GC-MS-Based Metabolite Profiling and Cytotoxic Activity of *Cymbopogon nardus* Essential Oil from Different Regions

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Abstract: *This study aimed to investigate the chemical profiles and anticancer activity of essential oil from citronella grass (*Cymbopogon nardus *L.) obtained from two regions and using two methods. The cytotoxic activity of each essential oil was assessed using the Alamar Blue assay against MCF-7 cells, while their chemical profiles were determined by gas chromatography-mass spectrometry and further analyzed using metabolomic analyses such as principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA). Based on the Alamar blue assay, citronella oil has an IC_{50} value ranging from 41.23 ± 6.09 to 179.65 ± 58.43 μg/mL. GC-MS analysis identified 102 metabolites in citronella oil. Based on the peak areas of the 102 metabolites, PCA determined the distribution of metabolites in each essential oil and grouped them based on their distribution into the Cibinong group, Solok hydrodistillation-sonication group, and Solok hydrodistillation without sonication group. Based on OPLS-DA, the compounds with potential as breast cancer agents were citronellal, geraniol, δ-guaiene, cedryl acetate, and elemol.*

Keywords: *anticancer;* Cymbopogon nardus *L.; essential oil; MCF-7 cell, metabolomic*

■ INTRODUCTION

Breast cancer is the most common cause of cancer-related death in women around the world [1]. Research on natural anticancer sources, such as essential oils, continues to be pursued because they have fewer side effects than synthetic drugs [2]. Several studies have reported the potential of essential oils as anticancer agents, including the potential of essential oils from

Cymbopogon flexuosus as an anticancer agent for breast cancer [3], essential oils from *Pittosporum glabratum* as an anticancer agent for lung cancer [4], and essential oils from *Cymbopogon winterianus* as an anticancer agent for cervical cancer [5]. Essential oils are volatile secondary metabolites extracted from plants and are one of Indonesia's main export commodities. Essential oils extracted from plants are a potential source of bioactive metabolites [6]. These oils can be extracted from the roots, flowers, fruits, leaves, and seeds of plants [7]. One of the plants that contain essential oils is citronella (*Cymbopogon nardus* L.). Citronella essential oil is widely used in food, cosmetics, and pharmaceutical industries. In addition, this essential oil has pharmacological effects, including antifungal, antimicrobial, antiviral, anti-inflammatory, anticancer, and enzyme-inhibitory activities [8-11].

Citronella oil contains a mixture of monoterpenes, with three main compounds: citronellal, citronellol, and geraniol [12]. These compounds are used extensively in the soap, aromatherapy candle, perfume, and flavoring industries [13]. In addition, geraniol has been reported to have potential for the treatment or prevention of various types of tumors, such as pancreatic, liver, and breast cancers [14]. The large number of metabolites in citronella oil enables synergistic effects among compounds, thereby increasing their biological activities [15]. Differences in metabolite composition and concentration in citronella oil can affect its toxicity against cancer cells. However, the concentration and composition of metabolites in citronella oil can be influenced by several factors, such as differences in postharvest processing, plant part, cultivation age, geographic conditions, extraction method, and growing locations [16]. Therefore, knowledge of effective cultivation sites and extraction methods is needed to obtain high-quality citronella essential oil with the highest toxicity against MCF-7 breast cancer cells.

The effect of different extraction methods and growing locations on the chemical composition of citronella oil was evaluated using a metabolomics approach based on GC-MS combined with chemometrics. GC-MS not only has high separation efficiency but can also detect hundreds to thousands of metabolites in citronella oil and their isomers [17]. In 2021, Feng et al. [16] reported that a combination of metabolomics approaches using GC-MS and chemometrics is an effective method for evaluating differences in the chemical characteristics of essential oils from different growing locations.

Several studies have reported the potential of essential oils from the *Cymbopogon* genus as anticancer agents, such as *C. citratus* [18], *C. schoenanthus* [19], *C. flexuosus* [3], and *C. winterianus* [5]. However, no research has examined the effect of growing location and extraction method on the anticancer potential of *C. nardus*. Therefore, in this study, we further explored this using metabolomic analysis to predict compounds with cytotoxic activity against MCF-7 breast cancer cells in citronella oil from the Mahapengiri variety of Solok and Cibinong, obtained using two different methods. The citronella oil was extracted by hydrodistillation, with and without sonication pretreatment. This study provides a preliminary overview of the potential of citronella essential oil as an anticancer agent and examines the effects of extraction method and cultivation location on its compound content and anticancer activity.

■ EXPERIMENTAL SECTION

Materials

The citronella plants of the Mahapengiri variety were obtained from Cibinong, Bogor, West Java, specifically from the experimental garden of the Research Institute for Spices and Medicinal Plants (Balittro). The other citronella plants were obtained from Solok Garden in Jarong Jambo Lipo, Lubuk Tarab Sijunjung, West Sumatra. The fragrant lemongrass species, Mahapengiri variety, from Balittro and Sumatra, was identified in the Balittro Cibinong Bogor experimental garden. Ten kilograms of citronella leaves from each location were harvested at the age of three months by cutting the leaves at a distance of 3 cm from the base of the stem. They were cut into 2-cm pieces and then air-dried at room temperature for 3 days. MCF-7 breast cancer cells (ATCC® HTB-22™), trypsin-EDTA,

penicillin-streptomycin solution, and PrestoBlue™ cell viability reagent were purchased from Sigma–Aldrich Co., distilled water, cisplatin, DMSO, Roswell Park Memorial Institute medium (RPMI), fetal bovine serum (10% FBS), and phosphate-buffered saline (10% PBS) were from Merck.

