

Secondary Metabolites and Antioxidants Activity from Citronella Grass Extract (*Cymbopogon nardus* L.)

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ABSTRACT

Background: *Cymbopogon nardus* L. is a rhizome plant that is widely used as traditional medicine. This research aimed to identify how the secondary metabolites of citronella grass including stems, leaves, and roots and other contents like the antioxidant and flavonoids compared to the ordinary citronella and reeds. **Materials and Methods:** GCMS (Gas Chromatography and Mass Spectroscopy) method was used to screen secondary metabolites along with methanol solvent. Then, the compounds could be identified through the existing library. Furthermore, DPPH and flavonoid test with aluminum chloride were used to analyze antioxidants. **Result:** indicated that secondary metabolites produced in *Cymbopogon nardus* L. stems were elemol, rosifolius, and ethyl oleate. In the root, it was found elemol, 1-Dodecamine, N,N- dimethyl-(CAS), 1-Tetradecanamine, N,N-dimethyl (CAS), while in the leaves it was found elemol, 9,2-octadecadienoic acid, methyl ester, (E,E)- (CAS), 1-dodecanamine, N,N-dimethyl;- (CAS). The highest level of flavonoid was found in the *Cymbopogon nardus* of 4%, in the *Cymbopogon citrasus*' stems of 2.84%, and in the *Imperata cylindrica* leaves of 1.28%. The highest antioxidant activity was found in *Cymbopogon nardus* of 79.5 µg/mL followed by *Cymbopogon citrasus* of 58.4 µg/mL and then the third was in *Imperata cylindrica* of 43.7 µg/mL. **Conclusion:** The content of secondary metabolites especially elemol is found in stems, roots, and leaves. The *Cymbopogon nardus* stem has the highest level of flavonoid and antioxidant activities among ordinary lemongrass and *Imperata cylindrica*.

Keywords: Antioxidants, *Cymbopogon nardus*, Flavonoids, Secondary metabolites.

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INTRODUCTION

Plants survive by carrying out metabolic processes which produce metabolites and function as defense. They are called secondary metabolites.¹ The secondary metabolites play important roles for the plants including for the plants' growth and defense mechanisms against various stressors from biotic (herbivores and pathogens) and abiotic (UV rays). Secondary metabolites are also widely used by humans as medicinal agents because they contain particular compounds for medication.²

Moreover, the secondary metabolites can increase immune system because they contain chemical compounds which have biological activities (bioactive substances). The content of secondary metabolites compounds can be qualitatively tested by phytochemical method³ and quantitatively by GCMS method⁴. The secondary metabolites play a direct role in the process of growth

in plants⁵ while the phytochemicals are used for protection and plants' growth. Recently, the secondary metabolites are proven to enhance immunity against Covid-19.⁶ In addition, some secondary metabolites perform the function of inflammatory modulators of intestinal disease.⁷

Citronella is a rhizome plant which is widely used and proven for its efficacy. Phytochemical screening of citronella grass showed that it contains flavonoids, terpenoids, saponin and tannin.⁸ Flavonoids are common secondary metabolites found in plants. Flavonoids reduce compounds which inhibit oxidation reactions.⁹ Flavonoids can be an antioxidant because they can transfer free radical compounds. Flavonoids have a powerful biological antioxidant effect which can inhibit the clotting of blood cell, inhibit the growth of cancer cells, and stimulate the production of nitric oxide (NO).¹⁰ On the other hand, lemongrass (*Cymbopogon nardus*) has the ability as a larvicide for *Aedes aegypti*.¹¹ This is because the citronella plant can be used as a vegetable fungicide to inhibit fungal growth on 84 rubber stems.¹² The citronella and geraniol content in citronella grass leaves can inhibit the growth of propionibacterium acnes bacteria.¹³

Antioxidants can counteract free radicals by reducing ROS in body. Consuming antioxidants and performing balanced diet and proper lifestyle can prevent or even reduce the occurrence



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of various degenerative diseases in the digestive system, cancers, and others.¹⁴⁻¹⁵ Antioxidant compounds include phenolic acid, flavonoid, carotene, vitamin E, uric acid, bilirubin, and albumin. This is a basic research which analyzed secondary metabolites and antioxidant activities of citronella grass (*Cymbopogon nardus* L.). It is hoped that the further research can be conducted to know the benefits of citronella grass in ethnobotany and pharmacognosy.

