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Chemical profiling of *Curcuma aeruginosa* Roxb. rhizome using different techniques of solvent extraction

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ABSTRACT

Objective: To investigate the possible phytochemical constituents of *Curcuma aeruginosa* Roxb. (*C. aeruginosa*) rhizome using two different techniques of direct solvent extraction.
Methods: Two different techniques of direct solvent extractions, *i.e.* methyl tert-butyl ether (MTBE) extraction and two-phase methanol/chloroform (M/C) system, were used in this study. The analysis of the phytochemical constituents in MTBE and M/C extracts was performed using gas chromatography-mass spectrometry/mass spectrometry. The mass spectra of the compounds was matched with the NIST 08 mass spectral library.
Results: The present study revealed that the extraction using two-phase M/C have resulted in higher metabolite coverage compared to the extraction with MTBE. Direct solvent extraction using MTBE revealed the presence of 27 compounds; whereas, M/C allowed the extraction of 18 and 36 compounds in polar (methanol) and nonpolar (chloroform) fractions respectively. The major compounds detected in the MTBE extract that based on the peak area percentage were methenolone (16.64%), cycloisolongifolene, 8,9-dehydro-9-formyl- (15.93%), labd-13-en-15-oic acid,8,12-epoxy-12-hydroxy-γ-lactone (10.77%), propiolic acid, 3-(1-hydroxy)-2 isopropyl-1,5-methylcyclohexyl) (7.84%), 4-oxo-β-isodamascol (5.17%), velleral (3.11%) and Z-α-farnesene (2.00%). The most prevailing major compounds identified in the polar fraction of the M/C extraction were α-D glucopyranoside, 1,3,4,6 tetrakis-O-(TMS) (trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS)- (38.08%), d-glucose, 2,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime (14.61%), D-fructose, 1,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime (5.28%), isocitric acid (TMS) (3.06%), oxalic acid, bis (TMS) ester (2.96%), hexadecanoic acid, TMS ester (2.16%), citric acid, ethyl ester, tri-TMS (1.91%) and butanedioic acid, [(TMS) oxy]-, bis (TMS) ester (1.14%); whereas in the nonpolar extract, among the major compounds detected were cycloisolongifolene, 8, 9-dehydro -9-formyl (15.70%), propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl) (11.09%), stearic acid, TMS ester (2.78%), hexadecanoic acid, TMS ester (2.33%), oleic acid, TMS ester (1.62%), curzerene (1.56%); Z-α-farnesene (1.52%), germacrone (1.41%) and β-elemene (1.33%).
Conclusions: It was evident from the results that *C. aeruginosa* rhizome extracted using two different techniques of solvent extractions (MTBE and M/C) contained various chemical classes of compounds including terpenoids, sterols, organic acids, fatty acids and sugars. Different methods of extraction have led to different compounds extraction for *C. aeruginosa* rhizome. The results also indicated that the plant was a source of phytochemical importance.

1. Introduction

Throughout history, plants have long been used traditionally for treatment of various diseases and ailments. In the last few decades, the trend of using plant-based medicine is increasing remarkably especially in the pharmaceutical industry. Pharmaceutical companies nowadays extensively explore into biodiversity-rich regions all over the world to develop their research towards plant materials that contain valuable medicinal properties. The tremendous attention given towards plant or plant-based products may be primarily due to the safety issues when dealing with human subject as well as the fact that they are more economical and easily available as compared to their synthetic counterparts[1]. A different

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class of phytochemicals present in plants such as flavonoids, phenylpropanoids and terpenoids contributes to the efficacy of plant extracts for their therapeutic functions.

