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In Vitro Biological Activity of *Tiliacora triandra* (Colebr.) Diels Root Extract

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Abstract

In this study, we explore the phytochemical properties of *Tiliacora triandra* (Colebr.) Diels, commonly known as Yanang, a herb traditionally used for treating fever and malaria. Our objective is to isolate and analyze bioactive compounds from *T. triandra*'s root extract, with a specific focus on tiliacorinine (T1), assessing its antioxidant, and anticancer capabilities. The antioxidant activity of *T. triandra* extract (TTE) was evident, with IC_{50} values of 196.32 ± 10.51 µg/mL and 88.22 ± 1.95 µg/mL in the DPPH and ABTS assays, respectively. The results also demonstrated that TTE and T1 had a significant cytotoxic effect, with IC_{50} values varying in a concentration-dependent manner against A549 with the IC_{50} of 48.25 µg/mL and 26.38 µg/mL, respectively. Additionally, both TTE and T1 displayed anti-inflammatory effects, with the most effective inhibition of nitric oxide production observed at a concentration of 40 µg/mL. This study underlines the potential of *T. triandra*, and its compound tiliacorinine in developing treatments with antioxidant, anti-inflammatory, and anticancer properties.

Keywords**:** *Tiliacora triandra; tiliacorinine; antioxidant; cytotoxic activity*

1. Introduction

In Thailand, edible flora forms a critical component of the local diet, with *Tiliacora triandra* (Colebr.) Diels, known as Yanang and part of the Menispermaceae family, being particularly popular. *T. triandra*, a species of considerable significance, possesses a variety of potential applications within the realm of traditional herbal medicine. This plant is a staple in the culinary heritage of Northeast Thailand and Laos, characterized by its vine-like growth, deep green foliage, and yellowish blossoms. Traditionally, the juice from its leaves has been used for its potential anticancer and immune-modulating effects. Studies have shown that the ethanolic extract from the leaves of *T. triandra* has hypoglycemic effects in animals (Katisart, & Rattana, 2017). Its root extract has been used traditionally to treat fever, diabetes, and malaria (Neamsuvan et al., 2015). Phytochemical analyses have revealed that the root of *T. triandra* is rich in a variety of secondary metabolites, including alkaloids, terpenoids, and flavonoids (Wongklom, & Panboon, 2024; Achmad et al., 2015). The presence of these bioactive compounds has led researchers to investigate the potential medicinal uses of this plant. One of the primary alkaloids found in the root of T. triandra is berberine, a well-known compound with a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, and antioxidant properties (Pradubyat et al., 2024; Ahmad et al., 2016). The root of *T. triandra* also contains a significant amount of terpenoids, such as tinosporide and tinosporidine, which have exhibited anti-diabetic,

anti-inflammatory, and immunomodulatory activities in previous studies (Chander, & Kumar, 2020). Furthermore, the root is a rich source of flavonoids, including apigenin, quercetin, and kaempferol, which have been linked to antioxidant, anti-cancer, and neuroprotective properties (Boonman et al., 2024; Chander, & Kumar, 2020). Although some research has identified bisbenzylisoquinoline alkaloids such as tiliacorine and tiliacorinine in its roots (Khamto et al., 2023; Sandhanam et al., 2019), comprehensive studies on the root's chemical makeup are lacking.

Furthermore, *T. triandra* is recognized for its anticancer, antipyretic, antifungal, antibacterial, and acetylcholinesterase inhibitory properties (Jongrungraungchok et al., 2023; Ingkaninan et al., 2003; Janeklang et al., 2014; Katisart, & Rattana, 2017; Mukherjee et al., 2007; Nutmakul et al., 2016a; Nutmakul et al., 2016b; Rattana et al., 2016), highlighting its potential as a source for pharmaceutical development. Given the increasing interest in natural products from plants, *T. triandra* is seen as a viable subject for medicinal research due to its varied bioactive and chemical characteristics. The importance of this research stems from the fact that, while many studies have focused on the leaves and crude extracts of *T. triandra*, very few have comprehensively investigated its root, particularly with regard to isolating and identifying specific bioactive compounds responsible for its medicinal effects. Previous research lacked depth in exploring the root's in vitro biological activities, particularly its antioxidant and anti-cancer potential. Tiliacorinine, a bisbenzylisoquinoline alkaloid from *T. triandra*, has recently gained attention for its anticancer properties, particularly against cholangiocarcinoma (CCA), a type of cancer that responds poorly to current chemotherapeutic agents and frequently develops drug resistance. Tiliacorinine has been shown to induce apoptosis in human CCA cell lines and inhibit tumour growth in mice (Detarya et al., 2023).

