Sarcouncil Journal of Biomedical Sciences

ISSN(Online): 2945-3666

Volume- 04| Issue- 05| 2025

Research Article

Received: 15-09-2025 | Accepted: 12-10-2025 | Published: 27-10-2025

Chemical Composition and Antioxidant Activity of (*Cinnamomum parthenoxylon* (Jack.) C.Nees) Essential Oil

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Abstract: *Cinnamomum parthenoxylon* is commonly referred to as mint, is a perennial herb and widely utilized in traditional medicine. It affects the lung and liver meridians, helping to clear wind-heat and reduce stress, and is used as a cooling medicine to treat wind-heat colds, headaches, conjunctivitis, rhinitis, nasal congestion, sore throat, measles, hives, and skin rashes. Although there have been several studies on the chemical composition of *Cinnamomum parthenoxylon* essential oil, the chemical composition of *Cinnamomum parthenoxylon* essential oil grown in Thanh Hoa, Vietnam, has not been investigated. However, the chemical composition and biological properties of the essential oil of *Cinnamomum parthenoxylon* collected in Thanh Hoa, Vietnam, have not been investigated. Gas chromatography-mass spectrometry (GC-MS) was used to identify the chemical composition of *Cinnamomum parthenoxylon* essential oil. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities of the essential oil, respectively. Among the different plant parts, the leaves, flowers, and bark all contain essential oils, with the bark having the highest content, ranging from 1–4%, while the leaves and fruits contain lower amounts (0.3–0.9%). The main components of the essential oil are as follows: the bark contains cinnamic aldehyde (77.3%) and cinnamic aldehyde (57.7%); the branches contain trans-cinnamyl acetate (27.6%); the leaves contain cinnamic aldehyde (53.5%) and (Z)-9-octadecenamide (19.5%); and the roots contain cinnamic aldehyde (58.3%) and *p*-methoxy cinnamic aldehyde (12.0%).The strong antioxidant and antibacterial effects of *Cinnamomum parthenoxylon* essential oil were demonstrated with an IC_{50} value of 6.75 ± 0.2 µg/mL. Our results indicate that the essential oil of *Cinnamomum parthenoxylon* collected in Thanh Hoa, Vietnam, has great potential in the pharmaceutical industry.

Keywords: Thanh Hoa, *Cinnamomum parthenoxylon*, essential oil, antioxidant, cinnamic aldehyde.

INTRODUCTION

Cinnamomum parthenoxylon is a tall tree, reaching up to 25 meters in height and 40–60 cm in diameter. The bark is dark greenish-brown at the base, deeply fissured longitudinally, and peels off in small flakes about 3–5 mm thick. The inner bark is light yellowish-brown. Buds are ovoid, with bud scales resembling bird's eyes and covered with rust-colored hairs. The whole tree emits a characteristic camphor scent. The leaves are simple and alternately arranged; the leaf blade is ovate, elliptic, or oblong, measuring 6–12 × 3–6 cm. The apex is shortly acuminate or extended, and the base is broadly cuneate. Both leaf surfaces are glabrous. There are 4–7 pairs of prominent lateral veins visible on both sides, and oil glands are present between the veins. The petiole is slender, 1.5–2.5 cm long, and smooth (Pham, N. A. 2023). This species is distributed in Cao Bang (Trung Khanh), Quang Ninh (Ha Coi), Tuyen Quang, Bac Giang, Ha Tinh (Vu Quang), Quang Tri (Dong Che), Thua Thien Hue (Bach Ma), and Da Nang, and also found in India and China (Yunnan, Guangxi, Guangdong, and Hainan) (Nguyen, K. D. 2010; Balijepalli, M. K. *et al.*, 2017).

The wood is used in construction; the tree yields essential oils applied in medicine and perfumery. The seeds contain oil used in industry, and the essential oils extracted from leaves and roots are utilized in pharmaceuticals, as flavoring agents, and in beverages (Song, X. *et al.*, 2014; Kim, E. C. *et al.*, 2015).