MATERIALS AND METHODS

Materials

The equipment used in this research was stirring rod, blender, funnel, Erlenmeyer flask, watch glass, beaker (Pyrex®), measuring flask, micro-pipet, oven (memmert®), tube clamp, pH meter, dropper pipette, measuring pipette, UV-vis spectrophotometer¹⁶ and GCMS.¹⁷ The materials used were distilled water, FeCl₃ 0,1%, ascorbic acid, 1% oxalic acid, 10% trichloroacetic acid, buffer phosphate 0,2 M pH 6.6, ethanol 96%, potassium ferricyanide, filter paper, *Cymbopogon nardus*.

Methods of Secondary Metabolites, Flavonoid Contents, and Antioxidant Levels

Extraction sample

The materials were collected and cleaned, and then they were cut into small pieces and dried in room temperature for 2 hours. After that, they were mashed into a powder, and stored in an airtight glass container and covered with aluminum foil. 50 g of powder sample was extracted with ethanol 96% as solvent of 300 ml for 2 hours, and then it was filtered using filter paper, repeat immersion 3 times. Finally, the extract was then evaporated using a rotatory evaporator.

Screening secondary metabolites by GCMS

The leaves, stems, and roots attached to the disease are 10 cm long, cut into small pieces, and then baked at 40°C. After drying, it was mashed using a blender and macerated using 70% ethanol in a ratio of 1:6 for 72 hours. In the maceration, the extract was evaporated at 60°C until thickened. The extracted result was analyzed using the GCMS method and presented in the form of molecular weight screening based on the library. GCMS data analysis was processed using Mass LynX v4.1 software.¹⁸

Total of Level Flavonoid

The total of flavonoid content was calculated using aluminum chloride test. Standard solutions with various concentrations of 30, 40, 50, 60, 70, 80, 90, 100 g/ml were prepared with 96% ethanol. Citronella extract as much 50 µl was mixed with 10 µl of 10% aluminum chloride solution, then another 150 µl ethanol 96% was added. All reagents were mixed and incubated for 40

min at room temperature, and then they were covered with aluminum foil to avoid exposure to sunlight. The absorbance value was measured with a wavelength of 415 nm. The unit of total flavonoid content used mg Quercetin Equivalent (QE) per g of citronella extract.¹⁹

Antioxidant Activity

The DPPH test was used to measure the activity of hydrogen atoms so that it could be used to calculate antioxidant activity by calculating the free radicals that occurs. Free radical testing with DPPH was marked with a purple color. The test was carried out by the TLC screening method using 0.2% DPPH and MeOH.²⁰ Wait for 30 minutes after spraying to see if the color change occurs. Then proceed with the spectrophotometric test, namely 30 µl of methanol was added to 3 ml of 0.004% DPPH-MeOH. Absorbance at a wavelength of 517 nm for 30 minutes (Perkin-Elmer-Lambda 11 spectrophotometer), and then calculate the percentage of antioxidant activity.²¹

RESULTS

Secondary metabolites in stems, roots, and leaves of *Cymbopogon nardus* L

The highest secondary metabolites of *Cymbopogon nardus* stems were elemol (Fig. 1) with area of 23.92%. The second highest was rosifoliol of 11.13%, and the third highest was 2-Naphthalenemethanol, 8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a)] with area 10.63%. Secondary metabolites in the root were elemol; 1-Dodecamine, N, N-dimethyl-(CAS); and 1-Tetradecanamine. The highest was elemol with area 20.87%, the second highest was 1- Dodecamine, N, N-dimethyl-(CAS) with area of 12.68%, and the third highest was 1-Tetradecanamine with area of 9.20% and the secondary metabolites in the leaves were elemol with area of 21.23%; the second highest was 9,2-octadecadienoic acid with area of 12.46%; methyl ester, (E, E)- (CAS), 1-dodecanamine, N, N-dimethyl-(CAS) with area of 8.53%.

In the three organs analyzed, in highest secondary metabolites in each organ was elemol. The content of elemol in the stems had the highest with area of 23.92% (Table 1), compared to the leaves 21.23% and roots 20.87%.

