

Bioactive constituents of *Goniothalamus tamirensis* essential oil and its anticancer activity

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Abstract

Background and objectives: Cancer is a major challenge, affecting health, society, and the economy. Natural therapies may help reduce side effects of conventional treatment. The present study aimed to determine phytochemical characterization and evaluation of *in vitro* anticancer activity of the essential oil (EO) obtained from *G. tamirensis* leaves. **Materials and methods:** The leaves of *G. tamirensis* were collected in Phong Dien, Thua Thien Hue, in August 2024. The chemical composition of the EO was identified using GC-MS, and the anticancer potential of the EO was evaluated using the SRB assay against human cell lines (HepG2, MCF7, A549). **Results:** The main compounds identified in *G. tamirensis* EO were oxygenated sesquiterpenes. The EO showed significant anticancer activity against MCF, A549 and HepG2 cell lines with IC_{50} of 8.79, 10.29 and 7.32 $\mu\text{g/mL}$, respectively. **Conclusion:** This study suggests that *G. tamirensis* leaf EO is a promising candidate for use as an anticancer agent.

Keywords: *Goniothalamus tamirensis*, chemical composition, anticancer.

1. BACKGROUND

Cancer remains one of the leading causes of mortality worldwide [1]. Although conventional treatments like chemotherapy have advanced over the years, they are still associated with significant side effects. Moreover, the development of drug resistance in cancer cells has diminished the efficacy of many chemotherapeutic agents, highlighting the urgent need for therapeutic alternatives. As a result, there is a pressing demand for natural products to be explored as potential cancer treatments. Several bioactive compounds derived from natural sources, including taxol, camptothecin, vincristine, and vinblastine, have already demonstrated significant therapeutic efficacy in oncology [2, 3]. These examples emphasize the immense potential of natural compounds as a rich and effective source of medicinal agents for cancer treatment [4, 5].

Among the diverse array of natural compounds, essential oils (EOs) have gained considerable attention for their broad range of bioactivities, which include antibacterial [6], antioxidant, anti-inflammatory [7], and anticancer effects [8-10]. EO is a mixture of many different chemical compounds. Many studies have reported that EOs are considered a potential anticancer agents. Besides their effectiveness, EOs also have the ability to limit side effects compared to conventional cancer treatments such as chemotherapy [11]. Therefore, research on the biological activity of Eos may open up opportunities for the development of safe, plant-

based cancer therapies.

The genus *Goniothalamus* shows potential as a source of medicinal compounds, although researches have focused on only a few of its species. Several studies have already demonstrated the composition [12–16] and anticancer potential of some *Goniothalamus* EOs on various cancer cell lines [17]. However, many researches have focused on other species within the genus, and relatively little is known about *Goniothalamus tamirensis*, a species native to Vietnam [18]. Given the lack of comprehensive studies on the chemical profile and anticancer activity of *G. tamirensis* EO, there is a significant gap in our understanding of its potential therapeutic applications. Research into the chemical composition and biological activity of *Goniothalamus tamirensis* EO is therefore crucial. Identifying and characterizing the bioactive compounds present in the EO could lead to the discovery of new anticancer agents, which could complement or even provide an alternative to conventional treatments. Additionally, understanding the phytochemical composition of *G. tamirensis* EO may contribute to the broader body of knowledge on the therapeutic potential of *Goniothalamus* species and their applicability in cancer treatment.

Given the promising results from related species and the limited research on *G. tamirensis*, this study aims to fill this knowledge gap by investigating the chemical composition and anticancer activity of *G. tamirensis* EO.

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2. MATERIALS AND METHODS

2.1. Plant materials

The leaves of *G. tamirensis* were collected in Phong Dien, Thua Thien Hue, in August 2024. A

voucher specimen has been deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Hue University, Vietnam.



Figure 1. *G. tamirensis* flower and leaf image

2.2. Methods

2.2.1. Distillation of the essential oil

The EO from *G. tamirensis* leaves was extracted by hydro-distillation. After extraction, the EO was dried over anhydrous sodium sulfate to remove any residual water and then stored under refrigeration until further analysis. The obtained EO was used to analyze its chemical composition and evaluate its biological activities. The yield of the EO was calculated using the formula: $H = E/M \times 100\%$, where E is the volume of the extracted EO (mL) and M is the initial rhizome biomass (g).