In traditional medicine, parts such as the leaves, buds, and bark of the *Cinnamomum parthenoxylon* are considered to have high medicinal value and have been used for a long time in folk remedies. Specifically, *Cinnamomum parthenoxylon* leaf water helps support digestion, stimulates digestive enzyme secretion, enhances appetite, reduces bloating, indigestion, and treats conditions like diarrhea and dysentery (Li, H. *et al.*, 2017). Additionally, extracts from the leaves and buds of the *Cinnamomum parthenoxylon* tree are used for antiseptic purposes, to treat open wounds, abscesses, and skin inflammation. The *Cinnamomum parthenoxylon* leaves applied to the skin help reduce inflammation, promote healing, and have anti-aging effects due to their natural antioxidant compounds (Li, H. *et al.*, 2017). Several traditional remedies also report benefits in supporting the treatment of diabetes, infections, joint pain, and weight management by regulating fat metabolism (Le, T. D. 2010).

Several publications report the chemical composition of *Cinnamomum parthenoxylon*, especially that of the essential oil (Sein, C. C., & Mitloehner, R. 2011; Dũng, N. Ñ. X. *et al.*, 1994; Quang, L. V. *et al.*, 2022). However, studies have also shown that the raw material from different

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DOI- https://doi.org/10.5281/zenodo.17455475

regions and different extraction methods also greatly affect the content of *Cinnamomum parthenoxylon*. Therefore, when testing its chemical activity, it is possible to discover different active constituents according to the above factors. This study aims to analyze and characterize the chemical composition and biological properties *Cinnamomum parthenoxylon* essential oil collected in Thanh Hoa, Vietnam, in support of its potential exploitation and effective utilization. Determination of the chemical composition of *Cinnamomum parthenoxylon* essential oil was conducted using gas chromatography-mass spectrometry (GC-MS). The the agar disc diffusion method were used to evaluate the antioxidant and antibacterial activities, respectively.

MATERIAL AND METHODS

Chemicals

Butylated hydroxytoluene (BHT), C7–C30 straight-chain hydrocarbons, reference chemicals for identification, Tween 80, and DPPH were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical analytical grades, the culture media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

Plant Material

The *Cinnamomum parthenoxylon* were collected from Thanh Hoa, Vietnam, in January 2022. A voucher specimen (No. RH-TH-01) was prepared and deposited at the Faculty of Natural Science and Technology, Hong Duc University, Thanh Hoa Provinve, Vietnam.

Figure 1. The **(***Cinnamomum parthenoxylon* (Jack.) C.Nees) collected in Thanh Hoa Province.

Extraction of Essential Oil

Collected leaves were cleaned, cut into small pieces, and subjected to steam distillation for 3.0 hours by a Clevenger-type apparatus. The essential oil obtained was then dehydrated using anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark prior to subsequent experiments.

Analysis of Essential Oil by GC-MS

To analyze the composition of the essential oil from the leaves of *Cinnamomum parthenoxylon*, a Trace GC Ultra-ITQ900 system (Thermo Fisher Scientific, MA, USA) was used. Data were interpreted by MassFinder 4.0 software. The separation was performed on a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The GC operational parameters included injector temperature 250 °C, detector temperature 260 °C, oven temperature program 60 to 260 °C at a heating rate of 4 °C/min, carrier gas helium at a flow rate of 1.0 mL/min, and sample injection volume 1 μL in split mode with a split ratio of 1:10. The mass spectrometer was operated in electron ionization (EI) mode with the following parameter values: ionization energy 70 eV, interface temperature 280 °C, ion source temperature 230 °C, MS quadrupole temperature 200 °C, and scan range 35–650 amu (Le, V. D. *et al.*, 2020). The retention indices of the essential oil constituents were determined using an HP-5 MS column and standard C7–C30 straight-chain hydrocarbon reference standards (Sigma-Aldrich Chemical Company, USA). The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology-NIST 08 and Wiley 09th version) and/or with published data. The relative percentages of the identified compounds were calculated based on GC peak areas without applying correction factors.