Instrumentation

Characterization of citronella oil was performed using the Agilent 7890B/5977A Series GC/MSD system to identify chemical compounds. An ELISA microplate reader was also used to measure cell color intensity and calculate cell viability (%) after interacting with the test sample solutions.

Procedure

Extraction of essential oil by hydrodistillation

The citronella oils were obtained by 2 methods. Hydrodistillation with sonication pretreatment was initiated by sonicating the citronella leaves in distilled water at a 1:20 (g sample/mL solvent) ratio for 1 h at 90% amplitude. Hydrodistillation was then performed for 6 h. On the other hand, for hydrodistillation without sonication, citronella leaves were distilled in distilled water at a 1:20 ratio for 6 h.

Cytotoxic activity determination of essential oil

The cytotoxic activity of citronella essential oil against MCF-7 breast cancer cells was determined *in vitro* using the Alamar Blue assay, following a previous method with some modifications [20]. The MCF-7 cancer cells used were derived from RPMI (Gibco 11875-093). The MCF-7 cell line was cultured in RPMI medium supplemented with 10% FBS and 50 µL/50 mL of penicillin-streptomycin solution. The test sample solutions were prepared by diluting them in the aforementioned medium to 8 different concentrations (7.81, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL). The cisplatin compound (EDQM C2210000) was used as a positive control. The cells were incubated at 37 °C with 95% humidity and 5% CO_2 for 3 days until the cell culture reached 70% density. The media were removed, the cells were washed with 1 mL of PBS twice, and the cells were suspended in trypsin-EDTA solution before being transferred to a new medium. The cells were then seeded in a 96-wellplate and incubated for 24 h at 37 °C. After incubation, the media were removed, 100 µL of test samples with previously prepared concentrations were added, and the cells were incubated for 48 h. The staining process was carried out by adding PrestoBlue™ Cell Viability Reagent solution and incubating it for 4 h. Cell color intensity was measured using an ELISA microplate reader at 570 nm, with cisplatin as the positive control. Percent viability was calculated by the following Eq. (1).

$$\%\text{viability} = \frac{\text{OD}_{\text{cell+sample}} - \text{OD}_{\text{negative control}}}{\text{OD}_{\text{cell}} - \text{OD}_{\text{negative control}}} \times 100\% \quad (1)$$

GC-MS analysis

Volatile compounds in citronella essential oil were separated using an Rtx-5 column (30 m × 0.25 µm × 0.25 µm; Shimadzu, JP) with electron impact (EI) as the ionization source. 1 µL of citronella essential oil was injected with a split ratio of 50 mL/min. Helium was used as the carrier gas. The operating parameters of the GC were set as follows: initial temperature of 50 °C, held for 5 min, then increased to 280 °C at 5 °C/min. The injector temperature was set to 280 °C. The parameters of the MS system were set as follows: the ionization source and interface temperatures were 250 and 280 °C, respectively. The fragment scan range was set at *m/z* 33–600, and the scan time range was 3 min–71 min. The data analyzed by GC-MS were further processed with MS-Dial using the GCMS-DB-Public-KovatsRI-VS3 database; the metabolomic models were then constructed using SIMCA version 14 (Umetrics, Umeå, Sweden). The compounds were identified by their mass spectra and retention index values, calculated relative to the alkane standard.

Statistical analysis

Cytotoxic activity was expressed as the mean ± standard deviation (SD) of four replicates. To determine whether there was a significant difference, one-way analysis of variance (ANOVA) was used, followed by Duncan's test if $p < 0.05$. Principal component analysis (PCA) was used to distinguish the citronella essential oils based on their hydrodistillation methods and

growing locations. Meanwhile, orthogonal partial least squares discriminant analysis (OPLS-DA) was used to predict the compounds unique to each citronella essential oil. The PCA and OPLS-DA models were constructed using SIMCA version 14 (Umetrics, Umeå, Sweden). Both PCA and OPLS-DA will show us all compounds unique to each sample that may influence their cytotoxic activity. Therefore, Pearson correlation analysis was conducted between the specific compounds and the cytotoxic activity of all samples to assess their correlation.

■ RESULTS AND DISCUSSION

Chemical Compounds of Citronella Oil

The metabolomics approach is a study that can describe both qualitatively and quantitatively all metabolites and their variations in biological systems. This study can also be used to evaluate changes in plant metabolism caused by environmental factors and post-harvest processes. Environmental factors, such as differences in soil characteristics across locations, can alter the composition of citronella essential oil. In addition, using different extraction methods can also increase or decrease the levels of extracted compounds [21]. Differences in metabolites contained in citronella essential oil were analyzed using GC-MS. The chromatograms of each citronella essential oil sample are shown in Fig. 1. The citronella essential oils from Cibinong and Solok have different chromatogram profiles, indicating that there are differences in metabolites in the two samples. However, for the hydrodistillation process with sonication and without sonication, there is no significant difference in the chromatogram profiles for the two growing locations. The pretreatment affected only the peak intensity of the chromatogram, indicating differences in metabolite concentrations.

