*Original Article*

# Essential oils from *Elettaria cardamomum* (L.) Maton grains and *Cinnamomum verum* J. Presl barks: Chemical examination and bioactivity studies

[Aceites esenciales de granos de *Elettaria cardamomum* (L) Maton y cortezas de *Cinnamomum verum* J. Presl: Examen químico y estudios de bioactividad]

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**Abstract**

*Context*: *Elettaria cardamomum* (L.) Maton grains and *Cinnamomum verum* J. Presl barks are immensely consumed by Jordanian population as food flavoring and remedies in treating different disorders without awareness of their effect or dose toxicity.

*Aims*: To evaluate the antibacterial, antioxidant and cytotoxic effects of essential oils (EOs) hydro-distilled from *E. cardamomum* grains and *C. verum* barks.

*Methods*: Hydro-distillation was carried out using Clevenger apparatus; the EOs were analyzed using GC/MS, and their constituents were quantified using GC/FID. The antibacterial activity was determined by agar diffusion test and micro-broth dilution assay, while the antioxidant activity was evaluated by DPPH and ABTS scavenging assays. The cytotoxic activity was evaluated against the MDA-MB-231 breast cancer cell line.

*Results*: *E. cardamomum* EO was most effective against *Bacillus subtilis* with MIC 3.75 µL/mL and exhibited antioxidant activity in DPPH assay with $IC_{50}$ 0.057 ± 0.013 µL/mL (0.076 ± 0.017 µL EO/µg Trolox®) but was ineffective in the ABTS test up to 5 µL/mL; 86.22% of its EO constituent are oxygenated monoterpenes with *a*-terpinyl acetate as the major component. Whilst *C. verum* EO was active against both tested Gram-positive and negative bacterial strains with MIC 1.25-5.00 µL/mL but was devoid of significant antioxidant activity up to 5 µL/mL; phenylpropanoids constituted 69.48% of its EO with *E*-cinnamaldehyde as the major component. Both EOs showed cytotoxic activity against the breast cancer cell line with $IC_{50}$ 0.14-0.46 µL/mL.

*Conclusions*: The current results unveil the potential application of *C. verum* and *E. cardamomum* in complementary and alternative medicine as biosafe remedies besides their usage as flavoring and seasoning sources.

*Keywords*: bioactivity; *Cinnamomum verum*; *Elettaria cardamomum*; essential oil.

**Resumen**

*Contexto*: Los granos de *Elettaria cardamomum* (L.) Maton y la corteza de *Cinnamomum verum* J. Presl son consumidos por la población jordana como aromatizantes alimentarios y remedios para el tratamiento de diferentes trastornos sin ser conscientes de su efecto o toxicidad por dosis.

*Objetivos*: Evaluar los efectos antibacterianos, antioxidantes y citotóxicos de los aceites esenciales (OE) hidrodestilados de granos de *E. cardamomum* y cortezas de *C. verum*.

*Métodos:* La hidrodestilación se llevó a cabo utilizando un aparato Clevenger; los OE se analizaron mediante GC/MS y sus constituyentes se cuantificaron mediante GC/FID. La actividad antibacteriana se determinó mediante una prueba de difusión en agar y un ensayo de dilución en microcaldo, mientras que la actividad antioxidante se evaluó mediante ensayos de barrido de DPPH y ABTS. La actividad citotóxica se evaluó frente a la línea celular de cáncer de mama MDA-MB-231.

*Resultados*: *E. cardamomum* EO fue más efectivo contra *Bacillus subtilis* con MIC 3,75 µL/mL y mostró actividad antioxidante en el ensayo DPPH con $IC_{50}$ 0,057 ± 0,013 µL/mL (0,076 ± 0,017 µL EO/µg Trolox®) pero fue ineficaz en el ABTS pruebe hasta 5 µL/mL; El 86,22% de su constituyente EO son monoterpenos oxigenados con acetato de *a*-terpinilo como componente principal. Mientras que, *C. verum* EO fue activo contra las cepas bacterianas Gram positivas y negativas probadas con MIC 1,25-5,00 µL/mL, pero careció de actividad antioxidante significativa hasta 5 µL/mL; los fenilpropanoides constituyeron el 69,48% de su OE con E-cinnamaldehído como componente principal. Ambos AE mostraron actividad citotóxica contra la línea celular de cáncer de mama con $IC_{50}$ 0,14-0,46 µL/mL.

*Conclusiones*: Los resultados actuales revelan la aplicación potencial de *C. verum* y *E. cardamomum* en la medicina complementaria y alternativa como remedios bioseguros, además de su uso como fuentes de sabor y condimentos.

*Palabras Clave*: aceite esencial; bioactividad; *Cinnamomum verum*; *Elettaria cardamomum*.

## INTRODUCTION

Plants represent an integral part of healthcare in developing countries. Plant-based remedies have a range of biomedical values, including treatment of microbial infections, inflammation, cancer, and system disorders. Their activity is attributed to the possession of secondary metabolites that affect more than a biological target, individually or synergistically. Moreover, based on their global availability, cost-effectiveness, and biosafety compared to currently available drugs, more than 80% of developing countries' individuals rely on plants (Murugesan and Deviponnuswamy, 2014), and tremendous surveys on the bioactivity of their metabolites have emerged.

