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EFFECT OF SOLVENT POLARITY ON THE PHYTOCHEMICAL COMPOSITION AND BIOACTIVITY OF *Clerodendrum fragrans* Vent. EXTRACTS COLLECTED FROM HUE CITY, VIET NAM

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GC-MS

ABSTRACT

Clerodendrum fragrans Vent. (C. fragrans), is a valuable medicinal plant found in Vietnam. This study aims to investigate the chemical composition and biological activities of the aerial parts (stem and leaves) of *C. fragrans*. The chemical composition was determined using four different solvents: methanol, water, ethyl acetate, and n-hexane. The study also examined biological activities, including antioxidant properties and cytotoxicity. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze compounds with varying polarities in the extracts. Antioxidant activity was assessed using the DPPH method, while cytotoxic activity was measured by analyzing the optical density between total protein and stained Sulforhodamine B. Qualitative analysis of the extracts identified a total of 46 compounds across the four solvent extracts. The methanol, water, and ethyl acetate extracts yielded the highest number of compounds, ranging from 16 to 17 compounds each, whereas the n-hexane extract produced the fewest, with only 6 compounds identified. The DPPH scavenging activity demonstrated a range of IC_{50} values from 206.350 to 420.974 µg/mL, with the ethyl acetate extract showing the highest activity at 206.350 µg/mL. In terms of cytotoxic activity against HeLa cells, IC_{50} values ranged from 57.89 to 137.381 µg/mL, with the ethyl acetate extract again demonstrating the most potent effect, recording an IC_{50} of 137.381 µg/mL. These findings indicate that the above-ground parts of *C. fragrans* possess significant antioxidant and cytotoxic properties. Therefore, further research is warranted to explore its potential pharmacological applications in human health.

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1 Introduction

The genus *Clerodendrum* L. belongs to the Lamiaceae family, which comprises a diverse array of plants, including herbs, shrubs, climbers, and trees. These plants are primarily found in subtropical and tropical regions worldwide (Ogunlakin et al. 2023; Ashrafuzzaman et al. 2024). One notable species within this genus is *Clerodendrum fragrans* Vent. (*C. fragrans*). Members of this genus are well-known for their various applications in traditional medicine and pharmacological research. Extracts from *C. fragrans* contain a variety of phytochemicals with significant biological potential, including flavonoids, phenolics, alkaloids, terpenoids, and steroids (Kalonio et al. 2022). The composition and bioactivity of these extracts can be significantly influenced by the polarity of the solvent used during the extraction process (Nawaz et al. 2020; Simorangkir et al. 2021; Kalonio et al. 2022). In plant extraction studies, selecting the appropriate solvent is essential for obtaining a wide range of active compounds and enhancing their activity. This selection is guided by the principle of "like dissolves like," meaning that polar compounds typically dissolve in polar solvents, while non-polar compounds dissolve in non-polar solvents. Common solvents used for phytochemical extraction include methanol (MeOH), ethanol (EtOH), and isopropyl alcohol (IPA), each chosen for its varying polarities to optimize extraction results (Simorangkir et al. 2019; Puthongking et al. 2023; Ogunlakin et al. 2023; Rguez et al. 2023; Ho et al. 2025).

In Vietnam, particularly in Hue City, *C. fragrans* is extensively used in traditional medicine to address various health issues, including irregular menstruation, uterine ulcers, gynecological disorders, boils, jaundice, high blood pressure, menstrual problems, asthma, and other respiratory diseases (Chi 2021). Recent research has revealed that *C. fragrans* contains a diverse array of secondary metabolites, including alkaloids, steroids, flavonoids, triterpenoids, saponins, tannins, and quinones. These compounds have been identified through the extraction process using various solvents, including n-hexane, ethyl acetate, ethanol, and methanol (Simorangkir et al. 2019). The extracts derived from *C. fragrans* exhibit various pharmacological activities, including free radical scavenging, anticancer properties, and antibacterial effects (Simorangkir et al. 2019; Sapiun et al. 2020; Simorangkir et al. 2020, 2021, 2022; Ogunlakin et al. 2023).

Despite these findings, a lack of systematic studies remains regarding how solvent polarity affects the phytochemical composition and biological activity of extracts from *C. fragrans*. Therefore, this study aims to investigate and analyze extracts from *C. fragrans* collected in Hue, using solvents of different polarities (n-hexane, water, ethyl acetate, and methanol) to identify differences in their chemical composition and biological activity. The study will employ modern methods such as Gas Chromatography-Mass Spectrometry (GC-MS) and bioactivity analysis to evaluate the extracts. The results will not only provide valuable insights into the chemical composition of *C. fragrans* but also pave the way for the development of pharmaceutical products derived from natural sources in Vietnam.

2 Materials and Methods

2.1 Materials plant

The *C. fragrans* samples were collected from Hue City, Vietnam, and washed under running water. After being air-dried in the shade, they were dried to a constant mass in a drying oven (Memmert model UN110, Germany) and then ground into a fine powder. The powder was stored in polyethylene bags in a dark environment (Figure 1).

2.2 Sample preparation

A sample of dried powder (100 g) was extracted by immersing it in 500 mL of each solvent, i.e., methanol, water, ethyl acetate, and n-hexane, for 24 hours. This extraction process was repeated three times for each solvent. The solution obtained after each extraction was combined, filtered through Whatman paper No. 41, and concentrated using the Hei-VAP Value system from Heidolph (Germany). The resulting extracts were stored at 4°C in a refrigerator to serve as raw materials for subsequent experiments.