2.2.2. Gas Chromatography–Mass Spectrometry (GC-MS) analysis

The chemical composition of the volatile compounds was determined using gas chromatography coupled with mass spectrometry (GC-MS). The GC-MS analysis was performed on a Shimadzu GCMS-QP2010 Plus system (Kyoto, Japan) equipped with an Equity-5 capillary column (30 m \times 0.25 mm, 0.25 μ m film thickness), and a mass spectrometer (MSD QP2010 Plus). The EO (1 mg) was diluted with *n*-hexane, and 1 μ L was injected for analysis. The oven temperature was initially set to 60 $^{\circ}$ C for 2 min, followed by a temperature ramp to 280 $^{\circ}$ C at a rate of 2 $^{\circ}$ C/min, with a total analysis time of 110 min. Helium was used as the carrier at a flow rate of 1.8 mL/min. The mass spectrometer was operated with an interface temperature of

280 $^{\circ}$ C, the EI-mode was 70 eV and the mass acquisition range was set from 40 to 500 m/z. A splitless injection mode was employed. Compound identification was performed by comparing the obtained mass spectra with the Wiley 7 and National Institute of Standards and Technology (NIST 11) libraries. In addition, a standard solution of C₈–C₃₈ alkanes was used to determine the retention index of compounds and to compare them with literature data [19]. The relative amounts of individual components were calculated based on the GC peak area without correction.

2.2.3. SRB assay for evaluating cytotoxic activity

The cytotoxic activity of the EO was evaluated using the sulforhodamine B (SRB) assay, as outlined by Monks et al., which quantifies the binding of SRB dye to cellular proteins [20]. The cancer cell lines MCF7 (breast cancer), A549 (human lung adenocarcinoma), and HepG2 (hepatocarcinoma) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 2 mM L-glutamine, 1.5 g/L Na₂CO₃, and 10% fetal bovine serum, with medium changes every 48 hours. The cells were detached using 0.05% trypsin-EDTA solution, subcultured at a 1:3 ratio every 3 to 5 days, and incubated at 37 $^{\circ}$ C in a humidified 5% CO₂ atmosphere for 48 hours. For the assay, 4×10^4 cells were seeded in each well of a 96-well microplate with 180 μ L of growth medium. After adding the

test samples (10 μ L), the plates were incubated under the same conditions. Following 72 hours of treatment with the EO, the medium was discarded, and the cell monolayers were fixed with 20% (w/v) cold trichloroacetic acid for 1 hour at 4 $^{\circ}$ C. SRB solution was then added to each well, and the plates were incubated at room temperature for 30 minutes. The wells were washed with 1% (v/v) aqueous acetic acid to remove excess dye, and the protein-bound dye was solubilized in 10 mM Tris base solution. Absorbance was measured at 515 nm for both control and treated wells. Dimethyl sulfoxide (DMSO) at 10% (DMSO concentration in the well is 0.5%) served as the blank, and ellipticine was used as the positive control. Cytotoxicity was assessed at concentrations of 100, 20, 4, and 0.8 μ g/mL, with IC_{50} value calculated using TableCurve version 4.0. The inhibition rate of cancer cells calculated by the following formula:

$$IR\% = (100\% - [(Abs_t - Abs_o) / (Abs_c - Abs_o)]) \times 100$$

Where, IR: inhibition rate of cell growth, Abs_t : average optical density value at day 3; Abs_o : average optical density value at time-zero; Abs_c : average optical density value of the blank DMSO control sample.

All experiments were set up in triplicate. The IC_{50} value were presented as mean \pm standard deviation. Cytotoxicity categories based on the US National Cancer Institute [21,22]: very toxic $IC_{50} \leq 20$ μ g/mL, moderate/toxic IC_{50} 21-200 μ g/mL, weak IC_{50} 201-500 μ g/mL, and non toxic $IC_{50} \geq 500$ μ g/mL.

3. RESULTS

3.1. Extraction and profiling

The yield of EO extraction from leaves of *G. tamirensis* was 0.04% (v/w), calculated on a dry weight basis. Table 1 displays the result obtained from the chemical composition analysis of this EO through GC-MS.

