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Original Article

Chemical profile and alpha-glucosidase inhibitory, cytotoxic, and anti-inflammatory properties of (*E*)-anethole-rich essential oil from *Limnophila rugosa* cultivated in Vietnam

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Abstract

Essential oils extracted from the leaves and stems of *Limnophila rugosa*, collected in Aluoi district, Hue city, Vietnam, were analyzed for their chemical composition and evaluated for *α*-glucosidase inhibitory, cytotoxic, and anti-inflammatory activities. The oils, with yields of 0.6 ± 0.02% (leaves) and 0.4 ± 0.01% (stems) (w/w, fresh weight), were characterized as milky white liquids with a distinct aromatic odor and lower density than water. GC-MS analysis identified 14 volatile compounds in the leaf oil (99.83% of total content) and seven in the stem oil (99.03% of total content), with (*E*)-anethole as the predominant constituent (77.53% in leaf oil, 94.30% in stem oil). Other notable compounds in the leaf oil included 1-octen-3-ol (8.19%) and 2-octanol (7.94%), which were less abundant in the stem oil (1.93% and 1.24%, respectively). The stem oil exhibited moderate *α*-glucosidase inhibitory activity (IC_{50} = 322.26 ± 15.16 µg/mL), while the leaf oil showed negligible activity (IC_{50} > 500.00 µg/mL), compared to acarbose (IC_{50} = 117.84 ± 7.25 µg/mL). Both oils demonstrated moderate cytotoxicity against HEK-293A, HepG2, and SK-LU-1 cell lines, with IC_{50} values ranging from 35.56 ± 2.04 to 60.47 ± 2.53 µg/mL, compared to ellipticine (IC_{50} = 0.34–0.38 µg/mL). Anti-inflammatory activity was assessed via nitric oxide (NO) production inhibition in RAW 264.7 macrophages, with both oils showing significant NO inhibition but IC_{50} values exceeding 100 µg/mL, compared to dexamethasone (IC_{50} = 13.55 ± 1.26 µg/mL). The high (*E*)-anethole content likely contributes to these bioactivities, though the presence of 1-octen-3-ol and 2-octanol in the leaf oil may reduce its *α*-glucosidase inhibitory efficacy. This study establishes the foundation for future investigations into the medicinal potential of *L. rugosa* essential oils from Hue city, Vietnam, by being the first to report their chemical composition and *α*-glucosidase inhibitory, cytotoxic, and anti-inflammatory actions.

Keywords: *Limnophila rugosa*, Essential oil, Cytotoxic activity, Alpha-glucosidase inhibition, Anti-inflammatory

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Introduction

Essential oils (EOs) are intricate blends of volatile compounds, predominantly terpenoids and phenolic constituents, extracted from plant sources. Extensive research has demonstrated their wide-ranging biological activities, encompassing antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, and anti-glucosidase effects, among others (Ben Miri, 2025; Nguyen et al., 2024). Derived primarily from plant materials, EOs exemplify multicomponent mixtures, comprising up to several hundred distinct compounds. The complex composition of these molecules, with their diverse functional groups, contributes to a broad spectrum of physical and chemical properties, enabling multi-target activities that distinguish EOs from single isolated compounds (Bunse et al., 2022).

About 40 species of the genus *Limnophila* (Scrophulariaceae) are found throughout the world, primarily in tropical and subtropical areas such as North America, Southeast Asia, South Asia, and the Pacific Islands. These herbaceous plants typically inhabit aquatic or semi-aquatic environments such as marshes, riversides, forest paths, and other wetland habitats.(Shi, 1998) Several *Limnophila* species, notably *L. aromatica*, *L. conferta*, *L. gratissima*, *L. indica*, *L. micrantha*, and *L. rugosa* are widely utilized in traditional cuisine and medicine across Asian countries. According to recent research, *Limnophila* species have a variety of biological activities, such as wound-healing, cytotoxic, antitubercular, antibacterial, anti-inflammatory, and antioxidant qualities. (Brahmachari, 2008).

In Vietnam, 17 *Limnophila* species have been documented, with species such as *L. aromatica* and *L. rugosa* commonly used as vegetables and spices in culinary practices (Pham, 1999). Nonetheless, little is known about these species' biological activity and chemical constituents in Vietnam. The chemical profiles of *L. rugosa* essential oils have been examined in earlier research using samples gathered in China, India, and southern and northern Vietnam. Traditional medicine uses this species to cure ailments like stomach pain, indigestion, and diarrhea (Tran, 2003; Verma et al., 2014; Yu and Cheng, 1986). Thus far, no research has examined the chemical constitution or biological properties of *L. rugosa* essential oils from Hue city, Vietnam, particularly their α-glucosidase inhibitory, cytotoxic, and anti-inflammatory properties.