This study seeks to fill the gap in existing literature by investigating the *in vitro* biological activities of *T. triandra* root extract, focusing on its potential to overcome drug resistance in cancers and its broader therapeutic benefits. This research focused on *T. triandra* samples gathered from Phra Nakhon Si Ayutthaya Province in Thailand, aiming to examine the secondary metabolites from its roots using proven isolation and identification methods. The study encompassed the extraction, structural determination, and analysis of the biological effects of these secondary metabolites.

2. Objectives

The primary objectives of this research were twofold: firstly, to isolate and purify secondary metabolites from the roots of *T. triandra*, and secondly, to assess the antioxidant capacity of these metabolites. Additionally, this study aimed to evaluate the anticancer activities of both the crude extracts and the isolated compound, specifically targeting non-small cell lung carcinoma (A549 cell line). Through these objectives, the research seeks to contribute to the understanding of the pharmacological properties of *T. triandra*, potentially providing a foundation for the development of novel therapeutic agents.

3. Materials and Methods

Plant Material and Extraction Methodology

The roots of *Tiliacora triandra* (1000.5 g) were collected from Phra Nakhon Si Ayutthaya Province, Thailand, and authenticated by the authors. The collected samples underwent a cleaning process, followed by drying at temperatures ranging from 45-50ºC, and were subsequently crushed to yield a coarse powder suitable for extraction. The powdered material was then subjected to maceration, initially with hexane (4 liters) and subsequently with dichloromethane (8 liters). The dichloromethane extract (TTE) was concentrated under reduced pressure and subsequently suspended in a 0.01 M HCl solution. To remove any impurities, the acidic extract was filtered through Whatman filter paper, and the filtrate was then extracted with ethyl acetate to separate non-alkaloidal components. The HCl solution, enriched with alkaloids, was neutralized using $NaHCO_3$ and further extracted with dichloromethane to yield an alkaloid mixture (500 mg). This mixture was then subjected to column chromatography using silica gel, eluted with a mixture of dichloromethane and methanol. Fractions Fr-6 to Fr-7 were pooled and further fractionated by column chromatography on silica gel (1.5 × 20 cm), eluted with a CH_2Cl_2: MeOH (95:5) mixture, resulting in the isolation of tiliacorinine (20.02 mg, T1). The structure was established based on an extensive spectroscopic analysis, including 1D and 2D mass spectrometric and NMR techniques. The Mass Spectrometry (MS) data were obtained using an electrospray ionization (ESI) coupled Orbitrap Elite Mass spectrometer (Thermo Scientific Orbitrap Elite MS, USA). The Nuclear Magnetic Resonance (NMR) spectra were recorded in $CDCl_3$ using a Bruker Advance 400 NMR

Spectrometer (Rheinstetten, Germany) with TMS as the internal standard.

Preparation of Test Samples

TTE and T1 were each solubilized in dimethyl sulfoxide (DMSO) to create a stock solution with a concentration of 50 mg/mL. To produce the working solution, this stock was serially diluted (1:2) in complete media to obtain the required concentrations. For all experiments, the extracts were mixed with DMSO, maintaining the final DMSO concentration in the media below 0.2%.

Determination of Antioxidant Activity via DPPH Assay

The antioxidant capacities of TTE and T1 were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, following the protocol established by Jongrungruangchok et al. (2023). In this process, six different concentrations of TTE and T1 (ranging from 0 to 400 μg/mL) were each tested in triplicate by mixing with 0.1 mL of DPPH solution (provided by Sigma-Aldrich, St. Louis, MO, USA) in ethanol, placed in a 96-well plate. The plate was covered with aluminum foil and incubated at 37°C for 30 minutes. The absorbance was measured at 517 nm using a Bio-Rad Benchmark Plus Microplate Spectrophotometer. The antioxidant activity was determined by comparing the sample's ability to neutralize the DPPH radical to a standard ascorbic acid solution, with results reported as the percentage of antioxidant activity. The DPPH free radical scavenging capacity was calculated using a specific formula.

$$\text{Antioxidant activity (\%)} = \frac{\text{OD control - OD sample} \times 100}{\text{OD control}}$$

In this study, the optical density (OD) of the control, referred to as OD_control, refers to the absorbance value derived from the control sample. On the other hand, OD_sample indicates the absorbance value associated with the test sample. Ascorbic acid was used as the positive control for the purpose of comparison. The IC_{50} values, representing the concentration needed to neutralize 50% of DPPH free radicals, were determined using linear regression analysis.