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Antioxidant Assay

The DPPH assay was used to evaluate the antioxidant activity of the essential oil from *Amomum villosum* leaf and stems (Le, V. D. *et al.*, 2020).The essential oil was dissolved in methanol to different concentrations (0.3125, 0.625, 1.25, 2.5, 5.0, and 10 µg/mL), and the positive control BHT was mixed with 200 µL of a methanolic solution (containing DPPH radicals at a concentration of 150 µmol/L). The mixture was then vigorously shaken and left in the dark for 30 minutes to complete the reaction. The absorbance solutions were measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm. A blank sample (a control solution without extract or BHT) was used for comparison. The scavenging ability of the essential oil was calculated as in Equation 1.

$$Scavenging\ acbility\ (\%) = \frac{A_{517}\ of\ control - A_{517}\ of\ sample}{A_{517}\ of\ control}\ x\ 100 \quad (1)$$

Statistical Analysis

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA) were utilized to analyze the results. The results are presented as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Investigation of Factors Affecting Essential Oil Yield

During the essential oil extraction process, several factors can influence both the yield and quality of the obtained oil, such as the type of raw material, solvent, temperature, and extraction time. However, in this study, we focused on examining two main factors: extraction time and the ratio of raw material to solvent (water).

Ratio of Raw Material to Water

The ratio between the mass of raw material and the volume of solvent (water) is a key factor affecting extraction efficiency. If the ratio of raw material is too low- meaning the amount of plant material is insufficient to provide a significant amount of essential oil- the yield will be low. Conversely, if too much raw material is used compared with the volume of solvent, the plant material may not be adequately exposed to the solvent, reducing the ability of the essential oil to diffuse into the extraction medium.

An appropriate ratio helps optimize the diffusion process of essential oil from plant tissues into the solvent, thereby increasing the extraction efficiency. The selection of a suitable ratio should be carefully considered based on the characteristics of the material, the extraction method, and the goal of maximizing oil recovery.

Table 1. Essential oil yield at different raw material/water ratios

Raw material/water ratio (g/mL)	1/9	1/11	1/13	1/15
Essential oil content (%)	0.213	0.265	0.269	0.214

The results presented in Table 1 indicate that the water-to-material ratio has a significant impact on the essential oil yield obtained from the distillation process. Specifically, when the water-to-material ratio increased from 1/9 to 1/13 (g/mL), the essential oil yield markedly rose from 0.213% to 0.269%, which represents the highest value among the experimental conditions. This finding suggests that increasing the solvent volume to an appropriate level enhances the diffusion and release of essential oils from plant cells into the solvent, thereby improving extraction efficiency. However, when the ratio continued to increase to 1/15, the essential oil yield slightly decreased to 0.214%. This reduction may be attributed to the excessive dilution of the mixture, which lowers the efficiency of contact between the solvent and the plant material, and may also cause difficulties in recovering the total amount of essential oil due to dispersion.

Therefore, within the scope of this study, a water-to-material ratio of 1/13 (g/mL) was determined as the optimal condition for achieving the highest essential oil yield from *Syzygium nervosum* leaves.

Extraction time is an important factor that directly affects the recovery efficiency of essential oils from plant materials. If the extraction time is too short, the essential oils may not be completely released from the plant cells, resulting in a lower yield. Conversely, an excessively long extraction period may lead to the degradation or evaporation of some volatile compounds due to prolonged exposure to high temperatures, thereby reducing the oil quality and causing the loss of valuable constituents. Therefore, determining the optimal extraction time is essential to balance extraction efficiency and oil quality. In general, the extraction time is typically selected within the range of 30 to 120 minutes, depending on the extraction method and the characteristics of the raw material.

