Anticancer Potential of Cardamom (*Elettaria cardamomum*) Essential Oil Against TNBC, Glioma and Kidney Cancer *In Vitro*

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Abstract

Background: Green cardamom (Elettaria cardamomum (L.) Maton), a member of the Zingiberaceae family, is widely recognized for its use as a spice and traditional medicine. It is commonly used for treating gastric, cardiac, and kidney disorders, as well as infections and inflammatory conditions. Cardamom essential oil, known for its high content of bioactive compounds such as 1,8-cineole and α-terpinyl acetate, has been studied for its diverse biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer effects. However, little is known about its anticancer potential against MDA-MB-231 (triple-negative breast cancer), U87 (glioblastoma), and HEK 293 (kidney) cell lines, especially in the context of cardamom oil sourced from Iraq. Methods: Cardamom essential oil was extracted via hydro-distillation from cardamom fruits collected in Erbil, Iraq, and its chemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The oil's anticancer activity was evaluated using the MTT assay against MDA-MB-231, U87, and HEK 293 cell lines. The cells were treated with various concentrations of cardamom essential oil, and cell viability was measured after 24 hours. The IC50 values were calculated to determine the oil's significantly reduced cell viability in MDA-MB-231 and HEK 293 cell lines in a dose-dependent manner, with IC50 values efficacy. Results: GC-MS analysis revealed 33 compounds in the essential oil, with 1,8-cineole (42.37%) and α-terpinyl acetate (22.14%) being the predominant constituents. The MTT assay demonstrated that cardamom essential oil of 96.95 µl/ml and 157.44 µl/ml, respectively. However, the oil was less effective against U87 cells, showing only 40.67% inhibition at the highest concentration (500 µl/ml). Conclusion: Cardamom essential oil, rich in 1,8-cineole and α-terpinyl acetate, exhibited strong anticancer activity against MDA-MB-231 and HEK 293 cell lines, highlighting its potential as a therapeutic agent for triple-negative breast cancer. Further studies are needed to explore its full potential and mechanisms of action against different cancer types.

Keywords: Cardamom essential oil, MDA-MB-231, anticancer activity, GC-MS, TNBC cells

Significance | This study determined the cardamom essential oil's bioactive components and its promising anticancer effects against TNBC, glioma and kidney cells.

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1. Introduction

Green cardamom (*Elettaria cardamomum* (L.) Maton; Family: Zingiberaceae) is a widely recognized spice and condiment, commonly used to enhance the flavor and aroma of various food products. Beyond its culinary applications, cardamom has long been valued in traditional medicine for its therapeutic benefits in treating gastric, heart, and kidney disorders, as well as conditions such as teeth and gum infections, asthma, cataracts, nausea, and diarrhea (Ashokkumar et al., 2020; Kumar & Kumari, 2021). The medicinal properties of cardamom are primarily attributed to its rich array of bioactive compounds, with essential oils being the

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dominant constituents. Other important compounds found in cardamom include lipids, resins, and flavonoids.

Cardamom essential oil is particularly rich in several key components, including 1,8-cineol, α-terpinyl acetate, α-pinene, sabinene, β-myrcene, and linalool (Kumar et al., 2005; Savan & Küçükbay, 2013; Husain & Ali, 2014; Özkan et al., 2018; Vukovic et al., 2022; Tarfaoui et al., 2022). These compounds are believed to be responsible for cardamom's extensive medicinal applications, which include antioxidant (Elguindy et al., 2018), antibacterial (Kaushik et al., 2010), anti-inflammatory (Souissi et al., 2020), anticancer (Vutakuri & Somara, 2018; Qiblawi et al., 2020), antidiabetic (Yahyazadeh et al., 2021), anticonvulsant (Masoumi-Ardakani et al., 2016), and hepatoprotective activities (Khattab et al., 2020).