Total of Flavonoid Contents and Levels

Total flavonoid contents and flavonoid levels in the three plants were *Cymbopogon nardus* (Citronella grass), *Cymbopogon citratus* (Lemongrass), and *Imperata cylindrica* (Reeds). It was analyzed using DPPH and then using DMRT as a follow-up test because there were significant differences in the analysis. The highest flavonoid content (Table 2) was found in *Cymbopogon nardus* (Citronella grass) with levels of 4%, 0.04 g QE/100 g, then followed by *Cymbopogon citratus* containing 2.84 g QE/100 g.

Table 1: Secondary Metabolites from *Cymbopogon nardus* Stems.

Peak #	R.Time	Area	Height	Name
1	6.239	12839909	1535710	Benzyl chloride
2	7.658	12376216	1802999	Phenol, 2-methoxy- (CAS)
3	11.668	125111700	6418871	2,3-DIHYDRO-BENZOFURAN
4	13.422	51648715	6874266	2-Methoxy-4-vinylphenol
5	14.391	23351728	2674771	Phenol, 2,6-dimethoxy-(CAS)
6	17.070	16920620	1871655	Phenol,2-methoxy-4-(1-propenyl)-, (E)- (CAS)
7	18.150	233707571	19829723	Methanamine, N,N-dimethyl-(CAS)
8	18.480	13353465	1870101	NAPHTHALENE, 8A-OCTAHYDRO-7-METHYL-4-METHYLENE-1-(1-METHYLE
9	18.753	60165972	7025254	Elemol
10	18.971	14150947	1077792	Spathulanol
11	19.842	1090616460	63453411	Elemol
12	20.079	42195436	7238649	3',5'-Dimethoxyacetophenone
13	20.266	58979527	11540858	endo-1-bourbonanol
14	20.906	29524267	4220638	Phenol, 2,6-dimethoxy-4-(2-propenyl)-(CAS)
15	21.580	100578054	13986433	10-epi-.gamma.-eudesmol
16	21.807	75532999	6591457	Torreyol
17	22.289	484747402	43586224	2-Naphthalenemethanol, 8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a]
18	22.937	142614810	14774030	1-Hexadecanaminium, N,N,N-trimethyl-, bromide
19	23.261	45190435	5450550	Phenol, 2,6-dimethoxy-4-(2-propenyl)-(CAS)
20	24.089	30274224	5233279	1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)
21	24.593	24019709	1693753	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- (CAS)
22	24.855	25474673	3979515	Heptadecanoic acid, ethyl ester (CAS)
23	25.009	34826529	4036607	Rosifoliol
24	26.210	507687148	27085934	Rosifoliol
25	27.002	56246996	3660930	1-Naphthalenamine, 4-bromo- (CAS)
26	27.236	61817965	5053270	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol
27	27.717	125429369	13659133	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]m
28	28.655	13646759	1071041	2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol
29	29.144	170442054	26518320	Hexadecanoic acid, ethyl ester (CAS)
30	29.743	95243121	4346944	LONGIFOLENALDEHYDE
31	30.264	21218082	1471896	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butyric acid
32	30.519	32876405	2984750	2-METHYL-5-(2',6',6'-TRIMETHYL-CYCLOHEX-1'-EN-1'-YL)-PENTAN-2,3-DIOL
33	31.251	141733549	11026770	2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans-(CAS)
34	31.560	131578848	20725591	1-Allyl-3-methylcyclohex-2-enol
35	32.543	351145928	30915424	Ethyl Oleate
36	32.912	28896799	6017358	Heptadecanoic acid, ethyl ester (CAS)
37	35.272	46824901	10860091	1-Phenyl-2-propanone

Peak #	R.Time	Area	Height	Name
38	38.596	8047403	1163438	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)
39	38.990	9384300	1536672	N-(2,6-DIMETHYL-PHENYL)-N-(2-MORPHOLIN-4-YL-2-PHENYL-ACETYL)-2-PHENYL-ACET
40	39.505	9636334	2198886	Dodecanoic acid, phenylmethyl ester (CAS)
		4560057329	407062994	

Table 2: Level flavonoids total of *Cymbopogon nardus*.