Figure 1 The *Clerodendrum fragrans*

2.3 Determining total phenolic content

The total phenolic content was determined using Gallic acid standards (Sigma) diluted to a concentration range of 0.002 to 0.014 mg/mL in 70% ethanol, along with the Folin–Ciocalteu reagent, as described by Molole et al. (2022). The reaction mixture consisted of 0.5 mL of the extract or each concentration of the standard, 3 mL of distilled water, and 0.25 mL of Folin–Ciocalteu's reagent, all combined in a 10 mL volumetric flask. The mixture was shaken thoroughly and left to stand for 5 minutes. After that, 0.75 mL of 20% Na_2CO_3 was added, and the volume was adjusted to 5 mL with distilled water. Following 45 minutes of incubation at room temperature, the reaction solutions were measured spectrophotometrically at 758 nm using a Multiskan SKY Microplate Spectrophotometer (Thermo Scientific). The total phenolic content of each crude extract was calculated and expressed in mg of gallic acid equivalents (GAE) per gram of crude extract.

2.4 Determining total flavonoid content

The total flavonoid content was determined using a colorimetric method with $AlCl_3$ reagent, as described by Long et al. (2024). For the analysis, a sample or Catechin standard solution was prepared at concentrations ranging from 0.02 to 1.0 mg/mL. Each concentration (0.5 mL) was combined with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite ($NaNO_2$). The mixture was shaken and incubated for 5 minutes. Following this, 0.15 mL of 10% $AlCl_3$ was added, and the mixture was incubated for an additional 6 minutes. After incubation, 2 mL of 1 M NaOH was added to the reaction mixture, and the total volume was adjusted to 5 mL with distilled water. The mixture was thoroughly shaken, and the optical density was measured at 510 nm using a Multiskan SKY Microplate Spectrophotometer (Thermo Scientific). The total flavonoid content of each crude extract was calculated in milligrams of Catechin standard equivalents per gram of extract.

2.5 Determining total Tanin content

Tannin content was quantified using the method described by Atanassova and Christova-Bagdassarian (2009). For the analysis, 5 mL of the extract solution was placed in a 200 mL volumetric flask, and then 2.5 mL of indigo carmine solution and 75 mL of sterile distilled water were added. The mixture was titrated with 0.1N $KMnO_4$ until the blue solution changed to yellow, with the result recorded based on the amount of 0.1N $KMnO_4$ used. The indigo carmine solution was prepared by dissolving 6 g of indigo carmine in 500 mL of deionized distilled water using a heating method. After cooling the solution to room temperature, 50 mL of 97% H_2SO_4 was added, and the mixture was then brought to a total volume of 1 liter and filtered through Whatman paper. Blank samples were prepared by titrating a mixture of 2.5 mL of indigo carmine solution and 75 mL of distilled water. All samples were analyzed in triplicate. Tannin content (T, %) is calculated based on the following formula

$$T\% = \frac{(V - V_o) \times 0.004157 \times 200 \times 100}{g \times V_1} \quad (1)$$

Here: V (mL) is the volume of 0.1N $KMnO_4$ solution used to titrate the sample; V_o (mL) is the volume of 0.1N $KMnO_4$ solution used to titrate the blank sample; 0.004157 is the converted tannin content in 1 mL of 0.1N $KMnO_4$; g (gram) is the mass of sample used for analysis; 200 mL is the volume of the volumetric flask; 100 is the conversion to %; V_1: volume of reaction sample.

2.6 Determining total alkaloid content

The alkaloid content was determined following the method outlined by John et al. (2014) for a caffeine standard. This involved creating a concentration series with volumes of 0.5, 1.0, 1.5, 2.0, and 2.5 mL, accompanied by a completion reaction using Bromocresol Green (BCG). For the extraction process, the sample was dissolved in 2 N HCl and filtered through Whatman paper to remove any residues. From this solution, 1 mL was transferred to a separating funnel and washed three times with 10 mL of chloroform. Following this, the pH of the solution was adjusted to neutral using 0.1 N NaOH. After adjusting the pH, 5 mL of BCG solution and 5 mL of phosphate buffer solution were added to the mixture. This solution was shaken vigorously and then extracted with an additional 1, 2, 3, and 4 mL of chloroform. The resulting extracts were collected and transferred to a volumetric flask, where the volume was adjusted to 10 mL with chloroform. The absorbance was then measured at 470 nm. A blank was prepared following the same procedure, replacing the sample with distilled water.

$$A = \frac{C.V.a}{m} \quad (2)$$

Here: A: total alkaloid content in the sample; C: caffeine concentration converted from the standard regression equation; V: initial volume (mL); a: mass of 100 (g) medicinal powder; m: mass of 100 (g) medicinal powder.

2.7 GC-MS analysis method

The crude extract sample (1 mg) was dissolved in 1 mL of hexane solvent. The sample was then centrifuged to collect the supernatant, which was subsequently filtered through a PTFE filter. The supernatant was analyzed using GC-MS on a TSQ 9000 Triple Quadrupole GC-MS/MS (Thermo) equipped with a DB column (5 µm, 30 m x 0.25 mm, 0.25 µm), with a flow rate of 1 mL/min and helium as the carrier gas. The sample was analyzed using a gradient program with the following conditions: starting at 60°C (held for 5 minutes) and then increasing the temperature by 10°C per minute until it reached 310°C (held for 30 minutes).