Essential oils from medicinal plants, including cardamom, have been widely reported for their health-promoting properties. They are frequently utilized in herbal medicines for the prevention and treatment of a variety of diseases, including various forms of cancer (Ahmad et al., 2024; Ahamad et al., 2019, 2020, 2021). Several preclinical studies have demonstrated the anticancer potential of cardamom essential oil, particularly against different types of cancer (Makhija et al., 2022; Vutakuri & Somara, 2018; Qiblawi et al., 2020; Manjunath & Mahurkar, 2021; Vahabi et al., 2023).

However, despite these promising findings, a review of the literature reveals that there have been no studies investigating the effects of cardamom essential oil on MDA-MB-231 (triple-negative breast cancer), U87 (glioblastoma), and HEK 293 (kidney) cell lines, particularly using samples collected from Erbil, Iraq. To address this gap, the present study aims to characterize the chemical composition of cardamom essential oil via gas chromatography-mass spectrometry (GC-MS) and evaluate its anticancer activity using the MTT assay on MDA-MB-231, U87, and HEK 293 cell lines. This research seeks to expand the understanding of cardamom's potential therapeutic applications, particularly in cancer treatment.

2. Material and Methods

2.1. Plant Material and Chemicals

Cardamom fruits (*Elettaria cardamomum* (L.) Maton; Family: Zingiberaceae) weighing 500 g were sourced from a local market in Erbil, Kurdistan Region, Iraq. The collected plant material was authenticated based on morphological characteristics and preserved as a voucher specimen in the department for future reference. The MDA-MB-231 (triple-negative breast cancer), U87 (glioblastoma), and HEK 293 (human embryonic kidney) cell lines were acquired from the National Centre for Cell Science (NCCS) in Pune, India. For cell culture, fetal bovine serum (FBS), DMEM (Dulbecco's Modified Eagle Medium), and an antibiotic solution were obtained from Gibco (USA). The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was supplied by Sigma (USA), while Himedia (India) provided dimethyl sulfoxide (DMSO). Additionally, 96-well tissue culture plates were procured from Tarson (India).

2.2. Isolation of Cardamom Essential Oil

The essential oil from cardamom fruits was isolated using the hydro-distillation method. Initially, the cardamom fruits (500 g) were thoroughly washed with distilled water to remove any impurities. The fruits were then placed in a round-bottom flask along with deionized water and subjected to hydro-distillation using a Clevenger apparatus. The distillation process lasted approximately six hours to ensure complete extraction of the essential oil. After the extraction period, the essential oil was collected, filtered to remove any residual plant material, and stored at 4 °C in a refrigerator until further analysis.

2.3. GC-MS Instrumentation and Identification of Chemical Constituents

The chemical composition of the cardamom essential oil was analyzed using an Agilent Bench Top Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent Technologies, Wilmington, DE, USA). The GC-MS setup included a DB-5 glass column (30 m × 0.25 mm i.d.; film thickness of 0.25 µm). The oven temperature was programmed to start at 50 °C for 1 minute, followed by an increase to 320 °C, which was maintained for 2 minutes. The injector port temperature was set at 280 °C, with helium gas serving as the carrier gas at a flow rate of 1 ml/min. A split ratio of 1:5 was maintained, and data acquisition occurred at an electron ionization voltage of 70 eV, with a scanning rate of 1.5 seconds. The mass scan range was set between 50 and 1000 atomic mass units (amu), with a total run time of 45 minutes. Control of the GC-MS operations was performed using ChemStation software (Agilent Technologies, Wilmington, DE, USA).

Identification of the chemical constituents was accomplished by comparing their retention indices (Kovats indices) to those reported in the literature. Additionally, mass fragmentation patterns obtained through GC-MS analysis were compared with data stored in databases such as the National Institute of Standards and Technology (NIST), NBS 54 K.L., and WILEY8 libraries, as well as published literature (Adams, 2007; Ali, 2001; Kumar et al., 2005; Savan and Küçükbay, 2013; Husain and Ali, 2014; Özkan et al., 2018; Vukovic et al., 2022; Tarfaoui et al., 2022).