Assessment of Antioxidant Capacity using the ABTS Assay

The antioxidative capacity of the *T. triandra* extract (TTE) and its isolated compound T1 was determined using the ABTS assay. In this method, the ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting ABTS with potassium persulfate. Separate solutions of 7 mM ABTS and 4.95 mM potassium persulfate were prepared and mixed in equal parts, then left to stand in darkness at room temperature for 16 hours to complete the reaction. The resulting mixture was diluted with methanol to achieve an absorbance range of 0.72 to 0.82 at 734 nm. The samples were serially diluted to concentrations of 25, 50, 100, 200, and 400 μg/mL, and 200 μL of the ABTS solution was combined with 20 μL of each sample concentration in a 96-well plate. After incubating for six minutes, the absorbance was measured at 734 nm using a UV spectrophotometer. In this setup, the $ABTS^{\bullet+}$ solution served as the blank, and trolox was used as the standard reference for positive control. The capacity of the samples to scavenge the ABTS radicals was calculated using a specific formula tailored for this analysis.

$$\text{Scavenging activity (\%)} = \frac{\text{OD control - OD sample} \times 100}{\text{OD control}}$$

The concentration causing 50% inhibition of the ABTS radical (IC_{50}; μg/mL) was determined graphically.

Inhibition of Nitric Oxide (NO) Production

Mouse macrophage cells (RAW 264.7) were grown in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, in a humidified environment with 5% CO_2 at 37°C. Before the tests, the cells were plated at a density of 1×10^6 cells/mL in 96-well plates and left to attach overnight.

To provoke nitric oxide synthesis, lipopolysaccharide (LPS) from *Escherichia coli* strain 0111:B4 was applied to stimulate the RAW 264.7 cells. The cells were exposed to LPS (1 μg/mL) alongside different doses of *T. triandra* extract (TTE) and its isolated compound (T1) for a period of 24 hours, with the treatment concentrations of TTE and T1 varying from 25 to 200 μg/mL.

Nitric oxide production was determined by measuring the nitrite levels in the cell culture supernatant through the Griess Reagent System (Wunnakup et al., 2024). Following the 24-hour exposure, 100 μL of the supernatant from each well was transferred to a fresh 96-well plate. An equal volume of Griess reagent (1% sulfanilamide, 0.1%

naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) was added to each well, and the plate was incubated for 10 minutes at room temperature, protected from light. The absorbance at 540 nm was read using a microplate reader. A standard curve was established using sodium nitrite to calculate the nitrite levels.

Cytotoxic and Antiproliferative Activities Testing

The A549 cell line, which is a type of human lung carcinoma epithelial cell, was grown in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mixture. These cells were kept in a humidified incubator at 37°C with 5% CO_2. Stock solutions of the TTE and T1 were prepared in dimethyl sulfoxide (DMSO), ensuring the DMSO concentration in the culture media remained below 0.1% to prevent toxic effects. The A549 cells were plated at 5×10^3 cells per well in 96-well plates and left overnight to adhere. They were then exposed to various concentrations of TTE and T1 (from 1 to 100 µg/mL) for 48 hours to observe both immediate and prolonged impacts (Ketkomol et al., 2014).

To determine the cytotoxicity of TTE and T1 on A549 cells, the MTT assay was employed. Following the exposure period, each well received MTT solution (5 mg/mL in PBS), and the plates were incubated for 4 hours at 37°C. The resulting formazan crystals were dissolved in DMSO, and absorbance was read at 570 nm using a microplate reader. The viability of the cells was assessed in comparison to the control group that was not treated.

Statistical Analysis

The results are shown as average values from experiments done in triplicate. Statistical significance of differences among the groups exposed to the extracts was determined using one-way analysis of variance (ANOVA). For multiple comparisons, post-hoc analysis was carried out with the Tukey test. Statistical significance was set at a p-value below 0.05.

4. Results

The root material, once dried, underwent extraction utilizing dichloromethane (CH_2Cl_2) as the solvent. Subsequent to the extraction, the crude extract was subjected to column chromatography for the purpose of isolation and purification, culminating in the acquisition of a pure alkaloidal entity. The resultant compound, designated as T1 (tiliacorinine, 20.02 mg), was characterized as an amorphous powder. Its molecular composition, $C_{36}H_{36}O_5N_2$, was elucidated through Electrospray Ionization Time-of-Flight Mass Spectrometry (ESI-TOFMS), adapted from Sureram et al. (2012), that revealed a mass-to-charge ratio (m/z) of 575.3 $[M\text{-}H]^-$, as depicted in Figure 1 (Sureram et al., 2012; Patra et al., 2010; Phuriyakorn, 1981). ^1H and ^{13}C NMR spectra are shown in Table 1, Figure 2 and 3, respectively.

