



GC-MS PROFILE AND CHEMOPROTECTIVE EFFECT OF *Cinnamomum zeylanicum* BARK ESSENTIAL OIL AGAINST TRIPLE-NEGATIVE BREAST CANCER CELL LINE MDA-MB-231

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ABSTRACT

Cinnamomum zeylanicum Blume (Lauraceae) bark is one of the most important spices used to enhance the taste and flavour of Indian foods. *C. zeylanicum* bark, besides its use as a spice, has potential antioxidants, antimicrobial, antifungal, anti-inflammatory, and chemoprotective potential. The present study was aimed to characterize key chemical constituents of *C. zeylanicum* bark essential oil and determine its chemoprotective effect against triple-negative breast cancer cells (TNBC). The chemical composition of *C. zeylanicum* bark essential oil was determined by gas chromatography mass spectroscopy (GC-MS). The analysis identified 29 chemical compounds, amongst which (*E*)-cinnamaldehyde (69.89%), eugenol (14.27%), δ -cadinene (2.80%), and (*Z*)-caryophyllene (2.33%) were the key constituents. *C. zeylanicum* bark essential oil produced concentration- and time-dependent inhibition of TNBC (MDA-MB-231) cells. *C. zeylanicum* bark essential oil at 15.62 $\mu\text{L mL}^{-1}$ concentration produced maximum inhibition of MDA-MB-231 cells which was about 90.6%. The aroma profile of *C. zeylanicum* bark essential oil and *in vitro* anticancer activity provide a scientific basis for its possible use as a cancer remedy.

Keywords: Aroma profile, breast cancer, *Cinnamomum zeylanicum*, cinnamon, essential oil, TNBC

INTRODUCTION

Cinnamon has been used as a culinary herb and spice since ancient times due to its pleasant aroma and sweet taste (Rao and Gan, 2014). The *Cinnamomum* genus consists of many species, with *C. zeylanicum* (*C. verum*), *C. cassia*, *C. loureiroi*, and *C. burmannii* as prominent species (Ranasinghe *et al.*, 2013). *Cinnamomum zeylanicum* Blume (family: Lauraceae) is preferred as a spice due to its characteristic aroma and taste. *C. zeylanicum* bark is a rich source of bioactive essential oil compounds responsible for its aroma (Kazemi and Mokhtariniya, 2016; Anand *et al.*, 2016). *C. zeylanicum* essential oil has been reported as a potent antioxidant, antimicrobial, and antifungal (Jayaprakasha *et al.*, 2003; Alizadeh Behbahani *et al.*, 2020; Tran *et al.*, 2020). Many studies have shown that *C. zeylanicum* essential oil has a potential chemoprotective effect against certain types of human cancer (Varalakshmi *et al.*, 2014; Aggarwal *et al.*, 2022; Cappelli *et al.*, 2023). *In vitro* and *in vivo* studies have shown the potential of *C. zeylanicum* essential oil against breast cancer cell lines (Abd Wahab *et al.*, 2017; Kubatka *et al.*, 2020).

Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer with a high mortality rate. Traditional chemotherapy and hormonal therapies are ineffective against TNBC because this type of breast cancer lacks estrogen, progesterone, and HER2 receptors (Foulkes *et al.*, 2010; Ahmad *et al.*, 2024). A thorough literature review has revealed that *C. zeylanicum* essential oil is effective against

many types of human cancers, including breast cancer. In recent decades, preclinical studies have shown that essential oils from many plants have potential against TNBC (Zito *et al.*, 2019; Lauricella *et al.*, 2022; Zhang *et al.*, 2022; Benedetti *et al.*, 2023;). Hameed *et al.* (2016) have explored the chemical composition of the methanolic extract of *C. zeylanicum* bark by GC-MS and reported the presence of many volatile and non-volatile chemical constituents but did not explore the major well-known chemical compounds of *C. zeylanicum* bark, like (*E*)-cinnamaldehyde, eugenol, benzaldehyde, and (*Z*)-caryophyllene. Another GC-MS study by Al-fekaiki *et al.* (2017) on the essential oil of *C. zeylanicum* bark from Basra (Iraq) also did not explore its major chemical compounds, and the results showed that the essential oil consists of cinnamaldehyde (57.83%), cyclohexane carboxylic acid (9.29%), and 6-octadecanoic acid (8.41%). The above studies did not accurately explore the chemical composition in the essential oil of *C. zeylanicum* bark as reported by many workers (Unlu *et al.*, 2010; Alizadeh Behbahani *et al.*, 2020). Hence, the present study was designed to characterize the aroma constituents present in the essential oil of *C. zeylanicum* bark available in Erbil (Iraq). The present study aimed to determine the key chemical constituents using GC-MS and evaluate the cytotoxic activity of the essential oil of *C. zeylanicum* bark against MDA-MB-231 TNBC cell lines.