In this work, we examined the composition of chemicals and assessed the cytotoxic, anti-inflammatory, and *α*-glucosidase inhibitory properties of essential oils that were extracted from *L. rugosa* leaves and stems that were collected in Hue, Vietnam's A Luoi district.

Material and Methods

Plant materials

Fresh leaves (1.0 kg) and stems (1.0 kg) of *L. rugosa* were harvested in April 2024 from A Luoi district, Hue city (16°09’56.94”N, 107°31’10.96”E). The plant species was authenticated by Dr. Tran Minh Duc from the University of Agriculture and Forestry, Hue University (Figure 1). A voucher specimen (HN-202404) is preserved at the Faculty of Biology, University of Education, Hue University, Vietnam.

Figure-1. *Limnophila rugosa* plants in their habitat.

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Hydrodistillation of the essential oils

Following protocols described in prior studies (Huong et al., 2022; Huong et al., 2023; Luyen et al., 2025; Pham et al., 2025b), fresh leaves and stems of *L. rugosa* were chopped before undergoing hydrodistillation. The process was conducted using a Clevenger apparatus with a 3.5 L distillation flask for 4 hours under atmospheric pressure, in accordance with the Vietnamese Pharmacopoeia V (2019) (Health, 2018). After that, the essential oils were gathered, dried with anhydrous sodium sulfate (Na_2SO_4), and refrigerated at 6°C. All experiments were performed in triplicate.

Analysis of the essential oil

An Equity-5 capillary column connected to a mass spectrometer (MSD QP2010 Plus) for GC/MS analysis was part of the GCMS-QP2010 Plus system (Shimadzu, Kyoto, Japan) that was used to determine the essential oil's chemical composition (Luyen et al., 2025). After diluting the oil in *n*-hexane at a ratio of 1:50, 1 µL of the diluted sample was injected. The carrier gas, helium, was used at a flow rate of 1.2 mL/min while the injector and interface temperatures were kept at 280°C. The temperature of the column was set to begin at 50°C (held for 1 minutes), climb to 240°C at 3°C/min (held for 15 minutes), and culminate at 280°C at 5°C/min (kept for 30 minutes). Injecting samples was done in splitless mode. With a sampling rate of 1.1 scan/s and a scan range of 45–550 *m/z*, the mass spectrometer functioned at an ionization voltage of 70 eV.

By contrasting their mass spectral data with fragmentation patterns of recognized compounds published in recent literature, individual constituents were identified (Adams, 2017) and by matching mass spectra against the NIST 11 and WILEY 7 GC/MS databases. Retention indices (RI) were calculated relative to a homologous series of *n*-alkanes (C_7–C_{40}) analyzed under identical conditions. Co-injection with standard compounds under the same GC conditions was also employed to confirm the identity of select components. The relative peak area percentage was used as the basis for quantification. Three runs of each sample were performed.

Alpha-glucosidase inhibitory activity

Using a previously developed methodology from our lab, the α-glucosidase inhibitory activity of essential oils isolated from *L. rugosa* leaves and stems was evaluated. A 96-well plate format was used for the assay. Each well included 130 µL of 100 mM phosphate buffer (pH 6.8), 20 µL of 0.5 U/mL α-glucosidase solution, and 50 µL of the test sample at various concentrations. For fifteen minutes, the mixture was incubated at 37°C. 50 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) was then added as the substrate, and the mixture was incubated for an additional 5 minutes at 37°C. A BioTek ELx800 Microplate Reader (BioTek, USA) was used to detect absorbance at 405 nm. Sigma-Aldrich, USA's acarbose was used as the positive control. The essential oils were tested at concentrations of 4, 20, 100, and 500 µg/mL. The formula α-glucosidase inhibition (%) = $(1 - A/A_0) \times 100$ was used to determine the percentage of α-glucosidase inhibition, where A is the absorbance of the test sample and A_0 is the absorbance of the control. Three replicate wells per concentration were used in each experiment, which was carried out in triplicate (n = 3) (Nguyen et al., 2023; Tran et al., 2023).

Cytotoxicity activity

The cytotoxicity assay was conducted as described in our prior studies. The samples of *L. arugosa* were evaluated for cytotoxic activity using a spectrophotometric assay with Sulforhodamine B (SRB) dye. Three human cancer cell lines were tested: HEK-293A (embryonic kidney), HepG2 (hepatocellular carcinoma), and SK-LU-1 (lung carcinoma).