4.1 Evaluation of Antioxidant Activity through DPPH and ABTS Assays

This investigation aimed to quantify the antioxidant potential of (TTE) and its isolated compound T1 by determining their half-maximal inhibitory concentration (IC_{50}) values via DPPH and ABTS assays. The findings revealed that both TTE and T1 exhibited antioxidant activities, with IC_{50} values recorded at 196.32 ± 10.51 µg/mL and 88.22 ± 1.95 µg/mL for the DPPH and ABTS assays, respectively, as summarized in Table 2. It was observed, however, that the antioxidant efficacies of TTE and T1 did not match the potency of the standard antioxidant's ascorbic acid and Trolox, which served as positive controls, detailed in Table 2.
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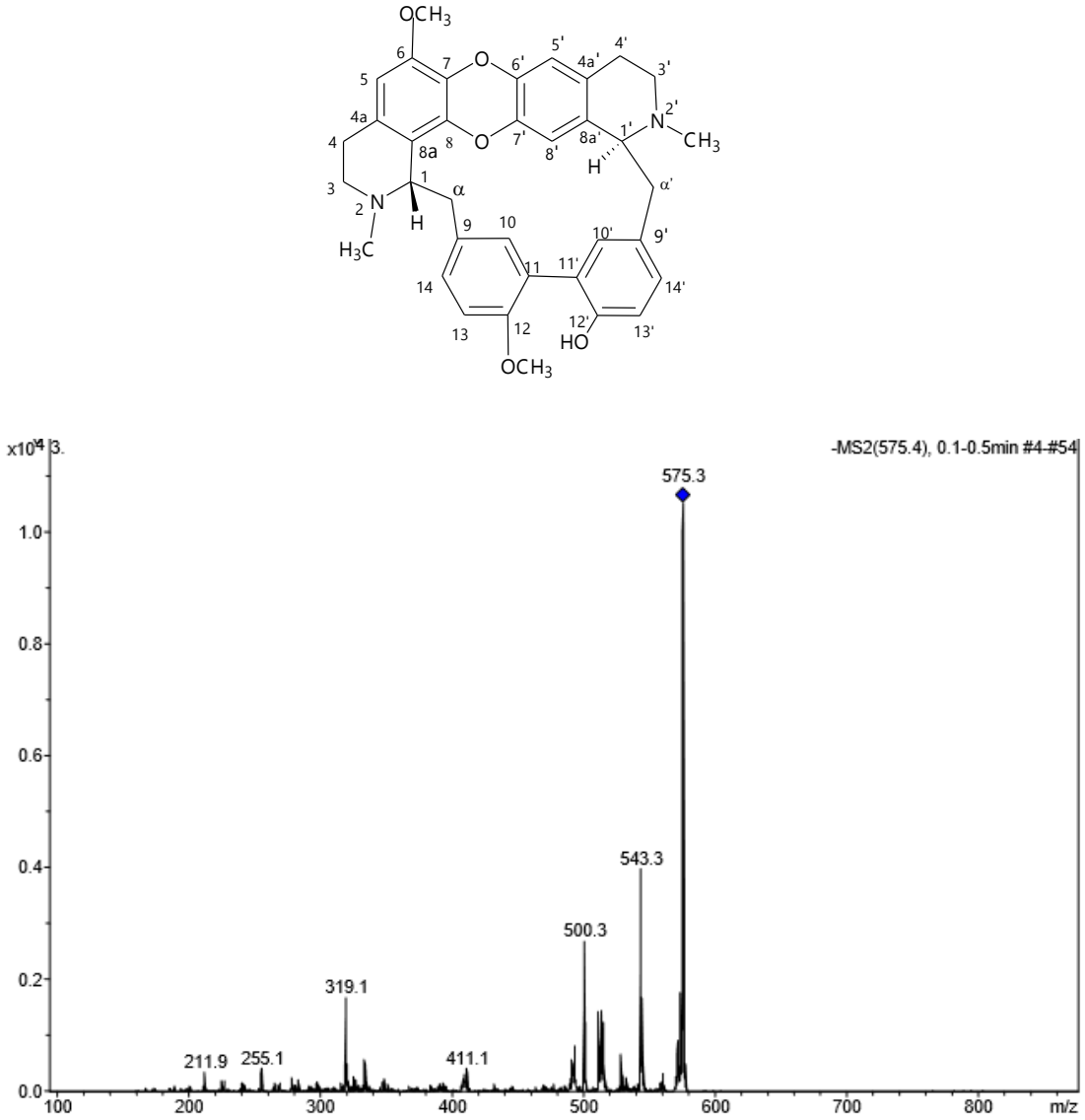

Figure 1 The structure and mass spectrum of T1

Table 1 The ^{1}H and ^{13}C NMR spectra of compound T1 and tiliacorinine

C	Compound T1		Tiliacorinine	
	δc, mult.[a]	**δ_H, mult., J in Hz**	**δc, mult.[a]**	**δ_H, mult**
1	62.1	3.70, *d*, (7.5 Hz)	62.7 CH	3.66, *d*, (7.5 Hz)
(2)*N*-Me	41.9	2.30, s, 3H	41.9 CH_3	2.30, *s*
3	43.1	3.08, *m*, H-3a	43.7 CH_2	3.00, *m*
		3.40, *m*, H-3b		2.84 *m*,
4	20.6	2.38, *m*, H-4a	21.2 CH_2	2.38, *m*
		2.95, m, H-4b		
4a	128.1		128.8 C	
5	105.6	6.32, *s*	106.3 CH	6.32 *s*

Table 1 Cont.