Species	Variabel	Flavonoid content (g QE/100 g)	Flavonoid levels (%)
<i>Cymbopogon nardus</i> (citronella grass)	Root	0.0115 ^{cd}	1.15 ^{cd}
	Stem	0.0400 ^a	4 ^a
	Leaf	0.0081 ^d	0.8 ^d
<i>Cymbopogon citratus</i> (lemongrass)	Root	0.0011 ^{ef}	0.11 ^{ef}
	Stem	0.2840 ^b	2.84 ^b
	Leaf	0.0218 ^{bc}	2.18 ^{bc}
<i>Imperata cylindrica</i> (reeds)	Root	0.0021 ^e	0.21 ^e
	Stem	0.0065 ^{de}	0.65 ^{de}
	Leaf	0.0128 ^c	1.28 ^c

Note: Each number followed by a different letter indicates a significant difference which has been calculated by the 95% DMRT test.

Table 3: DPPH Antioxidant Activity (µg/mL).

	Root	Stem	Leaf
<i>Cymbopogon nardus</i> (Citronella grass)	30.8 ^{de}	79.5 ^a	56.4 ^{bc}
<i>Cymbopogon citratus</i> (lemongrass)	20.9 ^e	58.4 ^b	38.3 ^d
<i>Imperata cylindrica</i> (reeds)	11.7 ^{fg}	17.3 ^f	43.7 ^c

Note: Each number followed by a different letter indicates a significant difference which has been calculated by the 95% DMRT test.

Furthermore, the flavonoid level of *Cymbopogon nardus* (citronella grass) in roots was 0.11%, in stems was 2.84%, and in leaves was 2.18%. The flavonoid level of *Imperata cylindrica* (reeds) in roots was 0.21%, in stem was 0.65%, and then in leaves was 1.28%. The flavonoid level of citronella grass' stems (Fig. 2) was more significant than in *Cymbopogon citratus* stems of 2.84%, and *Cymbopogon citratus* roots were 2.18%.

Antioxidant Activity

Cymbopogon nardus (citronella grass) had an antioxidant activity 30.8 µg/ml in root, 79.5 µg/ml in stem, and 56.4 µg/ml in leaf (Table 3). The citronella grass stems had the highest antioxidant activity compared to lemongrass leaves and roots. *Cymbopogon citratus* (lemongrass) had an antioxidant activity 20.9 µg/ml in roots, 58.4 µg/ml in stems, and 38.3 µg/ml in leaves. *Imperata cylindrica* (reeds) had an antioxidant activity of 11.7 µg/ml in root, 17.3 µg/ml in stem, and 43.7 µg/ml in leaf. From the results, it showed that the highest in citronella grass stems had 79.5 µg/

ml, followed by the antioxidant activity of lemongrass stems, namely 58.4 µg/ml, then the third-highest antioxidant activity was in impartial leaves, namely 43.7 µg/ml. The results indicated that antioxidant activity was correlated directly with flavonoid levels (Table 3). The antioxidant activity of *Cymbopogon nardus* was the highest. High flavonoid contents and high antioxidant activity were found in *Cymbopogon nardus* L.

DISCUSSION

In its biological activity, plants produce secondary metabolites. The functions of secondary metabolites are diverse, including those used in toxicity. Plants generally process this compound into certain parts such as apoplasts or certain organelles such as vacuoles, or parts of cells that are used as defense and tolerance mechanisms.²² Metabolomics is the study of comprehensive metabolic monitoring in plants, processing of metabolic compounds and pathways for processing compounds in plants.²³⁻²⁵ In the embryonic period, this interesting thing needs attention in