Based on the chromatogram profiles, 102 metabolites were identified in citronella essential oil (Table 1). Most of the identified metabolites belong to the monoterpenoid, diterpenoid, sesquiterpenoid, hydrocarbon, alcohol, and ketone groups. Among the 102

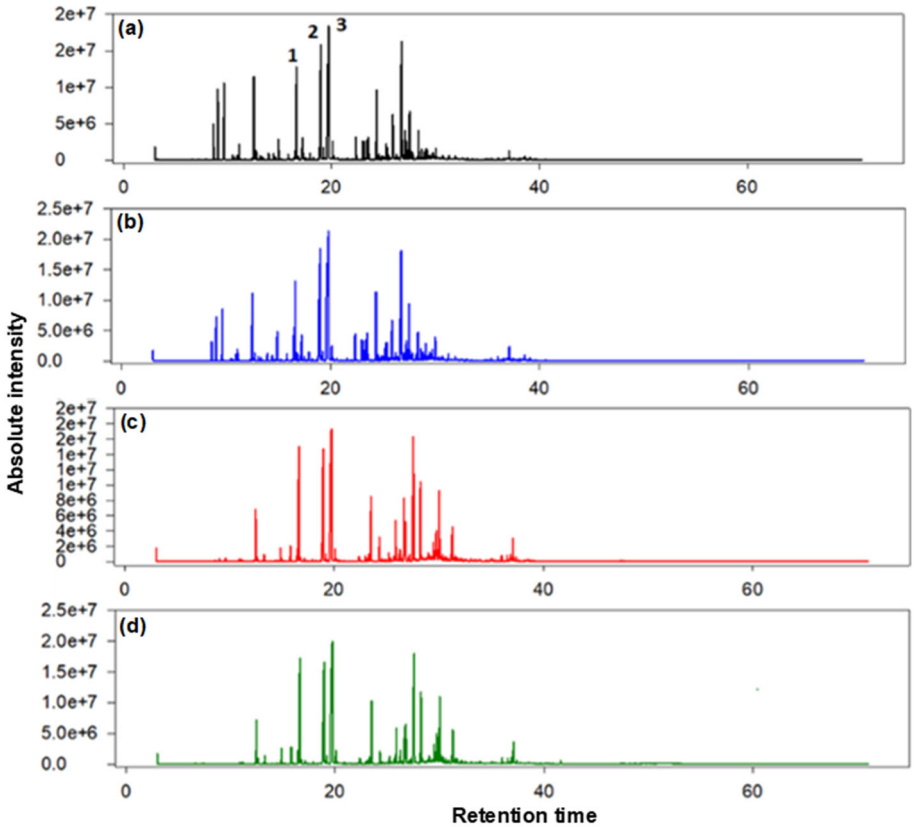


Fig 1. GC-MS chromatogram of citronella essential oils (a) S-HS, (b) S-HTS, (c) C-HS, and (d) C-HTS. (peak 1. citronellal, 2. citronellol, and 3. geraniol)

Table 1. Metabolites contained in citronella oil

No	RT (min)	RI	*m/z*	Molecular formula	Compound	Relative area (%)			
						S-HS	S-HTS	C-HS	C-HTS
1	27.226	1541	105	$C_{15}H_{24}$	(+)-α-muurolene	1.61	1.52	0.47	0.74
2	33.858	1837	68	$C_{13}H_{26}O$	(*Z*)-2-tridecen-1-ol	0.00	0.01	0.04	0.04
3	36.006	1944	69	$C_{17}H_{30}O_2$	(*Z*)-3,7-dimethyl-2,6-octadienyl heptanoate	0.11	0.20	0.44	0.51
4	15.397	1118	111	$C_{10}H_{18}O$	1,4-cineole	0.00	0.00	0.01	0.01
5	19.456	1248	96	$C_{11}H_{20}$	1-methylbicyclo(4.4.0) decane(*trans*)	0.08	0.07	0.03	0.04
6	22.43	1354	164	$C_{10}H_{12}O_2$	2-(1-propenyl)-6-methoxyphenol	0.00	0.07	0.86	0.87
7	28.99	1614	96	$C_{12}H_{22}O$	2-cyclododecenol	0.14	0.13	0.09	0.10
8	13.939	1074	71	$C_7H_{14}O$	4-heptanone	1.01	0.89	0.02	0.11
9	21.365	1316	150	$C_9H_{10}O_2$	4-vinylguaiacol	0.02	0.03	0.05	0.02
10	13.681	1066	105	C_8H_8O	Acetophenone	0.00	0.00	0.00	0.00
11	26.437	1509	189	$C_{15}H_{24}$	Alloakumadendrene	0.04	0.04	0.00	0.02
12	28.68	1600	159	$C_{15}H_{24}$	α-bergamotene	0.03	0.02	0.01	0.01
13	26.53	1513	93	$C_{15}H_{24}$	α-cedrene	0.02	0.02	0.13	0.12
14	26.425	1509	204	$C_{15}H_{24}$	α-cubebene	0.05	0.08	0.01	0.01
15	20.647	1291	95	$C_{12}H_{20}O_2$	α-fenchyl acetate	0.15	0.17	0.01	0.01
16	27.627	1557	96	$C_{15}H_{24}$	α-guaiene	0.04	0.04	0.74	0.55
17	26.891	1527	187	$C_{15}H_{24}$	α-gurjunene	0.00	0.00	0.01	0.00
18	25.556	1473	161	$C_{15}H_{24}$	Aristolochene (4,5-di-epi)	0.17	0.14	0.03	0.06
19	27.235	1542	138	$C_{15}H_{24}$	β-caryophyllene	0.01	0.01	0.00	0.00
20	26.228	1501	161	$C_{15}H_{24}$	β-gurjunene	0.30	0.35	0.39	0.39
21	23.291	1384	93	$C_{12}H_{20}O_2$	β-terpinyl acetate	0.08	0.09	0.41	0.37
22	26.388	1507	107	$C_{15}H_{24}$	Bulnesene (α-)	0.03	0.03	0.01	0.01
23	10.477	954	93	$C_{10}H_{16}$	Camphene	0.73	0.57	0.08	0.10
24	11.665	1005	93	$C_{10}H_{16}$	Carene (δ-3-)	0.04	0.05	0.01	0.01
25	19.749	1259	41	$C_{10}H_{16}$	Carvone	12.80	8.70	13.64	13.43
26	23.912	1407	133	$C_{15}H_{24}$	Caryophyllene (*Z*-)	0.02	0.02	0.00	0.00
27	30.656	1689	109	$C_{15}H_{26}O$	Cedrol	0.06	0.06	0.04	0.05
28	30.04	1662	95	$C_{17}H_{28}O_2$	Cedryl acetate	0.20	0.49	3.72	3.70
29	12.595	1033	59	$C_{10}H_{18}O$	Cineole (1,8-)	0.49	0.49	2.32	1.60
30	33.755	1832	69	$C_{17}H_{28}O_2$	*cis*-nerolidyl acetate	0.01	0.02	0.07	0.10
31	16.625	1155	69	$C_{10}H_{18}O$	Citronellal	7.36	6.41	10.61	10.00
32	18.966	1228	69	$C_{10}H_{20}O$	Citronellol	9.16	6.75	10.47	9.84
33	22.338	1350	81	$C_{11}H_{20}O_2$	Citronellyl formate	1.32	1.40	0.28	0.45
34	33.462	1817	69	$C_{16}H_{30}O_2$	Citronellyl hexanoate	0.02	0.03	0.09	0.09
35	26.859	1526	81	$C_{13}H_{24}O_2$	Citronellyl propionate	0.23	0.20	0.02	0.02
36	26.698	1520	161	$C_{15}H_{24}$	Clovene	12.20	9.76	3.08	6.03
37	22.97	1373	105	$C_{15}H_{24}$	Cubebene (α-)	0.97	0.81	0.09	0.31
38	26.064	1494	108	$C_{15}H_{24}$	Cumacrene	0.01	0.01	0.03	0.03
39	24.22	1419	150	$C_{10}H_{14}O$	Cuminyl alcohol	0.01	0.01	0.00	0.00
40	15.483	1121	139	$C_6H_{12}O$	Cyclopentylmethanol	0.00	0.00	0.00	0.01
41	17.668	1187	43	$C_{10}H_{14}O$	Cymen-9-ol (*para*-)	0.08	0.06	0.02	0.02
42	12.312	1025	119	$C_{10}H_{14}$	Cymene (*ortho*-)	0.32	0.23	0.16	0.15
43	18.243	1205	57	$C_{10}H_{20}O$	Decanal	0.09	0.10	0.03	0.04
44	26.144	1497	161	$C_{15}H_{24}$	δ-cadinene	0.08	0.14	0.17	0.15
45	27.534	1553	59	$C_{15}H_{24}$	δ-guaiene	2.26	2.73	6.66	6.32
46	22.015	1339	136	$C_{12}H_{20}O_2$	Dihydrocarvenyl acetate(equatorial)	0.01	0.01	0.00	0.01
47	40.555	2187	151	$C_{20}H_{32}O_4$	Dihydroflexibilide	0.01	0.01	0.00	0.00