C	Compound T1		Tiliacorinine	
	δc, mult.[a]	**δ_H, mult., J in Hz**	**δc, mult.[a]**	**δ_H, mult**
6	145.6		143.6 C	
6-*O*-Me	55.6	3.87, *s*	56.3 CH_3	3.87 *s*
7	129.6		129.5 C	-
8	137.1		137.7 C	-
8a	117.8		118.4 C	-
α	39.9	2.70, *m*	40.5 CH_2	2.84 m
		2.88, *m*		2.80 *m*
9	135.1	-	137.7 C	-
10	135.2	7.69, *d*, (1.6 Hz)	135.7 CH	7.64 *d*, (1.6 Hz)
11	127.2	-	127.7 C	-
12	152.5	-	153.1 C	-
12-*O*-Me	55.8	4.01, *s*	56.4 CH_3	4.01, *s*
13	110.5	7.00, *d*, (7.3 Hz)	111.1 CH	6.99, *d*, (7.3 Hz)
14	129.1	7.38, *m*	130.2 CH	7.38, *d*, (7.5,1.5 Hz)
1′	66.6	3.48, *m*	67.1 CH_2	3.44, *m*
(2′)*N*-Me	41.3	2.65, s	41.8 CH_3	2.66, s
3′	52.4	3.14, *m*, H-3′a	52.8 CH_2	3.13, *m*
		2.56, *m*, H-3′a		2.73, *m*
4′	26.5	2.73, *m*, H-4′a	26.9 CH_2	2.56, *m*
		3.00, *m*, H-4′b		
4a′	129.5	-	133.7 C	-
5′	114.6	6.67, *s*	115.3 CH	6.66, *s*
6′	139.3	-	135.1 C	-
7′	139.2	-	139.9 C	-
8′	113.6	8.10, *s*	113.9 C	7.97, *s*
8a′	129.9	-	129.9 C	-
α′	41.3	2.98, *m*	40.8 C	3.40, *m*
		3.40, *m*		
9′	133.3	-	*135.3* C	-
10′	133.6	7.69, *br*, *s*	133.3 CH	7.55, *s*
11′	125.7	-	127.1 C	-
12′	151.9	-	149.2 C	-
13′	117.8	7.04, *d*, (7.3 Hz)	118.4 CH	7.09, *d*, (9.3, Hz)
14′	129.5	7.35, *d*, (7.3 Hz)	133.3 CH	7.55, *s*

Source: Sureram et al., 2012; Patra et al., 2010.