Curcuma aeruginosa Roxb. (*C. aeruginosa*) (Zingiberaceae) known as pink and blue ginger in English is among the indigenous and underutilized ethno-medicinal plants in Southeast Asia which have been traditionally used to treat various ailments[2]. The plant is also commonly named as temu hitam in Malaysia or Waan-maa-haa-mek or kajeawdang in Thailand[3]. The rhizome of *C. aeruginosa* was traditionally used to treat gastrointestinal problems such as diarrhea and fungal infections[4]. The rhizome was also reported to have pharmacological activities in treating various diseases such as tumors, asthma and bronchitis[5]. In Malaysia, little work has been done on this herb to obtain a more complete phytochemical composition and understand their biological activities. The identification of the phytochemical composition including the bioactive compounds of these plants, is one of the necessary steps must be taken to explain their medicinal properties. As every plant has its own metabolite fingerprinting, profiling by some platform technology such as gas chromatography-mass spectrometer (GC-MS), liquid chromatograph-mass spectrometer and nuclear magnetic resonance is crucial to have a holistic overview of all metabolites present in the extracts. The present study was aimed to investigate the phytochemical constituents of *C. aeruginosa*by using two different techniques of solvent extraction and GC-MS analysis. The information obtained was essential as a preliminary step to proceed with a significant use of this herbal plant as the source of health-related products such as functional foods or pharmaceuticals to prevent or treat certain diseases.

2. Materials and methods

2.1. Chemical and reagents

Methanol and chloroform were purchased from Fisher Scientific (Leicester, UK), while ribitol, methylnonadecanoate and methyl tert-butyl ether (MTBE) were purchased from Sigma Aldrich (St. Louis, MO, USA). Methoxyamine hydrochloride and N-methyl-N-trimethylsilyl (TMS) trifluroacetamide were purchased from Acros Organics (New Jersey, USA). Pyridine was purchased from Merck (Darmstadt, Germany).

2.2. Collection, identification and preparation of plant material

The rhizomes of *C. aeruginosa* were collected from Temerloh, Pahang (Kiza Herbs), Malaysia in April 2013. The botanical identification on the basis of morphological features was performed by scientist Dr. Indu Bala Jaganath. The voucher specimens (No: MDI 12804) were deposited at Malaysian Agricultural Research and Development Institute herbarium. The rhizome buds were germinated and the plantlets were grown in the same plant house located in the institute. The rhizomes were cut into small pieces and immediately frozen in liquid nitrogen before being ground to a fine powder by using mortar and pestle. The samples were then freeze-dried for a few days and kept at -20 ℃ until further use.

2.3. Extraction of freeze-dried rhizome sample using MTBE

The extraction procedure was performed according to Jiang *et al.*[6] with a slight modification. Briefly, 10 mL MTBE was added to a glass vial containing 1.0 g of freeze-dried rhizomes and the mixtures were then incubated with shaking for 6 h at room temperature. After sonication for 10 min, the samples were centrifuged using the GSA centrifuge rotor at 5 400 r/min for 10 min. The supernatant obtained was then filtered using a 0.2 µm polytetrafluoroethylene filter membrane. A total of 200 µL of filtrate were injected directly to gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS).

2.4. Extraction of freeze-dried rhizome using two-phase methanol/chloroform (M/C) system

The extraction, methoximation of carbonyl moieties and derivatization of the samples prior to the GC-MS analysis were performed according to Roessner's method with minor modifications[7]. Methanol (8 mL) (including ribitol and methyl nonadecanoate as the internal standards for polar and nonpolar compounds respectively) was added to 1.0 g of freeze-dried rhizome samples and incubated for 15 min at 70 ℃ using water bath. The extracts were then mixed vigorously with 8.0 mL of distilled water. Lastly, 4.5 mL of chloroform was added to the mixture before the phase separation by centrifugation. Polar (methanol) and nonpolar (chloroform) supernatant were dried in a vacuum concentrator for 2-6 h. The dried polar extracts were re-dissolved in 50 µL of pure pyridine and 40 µL of 20 mg/mL methoxyamine hydrochloride in pyridine and then incubated for 90 min at 37 ℃. Finally, the silylation step for both polar and nonpolar extracts was performed by adding 250 µL N-methyl-N-TMS trifluroacetamide (Sigma Aldrich) to the extracts followed by incubation at 37 ℃ in water bath for 1 h. The polar (methanol) and nonpolar (chloroform) extracts were cooled down at room temperature for at least 1 h before GC-MS injection.