No	RT (min)	RI	*m/z*	Molecular formula	Compound	Relative area (%)			
						S-HS	S-HTS	C-HS	C-HTS
48	23.949	1409	56	$C_{12}H_{24}O$	Dodecanal	0.01	0.01	0.00	0.00
49	31.273	1717	69	$C_{15}H_{26}O$	Elemol	0.28	0.72	5.03	5.40
50	28.376	1588	93	$C_{15}H_{22}O_2$	Epoxyvulgarone A	0.92	0.60	0.08	0.18
51	28.07	1574	93	$C_{15}H_{24}$	Eremophilene	0.01	0.00	0.01	0.01
52	31.888	1744	147	$C_{15}H_{22}O$	Eudesma-4(14),7(11)-dien-8-one	0.20	0.12	0.02	0.03
53	21.415	1318	153	$C_{10}H_{18}O$	Fenchyl alcohol	0.01	0.01	0.02	0.01
54	27.583	1555	69	$C_{15}H_{24}$	γ-elemene	0.82	0.89	1.92	1.82
55	27.089	1537	161	$C_{15}H_{24}$	γ-muurolene	1.51	1.12	0.11	0.22
56	25.991	1491	57	$C_{11}H_{20}O_2$	γ-undecalactone	0.02	0.02	0.03	0.03
57	20.225	1275	69	$C_{10}H_{16}O$	Geranial	0.18	0.14	0.21	0.23
58	19.734	1258	69	$C_{10}H_{18}O$	Geraniol	19.42	19.05	23.99	22.23
59	24.923	1448	43	$C_{13}H_{22}O$	Geranyl acetone	0.05	0.05	0.14	0.12
60	29.234	1625	81	$C_{15}H_{24}$	Germacrene-B	0.44	0.43	0.05	0.08
61	28.889	1609	95	$C_{15}H_{24}$	Germacrene-D	0.20	0.20	0.04	0.06
62	32.425	1769	159	$C_{15}H_{22}O$	Germacrone	0.01	0.01	0.01	0.02
63	24.663	1438	161	$C_{15}H_{24}$	Guaiadiene (6,9-)	0.20	0.17	0.03	0.06
64	30.927	1701	57	$C_{17}H_{36}$	Heptadecane	0.01	0.01	0.02	0.02
65	10.913	974	43	$C_8H_{14}O$	Hepten-2-one (6-methyl-5-)	0.56	0.43	0.38	0.26
66	15.871	1134	69	$C_{10}H_{18}O_2$	Hexenyl Isobutanoate (3*Z*-)	0.31	0.41	0.02	0.01
67	25.942	1489	189	$C_{15}H_{24}$	*iso*-longifolene	0.08	0.08	0.06	0.06
68	20.492	1285	82	$C_{12}H_{20}O_2$	Lavandulyl acetate	0.01	0.01	0.06	0.04
69	12.503	1030	68	$C_{10}H_{16}$	Limonene	8.61	6.79	7.45	6.75
70	16.792	1160	41	$C_{10}H_{16}O_2$	Limonene dioxide	0.07	0.10	0.12	0.11
71	11.582	1002	56	$C_{10}H_{16}O$	Linalool oxide (dehydroxy-*cis*-)	0.01	0.01	0.00	0.00
72	10.932	975	99	$C_{10}H_{16}O$	Linalool oxide (dehydroxy-*trans*)	0.00	0.00	0.00	0.00
73	23.103	1378	69	$C_{12}H_{20}O_2$	Linalyl acetate	2.38	2.59	0.34	0.62
74	27.645	1558	69	$C_{14}H_{24}O_2$	Linalyl butyrate	0.83	0.89	0.01	0.48
75	20.93	1300	69	$C_{11}H_{18}O_2$	Linalyl formate	0.15	0.14	0.18	0.29
76	31.98	1749	68	$C_{16}H_{28}O_2$	Linalyl hexanoate	0.02	0.03	0.05	0.05
77	25.702	1479	161	$C_{15}H_{24}$	Longifolene	0.07	0.13	0.46	0.42
78	14.896	1103	71	$C_{10}H_{18}O$	Menth-2-en-1-ol (*cis-para*-)	1.68	1.76	1.58	1.50
79	16.979	1166	95	$C_{10}H_{18}O$	Menthone (*iso*-)	0.09	0.10	0.01	0.01
80	20.315	1278	69	$C_{12}H_{22}O_2$	Menthyl acetate	0.00	0.00	0.00	0.00
81	35.36	1912	81	$C_{17}H_{24}O_3$	Menthyl salicylate	0.05	0.06	0.04	0.05
82	17.844	1192	135	$C_8H_8O_2$	Meta-methoxybenzaldehyde	0.01	0.01	0.01	0.01
83	21.564	1323	69	$C_{11}H_{18}O_2$	Methyl geranate	0.23	0.28	0.01	0.03
84	11.1	982	93	$C_{10}H_{16}$	Myrcene	2.01	1.68	0.32	0.36
85	19.219	1240	41	$C_{10}H_{16}O$	Neral	1.12	0.93	0.75	0.73
86	22.584	1359	68	$C_{12}H_{20}O_2$	Neryl acetate	0.01	0.01	0.00	0.00
87	32.548	1775	91	$C_{15}H_{22}O$	Nootkatone	0.02	0.02	0.02	0.02
88	12.739	1038	93	$C_{10}H_{16}$	Ocimene (*e*-β-)	1.17	1.10	0.04	0.13
89	18.105	1200	115	$C_{10}H_{20}O_2$	Octanol acetate	0.00	0.00	0.00	0.00
90	11.112	983	81	$C_9H_{14}O$	Pentyl furan (2-)	0.04	0.03	0.03	0.02
91	12.949	1044	91	C_8H_8O	Phenylacetaldehyde	0.06	0.03	0.01	0.00
92	15.048	1107	57	$C_{10}H_{18}$	Pinane	0.03	0.02	0.05	0.03
93	10.633	961	93	$C_{10}H_{16}$	Sabinene	0.42	0.34	0.01	0.02
94	13.9	1073	111	$C_{10}H_{18}O$	Sabinene hydrate (*cis*-)	0.01	0.01	0.00	0.00
95	17.922	1195	59	$C_{12}H_{20}O_2$	Sabinene hydrate acetate (*cis*-)	0.65	0.71	0.22	0.25

