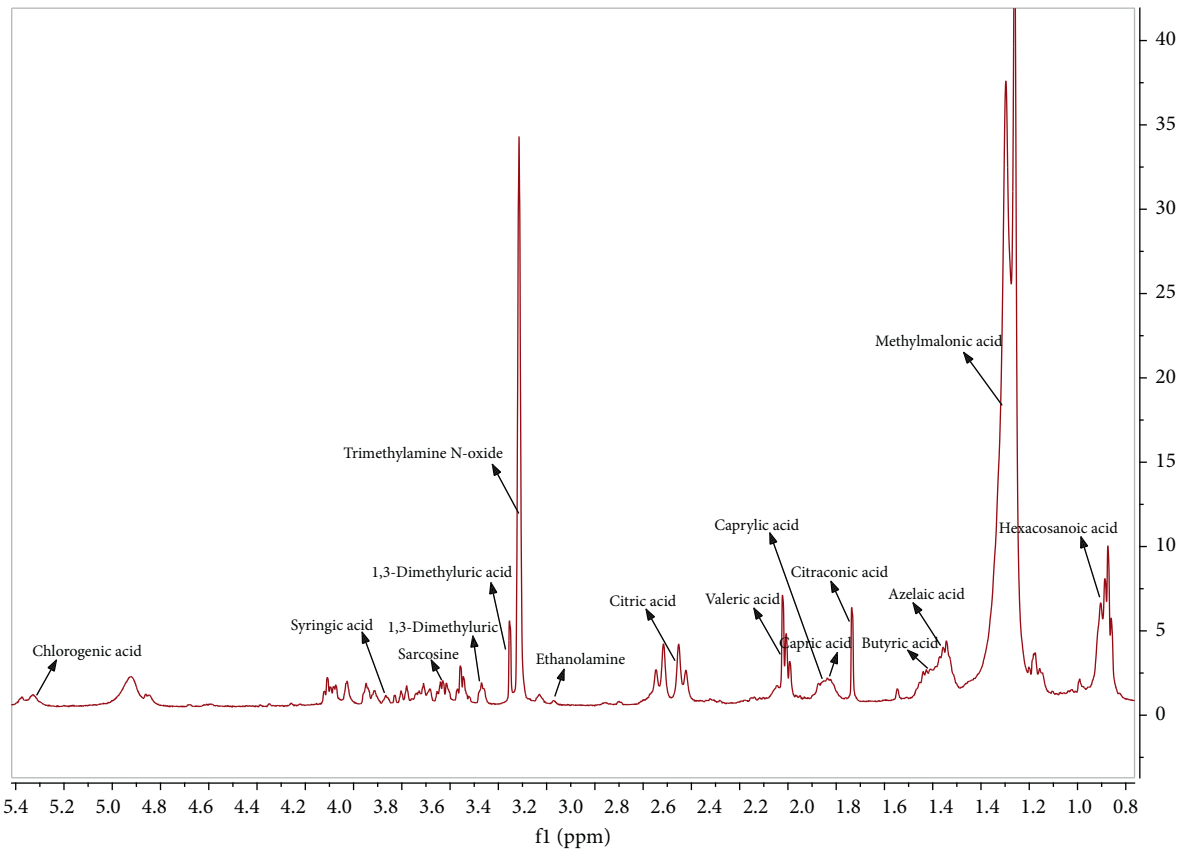

FIGURE 1: ^{1}H-NMR representative spectra of the identified metabolites of the oil extracted from torch ginger flower by the subcritical carbon dioxide method.

that demonstrated strong antimicrobial activity. Anderson et al. [49] identified several compounds of 6 oil samples using the combination of ^{1}H-NMR and the principle component analysis (PCA). In another study, NMR analysis was used to discriminate several oils including olive, hazelnut, and sunflower [50]. Moreover, interesting results were observed for the chilli, black pepper, and ginger oils extracted using subcritical CO_2 and analysed by NMR, and the results demonstrated higher concentration of the bioactive compounds in comparison to conventional extraction by organic solvents [51].

Torch ginger flower oil was subjected to storage to determine the stability of the antibacterial activity and the potential applications in pharmaceutical and food industries. In a previous study, oil was applied as natural preservative to extend the shelf life and prevented spoilage [52]. In this study, the extracted oil exhibited strong antibacterial activity against the tested pathogenic bacteria after being stored for 12 months at 8°C. Several previous studies recommended storing the oils at −20°C to reduce oxidation of the oil and maintain the biological activity [53, 54]. However, the results of this study demonstrated minimal effects of the storage at 8°C for 12 months on the stability of the antimicrobial activity of the oil extracted using subcritical CO_2. Scollard et al. [55] reported similar results for the oils extracted from thyme, oregano, and rosemary that maintained the antibacterial activity against *L. monocytogenes* during the storage at 4°C–8°C. The results indicated that the extraction of the oil using subcritical CO_2 was able to maintain the strong antimicrobial activity.

4. Conclusion

Subcritical carbon dioxide (CO_2) extraction was applied to extract oil from torch ginger flowers with minimum effect on the antibacterial activity. CO_2 extraction at low temperature prevented thermal degradation of the bioactive compounds. The oil of torch ginger flower contained bioactive compounds such as 1-dodecanol, saturated fatty acids, and organic acids that demonstrated a strong antimicrobial activity. The combination of GC-MS and NMR-based metabolomics profiling was used to identify the bioactive compounds in the oil. The oil of torch ginger flower extracted with subcritical CO_2 has a high potential for pharmaceutical and food applications as natural antibacterial agents. The antibacterial activity of the extracted oil was very stable after the storage for 12 months at 8°C. Further study is recommended to optimize the extraction conditions and enhance the yield of the extracted oil.

Data Availability

All data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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