2.5. GC-MS profiling of C. aeruginosa rhizome

Sample volumes of 1 µL were injected with a splitless mode into a GC-MS/MS system which consist of TSQ Quantum XLS GC-MS/MS (Thermo Scientific Co.). The GC column used for the analysis was TG-5MS with an inner diameter of 0.25 mm, 30 m length and 0.25 µm film thickness. Helium gas was used as carrier gas at a flow rate of 1 mL/min. The extracted sample using M/C technique was analyzed under the following oven temperature program: injection at 70 ℃ followed by 1 ℃/min oven temperature ramp to 76 ℃ and then by 6 ℃/min to 330 ℃ and finally with 10 min isothermal at 330 ℃. For the MTBE samples, the temperature program was set according to Jiang *et al*[6]. Mass spectra were acquired using full scan monitoring mode with a mass scan range of 50-700 m/z. The chromatogram and mass spectra were evaluated using the Xcalibur™ software embedded in the GC-MS/MS system.

2.6. Identification of phytochemical constituents

The identification of the compounds including their name, molecular formula and molecular weight was performed by

matching the spectra of all chromatogram peaks with the spectrum of the known compounds in the NIST 08 mass spectral library.

3. Results

3.1. GC-MS analysis of C. aeruginosa rhizome using MTBE technique

After successful extraction using MTBE as the extraction solvent, GC-MS analysis of *C. aeruginosa* rhizome revealed the presence of 27 different compounds. Compounds belonging to the class of monoterpenoids and sesquiterpenoids represented 75% of the total identified compounds in which oxygenated monoterpenoids and hydrocarbon sesquiterpenoids were the dominant compounds in their respective groups. Cineole, camphor and borneol were among the oxygenated monoterpenoids that were presented in the extract; whereas, for hydrocarbon sesquiterpenoids, β- and δ-elemene, caryophyllene and germacrene B were among the highlighted compounds. Diterpene lactone (labd-13-en-15-oic acid,8,12-epoxy-12-hydroxy-γ-lactone), plant sterol (β-sitosterol), naturally occurring steroid (methenolone)[8] and sesquiterpene dialdehyde (velleral) were also detected in the extract. Table 1 shows the molecular formula, molecular weight and the peak area percentage of the identified compounds. The major compounds which based on the peak area percentage were methenolone (16.64%), cycloisolongifolene, 8,9-dehydro-9-formyl- (15.93%), labd-13-en-15-oic acid,8,12-epoxy-12-hydroxy-γ-lactone (10.77%), propiolic acid, 3-(1-hydroxy)-2 isopropyl-1,5-methylcyclohexyl) (7.84%), 4-oxo-β-isodamascol (5.17%), velleral (3.11%) and Z-α-farnesene (2.00%). Oxygenated monoterpenoids detected in the extract included cineole, borneol and α-terpineol; whereas, sesquiterpene hydrocarbons included curzerene and germacrone B.

3.2. GC-MS analysis of C. aeruginosa rhizome using two-phase M/C technique

The GC-MS analysis of the *C. aeruginosa* rhizome extracts using methanol/chloroform/water as the extraction solvent enabled the identification of 18 and 36 different compounds from polar (methanol) and nonpolar (chloroform) fractions respectively, as shown in Tables 2 and 3. The compounds presented in the polar fraction differed from that in the nonpolar fraction. Metabolites in the group of sugars, organic acids and alkanes dominated the polar fraction; whereas, in the nonpolar fraction, *ca.* 50% of the compounds were dominated by terpenoids and fatty acid groups. In the polar extract, oxalic acid, bis (TMS) ester, malonic acid, bis (TMS) ester, butanoic acid, 4-[(TMS) oxy]-TMS ester were among the organic acid esters found in *C. aeruginosa* rhizome. Sugars represented by α-D lucopyranoside, 1,3,4,6 tetrakis-O-(TMS)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS)-, D-glucose, 2,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime and D-fructose, 1,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime were the most pronounced compounds that gave higher peaks compared to other compounds. Three alkanes (tetracosane, triacontane and tetratriacontane) and

Table 1

Phytochemical constituents identified in the MTBE extract of *C. aeruginosa* using GC-MS.