No	RT (min)	RI	*m/z*	Molecular formula	Compound	Relative area (%)			
						S-HS	S-HTS	C-HS	C-HTS
96	26.754	1523	41	$C_{15}H_{24}$	Sesquiphellandrene (β-)	1.75	1.46	0.53	0.50
97	13.49	1060	93	$C_{10}H_{16}$	Terpinene (γ-)	0.07	0.08	0.02	0.02
98	14.415	1088	93	$C_{10}H_{16}$	Terpinolene	0.48	0.48	0.08	0.11
99	19.803	1261	41	$C_7H_{14}O$	*trans*-3-heptenol	0.00	12.37	0.00	0.00
100	17.418	1179	84	$C_{10}H_{16}O$	*trans*-limonene-1,2-oxide	0.03	0.03	0.01	0.01
101	32.202	1759	137	$C_{16}H_{26}O_2$	*trans*-nerolidyl formate	0.01	0.02	0.00	0.00
102	23.583	1395	108	$C_{15}H_{24}$	Ylangene (α-)	0.29	0.25	0.02	0.08

identified metabolites, citronellal, citronellol, and geraniol were the most abundant compounds in citronella essential oil. This is shown by the chromatograms of the three compounds, which show the highest peaks across all citronella's essential oils tested. The results obtained are similar to those of a previous study, which reported that the main constituents of citronella essential oil were citronellal, citronellol, and geraniol [22]. The relative peak areas of the three compounds were 6.01–9.14, 6.34–9.02, and 17.86–20.70, respectively.

The identified compounds were confirmed as metabolites using mass spectrometry–data-independent analysis (MS-DIAL) software and data curation. Confirmation of metabolites using MS-DIAL was performed by matching retention index values and compound mass spectra against the AllPublic-KovatsRI-VS2 library. Meanwhile, data curation was evaluated by reducing compounds with the same retention times and those with different retention times. Compounds resulting from MS-DIAL processing were selected for the identification process based on the highest total score (750 to 1000), S/N greater than 3, and less than 10 Retention Indices (RI) unit differences compared to the reference RI [23-24]. The total score indicates how much the identified compound matches a specific compound in the library, while S/N indicates the signal-to-noise ratio of the compounds.

