Ind. J. Pharm. Edu. Res., 2025; 59(4):1623-1632.
https://www.ijper.org

Original Article

Identification and Comparison of Phytoconstituents of Essential Oil and Methanolic Extracts of Leaves and Rhizomes of *Elettariopsis curtisii* Grown in Malaysia via GCMS Analysis

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ABSTRACT

Introduction: *Elettariopsis curtisii* (Zingiberaceae) is a high-value medicinal plant that has been used by the locals in Southeast Asia mainly to treat bloating and for postnatal care purposes. It contains many valuable phytoconstituents, including various essential oils that might be used in the treatment of various diseases. All previous studies were mainly carried out on the essential oil of rhizomes, and no or very limited information is available on other parts of the plant and methanolic extracts. Thus, this current study was conducted to identify, characterize, and compare the phytoconstituents of rhizome and leaf essential oils and methanolic extracts of *E. curtisii* grown in Malaysia via GCMS analysis. **Materials and Methods:** The plant samples were collected from a nursery in Kuala Krai, Kelantan, Malaysia. The extraction of essential oils from rhizomes and leaves was carried out via hydrodistillation and simple cold maceration method using methanol as solvent, respectively. Later on, all samples were analysed via GCMS analysis, and phytoconstituent identification was done based on the comparison in the NIST08 library. **Results:** A number of compounds were identified by GCMS analysis in rhizome essential oil, leaf essential oil, methanol rhizome extract and methanol leaf extract of *E. curtisii*. The most abundant phytoconstituent reported in rhizome essential oil was (E)-2-Decenal. The compound (E)-2-octenal, is found to be present in highest concentration in leaf essential oil, methanol rhizome extract, and methanol leaf extract, with peak areas of 41.11%, 40.68%, and 54.14%, respectively. Literature shows the beneficial antimicrobial and antioxidant nature of these identified compounds. **Conclusion:** This study might provide valuable information about the phytoconstituents found in this plant and could help the researcher in the isolation of antimicrobial compounds. We also recommend performing LCMS analysis on the extracts prior to isolating the desired compounds.

Keywords: *Elettariopsis curtisii*, Essential oil, Methanolic extract, GCMS analysis.

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Received: 27-03-2025;
Revised: 19-05-2025;
Accepted: 02-07-2025.

INTRODUCTION

E. curtisii is known as Pokok Pepijat or Pokok Semomok belongs to the family *Zingiberaceae*, a native Southeast Asian plant, mostly found in countries such as Thailand, Malaysia, and Borneo. *E. curtisii* has rhizomes that are white, creepy and slender.[1] The leaves of *E. curtisii* are elliptic, glabrous but not prominently veined with height of approximately about 6.8 to 13.2 cm, and have a strong odour that similar to a stinking bug smell.[2] It grows in humid, damp and shady places.[3] The locals and indigenous people use the whole plant as medicinal, either in the form of decoction or freshly eaten, for the treatment of bloating and postnatal care.[3,4] Besides being used medicinally, it is also used in cuisine to remove the fish odor and enhance its flavour and sometimes it has been eaten freshly as vegetables, salad or as a sambal.[4] Plants are rich sources for secondary metabolites with a variety of structural arrangements and have remarkable biological properties. Natural products that come from the medicinal plants are important for pharmaceutical research and drug development. Its derivatives are often prepared from crude plant extracts, which comprise a complex mixture of different phytochemical constituents. The previous studies showed that *E. curtisii* consists mostly of monoterpenoids, which contain β-pinene and β-phellandrene in the essential oils of rhizomes and leaves and aldehydes, alcohols,

DOI: 10.5530/ijper.20251594

esters, and alkanes such as trans-2-decenal, (E)-2-decenoic acid, and (E)-2-octenal.[5]

Gas Chromatography and Mass Spectroscopy (GC-MS) is the best method for analysing the metabolites of different plant extracts, essentials oils, and the bioactive components like alcohols, acids, esters, alkaloids, long-chain hydrocarbons, etc.[6] It integrates two analytical methods into a single approach for examining chemical compound combinations. While mass spectroscopy examines each component independently, gas chromatography separates the mixture's constituents.[3,7] The essential oil of rhizomes was the primary focus of all prior research; little or no information is available regarding methanolic extracts and other plant parts. Thus, the objective of the study was to analyse and compare the phytoconstituents present in essential oil and methanolic extracts of rhizomes and leaves of *E. curtisii*, via GCMS analysis. Photographs of leaves and rhizomes of *E. curtisii* are shown in Figures 1(a) and (b), respectively.