Aromatic and spice plants are exotic sources of complex mixtures of volatile secondary metabolites (i.e., EOs), many of which have been approved by the US Food and Drug Administration (FDA) for human consumption (Bouhdid et al., 2010). They have been world widely used due to their health benefits and were involved in many applications, including flavors and fragrances, preservation of processed food, and pharmaceuticals (Khaosaad et al., 2006). *Elettaria cardamomum* (L.) Maton (green cardamom, family *Zingiberaceae*) and *Cinnamomum verum* J. Presl (syn. *C. zeylanicum* Blume, true cinnamon, family *Lauraceae*) are among the popularly used spices in Asia and Middle East countries.

*E. cardamomum* EO is used mainly for treating fevers, digestive complaints, and respiratory diseases (Gochev et al., 2012); it embraces aromatic compounds with stimulant, astringent, diuretic, carminative, anti-inflammatory, and antioxidant activities (Asghar et al., 2017). These pharmaceutical activities have been attributed to the presence of oxygenated monoterpenes (1,8-cineole, *a*-terpinyl acetate, and geraniol) and phenyl propanoids (eugenol) (Das et al., 2012; Tangjitjaroenkun et al., 2020). Meanwhile, *C. verum* extract is used to treat coughing, sore throats, arthritis, and microbial infections (Sharifan et al., 2016). Therefore, its extracts and oil have many uses in the pharmaceutical and cosmetics industries as well as in food processing. The antimicrobial activity of cinnamon EO is correlated with their anti-hypertriglyceridemia, anti-diabetic, anti-inflammatory, antioxidant and anticancer properties, especially the *E*-cinnamaldehyde (do Nascimento et al., 2020). Moreover, *E*-cinnamaldehyde, *E-O*-methoxycinnamaldehyde, and eugenol in cinnamon oils suppress immunomodulatory that stimulate cancer initiation and propagation (Najar et al., 2020).

Both spice sorts are being imported to Jordan from South Asia regions and are immensely used by the Jordanian population in the form of decoctions or infusions as a food flavoring, in daily hot drinks, and in treatment of dental, digestive, nervous, respiratory, and diabetic disorders. To date, studies conducted in Jordan on these plants were ethnopharmacological surveys concerned with their folkloric usage (Almuhur, 2019), their nutritional and microbiological composition (Ereifej et al., 2015), the effect of different organic extractants and temperatures on their antioxidant activities (Ereifej et al., 2016), or stability of their microencapsulated EOs in different materials (Al-Ismail et al., 2015). In contrast to other regions of the world, scientific investigations in Jordan that had dealt with studying their essential oil composition and awareness of their effect or dose toxicity are scarce or even none, which impose drawbacks in their medicinal benefits. In fact, importing routes, storage protocols, and durations implemented by herbalists might influence their pharmacological activity and health benefits. Therefore, herein the chemical composition, antibacterial, antioxidant, and anti-breast cancer activities of *E. cardamomum* and *C. verum* EOs were investigated, clarified, and related to results from other previously conducted studies.

## MATERIAL AND METHODS

### Plant samples and isolation of EOs

Five hundred grams of each cinnamon bark and cardamom grains were purchased from a local market for herbs in Al-Karak Governorate/South Jordan. Voucher specimens were deposited at the Department of Biology at Mutah University; identified and authenticated by Dr. Saleh Al-Quran, an expert botanist in the department. They were surface cleaned, air-dried in the shade for 1-2 weeks, and ground to a fine powder. One hundred grams of the pulverized material were soaked in 1 L of distilled water and were hydro-distilled using simple Clevenger apparatus for 6 h, which was enough time for satisfactory recovery of EOs. The extraction procedure was performed three times to obtain good volumes of EOs. All collected EOs from each sample were dried over anhydrous sodium sulfate ($Na_2SO_4$), combined, and stored in amber vials at 4°C until further analysis. Oil yield % was calculated in weight EO relative to absolute dry sample weight (% w/w).

## Gas Chromatography-Mass Spectrometry (GC-MS) analysis

For chemical analysis of the EOs, a Gas Chromatograph-Mass Spectrometer apparatus was used (GCMS-QP2010 Ultra, Shimadzu, Japan) with a splitless injection mode. Injected volume was (1 µL), and separation of EOs components was performed on HP-5Ms capillary column (30 m × 0.25 mm, 0.25 µm, Supelco Inc, USA). The chromatographic conditions were as follows: the column temperature was held at 60°C for 1 min, increased to 246°C at 3°C/min, then held for 3 min giving a total of 66.6 min run time. The temperature of the injector, transfer line, and ion source were set at 280°C, 250°C, and 200°C, respectively. Helium 5.0 (purity >99.99999%) was used as carrier gas at a constant flow of 1.0 mL/min. Mass spectra were scanned from 50 to 500 amu at 0.5 s scan rate in total ion chromatogram (TIC) mode and 70 eV ionization energy. Kovats Retention Indexes (KRIs) of oil components were calculated by injection of a series of n-alkanes ($C_8$–$C_{20}$) in the same column and conditions for gas chromatography analyses.

## Gas chromatography-Flame Ionization Detector (GC-FID) analysis

The quantitative analyses of EOs were performed using GC-FID (Agilent 6890N, Palo Alto, USA) operated under similar conditions to the GC-MS. The instrument was equipped with a 5 % phenyl-95 % methylsilicone (HP5, 30 m × 0.25 mm × 0.25 µm) fused silica capillary column, a split-mode injector (split ratio 1:50), and a flame ionization detector (FID); the injection volume was 1 µL. Injector and detector temperatures were 250°C and 280°C, respectively. In this study, internal standards (n-alkanes, $C_8$-$C_{20}$, % w/w) were added to each sample to help in the standardization of retention times and to calculate the concentration of each individual constituent (% w/w). The response factor for each constituent was estimated and found in the range of 0.93-1.09.