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Table 2. Essential oil yield obtained at different distillation times

Distillation time (h)	0.5	1.0	1.5	2.0	2.5	3.0
Essential oil content (%)	0.073	0.147	0.198	0.285	0.257	0.224

The results presented in Table 2 indicate that distillation time is a crucial factor affecting the extraction efficiency of essential oil from *Syzygium nervosum* leaves. The essential oil yield increased progressively during the initial phase of the distillation process. Specifically, as the distillation time increased from 0.5 h to 2.0 h, the essential oil yield rose from 0.073% to a maximum value of 0.285%. This trend suggests that, during this period, the cellular structures containing essential oil were effectively disrupted by heat, facilitating the release and recovery of the volatile components.

However, beyond 2.0 h, when the distillation time was further extended to 2.5 and 3.0 h, the essential oil yield showed a slight decline, dropping to 0.257% and 0.224%, respectively. This reduction may be attributed to two main causes: (i) partial degradation or evaporation of thermolabile and highly volatile compounds under prolonged heating, and (ii) limited additional oil release at extended times, while losses due to vaporization or adsorption on the apparatus surfaces become more significant.

Based on these findings, a distillation time of 2.0 hours was determined to be the optimal condition under the experimental setup, ensuring the highest essential oil yield while minimizing unnecessary energy consumption and product loss.

Essential oil was extracted from the leaves and stems of *Cinnamomum parthenoxylon* via hydrodistillation, with a 2.0% yield (w/w, based on fresh weight). The total ion chromatogram obtained from GC-MS analysis is shown in Figure 4.

