

Seasonal chemical composition and antioxidant activity of the essential oil from *Cinnamomum zeylanicum* Nees leaves in a Brazilian Cerrado region

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Abstract

Cinnamomum zeylanicum is an aromatic species with essential oil in its leaves, stems, and roots, widely used in traditional medicine and culinary applications. The essential oil of *C. zeylanicum* exhibits various biological activities, such as antioxidant and antifungal properties. This study aimed to evaluate the essential oil extracted from the leaves of *C. zeylanicum* collected during two seasonal periods in the Brazilian Cerrado, focusing on its volatile chemical profile, and antioxidant, and antifungal activities. Leaves of *C. zeylanicum* were collected in July and December 2023. The essential oil was extracted through hydro distillation. The volatile chemical profile was determined by gas chromatography-mass spectrometry (GC-MS), antioxidant activity was assessed by DPPH free radical reduction, and antifungal activity was evaluated based on the inhibition of *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides*, and *C. acutatum*. Two major compounds were identified: eugenol (72% and 63%) and eugenyl acetate (9% and 15%) for the dry and hot seasons (July) and the wet and hot seasons (December), respectively. DPPH free radical reduction showed maximum inhibition of 89% and 65% for July and December, respectively. Antifungal activity demonstrated maximum inhibition rates of 65% and 53% for *S. sclerotiorum*, 82% and 71% for *C. gloeosporioides*, and 56% and 27% for *C. acutatum* during the same periods. This study highlights seasonal variations in the major compounds of essential oil, which influenced its production, DPPH radical scavenging activity, and inhibition of the evaluated fungal strains.

Keywords: *Cinnamomum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*.

Composição química sazonal e atividade antioxidante do óleo essencial das folhas de *Cinnamomum zeylanicum* Nees em área de Cerrado do Brasil

Resumo

Cinnamomum zeylanicum é uma espécie aromática que possui óleo essencial nas folhas, caule e raízes muito utilizada na medicina tradicional e na culinária. O óleo essencial de *C. zeylanicum* apresenta diversas atividades biológicas como antioxidante e antifúngica. Esse estudo teve por objetivo avaliar o óleo essencial foliar de *C. zeylanicum* coletado em dois períodos sazonais em área de Cerrado brasileiro e avaliar o perfil químico dos voláteis e quanto a sua atividade antioxidante e antifúngica. Folhas de *C. zeylanicum* foram coletadas em Julho e Dezembro de 2023. O óleo essencial foi extraído por hidrodestilação. O perfil químico dos voláteis foi determinado por cromatografia gasosa com emissão de massas (CG-MS), a atividade antioxidante determinada pela redução do radical livre DPPH e a atividade antifúngica na inibição de *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* e *C. acutatum*. Foram encontrados dois compostos majoritários eugenol com 72% e 63% e o acetato de eugenil com 9% e 15%, respectivamente para o período seco e quente (Julho) e úmido e quente (Dezembro); 89% e 65% na redução do radical livre DPPH para os meses de Julho e Dezembro e inibição máxima de 65% e 53% para *S. sclerotiorum*, 82% e 71% para *C. gloeosporioides* e de 56% e 27% para

C. acutatum para os meses de Julho e Dezembro respectivamente. Este estudo demonstrou que houve variação entre os compostos majoritários devido a sazonalidade sobre a coleta e produção de óleo essencial, bem como, sobre a redução do radical livre DPPH e inibição das cepas fúngicas avaliadas.

Palavras-chave: *Cinnamomum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Sclerotinia sclerotiorum*.

1. Introduction

Cinnamomum zeylanicum is a perennial, subtropical, and tropical plant species from the Lauraceae family, native to India, Sri Lanka, Indochina, and Madagascar. It is widely used in natural medicine, cooking, and as a flavoring agent. Commonly known as "cinnamon", its bark, branches, and leaves have been used by the Chinese for over 4,000 years to treat various ailments (Wong et al., 2014).