Cytotoxic Activity of Citronella Oil

Currently, breast cancer is the most common cancer and is the leading cause of death in women worldwide [1]. Several conventional methods, such as chemotherapy, surgery, and radiation therapy, have been used to treat breast cancer. However, these methods have many side effects and toxicity in patients [15]. To prevent this, research into natural sources of bioactive metabolites continues, including citronella essential oil. The cytotoxicity of citronella essential oil against MCF-7 breast cancer cells was determined using the Alamar Blue method. The cytotoxic results of citronella essential oils on MCF-7 cells are presented in Fig. 2. Differences in hydrodistillation methods and growing locations had significant effects on the cytotoxic activity of citronella essential oils ($p < 0.05$). C-HTS citronella essential oil was the most effective citronella essential oil for reducing the viability of MCF-7 breast cancer cells, with an IC_{50} value of 41.23 ± 6.09 µg/mL. S-HS citronella essential oil had the lowest cytotoxic activity, with an IC_{50} value of 179.65 ± 58.43 µg/mL. Cisplatin was used as a positive control with an IC_{50} value of 53 µM.

Currently, there is no research on the potential of citronella essential oil as an anticancer agent for breast cancer. However, several studies have reported the potential of the genus *Cymbopogon* as an anticancer agent against breast cancer. Maksimović et al. [18] reported that *C. citratus* essential oil exhibited cytotoxic effects on breast cancer cell lines at a concentration of 200 µg/mL. Several major compounds in *C. citratus* essential oil,

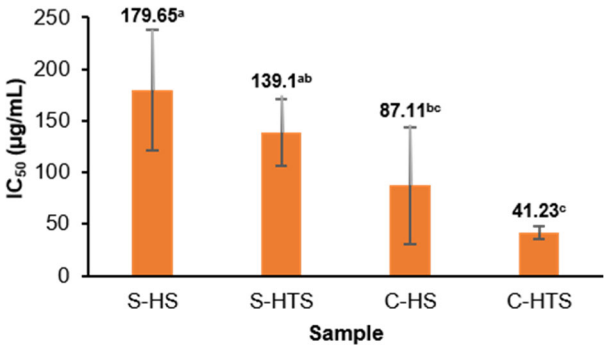


Fig 2. Cytotoxic activity of citronella oil against MCF-7 breast cancer

such as citral and geraniol, are predicted to exert antiproliferative effects on MCF7 breast cancer cells by inhibiting the HSP90 chaperone protein. Furthermore, citral exhibits selective cytotoxic activity against breast cancer cells [3]. The research results of Hago et al. [19] showed that the ethanol extract of *C. schoenanthus* had high selectivity and effectiveness against the MCF-7 breast cancer cell line, with an IC_{50} of 12.841 ± 0.21 µg/mL. In addition, Gaonkar et al. [3] reported another species of the genus *Cymbopogon*, namely *C. flexuosus*, which has potential as an anti-breast-cancer agent, and compared it with citral and geraniol. As a result, the oil was found to be more cytotoxic to MCF-7 cells, with IC_{50} values for the oil (69.33 µg/mL), citral (140.7 µg/mL), and geraniol (117 µg/mL). This study shows that *C. nardus* essential oil has greater anti-breast-cancer potential than those of *C. flexuosus* and *C. citratus*.

Metabolite Distribution in Citronella Oil

Differences in metabolite distribution in citronella essential oil, as influenced by hydrodistillation method and growth location, were evaluated using PCA. The results of the PCA are presented as a score plot in Fig. 3. The PCA score plot can clearly group the citronella essential oils based on their metabolites' distribution: (i) Cibinong, (ii) Solok hydrodistillation with sonication (S-HS), and (iii) Solok hydrodistillation without sonication (S-HTS), with a total PC of 82.2% (PC1 = 64.9% and PC2 = 17.3%). The results of PCA indicate that differences in growing locations affect the distribution of metabolites contained in the citronella essential oil. However, the hydrodistillation method, with or without sonication, only affected the metabolites of citronella essential oil obtained from Solok. This is shown by the PCA score plot, which does not reveal a difference in the metabolite distribution of citronella oil from Cibinong obtained with or without sonication pre-treatment. The PCA results also matched the cytotoxic activity of the Cibinong citronella essential oil, with the C-HS and C-HTS samples having IC_{50} values that were not significantly different ($p < 0.05$) (Fig. 2). In general, PCA can distinguish the samples into two groups: Cibinong and Solok. These results were also consistent with the cytotoxic activities of citronella essential oils from the two growing locations, which were significantly different ($p < 0.05$).

In addition to PCA, OPLS-DA was used to predict unique metabolites for each citronella oil sample obtained from different growing locations and hydrodistillation methods. The OPLS-DA score plot was able to distinguish samples into two groups, namely Cibinong and Solok (Fig. 4(a)), and S-HS and S-HTS (Fig. 4(c)). To assess the quality of the model, the values of R2 and Q2 were used. The model is acceptable if it has R2 > 0.5 and Q2 > 0.4 [25-26]. The OPLS-DA model for discrimination of citronella essential oil from Solok and Cibinong has values of R2X = 0.773, R2Y = 0.974, and Q2 = 0.964, while the OPLS-DA model for discrimination of S-HS and S-HTS has values of R2X = 0.777, R2Y = 0.996, and