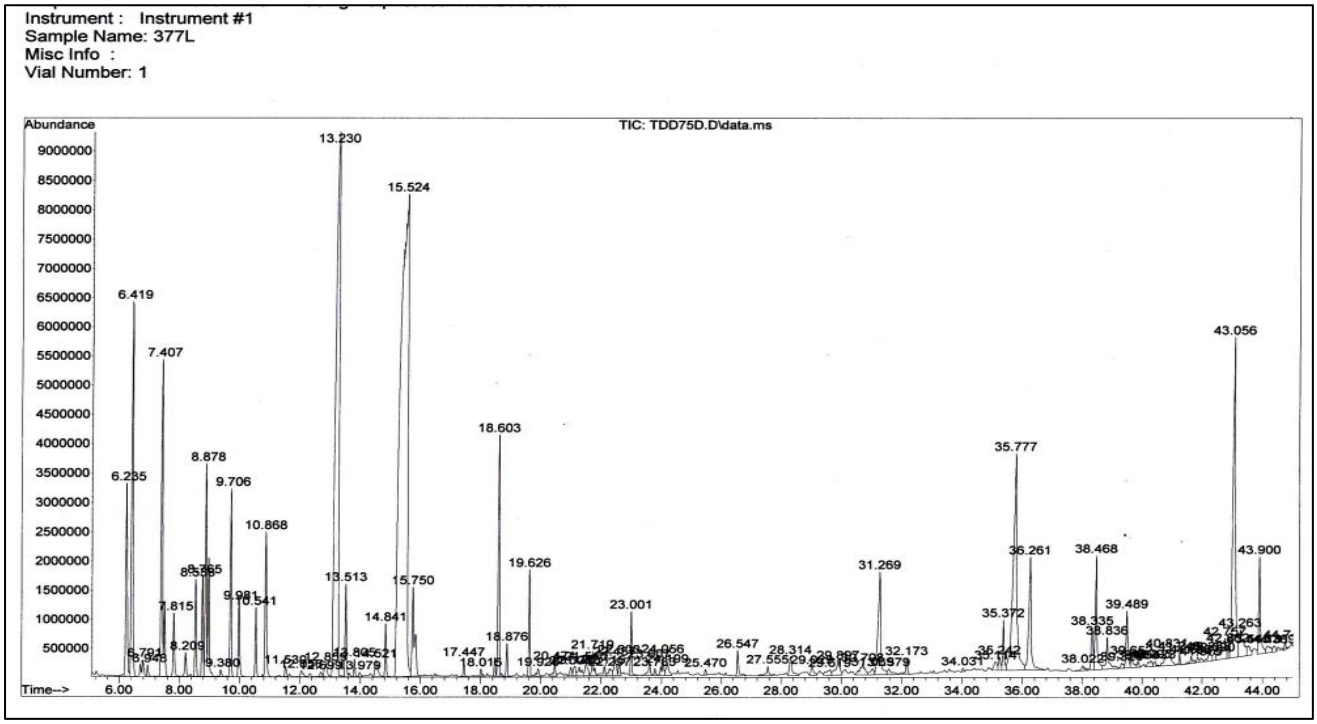

Figure 2. GC-MS total ion chromatogram of *Cinnamomum parthenoxylon* essential oil.

The wood and rhizome of *Cinnamomum parthenoxylon* contain about 1–2% of a light-yellow essential oil with a mild and pleasant aroma. The main constituents of the leaf essential oil include benzyl benzoate (29.4%), benzyl salicylate (24.3%), sabinene (16.2%), and farnesol (5.3%), accounting for approximately 60–75% of the total composition. This species is considered a valuable source of essential oil with high economic value, as its oil is among the most expensive in the market. The wood of *C. parthenoxylon* is classified as a rare and precious timber, commonly used in the production of statues, fine handicrafts, and high-end interior decorations, with prices ranging from 15 to 20 million VND per cubic meter. Due to overexploitation, natural populations of this species have become severely depleted, making it a scarce natural source of essential oil.

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Antioxidant Activity of Cinnamomum parthenoxylon Essential Oil

The antioxidant activity was evaluted by the DPPH radical scavenging assay, with the results summarized in Table 3. The IC_{50} value of *Cinnamomum parthenoxylon* essential oil was determined to be 5.95 ± 0.2µg/mL, compared to 6.1 ± 0.1 µg/mL for BHT, a well-known synthetic antioxidant. These results indicate that the essential oil of *Cinnamomum parthenoxylon* collected in Thanh Hoa has strong antioxidant activity comparable to BHT, highlighting its potential as a natural antioxidant agent.

Table 3. Antioxidant activity of *Cinnamomum parthenoxylon* essential oil

Essential oil (mg/mL)	% Inhibition			IC_{50} (mg/mL)
	1	2	3	
30	96.30	95.41	95.56	5.95 ± 0.2
15	68.21	66.25	65.51	
7.5	52.35	51.91	54.89	
3.75	45.19	47.04	43.49	
1.875	40.28	41.39	42.61	
BHT[a]				6.10 ± 0.1

[a]Butylated hydroxytoluene was used as positive control

CONCLUSION

This study is the first to investigate the essential oil from the leaves of *Cinnamomum parthenoxylon* collected in Thanh Hoa, Vietnam. GC-MS analysis identified a diverse chemical composition. The major constituents were benzyl benzoat (29.4%), benzyl salicylate (24.3%), sabinen (16.2%), and farnesol (5.3%). The essential oil exhibited notable biological activities, demonstrating strong antioxidant activity with an IC_{50} value of 5.95 ± 0.2µg/mL, surpassing the reference compound, BHT (6.1 ± 0.1 µg/mL). These findings underscore the potential of *Cinnamomum parthenoxylon* essential oil as a natural resource for pharmaceutical applications, particularly in antioxidant.

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Source of support: Nil; **Conflict of interest:** Nil.

Cite this article as:

Luong, N. X. "Chemical Composition and Antioxidant Activity of (*Cinnamomum parthenoxylon* (Jack.) C.Nees) Essential Oil." *Sarcouncil Journal of Biomedical Sciences* 4.5 (2025): pp 7-12.

Publisher: SARC Publisher