MATERIALS AND METHODS

Plant Materials

Plant leaves and rhizomes of *E. curtisii* were collected from Kuala Krai, Kelantan, during summer rainy season in December 2022. The plant parts were separated into rhizomes and leaves. The plant parts were cleaned and washed with clean water. The rhizomes and leaves were then sun-dried subsequently put into a grinder to make coarse powder. The coarse powder of plants was stored separately in a clean and closed airtight container and fully sealed with parafilm before extraction.

Extraction of Essential Oils from rhizome and leaves of *E. curtisii* using Clevenger Apparatus

The dried and coarse powder rhizomes (30 g) and leaves (30 g) has undergone the hydrodistillation method. The plant sample was separately introduced into a 1 L flask, after which distilled water was added until it covered the sample completely. The hydrodistillation method was carried out using Clevenger apparatus to obtain the essential oil. The distillation time was four hours and at normal pressure. The volatile oil distilled over the water was collected. The collected essential oil rhizomes (1.2 mL) and leaves (0.7 mL) were kept under refrigeration (8°C) until GCMS analysis process.

Preparation of methanolic extracts of rhizome and leaves of *E. curtisii*

The dried and coarse powder rhizomes (30 g) and leaves (30 g) were separately extracted with methanol (300 mL) by vigorously shaking for 1 hr and left open for 24 hr under the fume hood. After 24 hr, the mixture solution was filtered out into a new 1 L beaker, and the plant samples were added with a new 300 mL of methanol, and the same steps were repeated for 3 days. The empty and clean round-bottom flask was weighed before transferring the solvent into the flask. The rotary evaporator (Rotavapor) completely removed the solvent, yielding gummy exudates that were brownish-yellow from the rhizome and greenish from the leaves. The obtained crude extract was weighed, and their percentage yield was calculated by the formula:

$$\text{Percentage yield} = [\text{mass of the extract (g)} / \text{mass of the dried sample (g)}] \times 100$$

The crude extracts were stored at low temperature in a refrigerator for the GCMS analysis process.

GCMS analysis

The crude methanol extract of rhizomes (3 mg) and leaves (3 mg) was diluted separately with 3 mL methanol (HPLC grade) to prepare 1 mg/mL prior to GCMS analysis. Essential oil of rhizome and leaves samples was also made ready. All four extracts of rhizomes and leaves have then been put for the GCMS analysis. GCMS was performed using a gas chromatography mass selective detector (GC-MS) (GC 6890N; MS 5973N, Agilent Technologies, USA) with a HP-5MS Agilent column (30 m×0.25 mm diameter, 0.25 µm film thickness). The injector temperature was 250°C. Oven temperature was programmed at 60°C for 5 min, then heated to 250°C at 3°C/min and kept the temperature constant at 250°C for 10 min. The carrier gas was helium with a constant flow rate of 1 mL/min. The injector volume of the sample was 1.0 µL with a split ratio of 10:1 at a constant split flow of 10 mL/min. The spectrometer was run in electron impact mode, scanning at a mass range of 50-550 m/z with a solvent delay of 2.0 min. The total scan time was 78.333 min. The identification of components of the samples was based on a comparison of their mass spectra with those stored in the NIST08 library.

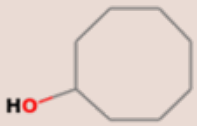
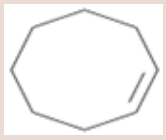
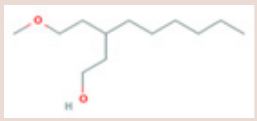
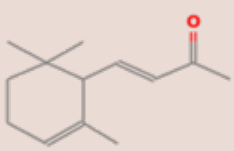
RESULTS

All extracts of rhizomes and leaves of *E. curtisii* afforded different appearances of extract with a strong odor. However, the leaf methanol extract gave the highest yield (18.9 % w/w). The rhizome essential oil, leaf essential oil, and rhizome methanol extract were afforded in lower yields of 4.0%, 2.3%, and 10.3%, respectively. The GCMS analysis identified 46 compounds in the rhizome methanolic extract, which is shown in Table 3. A total of 12 compounds were identified in rhizome essential oil (Table 1). Leaves essential oil and methanol leaves extract were found to contain a total of 4 (Table 2) and 9 (Table 4) compounds, respectively, which were identified in GCMS analysis. The chemical constituents and their retention time (RT) in min, molecular formula, molecular structure, and peak area (%) were presented in all Tables 1-4.