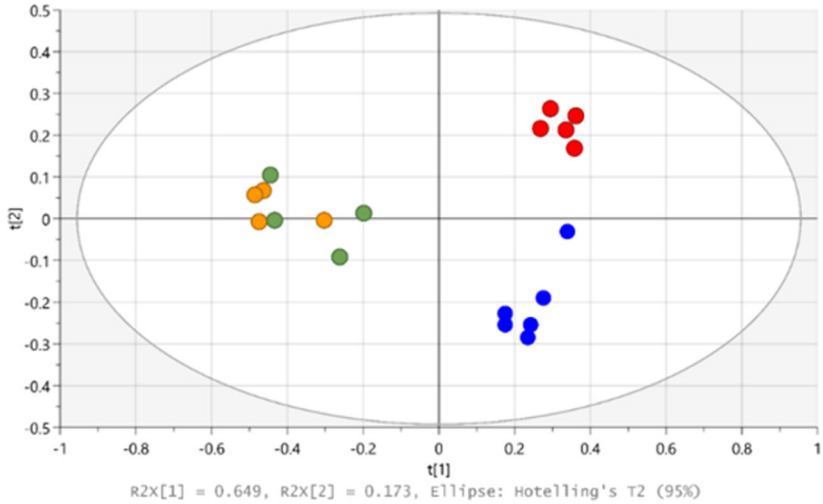


Fig 3. PCA score plot of citronella oil [S-HS (blue), S-HTS (red), C-HS (yellow) dan C-HTS (green)]

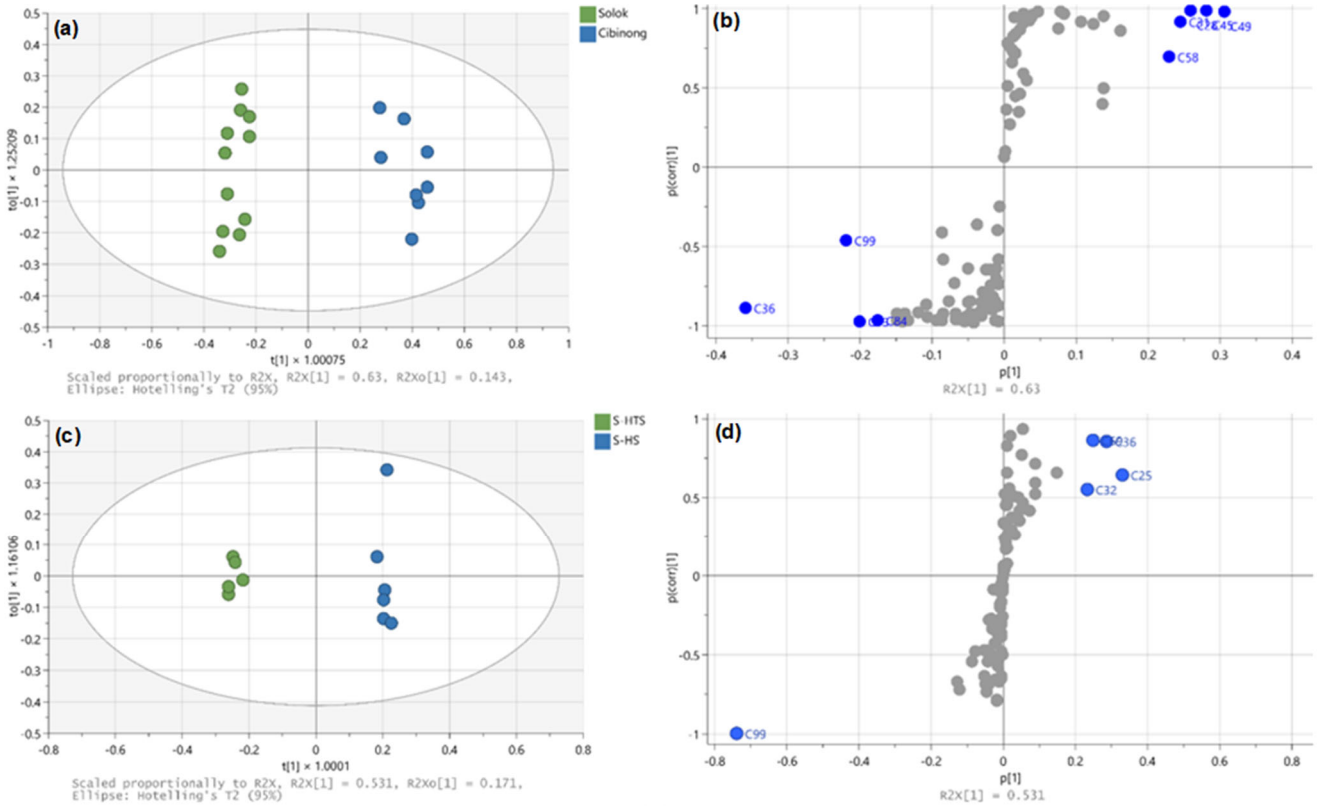


Fig 4. Score plot (a) and S-plot (b) of OPLS-DA citronella essential oil from Cibinong and Solok, and score plot (c) and S-plot (d) of OPLS-DA citronella essential oil of S-HS and S-HTS

Q2 = 0.958. Based on the R2X = 0.777, R2Y = 0.996, and Q2 = 0.958. Based on the resulting R2 and Q2 values, the two OPLS-DA models have a good fit and good predictive performance.

The unique compounds for each citronella essential oil were predicted using the S-plots. In the S-plot, the X-axis represents the variable contribution to the observed variance. In contrast, the Y-axis represents the correlation between the samples and the reliability of the results. Therefore, compounds that differ significantly between groups are usually located in the upper-right or lower-left corners of the plot [27]. In addition, compounds considered significant for distinguishing two groups of samples must have a variable importance in projection (VIP) value greater than 1 [28]. Based on the S-plot and VIP values, clovene (C36), linalyl acetate (73), myrcene (84), and *trans*-3-heptenol (99) are unique compounds found in citronella oil from Solok. In contrast, the compounds citronellal cedryl acetate (28), citronellal (31), δ-guaiene (45), elemol (49), and geraniol (58) are distinctive to citronella essential oil from Cibinong (Fig. 4(b)). Furthermore, to distinguish S-HS and S-HTS citronella essential oils, carvone (C25), citronellol (C32), clovene (C36), and limonene (C69) can be established to be unique to S-HS. In contrast, trans-3-heptenol (C99) is a compound exclusive to S-HTS (Fig. 4(d)).