2.8 Method for determining antioxidant activity

The antioxidant activity of the extracts was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method, as described by Long et al. (2020). The extract (1 mg) was dissolved in 1 mL of distilled water and then diluted to concentrations of 12.5, 25, 50, and 100 µg/mL. 0.5 mL of each dilution was reacted with 0.5 mL of a 0.2 mM DPPH solution diluted in 70% ethanol, mixed well, and incubated in the dark for 30 minutes. Then, the optical density (OD) was measured quantitatively at a wavelength of 517 nm using a Multiskan Sky Microplate Spectrophotometer (Thermo Scientific). The formula calculates the result of the DPPH free radical scavenging. The IC_{50} value was calculated based on the linear equation (y = ax + b), where y = 50%, to determine x (x is the value of IC50 that needs to be discovered) for different types of extracts.

$$\%SC = \frac{OD_c - OD_m}{OD_c} x\ 100 \quad (3)$$

Here: ODm: Values optical density of the test sample; ODc: Values blank optical density (without DPPH).

2.9 Method cytotoxic assay

The cytotoxic activity of the extracts was evaluated against selected cancer cell lines at the Institute of Biotechnology, Vietnam Academy of Science and Technology. The method employed in vitro cancer cell line culture as described by Long et al. (2020). The experiment aimed to determine the total cellular protein content based on the optical density (OD) values obtained using stained Sulforhodamine B (SRB, Sigma-Aldrich, USA). This approach is referenced in the work of Monks et al. (1991). The percentage of inhibited cell growth will be calculated using the following formula:

$$\%\ \text{Alive cells} = \frac{OD_{\text{reagent}} - OD_{\text{day 0}}}{OD_{\text{negatlvecontrol}} - OD_{\text{day 0}}} x\ 100 \quad (4)$$

$$\%\ \text{inhibited cells} = 100 - \%\ \text{alive cells} \quad (5)$$

2.10 Statistical analysis

The data analysis method employed in this research is ANOVA. The experimental values are represented as the mean of the measurements from three experimental replications, along with the standard deviation calculated using Excel 2010.

3 Results and Discussion

3.1 Compound composition analysis

The extraction of compounds from plants using various solvents yields compound groups with differing compositions. This variation can be attributed to the chemical and physical properties of both the compounds and the solvents. Natural compounds such as alkaloids, flavonoids, phenolics, and tannins demonstrate different solubilities in solvents, which depend on their molecular structures and the arrangement of functional groups (polar or non-polar) (Table 1). These characteristics influence the proportion of each compound group that is extracted when the same plant sample is used.

The results of the GC-MS analysis presented in Table 2 and Figure 1 show that the composition of compounds obtained from *C. fragrans* plants varies depending on the solvent used during extraction with an ultrasonic bath. A total of 46 compounds were identified across four different solvents (Table 2). The solvents methanol, water, and ethyl acetate yielded the highest number of compounds, ranging from 16 to 17 substances in the extracts. In contrast, n-Hexane produced the fewest compounds, totaling only 6. Several compounds were found in multiple solvents, including Hexadecanoic acid, methyl ester, and n-Hexadecanoic acid (identified in methanol, water, and ethyl acetate), as well as Phytol and 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) (found in methanol, water, and n-Hexane). Among the extracts from different solvents, certain compounds stood out due to their high concentrations. In the methanol extract, the dominant compounds were 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (30.94%) and 2,7-Dioxatricyclo[4.3.1.0(3,8)]decan-4-one (20.52%). For the water extract, the leading compounds were 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) (22.92%) and Phytol (20.69%). The n-Hexane extract featured Phytol (33.18%) and 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) (29.02%) as the predominant compounds. Lastly, in the ethyl acetate extract, the compound with the highest concentration was 2-Methoxy-4-vinylphenol (38.44%), followed by (E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol (15.65%), as shown in Table 2 and Figure 2.

Table 1 The composition of groups of substances in extracts in different solvents of *C. fragrans*

Solvents	Tanin	Phenolic	Alkaloid	Flavonoid
	% extract	mg/g extract		
Methanol	10.808	0.220	0.160	0.655
Water	11.640	0.152	0.135	5.026
n-Hexane	-	0.055	1.776	0.067
Ethyl Acetate	8.314	0.308	2.073	4.555

Note: "-": Not detected

Table 2 Compound composition in the extract from *C. fragrans* based on GC-MS analysis