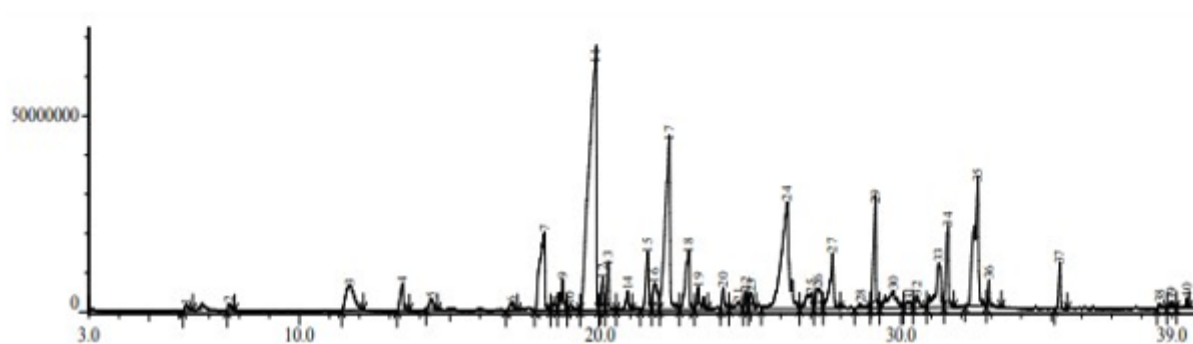
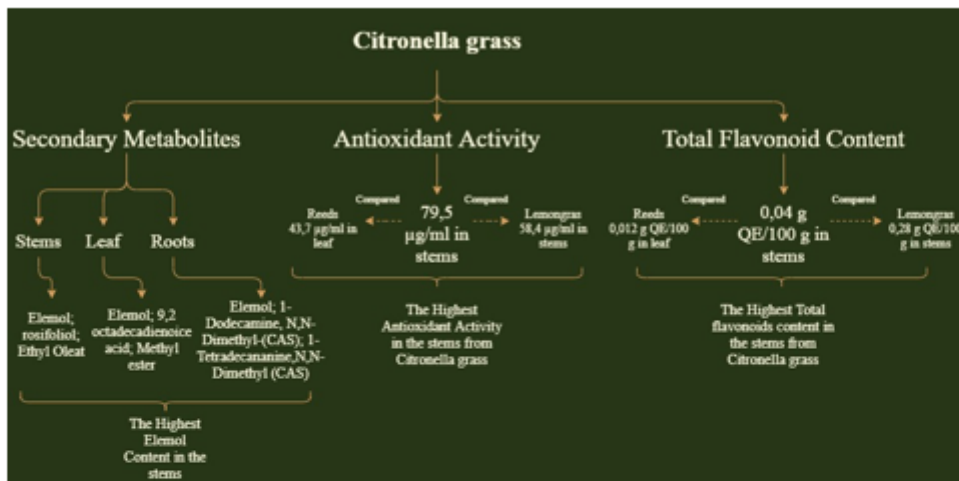


Figure 1: Secondary metabolites of Cymbopogon nardus' stems.

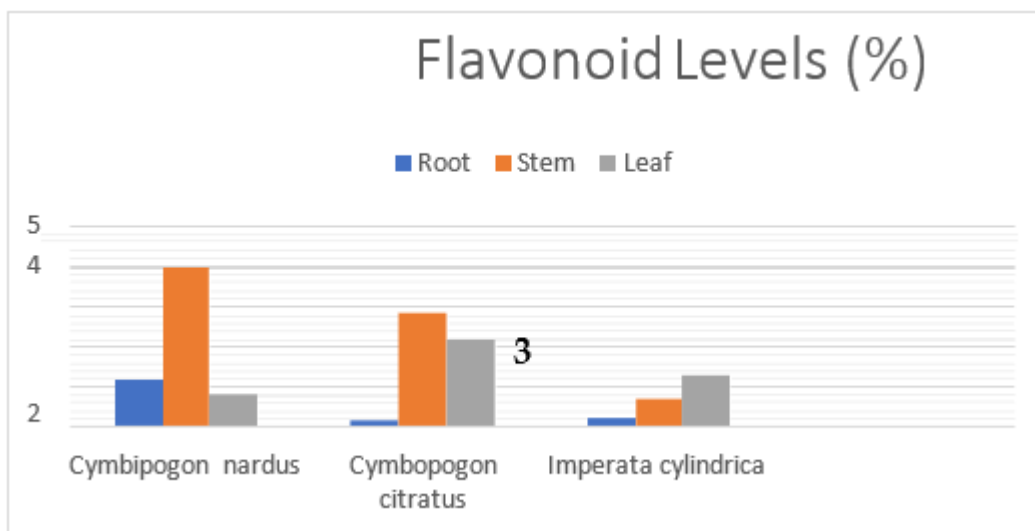


Figure 2: Flavonoid Levels Total.

understanding the regulation of metabolites globally.²⁶⁻²⁸ From this application, we can develop several applications which can provide valuable advanced information in the field of plant biotechnology, pharmaceutical industry,²⁹ food agrochemicals so as to increase food and agriculture production which has a positive impact on other industries.