MATERIALS AND METHODS

Plant material and chemicals

Cinnamomum zeylanicum bark (500 g) was procured in April 2024 from Majidi Hyper Market, Erbil, Kurdistan (Iraq). The plant sample was authenticated based on its organoleptic characteristics. *C. zeylanicum*, commonly called Ceylon cinnamon, has a sweet taste and characteristic aroma as compared to *C. cassia*, which has a slightly bitter taste, and a voucher specimen of plant sample was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Tishk International University, Erbil, Iraq (PRL/2025/03). Triple-negative breast cancer (MDA-MB-231) cell lines were purchased from NCCS, Pune (India). Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), and antibiotic solution were obtained from Gibco (USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-zolium bromide) at 5 mg mL⁻¹ concentration was purchased from Sigma (USA); dimethyl sulfoxide from Himedia (India). Wash beakers and 96-well tissue culture plates were procured from Tarson (India).

Isolation of essential oil

For isolation of essential oils from *C. zeylanicum* bark, hydro-distillation method was followed (Ahamad *et al.*, 2019). Small pieces of *C. zeylanicum* bark (250 g) were kept with distilled water in a Clevenger apparatus. The apparatus was run for 4 to 6 h to isolate maximum essential oils. After separation of essential oils from bark, the oil was collected in a glass bottle and passed through anhydrous sodium sulphate to remove the traces of water. The pure essential oil was stored at 4°C till further use.

GC-MS analysis and identification of chemical constituents

The bench-top Agilent GC-MS (Agilent Technologies, USA) was used to analyse the key chemical constituents present in *C. zeylanicum* bark. The essential oil was analysed on a non-polar DB-G glass column (30 m length × 0.25 mm internal diameter and 0.25 µm film thickness), and helium was used as carrier gas @ 1 mL min⁻¹. The operating temperature of oven was kept at 50°C for 1 min and then isothermally kept for 2 min at 320°C, and the injector port temperature was set at 280°C. Split ratio of 1:5, data was captured at 70 eV, and scanning time was 1.5 sec. The chemical compounds were monitored in mass range of 50 to 1000 atomic mass units, and the total run time was 42 min. The ChemStation (Agilent Technologies, USA) was used to monitor all the processes. For identification of individual peaks (chemical constituents), the data from GC-MS was compared with the reported Kovats index (Unlu *et al.*, 2010; Alizadeh Behbahani *et al.*, 2020) and with mass fragmentation patterns obtained by GC-MS analysis, and compared with those stored in databases like NIST, Wiley8 libraries, and published literature (Unlu *et al.*, 2010; Babushok *et al.*, 2011; Alizadeh Behbahani *et al.*, 2020).

Determination of cell viability by MTT assay

The cell viability assessed was performed by MTT assay as per Ahamad (2024) with slight modification. Briefly, the MDA-MB-231 cells were cultured in RPMI 1640 medium, and the medium was supplemented with 10% FBS and 1% penicillin. Cells were cultured at 37°C in a humidified 5% CO₂ incubator. For cell viability, cells were seeded in 96-well plates (10⁴ cells well⁻¹) and incubated at 37°C. After 24 h, the cell culture medium was removed and replaced with fresh medium containing different concentrations (0.98 to 500 µL mL⁻¹) of *C. zeylanicum* essential oils, which had previously dissolved in DMSO. After 24 h exposure, MTT solution (5 mg mL⁻¹ PBS) was added to each well. After 4 h, the formazan crystals were dissolved in DMSO, and optical density was read using a plate reader at 570 nm. GraphPad Prism (version 8) was used for statistical analysis and plotting data. Data is presented as mean ± standard deviation. One-way ANOVA was used to examine the significance between different groups, followed by Tukey's post hoc test. *p*-values < 0.05 were considered statistically significant (for *p* > 0.05; **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001).