L-glutamine, sodium pyruvate, $NaHCO_3$, 10% fetal bovine serum (FBS; Gibco, USA), 1% penicillin-streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin; Gibco, USA), and 0.05% trypsin-EDTA were added to Dulbecco's Modified Eagle Medium (DMEM) in order to support the cell culture. Cultures were maintained at 37°C with 5% CO_2, and subculturing was performed at a 1:3 ratio every 3–5 days, depending on cell growth. For the cytotoxicity assay, cells were seeded in 96-well plates and incubated for 72 hours. Following incubation, the media was discarded, and viable cells were stained with SRB dye and fixed with 20% trichloroacetic acid (TCA). To evaluate cell viability, optical density (OD) was measured at 515 nm using an ELISA Plate Reader (BioTek, USA).

The essential oils' cytotoxicity was assessed at doses of 100, 20, 4, and 0.8 µg/mL. Ellipticine served as positive control, and dimethyl sulfoxide was used as the negative control. The TableCurve 2D v4 program

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(Systat Software Inc., USA) was used to calculate the half-maximal inhibitory concentration (IC_{50}). Every experiment was carried out in triplicate to guarantee accuracy and reproducibility (Pham et al., 2023; Pham et al., 2025a).

Anti-inflammatory inhibition activity

Cell source and culture

The RAW 264.7 macrophage cell line was generously supplied by Prof. Dr. Domenico Delfino, University of Perugia, Italy. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (FBS; Gibco), 10 mM HEPES, and 1.0 mM sodium pyruvate was used to keep the cells alive. Subculturing was performed at a 1:3 ratio every 3–5 days, with cells incubated in a CO_2 incubator (Thermo Scientific, USA, Model: Heracell 150i) at 37°C with 5% CO_2 under stable conditions.

NO inhibition assay

RAW 264.7 macrophages were seeded in 96-well plates at a density of 2×10^5 cells/well and cultured for 24 hours at 37°C in a 5% CO_2 atmosphere to evaluate the suppression of nitric oxide (NO) generation. Following the substitution of FBS-free DMEM for the culture medium, 1 µg/mL lipopolysaccharide (LPS) was added to promote the generation of NO. Following the introduction of test samples at doses of 100, 20, 4, and 0.8 µg/mL, the samples were incubated for 24 hours under the same circumstances. The negative control was 1.0% dimethyl sulfoxide, while the positive control was dexamethasone (Sigma-Aldrich, USA). NO production was indirectly measured by quantifying nitrite levels using the Griess reagent (Promega Corporation, WI, USA). Specifically, 100 µL of culture supernatant was transferred to a new 96-well plate and combined with 100 µL of Griess reagent, comprising 50 µL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50 µL of 0.1% (w/v) *N*-1-naphthylethylenediamine dihydrochloride. A BioTek ELx800 Microplate Reader (BioTek, USA) was used to detect absorbance at 540 nm after the mixture had been incubated for 10 minutes at room temperature. Experiments were conducted in triplicate (n = 3) with three replicate wells per concentration. IC_{50} values, representing the concentration required for 50% inhibition, were determined using TableCurve 4.0 software (Systat Software Inc., USA) (Pham et al., 2025a).

MTT cell viability assay

The 96-well plate used for the NO inhibition experiment was supplemented with 10 µL of MTT solution (final concentration: 5 mg/mL) per well and 90 µL of fresh DMEM in order to assess cell viability. For four hours, the plate was incubated at 37°C. After incubation, the medium was aspirated, and 50 µL of 100% DMSO was added to each well to dissolve the formazan crystals. A BioTek ELx800 Microplate Reader (BioTek, USA) was used to measure absorbance at 540 nm. The following formula was used to calculate the cell viability in the presence of test substances.

$$\textbf{\textit{Inhibition}}\ (\%) = \frac{\boldsymbol{OD\ (sample) - OD\ (blank)}}{\boldsymbol{OD\ (DMSO) - OD\ (blank)}}$$

Statistical analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Nonlinear regression analysis was used to determine IC_{50} values by plotting the percentage of inhibition against the logarithm of sample concentrations to generate a dose-response curve, with the concentration producing 50% inhibition defined as the IC_{50} value. No statistical tests (e.g., ANOVA or *t*-test) were performed to compare differences between groups (e.g., leaf vs. stem essential oils or test samples vs. controls), as the study focused on descriptive characterization of bioactivity. Observed differences are reported qualitatively based on IC_{50} values and mean inhibition percentages.