Compound identified	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
3-Carene	$C_{10}H_{16}$	136	7.53	0.19
Camphene	$C_{10}H_{16}$	136	7.85	0.24
2-Thujene	$C_{10}H_{16}$	136	8.34	
β-Pinene	$C_{10}H_{16}$	136	8.42	0.54
Cineole (Eucalyptol)	$C_{10}H_{18}O$	154	9.51	1.90
Acetophenone	C_8H_8O	120	10.28	0.57
Camphor (1R,4R)	$C_{10}H_{16}O$	152	12.47	0.52
Borneol	$C_{10}H_{18}O$	154	12.90	0.12
Terpinen-4-ol	$C_{10}H_{18}O$	154	13.61	0.03
α-Terpineol	$C_{10}H_{18}O$	154	14.10	0.10
δ-Elemene	$C_{15}H_{24}$	204	19.87	0.20
β-Elemene	$C_{15}H_{24}$	204	22.08	0.83
Caryophyllene	$C_{15}H_{24}$	204	23.20	0.22
Germacrene B	$C_{15}H_{24}$	204	23.72	0.11
Z-α-farnesene	$C_{15}H_{24}$	204	24.65	2.00
Epi-bicyclosesquiphellandrene	$C_{15}H_{24}$	204	25.64	0.35
Curzerene	$C_{15}H_{20}O$	216	26.25	1.00
Cycloisolongifolene, 8,9-dehydro-9-formyl-	$C_{16}H_{22}O$	230	30.53	15.93
Germacrone	$C_{15}H_{22}O$	218	33.71	0.45
8,9 b-Dimethyl-4a,9b-dihydrodibenzo[b,d]furan-3(4H)-one	$C_{15}H_{22}O$	218	35.85	0.73
Propiolic acid, 3-(1-hydroxy)-2 isopropyl-1,5-methylcyclohexyl)	$C_{13}H_{20}O_3$	224	36.95	7.84
Velleral	$C_{15}H_{20}O_2$	232	37.21	3.11
4-Oxo-β-isodamascol	$C_{13}H_{20}O_2$	208	38.91	5.17
Methenolone	$C_{20}H_{30}O_2$	307	54.59	16.64
Labd-13-en-15-oic acid,8,12-epoxy-12-hydroxy-γ-lactone	$C_{20}H_{30}O_3$	318	59.26	10.77
Cholesta-22,24-dien-5-ol-4,4-dimethyl-	$C_{29}H_{48}O$	412	75.37	0.30
β-Sitosterol	$C_{29}H_{50}O$	414	76.85	0.67

Table 2

Phytochemical constituents identified in the polar (methanol) extract of *C. aeruginosa* using GC-MS analysis.

Compound identified	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
Oxalic acid, bis (TMS) ester	$C_8H_{18}O_4Si_3$	234	15.77	2.96
Malonic acid, bis (TMS) ester	$C_9H_{20}O_4Si_2$	248	17.67	0.05
Butanoic acid, 4-[(TMS) oxy]-TMS ester	$C_{10}H_{24}O_3Si_2$	248	18.49	0.15
L-alanine, N-octanyl-ethyl ester	$C_{13}H_{25}NO_3$	243	21.13	0.08
Butanedioic acid, [(TMS) oxy]-, bis (TMS) ester	$C_{13}H_{30}O_5Si_3$	350	25.32	1.14
Citric acid, ethyl ester, tri-TMS	$C_{17}H_{36}O_7Si_3$	436	31.80	1.91
Isocitric acid (TMS)	$C_{18}H_{40}O_7Si_4$	480	32.88	3.06
D-Fructose, 1,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime	$C_{22}H_{55}NO_6Si_5$	569	34.18	5.28
D-Glucose, 2,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime	$C_{22}H_{55}NO_6Si_5$	569	34.75	14.61
Hexadecanoic acid, TMS ester	$C_{19}H_{40}O_2Si$	328	36.78	2.16
myo-Inositol, 1,2,3,4,5,6-hexakis-O-(TMS)-	$C_{24}H_{60}O_6Si_6$	612	38.21	0.75
4,4-Dimethyl-N-(2-phenylethyl)-5α-androst-2-en-17-amine	$C_{29}H_{43}N$	405	39.97	0.21
Stearic acid, TMS ester	$C_{21}H_{44}O_2Si$	356	40.27	0.97
Tetracosane	$C_{24}H_{50}$	338	42.81	0.40
17-Hydroxy-3,20-dioxopregna-1,4,9 (11)-trien-21-yl acetate	$C_{23}H_{28}O_5$	384	43.80	0.23
Triacontane	C_3H_{62}	422	44.39	0.45
Tetratriacontane	$C_{34}H_{70}$	478	47.96	0.27
α-D Glucopyranoside, 1,3,4,6 tetrakis-O-(TMS)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS)-	$C_{36}H_{86}O_{11}Si_8$	918	48.36	38.08