No.	Synonyms	Molecular Formula	MW (g/mol)	RT (min)	%Area			
					Methanol	Water	n-Hexane	Ethyl Acetate
1	Phenol	C_6H_6O	94.042	4.732	-	-	-	3.350
2	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.068	8.481	-	-	-	38.440
3	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.167	9.859	-	1.240	-	-
4	3H-Cyclopenta[c]pyridazin-3-one, 2,5,6,7-tetrahydro-	$C_7H_8N_2O$	136.064	10.500	-	-	-	4.220
5	Benzaldehyde, 2-hydroxy-6-methyl-	$C_8H_8O_2$	136.052	12.579	1.720	-	-	-
6	Methylparaben	$C_8H_8O_3$	152.047	13.129	1.130	-	-	2.460
7	2,7-Dioxatricyclo[4.3.1.0(3,8)]decan-4-one	$C_8H_{10}O_3$	154.063	13.361	20.520	-	-	-
8	9-Oxabicyclo[3.3.1]nonan-2-one, 5-hydroxy-	$C_8H_{12}O_3$	156.079	13.636	7.750	-	-	-
9	Benzoic acid, 4-hydroxy-	$C_7H_6O_3$	138.031	14.175	3.370	-	-	-
10	2-Cyclohexen-1-one, 3-methyl-	$C_7H_{10}O$	110.073	14.903	-	-	-	2.400
11	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	430.133	15.644	-	-	17.990	-
12	Neophytadiene	$C_{20}H_{38}$	278.297	15.644	-	1.440	-	-
13	17-Pentatriacontene	$C_{35}H_{70}$	490.548	15.768	-	0.860	-	-
14	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	578.171	15.778	-	-	9.160	-
15	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	138.068	16.094	-	-	-	4.170
16	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.319	16.449	-	-	6.420	-
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.308	16.456	-	1.110	-	-
18	α-Methyl-D-mannopyranoside	$C_7H_{14}O_6$	194.079	16.536	2.930	-	-	-
19	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	374.318	17.264	-	-	4.220	-
20	Ethanone, 1-(2-hydroxyphenyl)-	$C_8H_8O_2$	136.052	17.271	-	-	-	3.980
21	3-[N-[2-Diethylaminoethyl]-1-cyclopentenylamino]propionitrile	$C_{14}H_{25}N_3$	235.205	17.687	2.990	-	-	-
22	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.256	18.552	0.730	2.430	-	2,470
23	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240	19.561	1.410	1.960	-	1,160

No.	Synonyms	Molecular Formula	MW (g/mol)	RT (min)	%Area			
					Methanol	Water	n-Hexane	Ethyl Acetate
24	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.256	20.339	-	1.040	-	-
25	9-Oxabicyclo[3.3.1]nonane-2,6-diol	$C_8H_{14}O_3$	158.094	20.353	-	-	-	2.260
26	Phytol	$C_{20}H_{40}O$	296.308	20.681	3.330	20.690	33.180	-
27	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	$C_{11}H_{14}O_3$	194.094	20.722	-	-	-	15.650
28	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.225	21.181	2.080	7.640	-	-
29	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	$C_{13}H_{22}O_2$	210.162	21.221	-	-	-	4.140
30	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	$C_{14}H_{24}O_3$	240.173	21.463	2.360	-	-	-
31	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196.110	22.519	-	-	-	0.920
32	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.240	22.73	4.640	22.290	29.020	-
33	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	$C_{12}H_{20}O$	180.151	27.201	-	-	-	0.85-
34	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.277	27.821	-	14.540	-	-
35	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.272	28.72	12.850	-	-	-
36	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336.303	28.951	1.230	-	-	-
37	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	236.178	30.021	-	-	-	7.330
38	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390.277	33.160	30.940			
39	Pregna-4,9(11)-dien-20-ol-3-on-19-oic acid lactone	$C_{21}H_{26}O_3$	326.188	33.895	-	5.540	-	-
40	6-Hydroxy-4-methyl-3-phenylcoumarin, trimethylsilyl ether	$C_{19}H_{20}O_3Si$	324.118	34.884	-	2.050	-	-
41	Propionic acid, 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-	$C_7H_{10}N_2O_3$	170.069	35.900	-	-	-	3.380
42	(5β)3-Acetoxypregnan-20-ol-6-one-18-oic acid lactone	$C_{23}H_{32}O_5$	388.225	36.373	-	1.730	-	-
43	Methyl cholate	$C_{25}H_{42}O_5$	422.303	37.151	-	3.090	-	-
44	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-	$C_{30}H_{48}O_2$	440.365	37.476	-	4.150	-	-
45	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	310.287	37.476	-	-	-	2.820
46	(2R)-2-(3-chloro-4-methylsulfonylphenyl)-3-[(1R)-3-oxocyclopentyl]-N-pyrazin-2-ylpropanamide	$C_{19}H_{20}C_lN_3O_4S$	421,086	39,344	-	8,210	-	-

Note: "-": Not detected; Peak areas (%) are calculated based on the number of compounds for each different type of extract; RT: Retention tim; MW: Molecular Weight