Results and Discussion

Chemical composition

The essential oils extracted from the leaves and stems of *L. rugosa* yielded an average of 0.6 ± 0.02% and 0.4 ± 0.01% (w/w, fresh weight), respectively. Both oils were milky white liquids, exhibiting a characteristic aromatic odor and a density lower than that of water. Fourteen volatile compounds, or 99.83% of the total amount of oil, were found in the leaf oil by GC-MS analysis, while seven compounds, or 99.03% of the total oil content, were found in the stem oil (Table 1, Figure 2, 3). The chemical profiles of both oils were predominantly composed of non-terpenic compounds, constituting 98.15% in the leaf oil and 97.47% in the stem oil. While oxygenated sesquiterpenes were not found in the leaf oil but were present in the stem oil at

a very low concentration (0.10%), monoterpene hydrocarbons were not found in either the leaf or stem oils. Oxygenated monoterpenes and sesquiterpene hydrocarbons were minor constituents in both oils, with the leaf oil containing 1.16% and 0.12%, respectively, and the stem oil containing 0.52% and 1.34%, respectively. The major constituent in both the leaf and stem essential oils of *L. rugosa* was (*E*)-anethole, comprising 77.53% of the leaf oil and 94.30% of the stem oil. Other notable components in the leaf oil included 1-octen-3-ol (8.19%) and 2-octanol (7.94%) (Figure 4), whereas these compounds were present in much lower amounts in the stem oil, at 1.93% and 1.24%, respectively.

Table-1. Chemical composition of the essential oil from the leaves and stems of *Limnophila rugose.*

No	RT	Compounds	RI_E	RI_L	Leaf	Stem	Classification
1	4.91	(2*E*)-Hexenal	848	846	0.14 ± 0.0002	—	NT
2	4.97	(3*Z*)-Hexenol	851	850	1.35 ± 0.0020	—	NT
3	5.22	(2*Z*)-Hexenol	862	859	0.18 ± 0.0003	—	NT
4	5.27	*n*-Hexanol	864	863	0.30 ± 0.0004	—	NT
5	8.72	1-Octen-3-ol	978	974	**8.19 ± 0.0120**	1.93 ± 0.0023	NT
6	8.96	3-Octanone	985	979	1.87 ± 0.0027	—	NT
7	9.32	2-Octanol	996	994	**7.94 ± 0.0116**	1.24 ± 0.0015	NT
8	13.62	Linalool	1100	1095	1.16 ± 0.0017	0.12 ± 0.0001	OM
9	18.01	Methyl chavicol	1199	1195	0.23 ± 0.0003	—	NT
10	18.33	*n*-Decanal	1206	1201	0.24 ± 0.0003	—	NT
11	20.45	(*Z*)-Anethole	1254	1249	0.18 ± 0.0003	—	NT
12	22.13	(*E*)-Anethole	1291	1282	**77.53 ± 0.1133**	**94.30 ± 0.1146**	NT
13	27.74	(*E*)-Caryophyllene	1422	1417	0.28 ± 0.0004	0.66 ± 0.0008	SH
14	29.15	*α*-Humulene	1456	1452	0.24 ± 0.0004	0.68 ± 0.0008	SH
15	36.48	Hinesol	1643	1640	—	0.10 ± 0.0001	OS
Total					99.83 ± 0.1459	99.03 ± 0.1204	
Oxygenated monoterpenes (OM)					1.16 ± 0.0017	0.12 ± 0.0001	
Sesquiterpene hydrocarbons (SH)					0.52 ± 0.0008	1.34 ± 0.0016	
Oxygenated sesquiterpenes (OS)					—	0.10 ± 0.0001	
Non-terpenic compounds (NT)					98.15 ± 0.1434	97.47 ± 0.1185	

RT: Retention time, RI_E: Experimental retention index, RI_L: Literature retention index, bold: major compounds.

Prior research on *L. rugosa* oil extracts from Vietnam and other areas shows both clear differences and commonalities. Methyl chavicol (73.5%) and (*E*)-anethole (25.6%) made up the majority of the essential oil extracted from the aerial portions of *L. rugosa* gathered in Cu Chi district, Ho Chi Minh City, Vietnam, according to Van et al. (2021). Minor compounds including *α*-caryophyllene (0.1%) and 1-hexen-3-ol (0.1%) were also present (Van et al., 2021). Similarly, Linh and Thach analyzed *L. rugosa* aerial parts from Trang Bang district, Tay Ninh province, Vietnam, at different growth stages and using varied extraction methods (conventional heating and microwave irradiation) (Linh and Thach, 2011). Their findings consistently identified methyl chavicol (70–71%) and (*E*)-anethole (24–27%) as the dominant components, with trace amounts of linalool (0.10–0.17%), (*E*)-caryophyllene (0.10–0.39%), caryophyllene oxide (0.10–0.18%), and limonene (0.18%). In contrast, our study shows a marked predominance of (*E*)-anethole over methyl chavicol, which was a minor constituent (0.23% in leaf oil and absent in stem oil), highlighting a significant compositional difference.