Elemol has been reported as an essential terpenoid constituent with insecticidal and anti-termite properties.³⁰⁻³² Elemol is one of the components of fragrance ingredients used in the beauty industry such as cosmetics, fabric softener, hand softener, perfume, shampoo, soap, as well as non-cosmetic products such as detergents, floor cleaners, and other household cleaners.³³ Elemol usage is 1-10 metric tons/year. the skin resistance threshold for the use of elemol that has been formulated into fragrance products is 00.07%.³⁴ Flavonoids are the result of metabolic pathways in plants that have many functions and structures. These functions range from plant growth, development, coloration (pigment) to plants, UV protection as well as in plant defense and signaling against various pathogenic stresses.³⁵

Cymbopogon nardus, both citronella and lemongrass, as herbal medicine or as a flavoring spice³⁶ is very effective because it has the highest flavonoid content than leaves or roots in both citronella and lemongrass. Flavonoids occur naturally in plants and have health human benefits. Studies on flavonoid derivatives have shown various activities of medicinal agents in reducing diseases caused by bacteria, viruses, cancer, allergies, and inflammation.³⁷

Flavonoids enter the defense signaling pathway of plant tissues under stress. Flavonoids are compounds which relied upon plants as a mechanism of counterattack against pathogenic microorganisms.³⁸ Flavonoids are very effective as oxidizing molecules from various free radicals causing disease. This is in line with the journal which indicated that extracts from herbal products can act as antioxidants.³⁹ Free radicals are compounds or molecules which contain one or more unpaired electrons in their outermost orbital. Since the unpaired electrons make the compounds to be very reactive looking for molecular pairs by binding electrons around it and attacking so that there is a molecular imbalance that triggers disease.⁴⁰

CONCLUSION

Secondary metabolites in *Cymbopogon nardus* (citronella grass) vary unlike other rhizome plants which contain lots of flavonoids. *Cymbopogon nardus* (citronella grass) has the main component of elemol. The highest elemol was found in stems which is often used in cosmetics and perfumery because it has a distinctive fragrance. The flavonoid content in *Cymbopogon nardus* (citronella grass) is also the highest among *Cymbopogon citratus* (lemongrass) and *Imperata Cylindrica* (reeds), so the potential for free radicals is also high. The potential for free radicals is significant with a higher antioxidant content than *Cymbopogon citrus* and *Imperata cylindrica*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GCMS: Gas Chromatography Mass Spectrofotometry; **DPPH:** 2,2-difenil-1-pikrilhidrazil; **DMRT:** Duncan Multiple Range Test.

SUMMARY

Citronella grass is a rhizome plant which is widely used and has proven for its efficacy. People benefit lemongrass for several uses according to their needs. Therefore, we know how the secondary metabolites of citronella which include stems, leaves and roots as well as the antioxidant and flavonoids content of citronella grass compared to ordinary citronella and reeds. GCMS (Gas Chromatography and Mass Spectroscopy) was used to screen along with methanol solvent. Then, the compounds can be identified through the existing library. DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to analyse antioxidants and flavonoid test with aluminium chloride. The secondary metabolites produced in *Cymbopogon nardus* L. stems are elemol, rosifoliol, and ethyl oleate; in the roots it was found elemol, 1-Dodecamine, N,N- dimethyl-(CAS), 1-Tetradecanamine, N,N-dimethyl (CAS); and in the leaves it was found elemol, 9.2-octadecadienoic acid, methyl ester, (E,E)-(CAS), 1-dodecanamine, N,N-dimethyl-(CAS). The highest level of flavonoid was in the *Cymbopogon nardus* of 4%, the stem of *Cimbopogon citrasus* was 2.84%, and then the third was in *Imperata cylindrica* leaves of 1.28%. The highest antioxidant activity was found in *Cymbopogon nardus* of 79.5 µg/ml followed by *Cimbopogon citrasus* of 58.4 µg/ml, and then the third was in *Imperata cylindrica* of 43.7 µg/ml.

In its biological activity, plants produce secondary metabolites. The functions of secondary metabolites are diverse including used in toxicity. Plants generally process this compound into certain parts such as apoplasts or certain organelles such as vacuoles, or parts of cells that are used as defense and tolerance mechanisms. Metabolomics is the study of comprehensive metabolic monitoring in plants, processing of metabolic compounds and pathways for processing compounds in plants. Still in the embryonic period, this interesting thing needs attention in understanding the regulation of metabolites globally.²⁶⁻²⁸ From this application, we can develop several applications that can provide valuable advanced information in the field of plant biotechnology, pharmaceutical industry, food agrochemicals to increase food and agriculture production which has a positive impact on other industries.

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