DISCUSSION

Phytochemical screening of essential oil from rhizomes and leaves of *E. curtisii* revealed the presence of different types of phytoconstituents. The main classes of compounds that have been detected in all extracts of rhizomes and leaves were aldehydes, alcohols, esters, alkanes and alkanes. Aldehyde (E)-2-decenal and (E)-2-octenal were the major compounds detected by GCMS analysis. The compound (E)-2-decenal was identified as the highest peak area in rhizome essential oil of *E. curtisii* (Table 1). Another aldehyde compound, (E)-2-octenal, was also found to be reported in the highest concentration in

Table 1: Phytochemical compounds identified in rhizome essential oil of *E. curtisii* via GCMS analysis.

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
7.076	Bicyclo[3.1.1]hept-2-ene, 2, 6, 6-trimethyl- (.+/-.)-	$C_{10}H_{16}$	136.23	2.59	
10.000	Octanal	$C_8H_{16}O$	128.21	0.43	
13.239	2-Octenal, (E)-	$C_8H_{14}O$	126.20	3.08	
13.537	2-Octenal, (E)-	$C_8H_{14}O$	126.20	0.96	
14.824	Cyclooctyl alcohol	$C_4H_8O_2$	72.11	-0.11	
14.858	Cyclooctane	C_8H_{16}	112.22	0.49	
20.294	Cyclooctene	C_8H_{14}	110.20	6.57	
23.487	(E)-2-Decenal	$C_{10}H_{18}O$	154.25	47.39	
23.865	(E)-2-Decenal	$C_{10}H_{18}O$	154.25	21.00	
24.288	1-Methoxy-3-(2-hydroxyethyl)nonane	$C_{12}H_{26}O_2$	296.50	9.67	
29.278	Cyclodecene, (Z)-	$C_{10}H_{18}$	138.25	6.63	
29.884	3-Buten-2-one, 4-(2, 6, 6-trimethyl-2-cyclohexen-1-yl)-, (E)-	$C_{13}H_{20}O$	192.30	1.30	

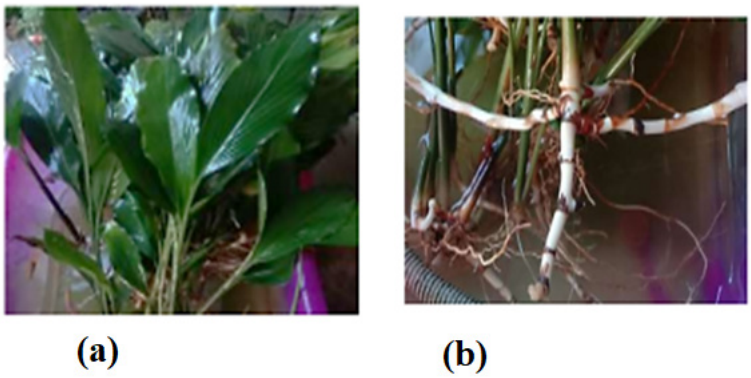
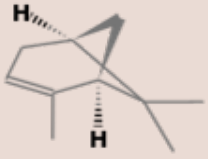


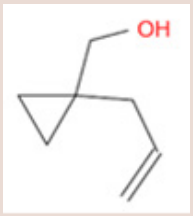
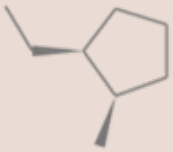
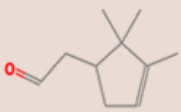
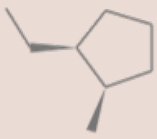
Figure 1: Photographs of leaves (a) and rhizomes (b) of *E. curtisii*.