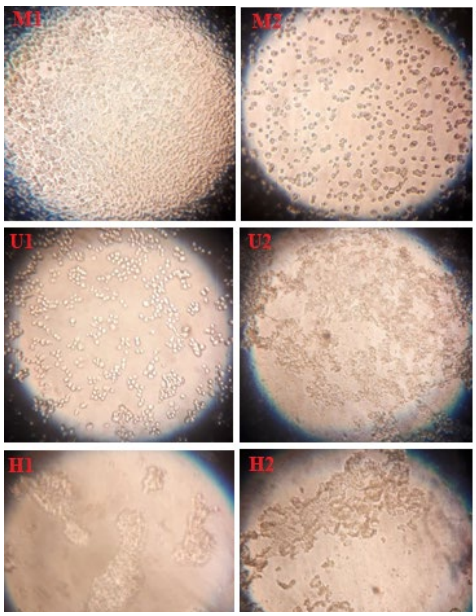
2.4. Evaluation of Anticancer Activity by MTT Assay

The anticancer activity of cardamom essential oil was evaluated against MDA-MB-231, U87, and HEK 293 cell lines using the MTT assay, following the methodology described by Tohkayomatee et al. (2022). U87 and HEK 293 cells were cultured in DMEM supplemented with high glucose, while MDA-MB-231 cells were grown in RPMI 1640 medium, both supplemented with 10%

Table 1. Chemical constituents in the essential oil of cardamom

S. No.	Chemical constituents	RT	KI	Area (%)
1.	α-Thujene	6.877	930	0.37
2.	α-Pinene	7.129	943	2.01
3.	Camphene	7.685	953	0.03
4.	Sabinene	8.617	975	4.76
5.	β-Pinene	8.741	981	0.56
6.	β-Myrcene	9.303	990	3.04
7.	3-Carene	9.905	1011	0.49
8.	α-Terpinene	10.361	1018	1.06
9.	1,8-Cineole	11.324	1031	42.37
10.	*trans*-β-Ocimene	11.711	1050	0.11
11.	γ-Terpinene	12.197	1059	1.96
12.	4-Thujanol	12.768	1070	0.52
13.	*trans*-Linalool oxide	13.380	1074	1.99
14.	6,7-Epoxymyrcene	13.678	1091	0.06
15.	Linalool	14.220	1098	2.09
16.	*cis*-, *p*-Menth-2-en-1-ol	15.187	1123	0.26
17.	δ-Terpineol	17.361	1146	0.18
18.	Terpinen-4-ol	17.834	1178	5.05
19.	α-Terpineol	18.611	1189	5.56
20.	Octyl acetate	19.177	1211	0.17
21.	Neral	20.403	1217	0.22
22.	Linalyl acetate	20.921	1231	0.31
23.	Geraniol	21.145	1237	0.98
24.	Citral	21.766	1267	0.23
25.	Bornyl acetate	22.350	1270	0.06
26.	δ-Terpinyl acetate	23.670	1313	0.19
27.	Geranic acid methyl-ester	24.126	1319	0.12
28.	α-Terpinyl acetate	25.434	1333	22.14
29.	Chavebetol	25.558	1350	0.09
30.	Geranyl acetate	26.600	1361	0.27
31.	Caryophyllene	28.098	1405	0.65
32.	Caryophyllene oxide	36.664	1581	0.24
33.	α-Eudesmol	37.548	2229	1.21