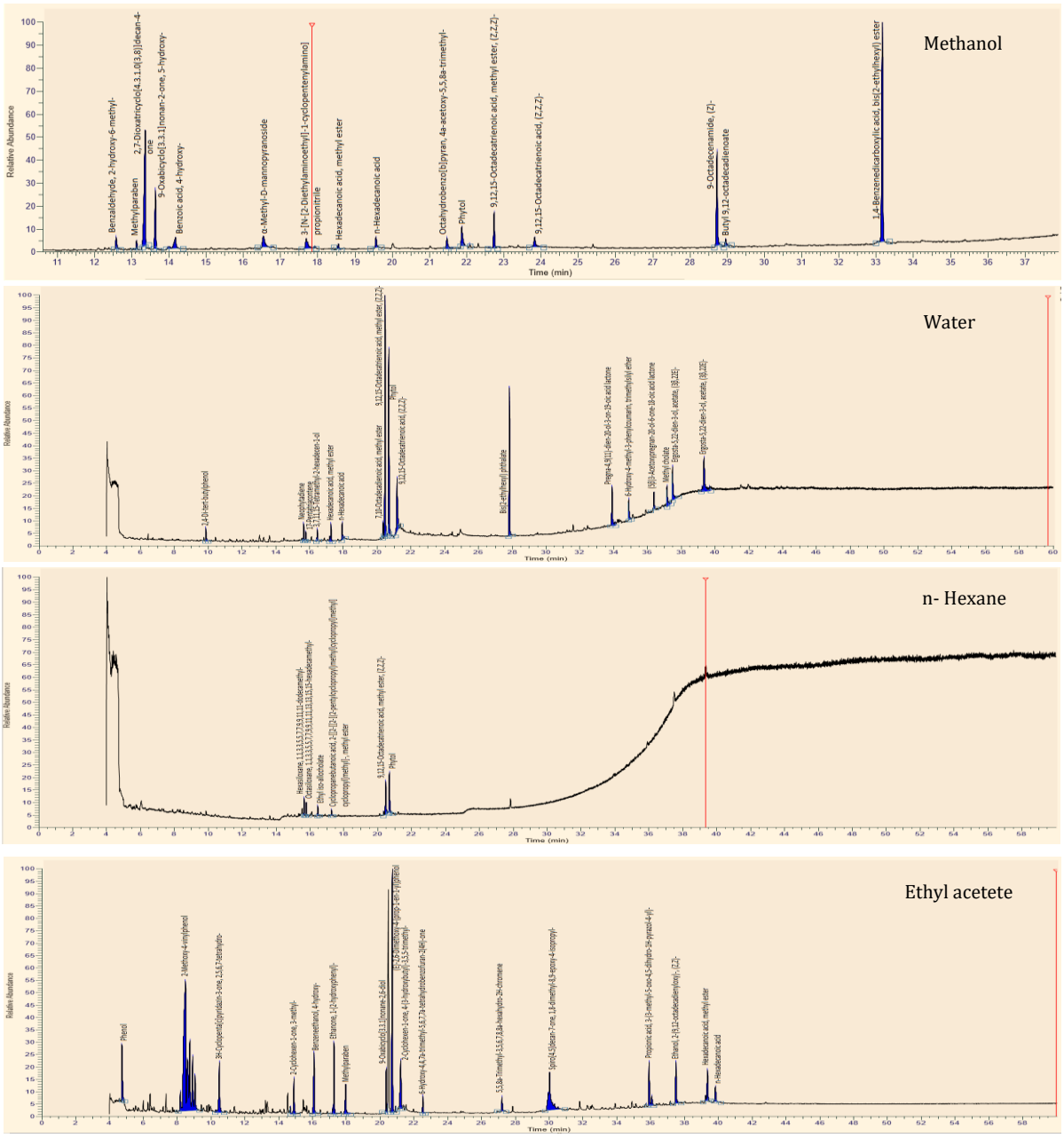

Figure 2 The chromatogram GC-MS from extracts of different parts of *C. fragrans*

3.2 Antioxidant activity analysis

The results of the DPPH free radical scavenging activity of four extracts from *C. fragrans* demonstrated that at a concentration of 500 µg/mL, the ethyl acetate extract exhibited the highest activity at 84.73%. This was followed by the water extract at 73.99%, the methanol extract at 74.23%, and the n-hexane extract, which showed the lowest activity at 52.51%. The concentration required to inhibit DPPH free radicals by 50% (IC_{50}) was found to be lowest for the ethyl acetate extract at 206.35 µg/mL, while the n-hexane extract required the highest concentration at 420.97 µg/mL. The methanol extract had an IC_{50} value of 277.22 µg/mL, and the water extract had an IC_{50} value of 230.59 µg/mL, with both showing no significant differences in their inhibitory effects on DPPH free radicals. The linear relationship between the concentrations investigated showed high reliability, with R^2 values ranging from 0.930 to 0.944. Ascorbic acid, a commonly used standard free radical scavenger, yielded significantly better results than the extracts tested (Figure 3 and Table 3).