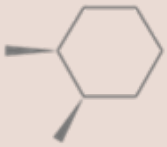
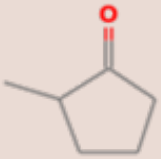
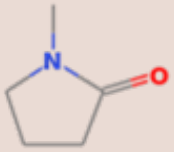
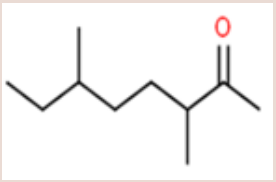
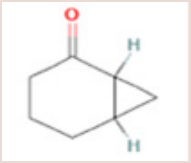
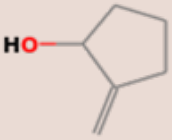
Table 2: **Phytochemical compounds identified in leaf essential oil of *E. curtisii* via GCMS analysis**

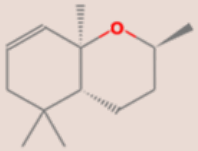
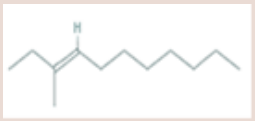
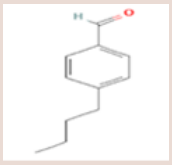
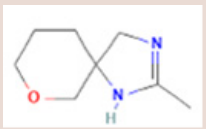
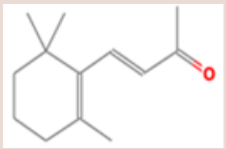
RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
12.375	2-Octenal, (E)-	$C_8H_{14}O$	126.20	41.11	
21.290	2H-1, 4-Benzodiazepin-2-one, 7-bromo-1, 3-dihydro-5-phenylpiperazin-1-yl) butyl	$C_{15}H_{12}N_2O$	236.27	2.56	
21.896	2-Decenal, (E)-	$C_8H_{14}O$	126.20	21.50	
30.147	Chloromethyl 6-chloro undecanoate	$C_{12}H_{22}Cl_2O_2$	269.20	34.84	

Table 3: **Phytochemical compounds identified in methanol rhizome extract of *E. curtisii* via GCMS analysis**

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
3.431	Hexanal	$C_6H_{12}O$	100.16	0.36	
4.524	2-Hexenal, (E)-	$C_6H_{10}O$	98.14	0.37	
5.903	Heptanal	$C_7H_{14}O$	114.19	0.61	
6.991	1R-.alpha.-Pinene	$C_{10}H_{16}$	136.23	0.98	

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
9.543	4-Nonenal, (E)-	$C_9H_{16}O$	140.23	0.35	
9.657	(1-Allylcyclopropyl) methanol	$C_7H_{12}O$	112.17	0.83	
9.886	Octanal	$C_8H_{16}O$	128.21	1.59	
11.866	Cyclopentane, 1-ethyl-2-methyl-, cis-	C_8H_{16}	112.21	0.93	
12.627	2-Octenal, (E)-	$C_8H_{14}O$	126.20	40.68	
12.976	2-Decen-1-ol	$C_{10}H_{20}O$	156.27	0.68	
13.090	1-Octanol	$C_8H_{18}O$	130.23	0.42	
13.931	Heptanoic acid	$C_7H_{12}O_2$	130.19	0.34	
14.080	Heptanoic acid	$C_7H_{12}O_2$	130.19	0.32	
14.555	Nonanal	$C_9H_{18}O$	142.24	0.30	
14.790	2, 4-Octadienal, (E,E)-	$C_8H_{12}O$	124.18	0.23	
15.516	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-	$C_{10}H_{16}O$	152.23	0.28	
16.523	Cyclopentane, 1-ethyl-2-methyl-, cis-	C_8H_{16}	112.21	0.38	

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
16.901	Cyclohexane, 1,2-dimethyl-, cis-	C_8H_{16}	112.21	0.19	
17.347	Cyclopentanone, 2-methyl-	$C_6H_{10}O$	98.14	0.68	
18.463	2-Pyrrolidinone, 1-methyl-	C_5H_9NO	99.13	1.37	
18.761	4-Nonenal, (E)-	$C_9H_{16}O$	140.23	0.53	
18.944	Octan-2-one, 3,6-dimethyl-	$C_{10}H_{20}O$	156.26	1.22	
19.167	Octanoic acid	$C_8H_{16}O_2$	144.21	1.03	
19.321	Decanal	$C_{10}H_{20}O$	156.26	0.57	
19.733	Bicyclo[4.1.0]heptan-2-one	$C_7H_{10}O$	110.15	2.38	
20.946	Methyldiallylamine	$C_7H_{13}N$	111.18	1.48	
21.284	2-Methylene cyclopentanol	$C_6H_{10}O$	98.14	1.66	
22.074	2-Decenal, (Z)-	$C_{10}H_{18}O$	154.25	24.33	