where, RT: Retention time (in minutes), and KI: Kovats index



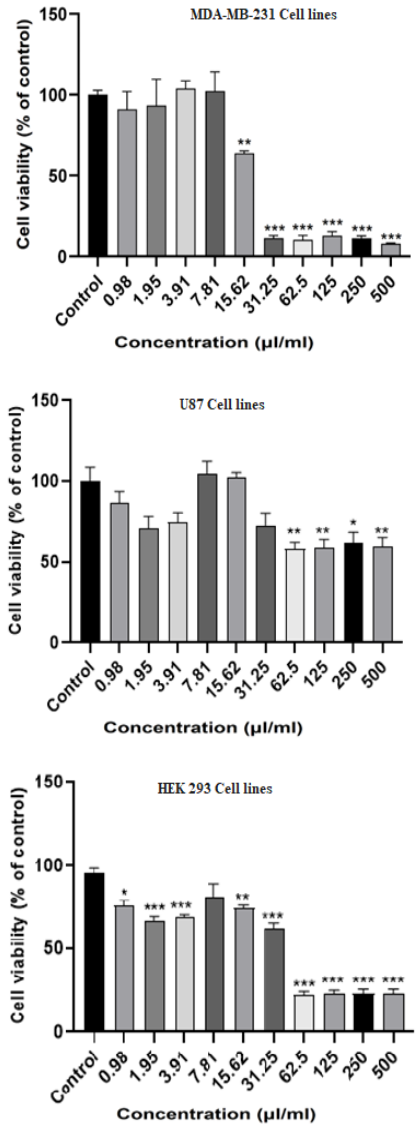


Figure 2. Anticancer activity of cardamom essential oil against MDA-MB-231, U87, and HEK 293 cell lines

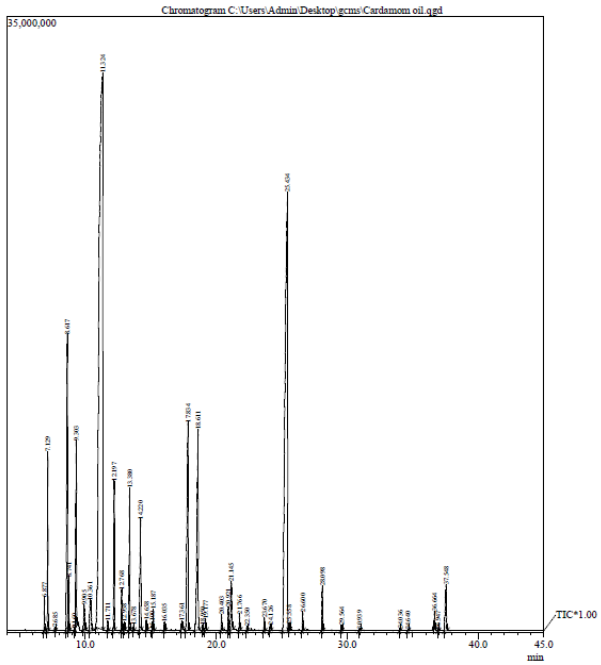


Figure 1. GC-MS chromatogram of cardamom essential oil

FBS and 1% antibiotic (penicillin/streptomycin). The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2. For the cell viability analysis, cells were seeded in 96-well plates at a density of 10^4 cells per well and allowed to adhere for 24 hours. Following this incubation, the culture medium was replaced with fresh medium containing varying concentrations of cardamom essential oil, previously dissolved in DMSO, ranging from 0.87 to 500 µl/ml, with three replicates for each concentration. After 24 hours of exposure, MTT solution (5 mg/ml in phosphate-buffered saline (PBS)) was added to each well. The plates were incubated for an additional 4 hours, allowing formazan crystals to form. Subsequently, the formazan crystals were dissolved in DMSO, and the optical density was measured at 570 nm using a microplate reader.

2.5 Statistical analysis

Statistical analysis and data plotting were performed using GraphPad Prism (version 8). Data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was utilized to assess the significance among different groups, followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant (for $p > 0.05$; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

3. Result and Discussion

3.1. GC-MS Analysis of Cardamom Essential Oil

In the current study, essential oil was isolated from cardamom capsules using the hydro-distillation method, yielding a light-yellow oil with a strong, pleasant aroma. The yield of cardamom essential oil was 8.76% (w/w). Gas chromatography-mass spectrometry (GC-MS) analysis identified thirty-three compounds, accounting for 99.35% of the total essential oil. The major components were 1,8-cineole (42.37%) and α-terpinyl acetate (22.14%), followed by α-terpineol (5.56%), terpinen-4-ol (5.05%), sabinene (4.76%), and β-myrcene (3.04%). Other significant compounds included linalool (2.09%), α-pinene (2.01%), trans-linalool oxide (1.99%), γ-terpinene (1.96%), α-eudesmol (1.21%), and α-terpinene (1.06%) (Table 1).