EO components were identified based on comparing the retention index of each constituent relative to ($C_8$–$C_{20}$) n-alkanes mixture and the mass spectrum obtained from authentic samples and/ or the NIST-14 library and those published by Adams (2007).

## Evaluation of biological activities

### Antibacterial activity

Antibacterial activity of spices' EOs was performed as described previously (Al-Zereini, 2014) and according to Laboratory Standards Institute guidelines (CLSI) by agar diffusion test and the minimum inhibitory concentration (MIC) was determined by micro-broth dilution assay. The Gram-positive *Staphylococcus aureus* ATCC 43300, *Bacillus subtilis* ATCC 6633, and the Gram-negative *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC13048 were used as test bacterial strains. An aliquot of an overnight culture of the test bacterial strain in nutrient broth (Oxoid, UK) was seeded on Muller Hinton agar plates (Oxoid, UK) at a final cell density of $10^6$ bacterial cells/mL. The test was performed in triplicate assays, and results were presented as means ± SD.

In the Agar diffusion test, 1, 3, and 5 µL/disc of EOs (in DMSO 1:1) were applied on sterile 6 mm blank discs that were placed on the top of bacterial seeded agar plates. 5 µL DMSO /disc and streptomycin (50 µg/disc, Bio Basic Inc, Canada) were used as negative and positive controls, respectively. While in micro-broth dilution assay, EOs and the positive control were tested at twofold diluted concentrations starting from 10 µL/mL (v/v) and 50 µg/mL, respectively. Type of inhibition, bacteriostatic or bacteriocidal, was determined by platting the preparations, where there were no visible growths, on agar plates with proper medium and with incubation for 24 h.

### Antioxidant activity test

The free radical scavenging activity of EOs was evaluated using 1,1–diphenyl–2-picrylhrzyl radical (DPPH, Sigma-Aldrich, Germany) and 2,20-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich, Germany) assays as mentioned in Al-Zereini (2014). Briefly, different volumes of EOs (0.01-5 µL/mL) were added into 1 mL methanol solution of 0.1 mM DPPH ($OD_{517nm}$ 0.7-0.8). The mixture was shaken vigorously and left to stand at 37°C for 30 min in the shade. The decrease in the absorbance of the resulting solution was measured at 517 nm by a visible spectrophotometer (Novaspec Model. 80-2088-64, Pharmacia Biotech, UK).

Meanwhile, in the ABTS assay, a final concentration of 7 mM ABTS and 2.45 mM potassium persulfate in distilled water were reacted together and incubated at room temperature in darkness for 16 h to generate ABTS•+ radical. The resulting ABTS•+ solution was diluted to an absorbance of 0.700 ± 0.005 at 734 nm UV/VIS Spectrophotometer (Novaspec Model. 80-2088-64, Pharmacia Biotech, UK). The decoloration of the ABTS•+ radicals was evaluated by adding different volumes of EOs (0.01-5.00 µL/mL) to 2 mL of diluted ABTS•+ with vigorous mixing, allowed to stand at room temperature for 6 min, and the absorbance was recorded immediately at 734 nm.

The scavenging activity of tested EOs and neutralization of DPPH or ABTS•+ radicals was calculated according to the formula [1].

Scavenging effect (%) = $\dfrac{A_{control} - A_{sample}}{A_{control}} \times 100$      [1]

Where $A_{control}$ is the absorbance of the radical solution, and $A_{sample}$ is the absorbance of radical solution after sample addition.

Trolox® standard (purity >97%, Sigma-Aldrich, Germany) at concentrations 0.1-1.5 µg/mL in methanol (99.5%) was prepared and assayed using the same conditions in both assays. All results were expressed in terms of mean values of Trolox® equivalent antioxidant capacity (TEAC). The assay was performed in triplicate for each sample and each concentration of standard. The $IC_{50}$ value was interpolated from a dose-response curve plotted for percentage inhibitions against respective EO concentrations.

*In vitro cytotoxicity assay*

The cytotoxicity of EOs to the human breast adenocarcinoma MDA-MB-231 (ATCC–HTB-26) and to the human dermal fibroblast (ATCC-PCS-201-012) cell lines was determined as described previously (Al-Zereini, 2017). Different concentrations of the samples (0.1-5.0 µL/mL) were applied into 24-well microtiter plates containing $5 \times 10^4$ cells/mL, incubated at 37°C in a humidified atmosphere containing 5.0% $CO_2$. Cultures of cell lines without tested substances were performed as negative controls and with different concentrations of doxorubicin hydrochloride (DOX, Sigma-Aldrich, Germany) as the positive control. The cytotoxic effect was determined microscopically in 24 h intervals and up to 72 h, quantitatively by using Giemsa stain.

The percentage of inhibition in the cell viability was determined using the following formula [2].

Cell proliferation inhibition (%) = $\dfrac{A_{untreated} - A_{treated}}{A_{untreated}} \times 100$    [2]

Where $A_{untreated}$ is the absorbance of untreated cell line, and $A_{treated}$ is the absorbance of cell line treated with EO.