Table 3

Phytochemical constituents identified in the nonpolar (chloroform) extract of *C. aeruginosa* using GC-MS analysis.

Compound identified	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
3-methyl cyclopentane-1-yl-TMS ether	$C_9H_{18}OSi$	170	11.42	0.17
Eucalyptol (cineole)	$C_{19}H_{18}O$	154	12.68	0.48
Thiosalicylic acid O, S-di-TMS-	$C_{13}H_{22}O_2SSi_2$	298	14.88	0.11
Camphor	$C_{10}H_{16}O$	152	15.86	0.67
Terpinne-4-ol	$C_{10}H_{18}O$	154	16.75	0.10
α-Terpineol	$C_{10}H_{18}O$	154	17.10	0.10
Butane-1,3-diol, 1-methylene-3-methyl-bis (TMS) ether	$C_{12}H_{28}O_2Si_2$	260	17.24	0.10
Glycine, N-(TMS)-, TMS ester	$C_7H_{12}F_3NO_3Si$	243	17.91	0.10
Borneol-TMS ether	$C_{13}H_{26}OSi$	226	18.01	0.72
Phenylethanolamine	$C_{17}H_{35}NOSi_3$	353	18.42	0.46
Tris (TMS) phosphate	$C_9H_{27}O_4PSi_3$	314	19.65	0.27
δ-Elemene	$C_{15}H_{24}$	204	21.11	0.43
β-Elemene	$C_{15}H_{24}$	204	22.57	1.33
Caryophyllene	$C_{15}H_{24}$	204	23.29	0.21
Z-α-farnesene	$C_{15}H_{24}$	204	24.14	1.52
β-Cubebene	$C_{15}H_{24}$	204	24.83	0.72
Curzerene	$C_{15}H_{20}O$	216	25.21	1.56
(2,6-ditert-butylphenoxy) (trimethyl) silane	$C_{17}H_{30}OSi$	278	26.37	0.58
Cycloisolongifolene, 8, 9-dehydro -9-formyl	$C_{16}H_{22}O$	230	27.94	15.70
Formic acid, 2-bromomethyl-4, 4-dimethyl-3-(3-oxobut-1-enyl) cyclohex-2-enyl ester	$C_{14}H_{19}O_4BrO_3$	314	29.15	2.46
Germacrone	$C_{15}H_{22}O$	218	29.87	1.41
Germacra-1(10), 4-diene-12-oic acid 6 alpha hydroxy gamma lactone	$C_{15}H_{22}O_{22}$	234	31.04	
Propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)	$C_{13}H_{20}O_3$	224	31.93	11.09
Velleral	$C_{15}H_{20}O_2$	232	33.21	0.33
Androst-4-en-17-one,3,16,bis ((TMS) oxy))-3α	$C_{25}H_{44}O_3Si_2$	448	34.34	1.38
2-Isopropenyl-2,3-dihydro-7H-furo (3,2-g) chromen-7-one	$C_{14}H_{12}O_3$	228	35.99	0.89
Hexadecanoic acid, TMS ester	$C_{19}H_{40}O_2Si$	328	36.79	2.33
Pregna-1,4,16-triene-3,20-dione,11,22-diacetoxy-	$C_{25}H_{30}O_6$	426	38.05	1.11
Heptadecanoic acid, TMS ester	$C_{20}H_{42}O_2Si$	342	38.55	0.47
Linoleic acid, TMS ester	$C_{21}H_{40}O_2Si$	352	39.73	0.53
Oleic acid, TMS ester	$C_{21}H_{42}O_2Si$	354	39.99	1.62
Stearic acid, TMS ester	$C_{21}H_{44}O_2Si$	356	40.32	2.78
4 alpha-methylandrostane-2,3-diol-17-dione	$C_{20}H_{30}O_4$	334	40.56	0.42
Anthiaergostan-5,7,9,22-tetraen-14-ol-15-one	$C_{28}H_{40}O_2$	408	41.54	0.22
19-Norpregn-4-en-20-yn-3-one, 17 (TMS) oxy	$C_{23}H_{34}O_2Si$	370	42.12	0.17
Androst-5-en 17-one, 3,16-bis [(TMS) oxy)], 0-methyloxime, (3β ,16α)	$C_{26}H_{47}NO_3O_2Si$	479	46.55	0.20