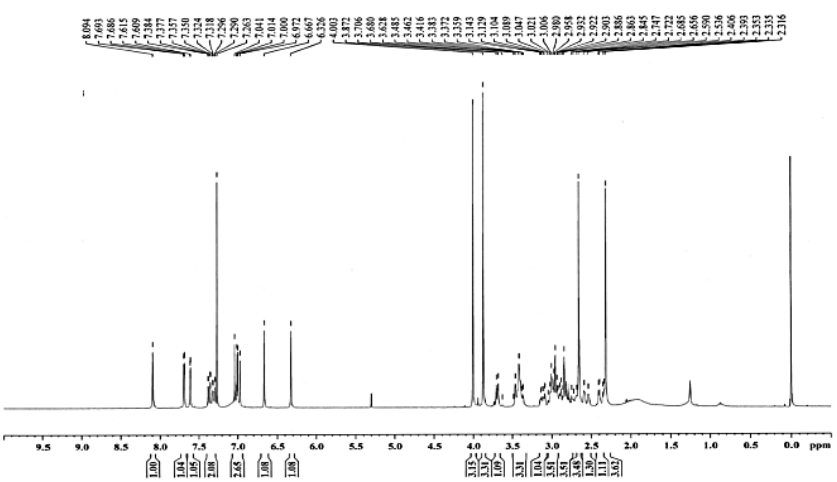

Figure 2 ^{1}H NMR ($CDCl_3$) spectrum of T1

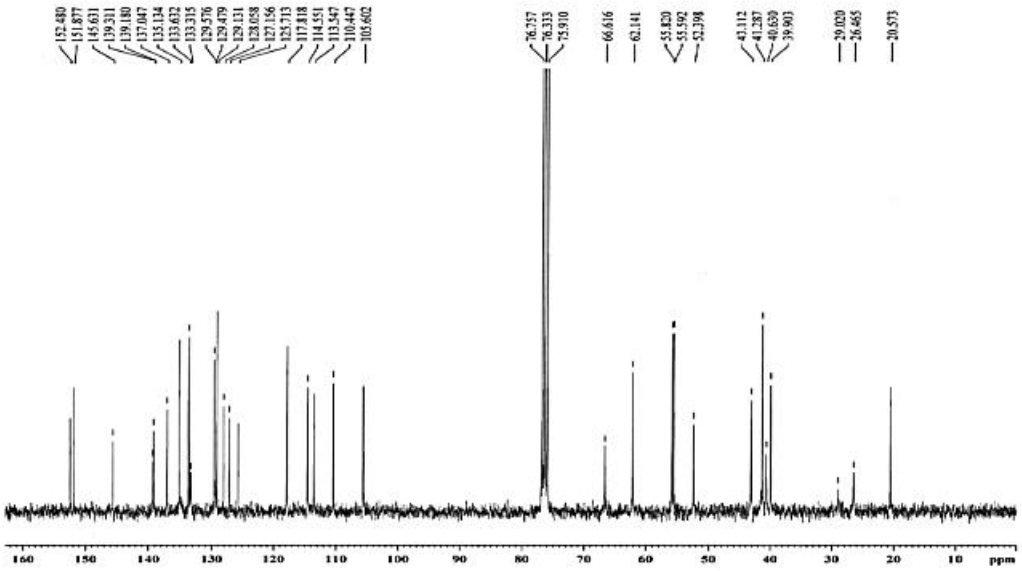

Figure 3 ^{13}C NMR ($CDCl_3$) spectra of T1

Table 2 Antioxidant activity of TTE and T1 determined against DPPH, ABTS

Sample	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
TTE	196.32 ± 10.51	98.50 ± 8.07
T1	88.22 ± 1.95	54.63 ± 5.08
Ascorbic acid	16.19 + 0.4523	0.15 + 0.01
Trolox	5.26 + 0.5739	1.37 + 0.14

Values are expressed as mean ± SD (n = 6). Values with different lowercase letters as superscripts within the same column indicate significant differences ($p < 0.05$).

Table 3 Inhibitory activity of TTE and T1 on the LPS-induced NO production in RAW 264.7 cells

TTE (µg/mL)	% NO inhibition	% Cell viability
0	3.86 ± 0.514	99.54 ± 0.215
10	20.54 ± 0.205	98.57 ± 0.361
20	45.52 ± 0.162	98.96 ± 0.212
40	95.91 ± 0.157*	96.21 ± 0.419
80	93.47 ± 0.467*	95.32 ± 0.992
160	92.45 ± 0.231*	94.41 ± 0.326

T1 (µg/mL)	% NO inhibition	% Cell viability
0	4.12 ± 0.535	99.67 ± 0.229
10	21.36 ± 0.217	98.76 ± 0.364
20	46.89 ± 0.162	99.03 ± 0.218
40	96.74 ± 0.153*	96.57 ± 0.425
80	94.29 ± 0.457*	95.68 ± 0.991
160	93.17 ± 0.23*	94.75 ± 0.336

Data were expressed as mean ± SEM values of 3 independent experiments (n = 3), with each experiment completed in triplicate. *P ≤ 0.05 compared to the control

4.2 ABTS Radical Scavenging Assay Outcomes

According to the data presented in **Table 2**, the compound T1 demonstrated superior ABTS radical scavenging capability (IC_{50}, 54.63 ± 5.08 µg/mL), surpassing that of TTE (IC_{50}, 98.50 ± 8.07 µg/mL). Nevertheless, when compared to Trolox, which exhibited a significantly lower IC_{50} value of 1.37 ± 0.14 µg/mL, the ABTS radical scavenging activities of both TTE and T1 were notably less potent.

Suppression of Nitric Oxide (NO) Synthesis

Nitric oxide (NO), a pivotal inflammatory mediator, is synthesized by RAW264.7 macrophage cells upon activation with lipopolysaccharide (LPS). The quantification of NO production was conducted by detecting nitrite ions (NO_2^-), a stable metabolite of NO, in the cell culture medium utilizing the Griess reagent. Observations at the 24-hour mark indicated a significant reduction in NO levels upon treatment with (TTE) and the isolated compound T1 at concentrations of 40, 80, and 160 µg/mL. The efficacy of TTE and T1 in inhibiting NO synthesis by LPS-stimulated RAW 264.7 cells is depicted in Table 3. Notably, at a concentration of 40 µg/mL, both TTE and T1 exhibited optimal suppressive effects on NO production, achieving 95.91% inhibition with a concomitant cell viability of 96.21%, as detailed in Table 3.