Data were expressed as mean ± SD of three independent experiments, and the concentration that caused 50% inhibition in cell proliferation was interpolated from a dose-response curve plotted for percentage inhibitions against respective EO concentrations.

The selectivity index (SI) of tested EOs or drug was determined based on the ratio of their cytotoxicity against normal dermal fibroblast cells to that against cancer cells. It was calculated according to the formula [3].

Selectivity index (SI)= $\dfrac{IC_{50 \ normal \ fibroblast \ cells}}{IC_{50 \ cancer \ cells}}$    [3]

## Statistical analysis

All data are reported as means ± standard deviation (SD). The dose-response relation was appraised using PROBIT regression analysis with a 95% confidence limit (Finney, 1978). Results were analyzed by one-way ANOVA and Tukey HSD post hoc test using the Statistical Package for the Social Sciences software (SPSS, version 16). Data were considered significant at $p<0.05$.

## RESULTS AND DISCUSSION

### Chemical analysis of *E. cardamomum* and *C. verum* EOs

Hydro-distillation of *E. cardamomum* grains give rise to a 0.35-0.5% (w/w) pale yellow colored EO with intense penetrating odor, while 1.1-1.3% (w/w) dark yellow, pleasant, cinnamon odorous EO was obtained from *C. verum* barks. GC-analysis of *E. cardamomum* EO resulted in the identification of 39 compounds that constituted 99.83% of the oil yield (Table 1, Figs. 1-2); 86.22% of identified compounds were oxygenated monoterpenes. *a*-Terpinyl acetate was the major component of cardamom EO (55.99%) followed by 1,8 cineole (8.82%), linalool (6.99%), dihydrocarveol (6.06%), geraniol (4.46%), Z-caryophyllene (3.82%), *E*-nerolidol (3.07%), eugenol (2.31%) and terpinen-4-ol (1.83%). Each of the remaining identified compounds constituted less than 1% of the oil yield. The dominance of oxygenated monoterpenes with *a*-terpinyl acetate, linalool, and 1,8-cineole as the dominant constituents was reported previously in different literature (Singh et al., 2008; Gochev et al., 2012). However, some studies had reported relatively lower proportions of *a*-terpinyl acetate, linalool with higher percentages of 1,8-cineole in EO of cardamom from several countries, 25.6%-26.71% of 1,8-cineole (Snoussi et al., 2015; Asghar et al., 2017). Interestingly, EO of *E. cardamomum* purchased from a local market in Saudi Arabia has 1,8-cineole as a major component (55.4%), *a*-terpinyl acetate (28.6%), and linalool (0.9%) (Noumi et al., 2018).

Meanwhile, 36 compounds were identified from *C. verum* EO, which represented 80.1% of total oil yield (Table 2), phenylpropanoid compounds account for 69.48% of detected substances. *E*-Cinnamaldehyde (55.75%) was the major component, followed by *E-O*-methoxycinnamaldehyde (6.8%), eugenol (4.6%), cubenol (1.97%), Z-cinnamaldehyde (1.57%), *a*-terpinyl acetate (1.42%). Each of the other identified compounds constituted less than 1% of the oil yield. The high content of phenyl propanoid compounds, particularly *E*-cinnamaldehyde (65%-97%), was documented in commercial *C. verum* sources as well as

from those in original habitats (Unlu et al., 2010; Choi et al., 2016; Catalá and Ferrer, 2017; Liyanage et al., 2017; Kallel et al., 2019; Najar et al., 2020), which is in line with our findings. However, inconsistent with the current results, some of these studies reported a higher quantity of *β*-caryophyllene and *E*-cinnamyl acetate with the absence of *O*-methoxycinnamaldehyde (Choi et al., 2016; Najar et al., 2020). Intriguingly, EO of *C. verum* bark collected from São Luís embraced *E*-

cinnamaldehyde (89.3%) as a main constituent and eugenol (2.8%) with a trace amount of *E*-cinnamyl acetate, while a sample collected from Santa Inês predominated by benzyl benzoate (23.3%), linalool (14.0%), *β*-caryophyllene (9.1%), and *E*-cinnamyl acetate (3.1%) with trace amounts of eugenol and *E*-cinnamaldehyde (<1%); a variation in chemical composition between two samples of same species collected from two locations in Brazil (Farias et al., 2020).
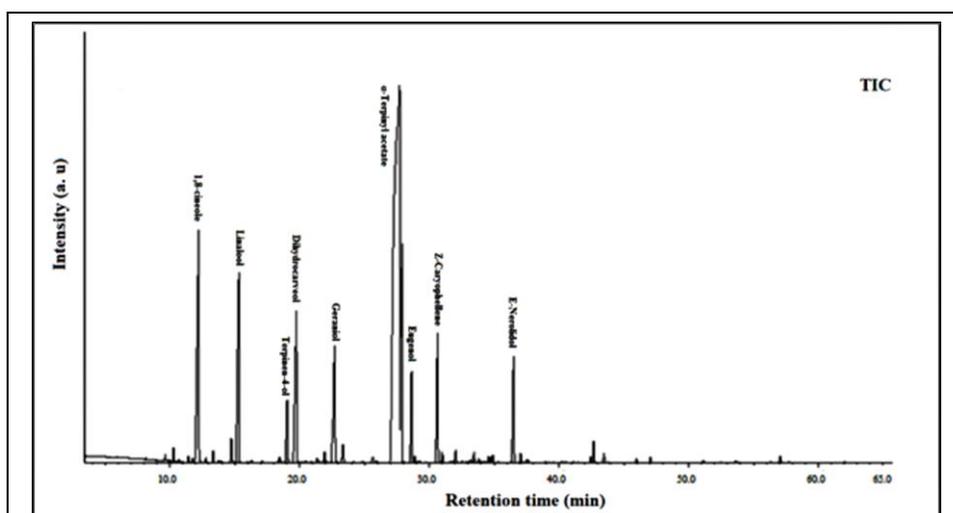