These results align closely with previous studies on the chemical composition of cardamom essential oil. For instance, Husain and Ali (2014) reported 1,8-cineole as the predominant compound in cardamom essential oil, a finding also supported by Özkan et al. (2018), Vukovic et al. (2022), and Tarfaoui et al. (2022). The variation in the proportion of compounds across studies could be attributed to differences in geographical origin, cultivation methods, and extraction techniques. Based on the present study's results, with 1,8-cineole comprising 42.37% of the essential oil, this cardamom variety can be classified as a 1,8-cineole chemotype (Ashokkumar et al., 2021).

3.2. Anticancer Activity of Cardamom Essential Oil

The anticancer potential of cardamom essential oil was evaluated using the MTT assay on MDA-MB-231 (triple-negative breast cancer), U87 (glioblastoma), and HEK 293 (kidney) cell lines. The results revealed a concentration-dependent and time-dependent inhibition of cell viability across the tested cell lines (Figure 2). Essential oil concentrations ranging from 31.25 to 500 µl/ml significantly reduced cell viability, while concentrations lower than 31.25 µl/ml exhibited minimal effects, except on MDA-MB-231 cells. At a concentration of 15.62 µl/ml, cardamom essential oil inhibited 36.3% of MDA-MB-231 cell viability.

The highest tested concentration, 500 µl/ml, resulted in a 92.2% reduction in MDA-MB-231 cell viability, while inhibition rates were 40.67% for U87 and 73.11% for HEK 293 cells. The half-maximal inhibitory concentration (IC50) values were 96.95 µl/ml for MDA-MB-231 cells and 157.44 µl/ml for HEK 293 cells, indicating stronger cytotoxicity toward MDA-MB-231 cells. In contrast, the U87 cell line showed lower sensitivity, with only 40.67% inhibition at the highest concentration, suggesting that cardamom essential oil may be less effective against glioblastoma cells under the conditions tested.

Morphological observations supported the MTT assay results. Following treatment with cardamom essential oil, MDA-MB-231 and HEK 293 cells lost their characteristic elongated spindle shapes within 24 hours, with some cells detaching and floating in the medium as suspension cells, a hallmark of cell death. By contrast, untreated cancer cells retained their original morphology and remained tightly adhered to each other. The reduced effectiveness of cardamom essential oil against U87 cells was further evidenced by the retention of the original cell morphology and lack of visible cell death even after prolonged treatment.

The MTT assay is widely recognized as a reliable method for evaluating cell viability and cytotoxicity, particularly in the preliminary screening of potential anticancer agents (Kumar et al., 2018; Ahamad, 2023). In this study, the MTT assay confirmed the promising anticancer activity of cardamom essential oil, particularly against MDA-MB-231 cells. These findings are consistent with prior studies that have reported the cytotoxic effects of cardamom essential oil on various cancer cell lines (Vutakuri & Somara, 2018; Qiblawi et al., 2020).

Several mechanisms could explain the anticancer effects of cardamom essential oil. The major compounds identified, particularly 1,8-cineole and α-terpinyl acetate, have been previously associated with anticancer properties. For instance, 1,8-cineole has been reported to induce apoptosis and inhibit cell proliferation in cancer cells (Ashokkumar et al., 2021), while α-terpinyl acetate has demonstrated similar bioactivity in preclinical studies (Makhija et al., 2022). The lower sensitivity of U87 cells may be due to the unique biological characteristics of glioblastoma cells, which are

known for their aggressive behavior and resistance to many chemotherapeutic agents (Vahabi et al., 2023).

4. Conclusion

In conclusion, this study highlights the potential of cardamom essential oil, particularly its major components 1,8-cineole and α-terpinyl acetate, as a source of natural compounds with anticancer activity. The essential oil exhibited significant cytotoxicity against MDA-MB-231 and HEK 293 cells, while its effectiveness against U87 glioblastoma cells was limited. Further studies are warranted to elucidate the underlying mechanisms and explore the therapeutic potential of cardamom essential oil in cancer treatment.

Author contributions

J.A. designed the study, prepared the manuscript, collected the plant material, isolated the essential oil, analyzed the GC-MS data, and drafted the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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