Moreover, the capability of TTE and T1 to inhibit NO production across a concentration range of 40 to 160 µg/mL was found to be significantly different from that of the control group. Importantly, at concentrations up to 160 µg/mL, TTE and T1 did not manifest any cytotoxic effects on RAW264.7 cells. However, at elevated concentrations ranging from 80 to 160 µg/mL, TTE and T1 exhibited minimal cytotoxicity towards RAW264.7 cells.

Cytotoxic and Antiproliferative Activities of TTE and T1 on A549 Cells

The present study aimed to evaluate the cytotoxic and antiproliferative effects of TTE and its isolated compound T1 on A549 lung carcinoma cells. The investigation utilized the MTT assay to assess cell viability and proliferation following treatment with various concentrations (0-160 µg/mL) of TTE and T1. The MTT assay results revealed a concentration-dependent cytotoxic effect of TTE and T1 on A549 cells. At lower concentrations, TTE and T1 exhibited minimal cytotoxicity, maintaining over 90% cell viability. However, as the concentration increased to 20 µg/mL and beyond, a significant reduction in cell viability was observed. The IC_{50} values for TTE and T1 were calculated to be 48.25 ± 0.164 µg/mL and 26.38 ± 0.289 µg/mL, respectively, indicating a higher cytotoxic potency of T1 compared to TTE (Figure 4).

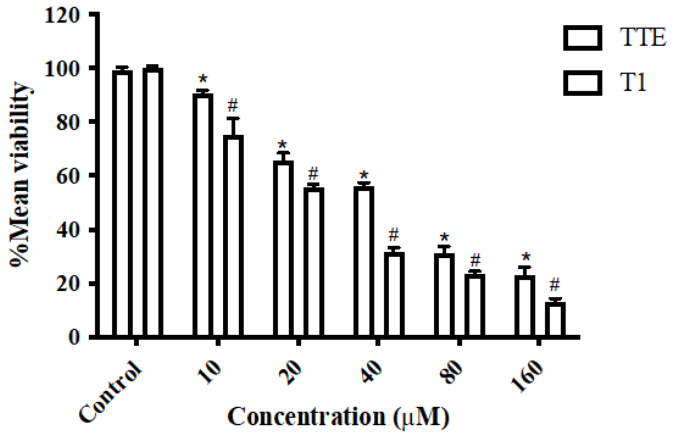

Figure 4 The cytotoxic activity of TTE and T1 against non-small cell lung cancer (A549) at 48 h incubation time. Data are represented as mean ± SEM values of 3 independent tests (n = 3), with each experiment completed in triplicate. *P ≤ 0.05 compared to the control of TTE and $^{\#}P \leq 0.05$ compared to the control T1

5. Discussion

T. triandra is recognized for its antioxidant, anti-inflammatory, and hepatoprotective attributes and has been historically employed in the treatment of various health conditions (Huang et al., 2021). Antioxidants are known to neutralize reactive oxygen species, hydroxyl radicals, and nitric oxide, while anti-inflammatory agents regulate the functions of pro-inflammatory enzymes and cytokines (Weerawatanakorn et al., 2018). The accumulation of free radicals is believed to contribute to cellular damage and is implicated in the etiology of numerous diseases (Weerawatanakorn et al., 2018). In this study, we explored the *in vitro* biological activities of *T. triandra* (Colebr.) Diels root extract, building upon its long-standing reputation for multiple biological properties (Huang et al., 2021). The significance of this research lies in the potential of *T. triandra* to address the limitations of current therapeutic agents, particularly in cancer treatment and inflammation management, areas where oxidative stress and nitric oxide production play a critical role. The novelty of this study is underscored by the growing interest in medicinal plants, with the root of *T. triandra* offering a diverse range of bioactive compounds that could serve as alternative therapies.

The DPPH and ABTS assays are well-established methods for evaluating the antioxidant capacity of compounds, particularly in natural products. Both assays measure the ability of an antioxidant to neutralize free radicals, but they do so through slightly different mechanisms. In the DPPH assay, the antioxidant donates an electron or a hydrogen atom to the DPPH radical, reducing it to a stable molecule and resulting in a color change from purple to yellow. This assay is especially valued for its simplicity and the ability to test both hydrophilic and lipophilic antioxidants. The ABTS assay, on the other hand, involves the generation of the ABTS radical cation, which is blue-green and absorbs at a specific wavelength. Antioxidants reduce the ABTS radical cation, leading to a decrease in absorbance, which is also measured spectrophotometrically. The ABTS assay is often preferred when testing hydrophilic compounds and can provide insights into the antioxidant's potential to neutralize a broader range of radicals compared to DPPH. In this investigation, we delved into the diverse biological properties of *T. triandra.* The assays conducted to evaluate antioxidative capacity indicated that both TTE and T1 exhibit significant antioxidant potential, likely due to their hydrogen-donating abilities which facilitate the scavenging of DPPH and ABTS radicals.