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
22.282	2-Decen-1-ol	$C_{10}H_{20}O$	156.27	2.64	
22.497	1-Dodecanol	$C_{12}H_{26}O$	186.33	0.30	
23.018	2H-1-Benzopyran, 3, 4, 4a, 5, 6, 8a-hexahydro-2, 5, 5, 8a-tetramethyl-(2.alpha., 4a.alpha., 8a.alpha.)-	$C_{13}H_{22}O$	194.31	0.27	
23.207	Nonanoic acid	$C_9H_{18}O_2$	158.24	0.45	
25.713	3-Undecene, 3-methyl-	$C_{12}H_{24}$	168.32	0.11	
26.108	Benzaldehyde, 4-butyl-	$C_{11}H_{14}O$	162.23	0.35	
26.605	1-Oxa-3, 4-Diazaspiro[4,5]dec-3-ene, 2-(acetyloxy)-2-methyl-	$C_8H_{14}N_2O$	154.21	0.50	
27.550	n-Decanoic acid	$C_{10}H_{20}O_2$	172.26	1.04	
28.482	Cyclodecene, (Z)-	$C_{10}H_{18}$	138.25	2.23	
29.198	3-Buten-2-one, 4-(2, 6, 6-trimethyl-2-cyclohexen-1-yl)	$C_{13}H_{20}O$	192.30	0.44	
29.827	trans-2-Decenoic acid	$C_{10}H_{18}O_2$	170.25	0.81	

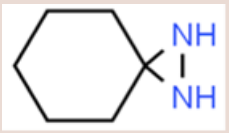
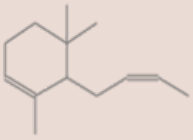
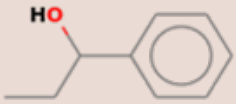
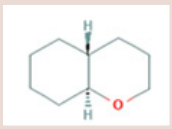
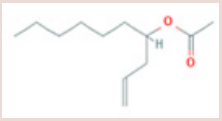
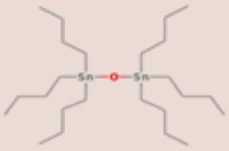
RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
30.119	1, 2-Diazaspiro(2.5) octane	$C_6H_{12}N_2$	112.17	0.79	
30.805	5-Dodecyne	$C_{12}H_{22}$	166.30	0.25	
31.647	Cyclohexane, 6-(2-butenyl)-1, 5,5-trimethyl-, (E)-	$C_{13}H_{20}$	176.30	0.35	
32.133	Pentadecane	$C_{15}H_{32}$	212.41	0.35	
40.201	Heptadecane	$C_{17}H_{36}$	240.47	2.13	
40.750	Benzenemethanol, .alpha.-ethyl-	$C_9H_{12}O$	136.19	1.10	
43.457	5, 6-Azulenedicarboxaldehyde, 1, 2, 3a, 8, 8a-hexahydro-2, 2, 8-trimethyl-, (3a.alpha., 8.alpha., 8a.alpha.)-(-)-			0.76	

Table 4: Phytochemical compounds identified in methanol leaf extract of *E. curtisii* via GCMS analysis.

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
9.875	Octanal	$C_8H_{16}O$	128.21	1.96	
12.409	2-Octenal, (E)-	$C_8H_{14}O$	126.20	54.14	
18.217	trans-2-oxabicyclo[4.4.0]decane	$C_9H_{16}O$	140.22	4.17	
19.705	Cyclooctene	C_8H_{14}	110.20	2.84	
21.902	2-Decenal, (E)-	$C_{10}H_{18}O$	154.25	29.32	