**Figure 1.** GC-MS chromatograms showing chemical compositions of *E. cardamomum* essential oil.

The run was done using (GC-MS-QP2010 Ultra) with HP-5Ms capillary column (30 m × 0.25 mm, 0.25 µm) and a splitless injection. Main components with area >1% are indicated; 1,8-cineole (Rt 12.21 min), linalool (Rt 15.33 min), terpinen-4-ol (Rt 19.04 min), dihydrocarveol (Rt 19.76 min), geraniol (Rt 22.71 min), *a*-terpinyl acetate (Rt 27.90 min), eugenol (Rt 28.90 min), *Z*-caryophyllene (Rt 30.65 min), and *E*-nerolidol (Rt 36.51 min).
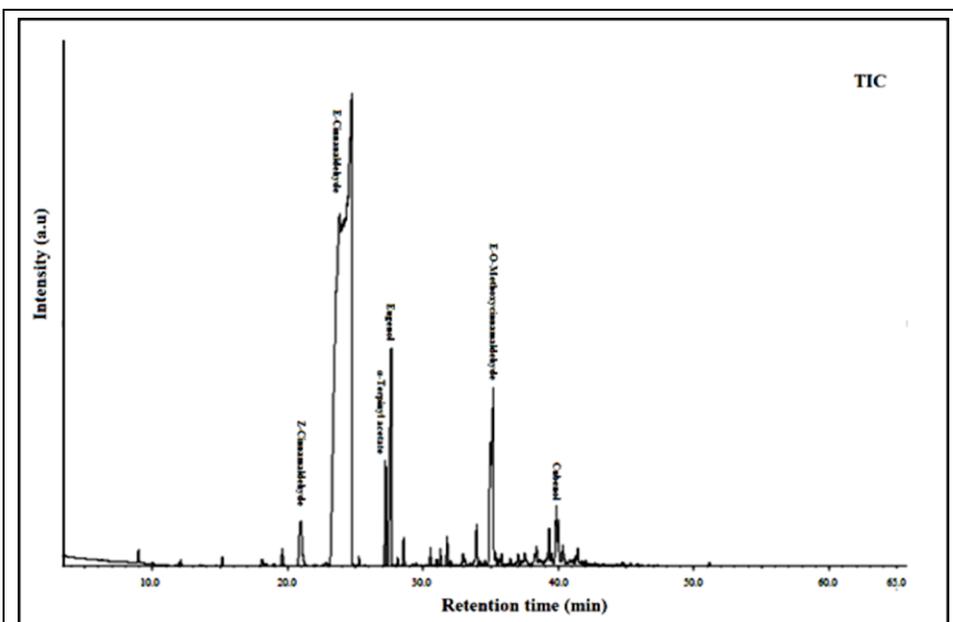


**Figure 2.** GC-MS chromatograms showing chemical compositions of *C. verum* essential oil.

The run was done using (GC-MS-QP2010 Ultra) with HP-5Ms capillary column (30 m × 0.25 mm, 0.25 µm) and a splitless injection. Main components with area >1% are indicated; Z-cinnamaldehyde (Rt 20.96 min), *E*-cinnanaldehyde (Rt 23.83 min), *a*-terpinyl acetate (Rt 27.24 min), eugenol (Rt 27.64 min), *E-O*-methoxycinnamaldehyde (Rt 35.19 min), and cubenol (Rt 39.85 min).