Table 1. Components of essential oil from leaves of *Goniiothalamus tamirensis*

No	RT	Compounds ^a	Type	RI ^b	RI ^c	Content (%)
1	17,63	Terpinen-4-ol	OM	1190	1174	1.29
2	18,67	<i>iso</i> -Dihydro carveol	OM	1213	1212	8.92
3	21,32	<i>neo</i> -3-Thujanol acetate	O	1273	1273	5.73
4	21,65	<i>p</i> -Ethyl acetophenone	O	1280	1279	1.54
5	23,07	δ -Terpinyl acetate	O	1313	1316	9.18
6	23,29	<i>neo</i> -Verbanol acetate	O	1318	1319	5.74
7	23,68	<i>cis</i> -Piperitol acetate	O	1327	1332	1.34
8	24,64	α -Longipinene	S	1349	1350	1.70
9	32,13	10- <i>epi</i> -Cubebol	OS	1530	1533	3.50
10	33,98	Allo-cedrol	OS	1577	1589	4.61
11	35,02	1,10-di- <i>epi</i> -Cubenol	OS	1604	1618	2.05
12	35,43	Ledol	OS	1614	1602	1.38
13	35,67	2,(7Z)-Bisaboladien-4-ol	OS	1621	1618	1.58
14	36,03	Hinesol	OS	1631	1640	1.80
15	37,02	<i>neo</i> -Intermedeol	OS	1657	1658	2.63
16	37,35	Intermedeol	OS	1666	1665	6.08
17	37,73	Eudesma-4(15),7-dien-1 β -ol	OS	1676	1687	2.45
18	38,91	14-hydroxy-4,5-dihydro Caryophyllene	OS	1708	1706	1.99
19	39,43	γ -Costol	OS	1723	1745	2.88
20	40,81	(Z)-Lanceol	OS	1762	1760	16.69
21	41,78	(E)-Isovalencenol	OS	1789	1793	1.97
22	43,29	β -Vetivone	OS	1833	1822	0.42
23	44,45	<i>cis</i> -Thujopsenic acid	O	1867	1863	3.72
24	46,17	Cembrene	D	1919	1937	3.54

Oxygenated monoterpene (OM)	10.21
Sesquiterpene hydrocarbon (S)	1.70
Oxygenated sesquiterpene (OS)	50.02
Dierpene (D)	3.54
Others (O)	27.25
Total	92.73

^a Elution order on Equity-5 column

^b Retention Indices on Equity-5 column

^c Literature retention indices

- Not identified

In this study, the majority of the identified compounds were oxygenated sesquiterpenes

(50.02%) and oxygenated monoterpenes (10.21%). A total of 24 compounds were detected, with (Z)-Lanceol representing the most abundant component in *G. tamirensis* EO at 16.69%, followed by δ -Terpinyl acetate (9.18%) and iso-dihydro carveol (8.92%).

3.2. Anticancer activity

The anticancer activity of the EO of *G. tamirensis* was determined using the SRB method, and their results are shown in Table 2.

Table 2. Cytotoxic activity of the leaves essential oil from *G. tamirensis*

C ($\mu\text{g/mL}$)	<i>G. tamirensis</i> essential oil			C ($\mu\text{g/mL}$)	Ellipticine		
	MCF7	A549	HepG2		MCF7	A549	HepG2
100	95.45 \pm 2.05	90.92 \pm 2.40	98.87 \pm 1.64	10	90.83 \pm 2.17	88.62 \pm 3.24	92.79 \pm 1.98
20	81.76 \pm 1.28	88.19 \pm 1.58	86.54 \pm 1.32	2	78.14 \pm 1.82	76.82 \pm 1.20	79.68 \pm 1.69
4	22.44 \pm 1.61	10.80 \pm 0.79	26.49 \pm 1.28	0.4	52.66 \pm 1.63	50.58 \pm 1.23	53.06 \pm 1.89
0.8	5.27 \pm 0.14	1.75 \pm 0.21	7.80 \pm 0.61	0.08	22.32 \pm 1.21	21.02 \pm 1.12	22.26 \pm 0.94
IC ₅₀	8.79 \pm 0.44	10.29 \pm 0.38	7.32 \pm 0.24	IC ₅₀	0.34 \pm 0.02	0.38 \pm 0.03	0.33 \pm 0.03

C: concentration

The IC₅₀ values demonstrated that the EO from the leaves exhibited significant cytotoxic effects on the MCF7, A549, and HepG2 cell lines, with IC₅₀ values of 8.79, 10.29, and 7.32 $\mu\text{g/mL}$, respectively.