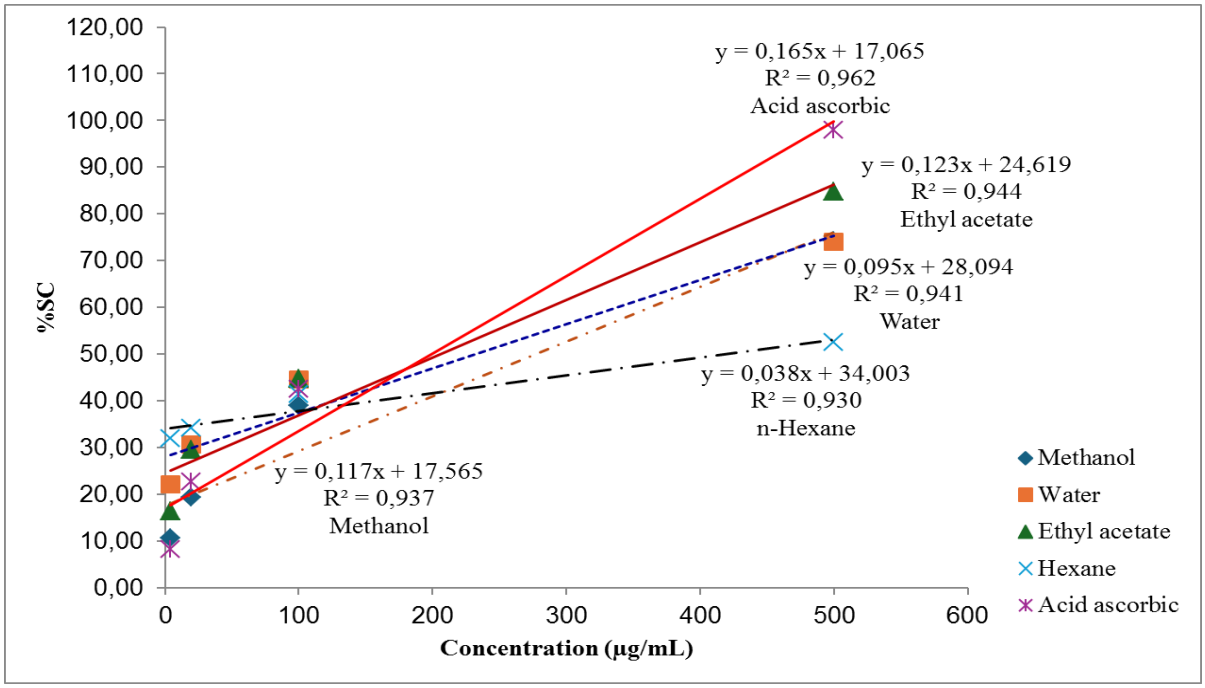

Figure 3 Linear equations showing the DPPH free radical scavenging activity of different extracts from *C. fragrans*

Table 3 DPPH free radical scavenging activity

Concentration (µg/mL)	Methanol	Water	Ethyl acetate	Hexane	Ascorbic acid
500	74.232 ± 0.395	73.993 ± 0.662	84.730 ± 0.361	52.510 ± 0.232	98. ±1.070
100	38.959 ± 0.188	44.577 ± 0.558	44.806 ± 0.708	41.329 ± 0.088	42.542 ± 1.120
20	19.418 ± 0.465	30.649 ± 0.440	29.584 ± 0.219	34.145 ± 0.044	22.745 ± 1.140
4	10.692 ± 0.699	22.162 ± 0.537	16.371 ± 2.877	31.944 ± 0.044	8.232 ± 2.030
IC_{50} (µg/mL)	277.222 ± 0.437	230.589 ± 0.549	206.350 ± 1.042	420.974 ± 0.102	90.492 ± 1.340

Table 4 Cytotoxic activity of extracts in different solvents of *C. fragrans* on human cervical carcinoma cell line (HeLa)

Concentration (µg/mL)	Inhibitory ability (%)				
	Methanol	Water	Ethyl acetete	n-Hexane	Ellipticine
100	47.32 ± 0.22	52.21 ± 1.27	72.32 ± 2.32	37.03 ± 0.66	100.13 ± 1.57
20	28.11 ± 0.19	21.74 ± 0.96	34.18 ± 2.17	16.15 ± 0.43	71.54 ± 2.12
4	12.20 ± 0.23	13.84 ± 0.24	21.47 ± 1.08	8.16 ± 0.27	58.75 ± 1.74
0.8	7.86 ± 0.30	6.38 ± 0.11	12.75 ± 0.92	1.04 ± 0.21	34.23 ± 1.03
IC_{50}	103.11 ± 0.23	93.34± 0.65	57.89± 1.62	137.38± 0.39	0.63± 0.02

Note: Ellipticine reference standard used in the test

3.3 Cytotoxic activity analysis

Table 4 presents the toxic effects of extracts from *C. fragrans* on the human cervical cancer cell line (HeLa), expressed as IC_{50} values (µg/mL). As shown in Table 4, the positive control, Ellipticine, remained stable throughout the experiment and demonstrated a significant toxic effect on the HeLa cell line, with an IC_{50} value of 0.63 µg/mL. The ethyl acetate extract also exhibited toxic effects, with an IC_{50} value of 57.89 µg/mL. This was followed by the water extract and methanol extract, which had IC_{50} values of 93.34 µg/mL and 103.11 µg/mL, respectively. The n-hexane extract showed the lowest cell inhibitory effect, with an IC_{50} value of 137.38 µg/mL.

4 Discussion

The world population has faced numerous challenges in healthcare in recent years, primarily due to various diseases that pose a significant threat to humanity (Singh and Kumar 2023). Plants play an indispensable role in our daily lives, and herbal medicines are becoming increasingly important. However, because of the vast variety and quantity of herbs used, many species remain unfamiliar to users or are not fully understood. Sarang Banua (*C. fragrans*), a plant commonly found in various regions of Vietnam, particularly in the Northern Delta and midland provinces, has been traditionally used by local communities as an ethnic remedy to treat various ailments (Kalonio et al. 2022). Some studies have shown that *C. fragrans* possesses antioxidant and antibacterial properties, helps reduce blood sugar levels, exhibits anti-cancer effects, and contains secondary metabolites with potential medicinal uses (Sapiun et al. 2020; Simorangkir et al. 2020, 2021; Barung et al. 2021). Research also suggests that the composition of compounds can vary depending on the solvent used with *C. fragrans* (Simorangkir et al. 2019).