**Table 1.** Essential oil composition of green cardamom (*E. cardamomum*).

| No | Compound | $R_{t\ (min)}$ | KI $_{(lit.)}$[a] | KI$_{(cal.)}$[b] | Concentration (% w/w) |
|---|---|---|---|---|---|
| 1 | Sabinene | 9.64 | 975 | 974 | 0.11 |
| 2 | Myrcene | 10.28 | 990 | 990 | 0.25 |
| 3 | Iso-sylvestrene | 11.44 | 1008 | 1007 | 0.12 |
| 4 | p-Cymene | 11.77 | 1024 | 1024 | 0.10 |
| 5 | 1,8-Cineole | 12.21 | 1031 | 1034 | 8.82 |
| 6 | *Z-β*-Ocimene | 12.31 | 1037 | 1036 | 0.07 |
| 7 | *E-β*-Ocimene | 12.78 | 1050 | 1046 | 0.11 |
| 8 | *γ*-Terpinene | 13.33 | 1059 | 1058 | 0.24 |
| 9 | n-Octanol | 13.72 | 1068 | 1067 | 0.07 |
| 10 | Terpinolene | 14.73 | 1088 | 1089 | 0.61 |
| 11 | Linalool | 15.33 | 1096 | 1102 | 6.99 |
| 12 | *cis*-p-Menth-2-en-1-ol | 16.29 | 1121 | 1122 | 0.05 |
| 13 | *trans*-p-Menth-2-en-1-ol | 17.12 | 1140 | 1139 | 0.05 |
| 14 | *δ*-Terpineol | 18.45 | 1166 | 1167 | 0.19 |
| 15 | Terpinen-4-ol | 19.04 | 1177 | 1179 | 1.83 |
| 16 | Dihydrocarveol | 19.76 | 1193 | 1194 | 6.06 |
| 17 | *trans*-Piperitol | 20.49 | 1208 | 1210 | 0.07 |
| 18 | *trans*-Carveol | 20.93 | 1216 | 1220 | 0.07 |
| 19 | Nerol | 21.35 | 1229 | 1229 | 0.21 |
| 20 | Neral | 21.94 | 1238 | 1243 | 0.36 |
| 21 | Geraniol | 22.71 | 1252 | 1260 | 4.46 |
| 22 | Geranial | 23.34 | 1267 | 1274 | 0.57 |
| 23 | Geranyl formate | 24.85 | 1298 | 1302 | 0.05 |
| 24 | *δ*-Terpinyl acetate | 25.64 | 1317 | 1319 | 0.22 |
| 25 | Methyl-geranate | 25.99 | 1324 | 1326 | 0.11 |
| 26 | *a*-Terpinyl acetate | 27.90 | 1349 | 1358 | 55.99 |
| 27 | Eugenol | 28.90 | 1359 | 1360 | 2.31 |
| 28 | Neryl acetate | 29.12 | 1361 | 1365 | 0.12 |
| 29 | Z-Caryophyllene | 30.65 | 1408 | 1409 | 3.82 |
| 30 | *a*-Humulene | 32.06 | 1454 | 1460 | 0.48 |
| 31 | Germacrene D | 32.82 | 1481 | 1479 | 0.24 |
| 32 | *cis-β*-Guaiene | 33.47 | 1493 | 1494 | 0.27 |
| 33 | Viridiflorene | 33.82 | 1496 | 1502 | 0.16 |
| 34 | *γ*-Cadinene | 34.58 | 1513 | 1515 | 0.19 |
| 35 | Chavibetol acetate | 34.78 | 1525 | 1525 | 0.32 |
| 36 | *E*-Nerolidol | 36.51 | 1536 | 1567 | 3.07 |
| 37 | Z-Dihydro-apofarnesol | 37.07 | 1572 | 1580 | 0.23 |
| 38 | Caryophyllene oxide | 37.56 | 1583 | 1592 | 0.19 |
| 39 | (2Z,6E)-Farnesol | 42.71 | 1698 | 1723 | 0.65 |
| **Total** | | | | | **99.83** |

**Table 1.** Essential oil composition of green cardamom (*E. cardamomum*) (continued...)

| No | Compound | R$_{t (min)}$ | KI $_{(lit.)}$[a] | KI$_{(cal.)}$[b] | Concentration (% w/w) |
|---|---|---|---|---|---|
| Monoterpene hydrocarbons (1-4,6-8,10) | | | | | 1.61 |
| Oxygenated monoterpenes (5,11-26,28) | | | | | 86.22 |
| Sesquiterpene hydrocarbons (29-34) | | | | | 5.16 |
| Oxygenated sesquiterpenes (36-39) | | | | | 4.14 |
| Phenylpropanoids (27,35) | | | | | 2.63 |
| Others (9) | | | | | 0.07 |

Quantitative analyses of EO were performed using GC-FID (Agilent 6890N) with HP5 capillary column (30 m × 0.25 mm × 0.25 μm), a split-mode injector, and a flame ionization detector (FID). The run was performed under similar conditions to the GC-MS. [a] Kovat retention index determined relative to the retention time of a series of n-alkanes (C$_8$–C$_{20}$).[b] Kovat retention index based on NIST-14 mass spectral reference library.

**Table 2.** Essential oil composition of cinnamon (C. *verum*).

| No | Compound | R$_{t (min)}$ | KI $_{(lit.)}$[a] | KI$_{(cal.)}$[b] | Concentration (% w/w) |
|---|---|---|---|---|---|
| 1 | Benzaldehyde | 8.97 | 960 | 956 | 0.12 |
| 2 | 1,8-Cineole | 12.10 | 1031 | 1031 | 0.10 |
| 3 | Linalool | 15.15 | 1096 | 1098 | 0.10 |
| 4 | *a*-Terpineol | 19.60 | 1188 | 1191 | 0.28 |
| 5 | Z-Cinnamaldehyde | 20.96 | 1219 | 1221 | 1.57 |
| 6 | *E*-Cinnanaldehyde | 23.83 | 1270 | 1280 | 55.75 |
| 7 | *E*-Cinnamyl alcohol | 25.27 | 1304 | 1311 | 0.10 |
| 8 | *a*-Terpinyl acetate | 27.24 | 1349 | 1354 | 1.42 |
| 9 | Eugenol | 27.64 | 1359 | 1362 | 4.60 |
| 10 | *cis*-Carvyl acetate | 28.12 | 1367 | 1373 | 0.13 |
| 11 | *a*-Copaene | 28.57 | 1383 | 1383 | 0.40 |
| 12 | *E*-Caryophyllene | 30.55 | 1419 | 1427 | 0.28 |
| 13 | Coumarin | 31.00 | 1434 | 1473 | 0.11 |
| 14 | *E*-Cinnamyl acetate | 31.29 | 1446 | 1444 | 0.25 |
| 15 | *E*-Cinnamic acid | 31.79 | 1454 | 1455 | 0.41 |
| 16 | *a*-Humulene | 32.04 | 1454 | 1461 | 0.10 |
| 17 | *δ*-Muurolene | 32.95 | 1479 | 1482 | 0.33 |
| 18 | *a*-Muurolene | 33.96 | 1500 | 1510 | 0.76 |
| 19 | *β*-Bisabolene | 34.23 | 1505 | 1516 | 0.11 |
| 20 | *δ*-Cadinene | 34.57 | 1523 | 1525 | 0.10 |
| 21 | *E*-*O*-Methoxycinnamaldehyde | 35.19 | 1528 | 1540 | 6.80 |
| 22 | *trans*-Cadina-1-4-diene | 35.37 | 1534 | 1544 | 0.18 |
| 23 | *δ*-Cuprenene | 35.62 | 1543 | 1545 | 0.12 |
| 24 | *a*-Calacorene | 35.80 | 1549 | 1549 | 0.18 |
| 25 | *E*-Nerolidol | 36.45 | 1563 | 1565 | 0.12 |
| 26 | Caryophyllenyl alcohol | 37.02 | 1572 | 1579 | 0.25 |
| 27 | Gleenol | 37.52 | 1587 | 1591 | 0.43 |
| 28 | Ledol | 38.39 | 1602 | 1612 | 0.61 |