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Table-2. Alpha-glucosidase inhibition activity of essential oil from leaves and stems of *Limnophila rugosa.*

C (µg/ml)	% I		
	Leaves oil	**Stems oil**	**Acarbose***
500	46.58 ± 1.24	98.08 ± 1.09	79.02 ± 1.59
100	8.53 ± 0.82	9.87 ± 0.82	51.11 ± 1.02
20	4.20 ± 0.22	4.75 ± 0.42	18.31 ± 0.82
4	2.32 ± 0.16	1.62 ± 0.12	8.12 ± 0.64
IC_{50}	**> 500.00**	**322.26± 15.16**	**117.84± 7.25**

% I: percentage of inhibition; *Positive control; Values are expressed as mean ± standard deviation (SD) (n = 3)

Further afield, Verma et al. investigated *L. rugosa* from two locations in northern India. The sample from Pantnagar (foothills) contained methyl chavicol (76.6%) and (*E*)-anethole (19.1%), while the Purara sample (mid-foothills) was dominated by (*E*)-anethole (88.5%), with methyl chavicol at only 0.7%, alongside linalool (0.3%), (*E*)-caryophyllene (0.1%), and 1-octen-3-ol (0.1%) (Verma et al., 2014). The high (*E*)-anethole content in our stem oil (94.3%) closely resembles the Purara sample, suggesting a potential chemotypic similarity influenced by environmental factors. Additionally, (*E*)-anethole as a major component is consistent with other plant species, such as *Clausena heptaphylla* leaf oils (98.2% in both flowering and fruiting stages) and *Pimpinella anisum* fruits from Turkey (90.81–96.64%), indicating its prevalence in certain aromatic plants (Avcı et al., 2023; Nath et al., 1996).

The prominence of 1-octen-3-ol and 2-octanol in our leaf oil (8.19% and 7.94%, respectively) is noteworthy, as these compounds were either absent or present in trace amounts in previous *L. rugosa* studies. These non-terpenic alcohols may contribute to the characteristic aromatic odor of the oil and could be linked to specific biosynthetic pathways active in the A Luoi region. The absence of monoterpene hydrocarbons in both oils and the minimal presence of oxygenated sesquiterpenes (e.g., hinesol at 0.10% in stem oil) further differentiate our samples from those reported by Linh and Thach, where limonene and caryophyllene oxide were detected (Linh and Thach, 2011).

These compositional variations likely arise from geographical, climatic, and soil differences between A Luoi district and other collection sites, as well as genetic factors influencing chemotype expression. The higher (*E*)-anethole content in the stem oil compared to the leaf oil may also reflect tissue-specific metabolic differences, with stems potentially favoring the biosynthesis of phenylpropanoids like (*E*)-anethole. Future studies should explore the environmental and genetic factors driving these differences, alongside the potential bioactivity contributions of minor constituents like 1-octen-3-ol and 2-octanol, to better understand the chemotypic diversity of *L. rugosa*.

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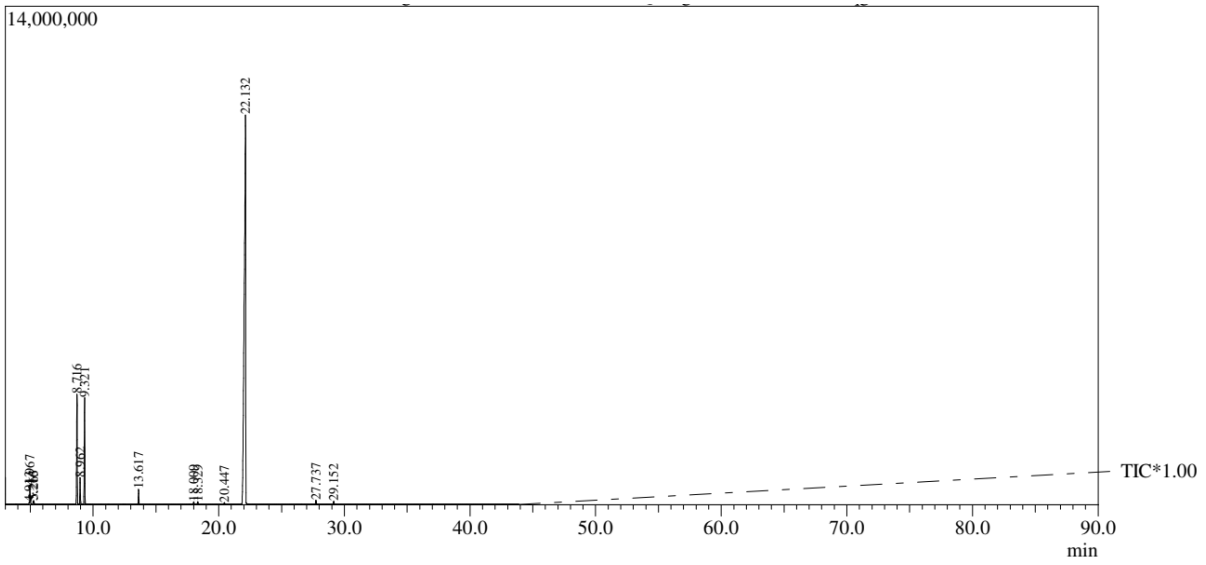

Figure-2. The GC chromatogram of *Limnophila rugosa* leaf essential oil.