In this study, we utilized the GC-MS method to identify 46 compounds in the above-ground parts of the *C. fragrans* plant extracted using four different solvents: methanol, ethyl acetate, n-hexane, and water. These samples were collected from the wild in Hue City, Vietnam. Each solvent, due to its differing polarity, yielded various compounds. For instance, methanol extracted 16 compounds, while water and ethyl acetate extracted 17 compounds each, and n-hexane extracted only 6 compounds (Table 2 and Figure 2). The extract obtained using ethyl acetate demonstrated notable activities, including DPPH free radical scavenging (IC_{50} = 206.350 µg/mL) and significant inhibitory effects on the human cervical cancer cell line HeLa, with an IC_{50} of 57.89 µg/mL.

According to the findings of Simorangkir et al. (2019), antioxidant activity results for *C. fragrans* leaves showed that extracts using ethanol, ethyl acetate, and n-hexane had stronger DPPH scavenging abilities (27.26 and 88.77 ppm, respectively) compared to the extracts from this study collected in Hue City (IC_{50} = 206.350 and 420.974 µg/mL, respectively). Moreover, for the *C. chinense* species, the ethanol extract from leaves exhibited a lower DPPH free radical scavenging ability, with an IC_{50} value of 334.2 µg/mL (Simorangkir et al. 2021). This result is quite similar to our findings, where the antioxidant activity for *C. fragrans*, using different solvents, yielded IC_{50} values ranging from 206.350 to 420.974 µg/mL (Table 3). Kalonio et al. (2017) reported that 12 plants from the genus *Clerodendrum* have demonstrated *both in vitro and in vivo* anticancer activities. The cytotoxic potential of *C. fragrans* extracts on various cancer cell lines has been investigated (Barung et al. 2021; Simorangkir et al. 2021). Barung et al. (2021) demonstrated that extracts from *C. fragrans*, including those in ethanol, hexane, ethyl acetate, and water-soluble fractions, exhibited anticancer activity against A549 lung cancer cells. The ethyl acetate fraction exhibited a moderate activity with an IC_{50} value of 191.165 ppm. In contrast, our experiment on the HeLa cancer cell line revealed even better results, with IC_{50} values ranging from 137.38 to 57.89 µg/mL, where the ethyl acetate extract provided the best cytotoxicity (IC_{50} = 57.89 µg/mL).

According to Chittasupho et al. (2023a, b), extracts from the leaves and flowers of *C. chinense* demonstrated anticancer activities against MCF-7, A549, SKOV-3, and HeLa cells, with IC_{50} values of 109.2, 206.9, 423, and 155.6 µg/mL, respectively. Notably, the cytotoxic activity against the HeLa cell line in their study was lower than that observed with the extracts from *C. fragrans* using various polar solvents. The results from this study align with previously published findings. Therefore, further in-depth *in vitro and in vivo* studies are necessary to evaluate and isolate the compounds found in *C. fragrans* plants for potential applications.

Conclusion

This study analyzes the composition of compounds in *C. fragrans* plants using the GC-MS technique on extracts obtained from solvents with varying polarities. The extract from the EtOAc solvent exhibited notable activities, including DPPH free radical scavenging and significant inhibitory effects on the human cervical cancer cell line HeLa. Therefore, further in-depth studies, both *in vitro and in vivo*, are necessary to evaluate and isolate the compounds found in *C. fragrans* plants for potential applications.

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Conflict of Interest Statement

There is no conflict of interest regarding the publication of this paper.

Ethical Clearance

No animal model was used in this study; therefore, no ethical clearance was required.

References

Ashrafuzzaman, M., Keya, K. K., & Golam Sarwar, A. K. M. (2024). Species Diversity of the genus *Clerodendrum* L. (Lamiaceae) in Bangladesh Agricultural University Campus and their use in traditional medicines. *Pleione*, *18*(1), 01 – 12.

Atanassova, M., & Christova-Bagdassarian, V. (2009). Determination of tannins content by titrimetric method for

comparison of different plant species. *Journal of the University of Chemical Technology and Metallurgy*, *44*(4), 413-415.

Barung, E. N., Kalonio, D. E., Banne, Y., & Kambuno, N. T. (2021). Anticancer Activities of Sesewanua Leaf Extracts (*Clerodendrum fragrans* (Vent.) Willd) Against A549 Lung Cancer Cell. *Open Access Macedonian Journal of Medical Sciences*, *9*(A), 1226-1230.

Chi, V. V. (2021). *Dictionary of Medicinal Plants in Vietnam*. Medical publishing house, Ha Noi, pp. 102-103.

Chittasupho C., Samee W., Tadtong S., Jittachai W., & Managit C. (2023a). Athikomkulchai S. Cytotoxicity, Apoptosis Induction, Oxidative Stress, and Cell Cycle Arrest of Clerodendrum chinense Flower Extract Nanoparticles in HeLa Cells. *Natural and Life Sciences Communications, 22*, e2023057.

Chittasupho, C., Athikomkulchai, S., Samee, W. N., Takuathung, M., Yooin, W., Sawangrat, K., & Saenjum, C. (2023b). Phenylethanoid Glycoside-Enriched Extract Prepared from *Clerodendrum chinense* Leaf Inhibits A549 Lung Cancer Cell Migration and Apoptosis Induction through Enhancing ROS Production. *Antioxidants*, *12*, 461.