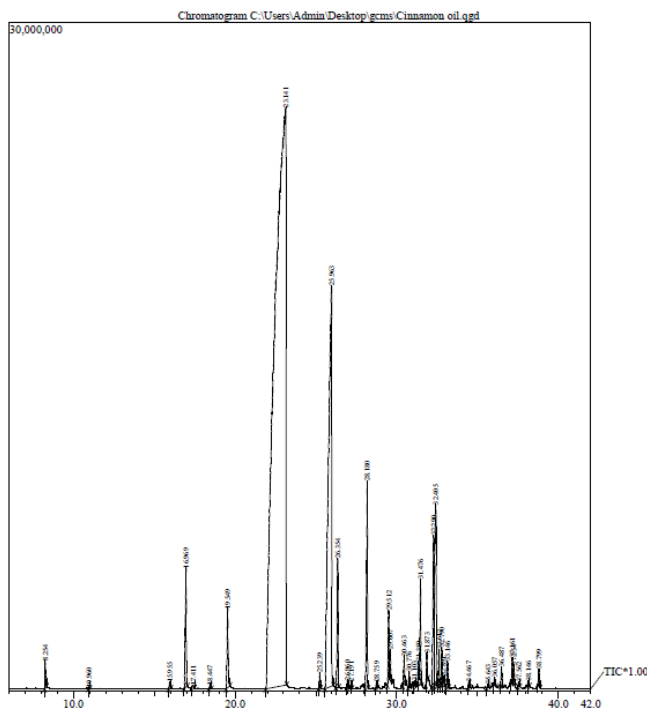
RESULTS AND DISCUSSION**GC-MS profile of *Cinnamomum zeylanicum* essential oil**

The *C. zeylanicum* bark essential oil was analysed by GC-MS and revealed the presence of 29 chemical constituents (Table 1; Fig. 1). GC-MS analysis enabled the identification of 99.4% of the chemical

Table 1: Chemical composition of *C. zeylanicum* bark essential oils determined by GC-MS

S. No.	Chemical constituents	M.F.	R.T. (min)	K.I.	Content (%)
1.	Benzaldehyde ^f	C ₇ H ₆ O	8.254	936	0.22
2.	2-Methyl-1-benzofuran ^f	C ₉ H ₈ O	15.955	1109	0.06
3.	α-Terpineol ^c	C ₁₀ H ₁₈ O	16.969	1175	1.37
4.	(Z)-Cinnamaldehyde ^a	C ₉ H ₈ O	19.549	1182	0.94
5.	(E)-Cinnamaldehyde ^a	C ₉ H ₈ O	23.141	1268	69.89
6.	α-Terpinyol acetate ^c	C ₁₂ H ₂₀ O ₂	25.239	1334	0.13
7.	Eugenol ^a	C ₁₀ H ₁₂ O ₂	25.963	1339	14.27
8.	α-Copaene ^b	C ₁₅ H ₂₄	26.354	1375	1.31
9.	(Z)-Caryophyllene ^b	C ₁₅ H ₂₄	28.180	1407	2.33
10.	Cinnamyl acetate ^d	C ₁₁ H ₁₂ O ₂	28.759	1408	0.05
11.	cis-α-Bergamotene ^b	C ₁₅ H ₂₄	29.512	1451	0.61
12.	(E)-Caryophyllene ^b	C ₁₅ H ₂₄	29.607	1454	0.21
13.	Viridiflorene ^b	C ₁₅ H ₂₄	30.463	1488	0.26
14.	Cadina-1(6),4-diene ^b	C ₁₅ H ₂₄	30.776	1442	0.15
15.	α-Murolene ^b	C ₁₅ H ₂₄	31.103	1491	0.06
16.	β-Bisabolene ^b	C ₁₅ H ₂₄	31.359	1499	0.12
17.	γ-Cadinene ^b	C ₁₅ H ₂₄	31.476	1505	0.88
18.	γ-Bisabolene ^b	C ₁₅ H ₂₄	31.873	1511	0.27
19.	β-Curcumene ^b	C ₁₅ H ₂₄	32.290	1512	1.83
20.	δ-Cadinene ^b	C ₁₅ H ₂₄	32.405	1513	2.80
21.	Eugenyl acetate ^a	C ₁₂ H ₁₄ O ₃	32.612	1524	0.34
22.	trans-Cadina-1,4-diene ^b	C ₁₅ H ₂₄	32.790	1532	0.32
23.	(E)-α-Bisabolene ^b	C ₁₅ H ₂₄	33.146	1534	0.30
24.	Tetradecanal ^f	C ₁₄ H ₂₈ O	34.467	1594	0.05
25.	Humulol ^c	C ₁₅ H ₂₆ O	36.057	1618	0.09
26.	β-Caryophyllene alcohol ^c	C ₁₅ H ₂₆ O	36.487	1625	0.21
27.	α-Murolol ^c	C ₁₅ H ₂₆ O	37.161	1627	0.20
28.	α-Cadinol ^c	C ₁₅ H ₂₆ O	37.254	1652	0.13
29.	α-Bisabolol ^c	C ₁₅ H ₂₆ O	38.799	1668	0.15