RT (min)	Compound Name	Molecular formula	Molecular Weight	Peak Area (%)	Molecular Structure
22.303	2-Decen-1-ol, (E)-	$C_{10}H_{20}O$	156.26	2.62	
27.109	4-Acetoxy-1-decene	$C_{12}H_{22}O_2$	198.30	1.69	
27.939	Distannoxane, hexabutyl-	$C_{24}H_{54}OSn_2$	596.10	0.54	
28.448	Cyclodecene	$C_{10}H_{18}$	138.25	2.72	

leaf essential oil (RT: 12.375 min, Peak area: 41.11%) (Table 2), methanol rhizome extract (RT: 12.627 min, Peak area: 40.68%) (Table 3), and methanolic leave extract (RT: 12.409 min, Peak area: 54.14%) (Table 4) of *E. curtisii*. The remaining constituents were identified in comparatively lower concentrations, as shown in Tables 1-4. Among the identified compounds, the aldehydes, the major phytoconstituents in all extracts of rhizomes and leaves including (E)-2-decenal and (E)-2-octenal were found in several other plants, including *Ailantus althissima*;[6] *Oleo europaea*.;[7] *Coriandrum sativum*[8] and *Halyomorpha halys*[9] had been well-documented in their antimicrobial activity. These aliphatic aldehydes were thought to show a potent antimicrobial substance.[10] These aliphatic aldehydes were thought to show a potent antimicrobial substance.[11]

In this study we have found that two peaks eluted from the same compound at two different retention times and have similar fragment patterns, like the compound (E)-2-Decenal in rhizome essential oil. Chromatographic alignment software and/or Kovats retention indices may be used in conjunction with MS data to ensure further accurate identification of these compounds. Currently, we considered (E)-2-Decenal as the main compound in rhizome essential oil, which provided a larger peak area.

There was a minor presence of monoterpenes (1R-α-pinene) which had a peak area of 0.98% in the methanol extract of rhizomes. However, these experimental results were different from the previous reports, which found out that monoterpenes, including β-pinene and β-phellandrene, were the most abundant phytoconstituents in the essential oil of the rhizomes and leaves, with 88.3% and 77.15%.[12,13] The α-pinene compound was the main secondary metabolite in many essential oils that had been extensively studied to have biological activity, including antimicrobial, anti-inflammatory and antioxidant.[14] Terpenes compounds were also reported to have a potent activity against gastric ulcers,[15] which is beneficial for the pharmaceutical industry. The presence of these phytoconstituents in the extract of *E. curtisii* leaves and rhizomes possibly indicated its medicinal properties, such as antioxidants and antimicrobials.

CONCLUSION

In conclusion, this study provides phytochemical analysis of the organic and oil extracts from the rhizomes and leaves of *E. curtisii*. The main identified compound like aldehydes (E)-2-decenal in rhizome essential oil and (E)-2-octenal in leaf essential oil, methanol rhizome extract and methanol leaf extract, could show antimicrobial activity. Researchers may target isolating these compounds, which could help in getting antimicrobial pharmaceutical entity in future.

This is the first-time study carried out for the identification and comparison of phytoconstituents of leaves and rhizomes of methanolic extract and essential oil of *E. curtisii*. We also recommend carrying out LCMS analysis of the extracts before proceeding to isolate the targeted compounds.

ACKNOWLEDGEMENT

The authors would like to thank Faculty of Pharmacy, UiTM for providing research facility to carry out FYP research study. The authors would also like to thank Universiti Teknologi MARA(UiTM) for providing financial support under reference no 600-RMC/KEPU 5/3 (008/2023).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GCMS: Gas chromatography mass spectroscopy; **RT:** Retention Time.

SUMMARY

Elettariopsis curtisii (Zingiberaceae) has been used by the locals in Southeast Asia mainly to treat bloating and postnatal care purposes. This study shows that different plant parts contain different metabolites. The most abundant phytoconstituents reported in this study were aldehydes (E)-2-decenal (47.39%) in rhizome essential oil and (E)-2-octenal in leaf essential oil, methanol rhizome extract and methanol leaf extract. Some compounds like aldehydes (E)-2-decenal are found to be identified in different parts of plant. Some metabolites show good antimicrobial activity as reported in literature.

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Cite this article: Najlaa EN, Sultan S, Ashraf K. Identification and Comparison of Phytoconstituents of Essential Oil and Methanolic Extracts of Leaves and Rhizomes of *Elettariopsis curtisii* Grown in Malaysia via GCMS Analysis. Indian J of Pharmaceutical Education and Research. 2025;59(4):1623-32.