**Table 2.** Essential oil composition of cinnamon (C. *verum*) (continued...)

| No | Compound | $R_{t\ (min)}$ | KI $_{(lit.)}$[a] | KI$_{(cal.)}$[b] | Concentration (% w/w) |
|---|---|---|---|---|---|
| 29 | *a*-Corocalene | 39.00 | 1623 | 1628 | 0.11 |
| 30 | 1-epi-Cubenol | 39.32 | 1628 | 1636 | 0.75 |
| 31 | *allo*-Aromadendrene epoxide | 39.51 | 1641 | 1641 | 0.22 |
| 32 | Cubenol | 39.85 | 1646 | 1649 | 1.97 |
| 33 | *a*-Cadinol | 40.34 | 1654 | 1662 | 0.45 |
| 34 | Selin-11-en-4-*a*-ol | 40.82 | 1659 | 1674 | 0.32 |
| 35 | epi-*a*-Bisabolol | 41.44 | 1671 | 1689 | 0.47 |
| 36 | Amorpha-4,9-dien-2-ol | 42.01 | 1700 | 1704 | 0.10 |
| **Total** | | | | | **80.10** |
| Oxygenated monoterpenes (2-4,8,10) | | | | | 2.03 |
| Sesquiterpene hydrocarbons (11,12,16-20, 22-24,29) | | | | | 2.67 |
| Oxygenated sesquiterpenes (25-28,30-36) | | | | | 5.69 |
| Phenylpropanoids (5-7,9,14,15,21) | | | | | 69.48 |
| Others (1,13) | | | | | 0.23 |

Quantitative analyses of EO were performed using GC-FID (Agilent 6890N) with HP5 capillary column (30 m × 0.25 mm × 0.25 µm), a split-mode injector, and a flame ionization detector (FID). The run was performed under similar conditions to the GC-MS. [a] Kovat retention index determined relative to the retention time of a series of n-alkanes ($C_8$–$C_{20}$).[b] Kovat retention index based on NIST-14 mass spectral reference library.

Taking all obtained analyses on both spices' EOs in consideration, variations in their composition with literature might be attributed to geographical origin, extraction and drying method, analytical chromatography conditions, and storage conditions; particularly as temperature and sunlight might influence the percentage and type of volatile compounds detected (Asghar et al., 2017).

**Biological activity of *E. cardamomum* and *C. verum* EOs**

*E. cardamomum* EO showed anti-Gram-positive potential with predominant activity against *B. subtilis* at all tested concentrations and MIC of 3.75 µL/mL; it caused inhibition zones ranged from 12-13.5 mm at the different tested concentrations. However, *S. aureus* was less susceptible at the highest applied concentration (i.e., 5 µL/disc, mg/disc) with a 6.5 mm inhibition zone. Intriguingly, none of the tested Gram-negative bacteria were inhibited up to the maximum applied concentration in agar diffusion (5 µL/disc) and micro-broth dilution assays (10 µL/mL) (Table 3). In general, the tested bacterial strains were significantly less susceptible to tested *E. cardamomum* EO than the positive control (streptomycin), which caused the formation of inhibition zones (21-31.7 mm) with significantly lower MIC values of 0.39-1.56 µg/mL ($p<0.05$). Although EO of *E. cardamomum* did not show scavenging activity to ABTS•+ radicals, it embraces components that render it a promising source of antioxidant agents; it was able to scavenge

DPPH radical starting from 0.03 µL/mL with $IC_{50}$ value of 0.057 ± 0.013 µL/mL (0.076 ± 0.017 µL EO/µg Trolox). The standard (Trolox) neutralized generated DPPH radicals at $IC_{50}$ value of 0.75 ± 0.05 µg/mL.