Inhibition of alpha-glucosidase activity

Essential oils isolated from *L. rugosa* leaves and stems were evaluated for their *α*-glucosidase inhibitory activities. The stem essential oil displayed moderate inhibitory activity, with an IC_{50} value of 322.26 ± 15.16 µg/mL, while the leaf essential oil exhibited minimal activity, with an IC_{50} value exceeding 500.00 µg/mL (Table 2). In comparison, the positive control, acarbose, showed an IC_{50} value of 117.84 ± 7.25 µg/mL, indicating that the stem essential oil was approximately three times less effective (Table 2). The lower activity of the leaf essential oil, despite its high (*E*)-anethole content (77.53% compared to 94.30% in the stem oil), may be influenced by differences in its chemical composition, such as the higher presence of 1-octen-3-ol (8.19%) and 2-octanol (7.94%) in the leaf oil (Table 1). It is hypothesized that these compounds could potentially modulate the bioactivity of (*E*)-anethole, possibly through synergistic or antagonistic interactions, though this requires further investigation due to the lack of specific studies on their effects in this context. Additional research is needed to elucidate the roles of individual components and their interactions in determining the *α*-glucosidase inhibitory activity of *L. rugosa* essential oils.

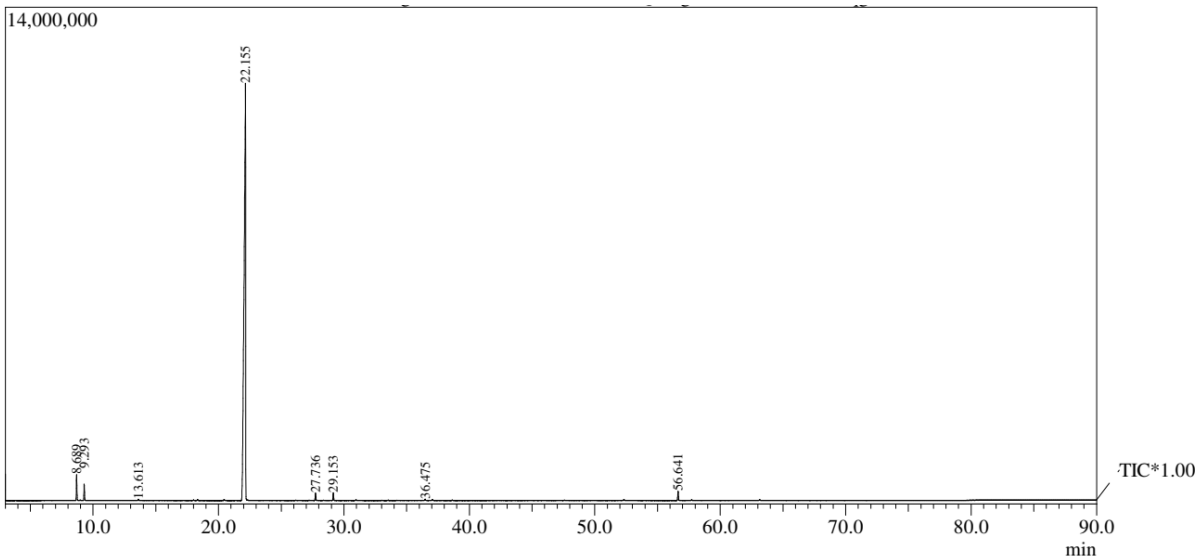

Figure-3. The GC chromatogram of *Limnophila rugosa* stem essential oil.

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The essential oil extracted from fresh *Clausena harmandiana* leaves, which is mainly made up of (*E*)-anethole (91.44%), estragole (2.98%), and (*Z*)-anethole (2.55%), showed *α*-glucosidase inhibitory activity with an IC_{50} value of 677.00 µg /mL in a related study. This is roughly twice as strong as acarbose (IC_{50} = 1260.00 µg/mL) (Peerakam et al., 2021). The high (*E*)-anethole content in both *L. rugosa* and *C. harmandiana* essential oils suggests that (*E*)-anethole is a key contributor to their *α*-glucosidase inhibitory activity.