Cinnamon exhibits numerous biological activities. This is primarily due to the essential oil's (EO) chemical composition, which determines its industrial value. The EO of *C. zeylanicum* contains various oxygenated and hydrocarbon compounds. The bark is rich in trans-cinnamaldehyde (70–90%), which has strong antibacterial, antifungal, antioxidant, and antimutagenic properties (Behbahani et al., 2020), as well as anti-Alzheimer, antidiabetic, and therapeutic effects for respiratory, digestive, and gynecological diseases (Tepe; Ozaslan, 2020; Shen et al., 2002; Gruenwald et al., 2010).

In addition, EO can be obtained from the leaves, which contain valuable volatile compounds such as (E)-cinnamaldehyde, (E)-cinnamyl acetate, and eugenol. The roots also provide essential oil with a high camphor content (Paranagama et al., 2010; Ranasinghe et al., 2013). EOs from the bark, leaves, and roots are extensively used in the food industry to flavor cereals, grain- and fruit-based dishes, and beverages, as well as in natural insecticides (Cheng et al., 2009, 2011; Tung et al., 2010; Ribeiro et al., 2020).

As demonstrated, *C. zeylanicum* EO exhibits antibacterial and antifungal properties and has significant potential as a free radical scavenger. Its antioxidant activity involves neutralizing free radicals, preventing damage to various oxidizing molecules that could degrade DNA and RNA in both animals and plants. This activity is attributed to specific groups of secondary metabolites, such as phenolic and polyphenolic compounds (Ranjbar et al., 2006; Ribeiro et al., 2020).

Over 300 volatile compounds have been identified in cinnamon EOs, many of which show positive effects against a wide range of fungal and bacterial strains. These pathogens annually cause severe human and animal health issues and significant agricultural losses, particularly in stored foods (Dongmo et al., 2007). Fungal problems create major economic challenges throughout the agricultural production chain, from grain cultivation—such as white mold (*Sclerotinia sclerotiorum*) (Hossain et al., 2023), which rots soybean stems—to fresh produce like strawberries, bananas, and papayas, which are attacked by spoilage fungi causing anthracnose (*Colletotrichum gloeosporioides* and *Colletotrichum acutatum*) (Dowling et al., 2020), among others. This study aimed to evaluate the chemical profile of *Cinnamomum zeylanicum* leaf essential oil collected during two seasonal periods in the Brazilian Cerrado region.

2. Materials and Methods

2.1 Reagents

2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Taiwan), acetone (Alphatech, Brazil), dimethyl sulfoxide (Dinâmica, Brazil), ethanol (Dinâmica, Brazil), potato dextrose agar (PDA) medium (Dinâmica, Brazil), quercetin (Sigma-Aldrich, USA).

2.2 Speciem collection and identification

Fresh leaves of *C. zeylanicum* were collected in July and December 2023 at Fazenda Antônio Menezes, Rio Verde, Goiás, Brazil, and identified at the Systematics and Herbarium Laboratory of the Federal Institute of Goiás, Rio Verde, Goiás, Brazil, where the voucher specimens are deposited (HRV 15901).

2.3 Essential oil extraction

The leaves were steam-distilled for about 6 h using a Clevenger apparatus. According to Dongmo et al. (2007),

the oils recovered were dried over anhydrous sodium sulfate and stored at -8 °C until used.

2.4 Chemical profile by gas chromatography and mass spectrometry (GC-MS)

GC-MS also evaluated the composition of the essential oil. In this experiment a PerkinElmer GC Clarus 580 equipment coupled with MS Clarus SQ 8S was used, the injector, interface, and source temperatures were 250, 270, and 270 °C respectively, using electron impact at 70 eV. The analysis was performed using the following heating method, 60 °C for 2 min., rising to 270 °C at a ratio of 12 °C min⁻¹ and remaining for 1 min, Helium gas flow was 1 mL min⁻¹, 1 µL of sample dissolved in Ketone was injected. The column used was a DB-5MS (30 m, 0.25 mm ID, 0.25 µm). The compounds were identified from the n-alkane calibration standard series (C₇-C₄₀), and the mass spectrum was compared to the literature of Adams (2007) and by the Nist 11 Spectroteca.