Prediction of Anticancer Active Compound in Citronella Oil

The potential of citronella essential oil as an anti-breast-cancer agent was evaluated in MCF-7 cells. MCF-7 cells are a breast cancer cell line commonly used to evaluate sample activity *in vitro*. In general, citronella essential oil from Cibinong has greater anti-breast-cancer potential than that from Solok. Based on the OPLS-DA S-plot, citronella essential oil from Cibinong contains citronellal, geraniol, δ-guaiene, cedryl acetate, and elemol at higher levels than those of Solok. The five compounds are predicted to be anti-breast-cancer compounds present in citronella essential oil. The chemical structure of the

compound that has the potential to be an anticancer agent is shown in Fig. 5. In addition, based on Pearson correlation results, the 6 compounds have a significant correlation ($p < 0.05$) with the IC_{50} value at $\alpha = 0.05$. The negative correlation coefficients for 6 compounds indicate that higher concentrations of these compounds in citronella essential oil will reduce the IC_{50} value. The correlation coefficients for the 6 compounds are shown in Table 2.

According to the literature, several compounds in citronella essential oil have the potential to reduce MCF-7 cell viability, including citronellal, geraniol, citronellol, neral, and geranial. Citronellal has been reported to be an anti-breast cancer agent, with an IC_{50} for MCF-7 cell viability of 0.091 mM [29]. Geraniol was reported to inhibit the growth of MCF-7 cells by inducing G1 phase arrest and reducing their viability [30]. In addition, geraniol showed specific activity against tumors without affecting the growth of f-10f normal breast epithelial cells [31]. Citronellol is an acyclic monoterpene that can induce apoptosis of MCF-7 cells at a concentration of 80 mM/mL. This compound causes apoptosis in MCF-7 cells by inducing oxidative damage and modulating the expression of various pro- and anti-apoptotic proteins [32]. Meanwhile, nerol and geraniol have been reported to induce apoptosis in MCF-7 cells, but to date no studies have explored the mechanism of this induction [33]. Furthermore, other compounds in citronella essential oil may also have anticancer potential through synergistic interactions.

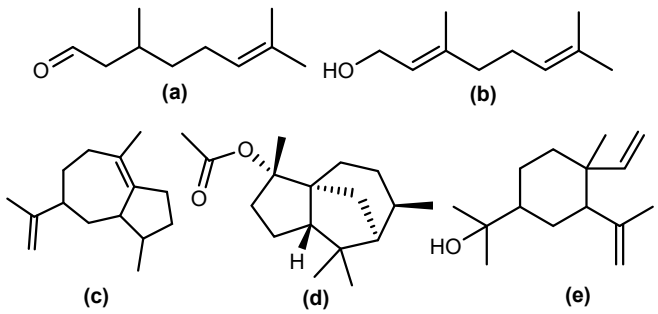


Fig 5. Chemical structures of (a) citronellal, (b) geraniol, (c) δ-guaiene, (d) cedryl acetate, and (e) elemol

Table 2. Pearson correlation coefficient of anticancer active compounds on IC_{50} values

Citronellal	Geraniol	δ-Guaiene	Cedryl acetate	Elemol
−0.582	−0.445	−0.758	−0.761	−0.803

■ CONCLUSION

Metabolomics studies were successfully used to evaluate the effect of different extraction methods and growing locations on the cytotoxic activity and metabolite profiles of citronella essential oil. Citronella essential oil from Cibinong has higher cytotoxicity against MCF-7 cells than citronella essential oil from Solok. In addition, differences in growing locations also resulted in significant differences ($p < 0.05$) in the GC-MS chromatogram profiles. However, pre-treatment with sonication did not give any significant effect ($p < 0.05$) on the chromatogram profiles of citronella essential oils from both growing locations. Analysis by GC-MS identified 102 metabolites across the monoterpenoid, diterpenoid, sesquiterpenoid, hydrocarbon, alcohol, and ketone groups. Based on the peak areas of the 102 metabolites, PCA was able to group the samples into: (i) Cibinong group, (ii) S-HS, and (iii) S-HTS, while OPLS-DA was able to determine compounds that are unique to each sample based on the compounds' abundance. Based on the OPLS-DA S-plot and Pearson correlation, citronellal, geraniol, δ-guaiene, cedryl acetate, and elemol are predicted to be the anti-breast cancer compounds.

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■ CONFLICT OF INTEREST

The authors of this work declare that they have no competing conflicts of interest.

■ AUTHOR CONTRIBUTIONS

Dede Sukandar: Supervision, study conception and design, analysis and interpretation of results, draft manuscript review and editing; Rudi Heryanto: Study conception and design, analysis and interpretation of

results; Dasumiati: review and editing; Anny Sulaswatty: supervision, review and editing; Egi Agustian: Data collection, analysis and interpretation of results, review and editing; Isty Anggraeni: data collection and interpretation of results; Saeful Rohman: data collection and interpretation of results; Alfi Hudatul Karomah: data collection and interpretation of result; Tarso Rudiana: Editing and review: Jefry Novrianto: data collection, interpretation of results, preparation manuscript. All the authors agreed to the final version of this manuscript.

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