The NO inhibition assay adds another dimension to the evaluation, focusing on the ability of *T. triandra* fractions to inhibit the production of nitric oxide, a reactive nitrogen species that contributes to oxidative stress and inflammation. In pathological conditions like diabetes, excessive nitric oxide production can lead to the formation of peroxynitrite, a potent oxidant that exacerbates cellular damage. The anti-inflammatory potential was assessed in RAW264.7 cells, following a preliminary cytotoxicity evaluation of TTE and T1 on the same cell line. TTE was found to be non-cytotoxic at concentrations up to 160 µg/mL, enabling the assessment of anti-inflammatory effects at non-toxic concentrations (0–160 µg/mL). Both TTE and T1 demonstrated notable

anti-inflammatory effects by reducing NO production without causing significant toxicity to the cells, as shown in the results. These observations suggest that the active compounds within TTE and T1 possess both antioxidant and anti-inflammatory properties, making them beneficial for reducing inflammation associated with NO release. The capability of these extracts to act as NO scavengers or inhibit its production, particularly with minimal cytotoxicity, presents a viable approach for mitigating inflammation. The reduction in NO production by these plant extracts might be linked to the inhibition of inducible nitric oxide synthase activity or expression. The inhibition of nitric oxide by the fractions indicates a potential anti-inflammatory effect, complementing the antioxidant activity observed in the DPPH and ABTS assays. Moreover, our research highlighted the pronounced cytotoxic and antiproliferative effects of TTE on non-small cell lung cancer line A549 in a concentration-dependent manner (Figure 4). The observation of T1's superior cytotoxic activity suggests a potential synergistic effect within the plant's crude extract on cell growth and survival. Notably, TTE, as a crude extract of *T. triandra*, displayed significant anticancer activity at very low concentrations. The cytotoxicity of polyphenols against various cancer cell lines has been established, alongside the exploration of their antioxidant capabilities.

Our study revealed that the fractions derived from the roots of *T. triandra* exhibit potent antioxidant activities across these assays, highlighting the plant's robust capacity to neutralize free radicals through multiple pathways. This multifaceted antioxidant potential is significant in the context of diabetes management, where oxidative stress plays a crucial role in the disease's progression. By mitigating oxidative damage, *T. triandra* fractions could potentially slow down or prevent the complications associated with diabetes, such as neuropathy, retinopathy, and cardiovascular issues. Moreover, the presence of multiple active components within the fractions likely contributes to its broad-spectrum antioxidant effects. A drawback of earlier studies was their limited focus on specific bioactive components within the plant. By using molecular techniques and various assays, we have identified key compounds responsible for these activities, laying the groundwork for future research on the development of novel antioxidant and anti-inflammatory therapies. Moreover, the observed cytotoxic effects of *T. triandra* fractions against cancer cell lines, particularly A549 lung cancer cells, suggest its potential role in cancer therapy. This aspect is especially significant given the limitations of current chemotherapeutic agents and the need for alternative treatments that can target oxidative stress in cancerous cells.

Overall, this study not only reinforces the traditional uses of *T. triandra* but also paves the way for future research into its bioactive compounds and therapeutic applications, particularly in oxidative stress-related diseases and cancer. Further phytochemical analyses could help identify specific compounds that contribute to the plant's diverse bioactivities, providing insights into the development of targeted therapies derived from *T. triandra*.

6. Conclusion

This study has elucidated the significant anticancer potential of TTE and T1, alongside their antioxidant and anti-inflammatory properties. The anti-inflammatory efficacy of TTE is likely related to its capacity to inhibit NO production and to act as a free radical scavenger. These effects may be attributed to the presence of various bioactive compounds such as bisbenzylisoquinoline alkaloids, flavonoids, phenolic compounds, and fatty acids found in the plant's roots. While further research is needed to elucidate the mechanisms of action and efficacy in humans, these findings indicate that TTE and T1 could serve as promising adjuncts in cancer therapy. The potential therapeutic and health-promoting effects of these compounds warrant additional investigation to fully understand their roles. Nonetheless, the current findings position *T. triandra* as a candidate for further research aimed at disease prevention and treatment, with ongoing efforts to identify and characterize the phytochemicals responsible for these beneficial activities and to evaluate their effects in animal models.

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8. References

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