2.5 Antioxidant activity using the DPPH method

The antioxidant activity assay was performed as described by Ribeiro et al. (2020) using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction method. Briefly, an EO sample was dissolved in ethanol at various concentrations (8, 20, 40, 80, and 100 µg mL⁻¹), and a DPPH solution in ethanol at 60 µM was subsequently prepared. The samples were prepared by mixing 50 µL of each concentration with 1950 µL of the DPPH solution. A control solution, with the same volume as the EO sample, was prepared by replacing 50 µL of ethanol for the EO sample. The samples were read using a UV-Vis spectrophotometer (BelPhotonics, Modl. M-51) at 517 nm after 30 min, kept in a dark place at a temperature of 20 °C. The DPPH inhibition percentage was calculated using Equation (1):

$$\% I = (S_a - C_a)/S_a * 100 \quad \text{Eq. (1)}$$

Where: S_a = absorbance of the sample, and C_a = absorbance of the control.

2.6 Antifungal activity

The antifungal assay was performed using isolates of *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides*, and *C. acutatum*, maintained on potato dextrose agar (PDA) medium. The antifungal activity of *C. zeylanicum* leaf EO on the mycelial growth of *S. sclerotiorum*, *C. gloeosporioides*, and *C. acutatum* was evaluated at different concentrations, starting from 100 (pure oil), 50, and 25 µL mL⁻¹. As a negative control, the absence of essential oil was used, along with the fungicide Frownicide500 SC[®] at a concentration of 10 µL mL⁻¹ and dimethyl sulfoxide (DMSO) as a positive control.

The EO concentrations were added to the PDA culture medium after sterilization and cooling, as well as the treatments with commercial fungicide and DMSO. After solidification of the medium, a 7 mm mycelial disc from each *S. sclerotiorum*, *C. gloeosporioides*, and *C. acutatum* strain was placed in the center of a 10 cm diameter Petri dish. They were then incubated at a temperature of 26 °C, as described by Garcia et al. (2012). The evaluation consisted of daily measurements of the colony diameter using a manual caliper, starting 24 h after incubation began and continuing until the fungal colonies in the control treatment completely covered the inner area of the plate. The percentage of growth inhibition (PGI) was determined according to Equation (2):

$$\text{PGI} = (\text{DCT} - \text{DCTr})/\text{DTT} * 100 \quad \text{Eq. (2)}$$

Where: PGI = percentage of growth inhibition, DCT = diameter in the control treatment, and DCTr = diameter in the chemical treatment.

2.7 Statistical analysis

Analysis was carried out in triplicate and the standard deviations (SD) among them were calculated. The data were collected and represented as the standard error of the mean. Following ANOVA, a Tukey's test was used to examine the statistical relevance of the mean differences at 5%, using Sisvar statistical software (Ferreira et al., 2019).

3. Results

3.1 Chemical profile of the essential oil

A total of 21 volatile compounds were identified in the EO of *C. zeylanicum* collected during two seasonal periods (Table 1). The major compounds were Eugenyl acetate, accounting for 9% and 15%, and Eugenol, representing 72% and 63%, for July and December, respectively, with a total area of 98%.

Table 1. Chemical profile of the essential oil from *Cinnamomum zeylanicum* leaves collected in two seasonal periods in a Brazilian Cerrado region.

Compound	Relative abundance (%)*	Relative abundance (%)**
α -Pinene	1.32	1.47
Camphene	0.60	0.35
Sabinene	0.11	0.00
α -Phellandrene	0.14	0.20
Myrcene	0.07	0.11
α -Terpinene	0.21	0.12
Linalool	0.16	0.05
<i>Trans</i> -Cinnamaldehyde	0.31	0.40
Terpinen-4-ol	0.34	1.11
α -Terpineol	0.31	0.30
Sabinol	0.18	0.22
1,8-Cineole	0.23	0.14
δ -Cadiene	0.76	2.90
(<i>Z</i>)-Cinnamyl acetate	1.14	4.16
Eugenyl acetate	9.57	15.59
Eugenol	72.15	63.57
α -Ylangene	1.03	0.15
β -Caryophyllene	6.28	4.88
Elemol	1.76	0.90
T-Cadinol	0.14	0.31
Benzyl benzoate	1.32	1.36
Total	98.13	98.29

Note: Analyses were carried out in July* and December**.