4. DISCUSSION

This study was conducted to explore the phytochemical composition and evaluate the potential biological activities of the EO derived from *Goniiothalamus tamirensis*. Our findings revealed that oxygenated sesquiterpenes were the predominant components of *G. tamirensis* EO, accounting for 50.02% of its composition. Among these, the sesquiterpenoid (Z)-lanceol was identified as the major constituent, comprising 16.69% of the EO. Previous research has provided valuable insights into the chemical composition of the EO from *G. tamirensis*, with variations reported between oils extracted from different parts of the plant. For instance, a study by T.D. Thang identified α -pinene (33.4%), viridiflorol (18.5%), and β -caryophyllene (12.4%) as the dominant components in the EO from

the leaves of *G. tamirensis*. In contrast, the stem oil was primarily composed of γ -gurjunene (11.2%), β -caryophyllene (10.9%), and δ -cadinene (10.3%) [18]. The chemical profile observed in our study differed from these previous findings, which could be attributed to several factors, such as variations in the plant's growing environment, differences in the extraction methods employed, and advances in the instrumental techniques used for analysis. These factors can significantly influence the chemical composition of EO, leading to discrepancies between studies, even when investigating the same plant species.

Extracts and isolated compounds from *G. tamirensis* have demonstrated inhibitory effects against various cancer cell lines. From the leaves of *G. tamirensis*, 15 compounds were isolated. Among them, (-)-5-acetoxylgoniothalamine and (Z)-6-styryl-5,6-dihydro-2-pyranone demonstrated inhibitory activity against colon cancer cells (HCT116), with IC₅₀ values of 8.6 μM and 22.1 μM , respectively. Other species in this genus may also exhibit anti cancer activity. For comparison, the positive control

doxorubicin showed an IC_{50} of 9.7 μ M [23]. The crude methanolic extract of *G. elegans* exhibited pronounced cytotoxic activity against SW-480 (colorectal), AGS (gastric), and SK-LU-1 (lung) cancer cell lines [24] with IC_{50} values of 14.51; 19.57 and 24.69 μ g/mL, respectively. Furthermore, two isolated alkaloids-lysicamine and liriodenine-demonstrated significant inhibitory effects against these cancer cell lines, with the IC_{50} ranging from 9.84 to 31.72 μ M, indicating their potential as lead compounds for anticancer drug development [24]. However, the anticancer potential of *G. tamirensis* EO has not yet been investigated. This study represents the first report on the cytotoxic activity of the EO from this species. The results of this study suggest that the EO from *G. tamirensis* possesses promising anticancer properties, which may contribute to its ability to inhibit the viability of cancer cells. The potent cytotoxic effects observed in this study highlight the potential of *G. tamirensis* EO as a candidate for further investigation in cancer drug discovery. This EO, with its bioactive compounds, may represent a valuable natural source for developing new therapies to treat cancer. The findings of this study provide scientific evidence supporting the use of *G. tamirensis* as a therapeutic agent in traditional medicine, as well as its potential applications in modern pharmaceutical research and development.

Although the results from this study are encouraging, further research is needed to expand our understanding of the anticancer potential of *G. tamirensis* EO. To gain a more comprehensive evaluation, it would be beneficial to test the oil on a broader range of cancer cell lines, including those derived from various tissue origins, to assess its efficacy across different cancer types. To further evaluate its therapeutic potential, it is recommended that the antiproliferative activity of the EO be tested on additional cancer cell lines from various tissue types. Additionally, *in vivo* studies using animal models are essential to confirm the observed anticancer effects and to explore the potential therapeutic benefits in a living organism. In-depth pharmacological investigations, including dose-response studies and toxicity profiling, are necessary steps to determine the safety and efficacy of this EO as a potential anticancer treatment. By expanding the scope of research, we can better understand the full therapeutic potential of *G. tamirensis* and its EO, paving the way for its possible use as a natural adjunct or alternative to conventional cancer therapies.

5. CONCLUSION

In conclusion, the chemical composition of *Goniiothalamus tamirensis* EO was analyzed using GC-MS, revealing that oxygenated sesquiterpenes (50.02%) and oxygenated monoterpenes (10.21%) were the major constituents. The EO demonstrated significant cytotoxic activity against the MCF7, A549 and HepG2 cancer cell lines with IC_{50} values of 8.79, 10.29, and 7.32 μ g/mL, respectively. These findings provide valuable insights into the potential of *G. tamirensis* as a source of bioactive compounds with anticancer properties.

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