Ho, Q. P., Nguyen, T. B. T., Tran, T. M., Nguyen, V. D., Lam-Phuc, T., Le, K. Q., Huynh, N., & Huynh, L. H. (2025). Effect of extraction solvents on yield, chemical composition and antioxidant activity of *Peperomia pellucida* (L.) Kunth seed extract. *CTU Journal of Innovation and Sustainable Development* , *17* (Special issue: ETMD), 75-80.

John, B., Sulaiman, C.T., George, S., & Reddy, V. R. K. (2014). Spectrophotometric estimation of total alkaloids in selected justicia. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(5), 647-648.

Kalonio, D. E., Barung, E. N., Rumagit, B. I., Wullur, A. C., & Timpalan, M. A. (2022). *Clerodendrum fragrans* (Vent) Willd: Review of Pharmacognosy-Phytochemical and Pharmacology: *Clerodendrum fragrans* (Vent) Willd: Tinjauan Farmakognosi-Fitokimia dan Farmakologi. *Ahmar Metastasis Health Journal*, *1*(4), 160-167.

Kalonio, D. E., Hendriani, R., & Barung, E. N., (2017). Aktivitas Antikanker Tanaman Genus *Clerodendrum* (Lamiaceae): Sebuah Kajian. *Traditional Medicine Journal*, *22*(3), 182–189.

Long, D. T., Hong, H. T. K., Tram, L. L. T., & Trang, N. T. Q. (2020). Study of the Procedure for Bath Ultrasound-Assisted Extraction of Total Flavonoid from Lotus Seeds and Testing Some Biological Activities. *Indian Journal of Agricultural Research*, *54*(5), 563-570.

Long, D. T., Le, V. T. M., Han, H. T. N., Hue, T. T., & Hong, H. T. K. (2024). Optimization of extraction of total flavonoid from leafs of *Helicteres hirsuta* Lour. based on bath ultrasoundassisted and testing some biological activities. *Research Journal of Biotechnology*, *19*(1), 71-78.

Molole, G. J., Gure, A., & Abdissa, N. (2022). Determination of total phenolic content and antioxidant activity of Commiphora mollis (Oliv.) Engl. *Resin. BMC Chemistry*, *16*(48), 1-11.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., et al. (1991). Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *Journal of the National Cancer Institute*, *83*(11), 757–766. https://doi.org/10.1093/jnci/83.11.757

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., & Ullah N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences, 56*, e17129.

Ogunlakin, A. D., Akinwumi, I. A., & Ambali, O. A. (2023). Ethnomedicinal application, phytochemistry andtherapeutic effects of genus clerodendrum. *Functional Food Science*, *3*(10), 228-247.

Puthongking, P., Ratha, J., Panyatip, P., Datham, S., Siriparu, P., & Yongram C. (2023). The Effect of Extraction Solvent on the Phytochemical Contents and Antioxidant and Acetylcholinesterase Inhibitory Activities of Extracts from the Leaves, Bark and Twig of Dipterocarpus alatus. *Tropical Journal of Natural Product Research*, *7*(12), 5595-5600.

Rguez, S., Yeddes, W., Hammami, M., & Sellami I. H. (2023). Effect of extraction solvent on the phytochemical composition and the antioxidant activity of *Cupressus sempervirens*. *European Journal of Biotechnology and Bioscience*, *11*(1): 6-10.

Sapiun, Z., Pangalo, P., Imran, A. K., Wicita, P. S., & Daud, R. P. A. (2020). Determination of total flavonoid levels of ethanol extract Sesewanua leaf (*Clerodendrum fragrans* Wild) with maceration method using UV-Vis spectrofotometry. *Pharmacognosy Journal*,*12*, 356-360.

Simorangkir, M., Hutabarat, W., Nainggolan, B., & Silaban, S. (2019). Antioxidant and antibacterial activities of nonpolar to polar solvent extracts of sarang banua (*Clerodenrum fragrans* vent willd) leaves. *Rasāyan Journal of Chemistry*, *12*(2), 959-965.

Simorangkir, M., Nainggolan, B., Doloksaribu, J. F., & Silaban, S. (2020). Effect of sarang banua (*Clerodendrum fragrans* Vent Willd) leaves extract on serum globulin levels of rabbit

(*Oryctolagus cuniculus*). *Journal of Physics: Conference Series*, *1485*, 012016.

Simorangkir, M., Nainggolan, B., Juwitaningsih, T. & Silaban, S. (2021). The Toxicity of n-Hexane, Ethyl Acetate and Ethanol Extracts of SarangBanua (*Clerodendrum fragrans* Vent Willd) Leaves by Brine Shrimp Lethality Test (BSLT) Method. *Journal of Physics: Conference Series*, *1811*, 012053.

Simorangkir, M., Sinaga E., Pasaribu R., & Silaban S. (2022). Antidiabetic activity of leaf extract of *Clerodenrum fragrans* Vent Willd in Rattus novergicus induced by Alloxan. *Jurnal Bioteknologi dan Biosains Indonesia* , *9*(1): 119-125.

Singh, V.K., & Kumar, A. (2023). Secondary metabolites from endophytic fungi: Production, methods of analysis, and diverse pharmaceutical potential. *Symbiosis*, *90*, 111–125.