two fatty acid ester (hexadecanoic acid-TMS ester and stearic acid-TMS ester) were also found in the polar extract. The most prevailing major compounds identified in the polar fraction were α-D glucopyranoside, 1,3,4,6 tetrakis-O-(TMS)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(TMS)- (38.08%), D-glucose, 2,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime (14.61%), D-fructose, 1,3,4,5,6-pentakis-O-(TMS)-, O-methyloxime (5.28%), isocitric acid (TMS) (3.06%), oxalic acid, bis (TMS) ester (2.96%), hexadecanoic acid, TMS ester (2.16%), citric acid, ethyl ester, tri-TMS (1.91%) and butanedioic acid, [(TMS) oxy]-, bis (TMS) ester (1.14%). The other compounds were detected in less concentration (less than 1%). Most of the monoterpenoids and sesquiterpenoids which were found in the nonpolar extract were also found in the MTBE extract. These included bicyclic monoterpenoids (cineole and camphor) and sesquiterpenoids (curzerene and germacrone). However, borneol was found in the form of ether in the nonpolar extract. Based on the peak area percentage, the major compounds detected in the nonpolar extract were cycloisolongifolene 8,9-dehydro-9-formyl (15.70%), Propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl) (11.09%), stearic acid, TMS ester (2.78%), hexadecanoic acid, TMS ester (2.33%), oleic acid, TMS ester (1.62%), curzerene (1.56%); Z-α-farnesene (1.52%), germacrone (1.41%) and β-elemene (1.33%). Several plant steroids including pregna-1,4,16-triene-3,20-dione,11,22-diacetoxy-, androst-4-en-17-one,3,16,bis [(TMS) oxy)]-3, 4-alpha-methylandrostane-2,3-diol-17-dione, 19-norpreg-4-en-20-yn-3-one 17 (TMS)-oxy and anthiaergostan-5,7,9,22-tetraen-14-ol-15-one were also detected in the extract. Five fatty acid esters were also found in the chloroform extract. These included unsaturated fatty acid esters *i.e.* linoleic acid and oleic acid TMS esters.

4. Discussion

The GC-MS analysis of *C. aeruginosa* rhizome extracted using two different techniques of solvent extractions(MTBE and M/C) confirmed the presence of different chemical classes of compounds including terpenoids, sterols, organic acids, fatty acids and sugars. These compounds were identified by comparing the spectra of all chromatogram peaks with the spectrum of the known compounds in the NIST 08 mass spectral database library. The results of the GC-MS analysis indicated that the different methods have led to different compounds extraction for *C. aeruginosa* rhizome which showed that the selection of extractive techniques is crucial for extraction of the specific compounds[9]. The present study also revealed that the extraction using two-phase M/C have resulted in higher metabolite coverage compared to the extraction with MTBE. This was due to the fact that the extraction using M/C resulted in polar and nonpolar fractions where different chemical classes of compounds including fatty acids, organic acids and sugars were also extracted as compared to the extraction with the MTBE in which most of the compounds extracted were terpenoids. Direct solvent extraction using MTBE revealed the occurrence of 27 compounds whereas M/C allowed the extraction of 18 and 36 compounds in polar (methanol) and nonpolar (chloroform) fractions, respectively.