This research is the first to evaluate the *α*-glucosidase inhibitory activity of essential oils from the *Limnophila* genus, providing a foundation for future research into their potential applications. The results indicate that the stem essential oil of *L. rugosa*, with an IC_{50} value of 322.26 ± 15.16 µg/mL, exhibits moderate *α*-glucosidase inhibitory activity compared to acarbose (IC_{50} = 117.84 ± 7.25 µg/mL), suggesting that it may not yet have sufficient potency for direct therapeutic use. However, the presence of (*E*)-anethole as a key contributor to this activity highlights the potential of *L. rugosa* essential oils as a starting point for developing natural *α*-glucosidase inhibitors for conditions such as type 2 diabetes. Further studies, including fractionation or combination with other bioactive compounds, are necessary to enhance the efficacy and therapeutic applicability of these essential oils.

Cytotoxicity activity

The cytotoxic effects of the essential oils extracted from *L. rugosa* leaves and stems were evaluated on three human cell lines: SK-LU-1 (lung carcinoma), HepG2 (hepatocellular carcinoma), and HEK-293A (embryonic kidney). The IC_{50} values ranged from 35.56 ± 2.04 to 60.47 ± 2.53 µg/mL (Table 3). Specifically, the leaf essential oil exhibited IC_{50} values of 56.53 ± 2.82 µg/mL (HEK-293A), 44.64 ± 1.62 µg/mL (HepG2), and 52.93 ± 1.92 µg/mL (SK-LU-1). The stem essential oil demonstrated IC_{50} values of 60.47 ± 2.53 µg/mL (HEK-293A), 35.56 ± 2.04 µg/mL (HepG2), and 40.14 ± 2.61 µg/mL (SK-LU-1). With IC_{50} values of 0.34 ± 0.03 µg/mL (HEK-293A), 0.34 ± 0.02 µg/mL (HepG2), and 0.38 ± 0.03 µg/mL (SK-LU-1), the positive control, ellipticine, demonstrated consistent cytotoxicity (Table 3). The cytotoxic action of essential oils from the *Limnophila* genus has never before been assessed, until now.

Table-3. Cytotoxicity of essential oil from leaves and stems of *Limnophila rugosa.*

Cell lines	IC50 (µg/mL)		
	Leaves oil	Stems oil	Ellipticine*
HEK-293A	56.53 ± 2.82	60.47 ± 2.53	0.34 ± 0.03
HepG2	44.64 ± 1.62	35.56 ± 2.04	0.34 ± 0.02
SK-LU-1	52.93 ± 1.92	40.14 ± 2.61	0.38 ± 0.03

*Positive control; Values are expressed as mean ± standard deviation (SD) (n = 3)

In a related investigation, essential oils from the aerial portions of *Limnophila aromatica* displayed considerable cytotoxicity against lung fibroblast cells, with an IC_{50} of 16.62 µg/mL and an IC_{80} of 50.78 µg/mL (Houdkova et al., 2018). Anethole, a major constituent of these essential oils, has demonstrated significant in vitro cytotoxicity against multiple cancer cell lines, including MCF-7, MDA-MB-231, and T47D (breast cancer) (Chen and deGraffenried, 2012; Shahbazian et al., 2015), Ca9-22 (oral cancer) (Contant et al., 2021), A2780, A2780cisR, and A2780ZD0473R (ovarian cancer) (Shahbazian et al., 2015), PC-3 (prostate cancer), (Elkady, 2018) CRL-6475 and HEMa-LP (skin cancer) (Li et al., 2023), AGS (gastric cancer) (Carrillo et al., 2019), MG-63 (bone tumor) (Pandit et al., 2021), and HCT 116 and HT-29 (colon cancer) (Asif et al., 2016). Furthermore, anethole exhibited potent *in vivo* anticancer activity in a xenograft model of non-small cell lung cancer (A549) in BALB/c nude mice (Kumar and Venkateswarulu, 2023). Nevertheless, *trans*-anethole (tAT) caused cytotoxicity in primary hepatocytes from cultured mice, and *α*-naphthoflavone pretreatment decreased the vulnerability to tAT-induced cytotoxicity (Shen et al., 2025).

The biological characteristics of *L. rugosa* essential oils are better understood thanks to this work, which also shows that they have anticancer potential, mainly

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because to anethole. These results set the stage for more research to maximize their therapeutic effectiveness.

Action of anti-inflammatory inhibition

In order to determine the anti-inflammatory potential of the essential oils that were isolated from the leaves and stems of *L. rugosa*, their effects on the viability of RAW 264.7 macrophage cells and the generation of nitric oxide (NO) were assessed. At concentrations of 100 and 20 µg/mL, neither the leaf nor stem essential oils significantly affected cell viability, as determined by the MTT assay, with cell viability ranging from 43.64% to 99.07%. Therefore, the ability of these essential oils to decrease NO generation at doses of 100, 20, 4, and 0.8 µg/mL was measured in order to examine their anti-inflammatory potential.