3.2 Antioxidant activity

Table 2 presents the values obtained for antioxidant activity in the reduction of the DPPH radical for different concentrations of *C. zeylanicum* leaf EO collected in two seasonal periods in the Brazilian Cerrado region. DPPH radical inhibition values were obtained for the EO collected in July, with a maximum inhibition activity of 89%. All the results showed strong DPPH radical reduction activity, although lower than the quercetin standard.

Table 2. Antioxidant activity in the reduction of the DPPH free radical by the essential oil of *Cinnamomum zeylanicum* leaves collected in two seasonal periods in the Cerrado region of Brazil.

$\mu\text{g mL}^{-1}$	Antioxidant activity (DPPH)		
	(% Inhibition activity)		
	July	December	Standard*
8	21.55 ± 0.63b	18.14 ± 0.30c	30.15 ± 0.10a
20	33.05 ± 0.21b	26.90 ± 0.18c	48.88 ± 0.11a
40	58.46 ± 0.34b	39.08 ± 0.71c	63.47 ± 0.92a
80	78.10 ± 0.50b	57.33 ± 0.96c	90.18 ± 0.44a
100	89.11 ± 0.65b	65.13 ± 0.90c	98.90 ± 0.19a

Note: *Quercetine. Means compared between lines using the standard. Means followed by different letters differ from each other according to the *Tukey* test with 5% probability. Source: Authors, 2024.

3.3 Antifungal assay

Table 3 presents the values obtained for the antifungal activity of *C. zeylanicum* EO collected in two seasonal periods. At the highest concentration of 100 $\mu\text{L mL}^{-1}$, the highest mycelial inhibition rates were observed, with 65% and 53% for *S. sclerotiorum*, 82% and 71% for *C. gloeosporioides*, and 56% and 27% for *C. acutatum*, respectively. All results were lower than the standard, which showed 100% inhibition *in vitro*.

Table 3. Antifungal activity of the essential oil from *Cinnamomum zeylanicum* leaves collected in two seasonal periods in the Brazilian Cerrado region.

Microorganism	Antifungal activity ($\mu\text{L mL}^{-1}$)					
	Inhibition Zone (mm)					
	100*	50*	25*	100**	50**	25**
<i>S. sclerotiorum</i>	65.15b	47.48c	37.07d	53.37b	31.11c	16.04d
<i>C. gloeosporioides</i>	82.56b	78.04c	54.44d	71.00b	45.07b	18.90c
<i>C. acutatum</i>	56.00b	36.85c	22.11d	27.77b	21.05cb	13.33d

Note: Analyses were carried out in July* and December**. The commercial antifungal Frowncide 500 SC[®] = 100% mycelial inhibition. Means followed by the same letter in the same row do not differ according to Tukey's test with 5% probability. Source: Authors, 2024.

4. Discussion

In a plant species, the volatile chemical composition of the essential oil can vary in terms of both the quantity extracted and its chemical constitution. Several studies show that climate, solar radiation, soil types, and regions affect these factors (Zouari et al., 2012; Sá Filho et al., 2022). Furthermore, different chemotypes within the same plant group can undergo drastic variations in their volatile chemical composition and other phytochemicals in the special metabolism (Zhang et al., 2023). Regarding the chemical profile of the essential oil from *C. zeylanicum* leaves collected in two seasonal periods (hot and dry) and (hot and humid), this study observed two major compounds: eugenol and eugenyl acetate.

However, when comparing our values with the literature, Ribeiro et al. (2020) found different results for the EO from *C. zeylanicum* leaves collected in the municipality of Boa Vista, Roraima state (Brazil). In that study, the authors found the major compounds to be benzyl benzoate (74.2%) and α -phellandrene (6.9%). In another study, Dongmo et al. (2007) found eugenol (89.1%) and benzyl benzoate (3.1%) as the major compounds in the EO from *C. zeylanicum* leaves collected in Cameroon, Africa. This variation in values is correlated with the biotic and abiotic factors discussed previously.