Where MF (molecular formula), RT (retention time, minutes), and KI (Kovats index); ^aPhenylpropanoids (04), ^bSesquiterpenes (14), ^cSesquiterpenoids (05), ^dStyrenes (01), ^eMonoterpenoids (02), and ^fMiscellaneous (03).



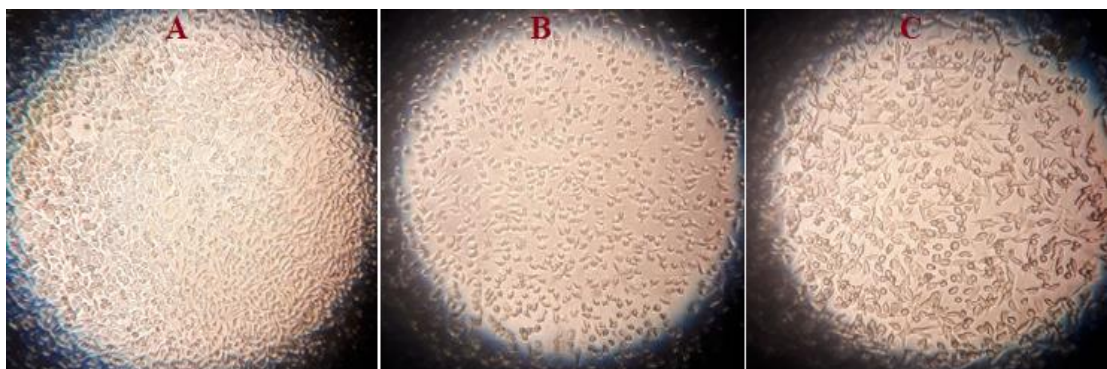


Fig. 3: Cell viability after treatment with *C. zeylanicum* bark essential oil against TNBC (MDA-MB-231) cell lines: A) Control, B) *C. zeylanicum* bark essential oil @ 3.91 µg mL⁻¹, and C) *C. zeylanicum* bark essential oil @ 15.62 µg mL⁻¹.

control. The study revealed that *C. zeylanicum* bark essential oil is a potential anticancer remedy. Our findings are in close agreement with previous studies performed on cinnamon. Abd Wahab and Adzmi (2017) showed that *C. zeylanicum* bark essential oil significantly produces cell toxicity with an IC₅₀ value of 58 µg mL⁻¹ against MCF-7 breast cancer cell lines. Husain *et al.* (2018) studied the anticancer activity of ethanolic extract of *C. zeylanicum*, and the study shows that its extract significantly inhibits cell viability of MDA-MB-231 cell lines with an IC₅₀ value of 25 µg mL⁻¹. Kubatka *et al.* (2020) explored that *C. zeylanicum* bark powder can significantly inhibit MCF-7 and MDA-MB-231 cell lines.

Conclusion: *C. zeylanicum* bark is a well-known traditional medicinal plant used as a spice and condiment. Its bark is a household remedy as it has carminative, stomachic, stimulant, and stringent properties. GC/MS analysis explores the chemical composition of *C. zeylanicum* bark available in local market of Iraq. The key constituents in essential oil were found as (*E*)-cinnamaldehyde and eugenol. The MTT assay revealed the potential chemoprotective effect of *C. zeylanicum* bark essential oil. The present study's finding on *C. zeylanicum* provides a scientific basis for further use as a possible remedy for breast cancer, especially TNBC.

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Ethical statement: In the present study, there is no use of animals or humans, so we do not need ethical approval.

Conflict of interest: The author declares that there is no conflict of interest.

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