Table-4. Nitric oxide (NO) inhibition activity of essential oil from leaves and stems of *Limnophila rugosa.*

C	Leaves oil		Stems oil		Dexamethasone	
(µg/mL)	% I	% CS	% I	% CS	% I	% CS
100	89.68 ± 3.07	43.64 ± 2.01	96.62 ± 0.48	74.07 ± 0.17	87.44 ± 1.25	96.22 ± 1.22
20	13.38 ± 0.24	90.87 ± 1.49	11.02 ± 1.20	80.93 ± 1.46	53.51 ± 0.98	99.07 ± 1.48
4	3.02 ± 0.25		5.88 ± 0.46		41.93 ± 1.01	
0.8	-3.05 ± 0.20		-2.71 ± 0.27		31.98 ± 0.64	
IC50	**NA**		**NA**		**13.55 ± 1.26**	

% I: percentage of inhibition, % CS: percentage of cell survival; *Positive control; Values are expressed as mean ± standard deviation (SD) (n = 3).

As presented in Table 4, both leaf and stem essential oils of *L. rugosa* exhibited significant NO inhibitory activity; however, IC_{50} values could not be determined. This was due to either cytotoxicity at higher concentrations or insufficient inhibitory activity, with IC_{50} values exceeding 100 µg/mL. Dexamethasone, the positive control, showed steady NO inhibition with an IC_{50} value of 13.55 ± 1.26 µg/mL. This study is the first to assess the anti-inflammatory qualities of essential oils from the *Limnophila* genus, setting the stage for future investigations into their possible medical uses.

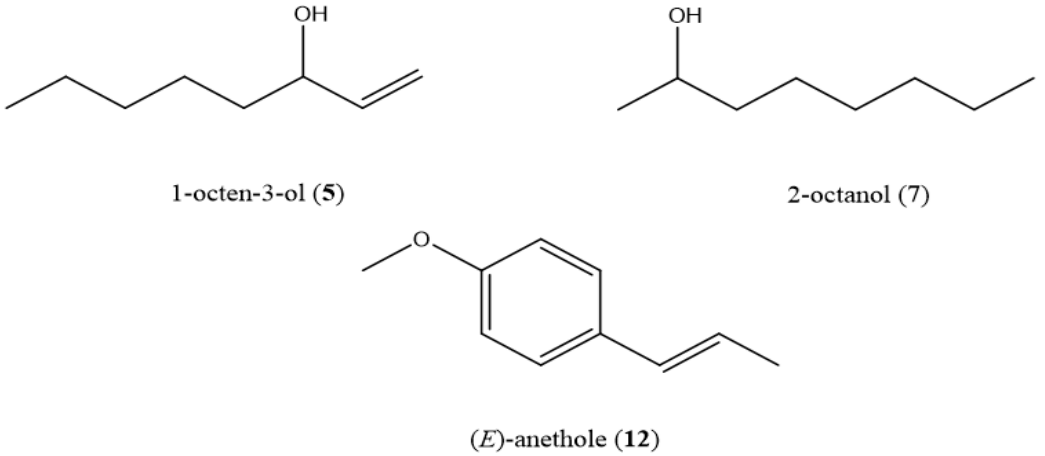

Figure-4. The major chemical constituents of *Limnophila rugosa* leaf essential oil.

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Although dexamethasone and ellipticine made good positive controls for the cytotoxic and NO inhibition tests, using them exclusively prevents direct comparisons between the bioactivity of *L. rugosa* essential oils and other natural products or essential oils. A reference essential oil with established cytotoxic or anti-inflammatory properties would have provided a more solid foundation for assessing the oils' efficacy. However, the current study's inadequate resources prevented this from happening. Such reference standards should be incorporated into future research in order to more thoroughly validate and contrast the biological effects of *L. rugosa* essential oils with those of related natural products.

Conclusion

The main constituents identified in the essential oils were (*E*)-anethole, 1-octen-3-ol, and 2-octanol. The stem essential oil displayed *α*-glucosidase inhibitory activity, while both leaf and stem oils exhibited moderate cytotoxic and anti-inflammatory effects. These results provide important new information about the biological characteristics and chemical makeup of *L. rugosa* essential oils, laying the groundwork for future studies.

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Contribution of Authors

Pham TV: Research methodology, investigation, data curation and article write up.
Duc TM, Danh LC and Tan NDN: Sample collection and analysis.
Phuoc NTM: Literature review and investigation.
Thang TN: Project administration.
Pham T: Article writing, editing and project administration.

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