Regarding antioxidant activity in the reduction of DPPH, the EO from *C. zeylanicum* leaves collected in two seasonal periods demonstrated strong reduction activity of this free radical, even when compared to quercetin as

the analytical purity reference. Ribeiro et al. (2020) found results similar to ours, with a high DPPH reduction capacity for EO from the stems and leaves of *C. zeylanicum*, showing maximum activities of 61.34% and 59.17%, respectively. We can suggest that the antioxidant potential of DPPH is related to the major compounds found; however, Wang et al. (2008) suggest that it is difficult to attribute the antioxidant effect of essential oil to a particular volatile constituent, as both major and minor compounds can significantly contribute to the reducing capacity in different free radical models such as DPPH, FRAP, and ABTS⁺. Behbahani et al. (2020) found significant results in the reduction of the DPPH free radical using the EO from the stem bark of *C. zeylanicum* as the antioxidant agent, with 71.12% inhibition. Dongmo et al. (2007) found the antioxidant activity of 4.5 mg L⁻¹ for the EO from *C. zeylanicum* leaves, compared to 7 mg L⁻¹ for the antioxidant standard butylated hydroxytoluene (BHT), demonstrating a special and important antioxidant activity against DPPH.

The EO from *C. zeylanicum* leaves extracted in two seasonal periods demonstrated strong antifungal activity at all tested concentrations. However, it was inferior to the control with the synthetic commercial antifungal. Although there was a statistical difference when compared to the antifungal standard, EO remains an excellent alternative option to minimize the fungal activity of the tested microorganisms. We suggest that to complement the antifungal action, a higher dose of EO and a lower dose of the standard antifungal agent should be used. This approach would result in savings on the acquisition of the standard antifungal, reducing the economic cost of these products (Van Vuuren et al., 2009; Serra et al., 2018).

Results evaluating the synergism between essential oils, plant extracts, and antifungal standards have already been tested with promising outcomes. In other studies, Tran et al. (2020) found significant inhibition activity in the development of *Candida albicans* and *Candida auris* with EO from the leaves and bark of *C. zeylanicum* acquired from the European market. Behbahani et al. (2020) described a high antibacterial potential against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Listeria innocua*, *Staphylococcus aureus*, and *Bacillus cereus* using the EO from the trunk bark of *C. zeylanicum* as the antibacterial agent. Dongmo et al. (2007) found that the EO from *C. zeylanicum* leaves collected in Cameroon, Africa, showed strong antifungal activity against *Aspergillus flavus* and *Fusarium moniliforme*, fungi that deteriorate stored food. Ranasinghe et al. (2002) reported in their study that the EO of *C. zeylanicum* exhibited strong antifungal activity against anthracnose isolates in bananas. The essential oil significantly inhibited the development of *Colletotrichum musae*, *Lasiodiplodia theobromae*, and *Fusarium proliferatum*.

5. Conclusions

The essential oil from *Cinnamomum zeylanicum* leaves collected in two seasonal periods showed variation in the volatile chemical profile, potential antioxidant activity using the DPPH free radical model, and antifungal activity against food spoilage fungi in raw products, stored grains, and soybean culture. We suggest that new biological assays be conducted to investigate the potential of using *C. zeylanicum* essential oil produced in different periods throughout the year to obtain similar results that could influence decision-making regarding the content of major compounds in the essential oil and their oscillatory effects on biological activities.

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7. Authors' Contributions

Lorena Gonçalves Lima: study development, sample collection, laboratory analyses, and article writing. *Jéssica Ferreira Sousa*: study development, laboratory analyses, article writing, and final revisions. *Antonio Carlos Pereira de Menezes Filho*: laboratory analyses, article writing, submission, final revisions, and publication. *Matheus Vinícius Abadia Ventura*: translation and final revisions. *Elizabeth Nunes da Rocha*: supervisor, final revisions, and publication.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

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