Cycloisolongifolene 8,9-dehydro-9-formyl, propiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl) and curzerene were among the major compounds identified in both MTBE extract and the nonpolar fraction of M/C. The result from this study also suggested that higher number of terpenoids could be extracted from MTBE as compared to M/C techniques. Most of the terpenoids identified in the nonpolar fraction by using M/C technique were also presented in the MTBE extract except for β-cubebene. In contrast, other terpenoids such as 3-carene, camphene, 2-thujene, β-pinene, germacrene B and epi-bicyclosesquiphellandrene could only be detected in the MTBE extract.

Several phytochemicals identified in the rhizome of *C. aeruginosa* in the present study have previously been reported by other researchers. These included compounds such as germacrone[5,10], cycloisolongifolene 8,9-dehydro-9-formyl, velleral and camphor[11], 1,8-cineole,β-pinene[12], curzerene[10] and β-elemene[13]. However, most of the above mentioned compounds were detected in the essential oil of *C. aeruginosa* rhizome. A phytochemical study on dried rhizome of the plant carried out by Sukari *et al.*[14] found three sesquiterpenoids *i.e.* zedoarol, curcumenol and isocurcumenol. However, the analysis was performed using nuclear magnetic resonance. Methenolone, the naturally occurring steroid that has been identified in the present study has been previously reported to occur in *Zingiber nimmonii*[15] and giant horsetail (*Equisetum giganteum* L.)[8].

The usage of plants or herbs for medicinal purposes relies on their phytochemical composition that exhibits some interesting and specific biological activities. Different phytochemicals identified in the present study have been found to possess a wide range of biological activities. It has long been reported that Zingiberaceae families contained a number of phytochemicals such as terpenoids, flavonoids, phenypropanoids and sesquiterpenes which exhibited anti-tumor activities[16,17]. It was also reported that essential oil of *C. rhizoma* and Ezhu You, a Chinese medicinal herb containing terpenoids as the main active compounds such as curzerene, cineole, germacrone and β-elemene showed anti-cancer activity towards gastric cancer cell lines[18]. The sesquiterpene, elemene which consists of β, α and δ-elemene has been approved by the Food and Drug Administration of China as anti-cancer adjuvant drug and consumed as part of cancer treatment in China[19]. Zhong *et al.* reported that sesquiterpenoid germacrone exhibited anti-cancer properties among others by inhibiting cell proliferation, increasing lactate dehydrogenase and mediating G1 and G2 cell cycle arrest in human breast cancers[20]. In this present study, one diterpene lactone, *i.e.* labd-13-en-15-oic acid,8,12-epoxy-12-hydroxy-γ-lactone, was found in the rhizome extract of *C. aeruginosa*. Diterpene lactones of labdane-type have been reported to possess a broad spectrum of biological activities. For instance, two novel labdane diterpenenoids isolated from *Alpinia calcarata* (Zingiberacae family) exhibited cytotoxicity activity towards human KB cell *in vitro*[21]. Several labdane diterpenes isolated from *Aster oharai* plant (Asteraceae) also showed anti-cancer activity against human colorectal cancer cell line (HCT115), human central nervous system cancer cell lines (SNB 19) and human skin cancer cells[22]. The study carried out by Cuadrado *et al.* on two labdane diterpenes *i.e.* dehydroisohispanolone and 8,9-dehydrohispanolone 15,16-lactol showed that the compounds exhibited anti-inflammatory and cryoprotective activities[23]. Fatty

acid compounds such as the compounds found in the nonpolar extract in this present study (fatty acid metyl ester, hexadecanoic acid methyl ester, linolenic acid) have been shown to posses antioxidant, antiproliferative, antimicrobial and anti-inflammatory activities[24,25].

The GC-MS analysis of *C. aeruginosa* rhizome extracted using two different techniques of solvent extractions (MTBE and M/C) confirmed the presence of various chemical classes of compounds including terpenoids, sterols, organic acids, fatty acids and sugars. The different methods of extraction have led to different compounds extraction for *C. aeruginosa* rhizome. The results also indicates that the plant is a source of phytochemical importance. The determination of the phytochemical composition by GC-MS in this species served to be only the preliminary steps in order to explore the biological potential of the compounds or the plant extract itself and for this, it warrants further investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

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