The herein reported antibacterial and antioxidant activities of *E. cardamomum* EO could be attributed to the presence of high content of oxygenated monoterpenes, mainly 1,8-cineole, *a*-terpinyl acetate (Das et al., 2012; Elguindy et al., 2016; Tangjitjaroenkun et al., 2020). Its EO was reported to inhibit the growth of Gram-positive and negative bacteria with MIC (0.4-0.8%, v/v) (Gochev et al., 2012). Remarkably, the anti-*S. aureus* activity of *E. cardamomum* EO and the anti-quorum sensing activity toward *Pseudomonas aeruginosa* PAO1 with significant inhibition in the production of virulence factors (elastase and protease) were ascribed to the 1,8-cineole content (Noumi et al., 2018); 1,8-cineole was more potent than the EO *per se*. In fact, 1,8-cineole influences the integrity of the cell membrane and causes alteration in the morphology of the bacterial cell wall (Li et al., 2014). Furthermore, in concomitance with our antioxidant results on *E. cardamomum* volatile constituents, EO extracted with tetrafluorethane and the methanol caused inhibition in DPPH radicals, but at $IC_{50}$ values thousand-fold (Gochev et al., 2012) and tenfold (Khalaf et al., 2008) higher than herein obtained result (0.057 ± 0.013 µL/mL, mg/mL); the $IC_{50}$ values were 63.3 mg/mL and 0.681 mg/mL, respectively. Such variation in effective concentration might imply the role of extractant on the EO activity. Worthy to note, *E. carda-*

*momum* EO up-regulated the antioxidant and detoxification enzymes, such as glutathione-S-transferase, glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase (Das et al., 2012; Elguindy et al., 2016).

In contrast to EO of *E. cardamomum,* EO of *C. verum* exhibited high antibacterial activities against tested bacterial strains (21-29 mm at 5 µL/disc and MIC 1.25-5.00 µL/mL) with the Gram-negative *E. aerogenes* being the least sensitive bacteria to the tested EO (Table 3), but it was devoid of antioxidant potential in both DPPH and ABTS assays. Our finding is consistent with reported activities of *C. verum* EO against *E. coli, P. aeruginosa,* and *B. subtilis* starting from 0.5-1.0 mg/mL (Sharifan et al., 2016), *Streptococcus mutans* and *S. sobrinus*, with MIC of 0.02% (v/v) (Choi et al., 2016), and the broad antibacterial activity of *C. zeylanicum* at MIC 0.78-6.25 mg/mL (Behbahani et al., 2020). Furthermore, *C. zeylanicum* EO at a concentration up to 2.5 mg/mL did not show any significant antioxidant activity in DPPH assay (Politeo et al., 2006); an activity controversial to the reported ability of *C. cassia* and *C. burmannii* EOs to reduce 50% and 98% of generated DPPH radical at 147.23 µg/L (Brodowska et al., 2016) and 100 µg/mL (Kuspradini et al., 2016), respectively.

Actually, the antioxidant and antimicrobial activities of cinnamon were correlated with the presence of a higher amount of *E*-cinnamaldehyde and eugenol (Behbahani et al., 2020; do Nascimento et al., 2020; Farias et al., 2020). It was proposed that these volatile compounds cause morphological changes in bacteria and thus modulate their growth (Choi et al., 2016); they, individually or within the plant EO, are able to trap free radicals and prevent oxidative damage (Kallel et al., 2019). The presence of a higher amount of eugenol in EO of collected *C. verum* chemotype from São Luís revealed superior antioxidant activity over the chemotype collected from Santa Inês tested at 10 µL/mL in DPPH assay (Farias et al., 2020); while *E*-cinnamaldehyde exhibited potent inhibitory activity for NO synthesis in murine macrophage RAW 246.7 and J774A.1 cell lines (Catalá and Ferrer, 2017). Consequently, the undetectable antioxidant activity of *C. verum* EO in the current study might be related to either presence of *E*-cinnamaldehyde and/or eugenol in lower percentages compared to those studies or evaluating the antioxidant activity of the oil at concentrations lower than that permit its pronounced ability in inhibiting generated radicals in employed assays.

**Table 3.** Antibacterial activity of *E. cardamomum* and *C. verum* essential oils against tested bacterial strains.

| Bacteria name | Concentration (µL/disc) | Diameter of inhibition zone (mm ± SD) | | | |
|---|---|---|---|---|---|
| | | *B. subtilis* | *S. aureus* | *E. aerogenes* | *E. coli* |
| *E. cardamomum* | | | | | |
| | 1 | 12.0 ± 2.8[a*] | - | - | - |
| | 3 | 13.5 ± 3.2[a*] | - | - | - |
| | 5 | 13.5 ± 3.2[a*] | 6.5 ± 0.7[b*] | - | - |
| MIC (µL/mL) | | 3.75[c*] | > 10 | > 10 | > 10 |
| *C. verum* | | | | | |
| | 1 | 17.5 ± 3.5[c*] | 16.5 ± 3.8[c*] | 9.0 ± 1.8[d*] | 13.0 ± 1.3[c*] |
| | 3 | 25.0 ± 1.4[e] | 22.5 ± 3.3[e] | 13.5 ± 2.1[f*] | 22.0 ± 2.8[e] |
| | 5 | 29.0 ± 1.5[g] | 29.0 ± 1.5[g] | 21.0 ± 3.1[g] | 24.0 ± 3.8[g] |
| MIC (µL/mL) | | 1.25[c*] | 1.25[c*] | 5[s*] | 1.25[c*] |
| **Streptomycin** (50 µg/disc) | | 31.7 ± 1.5[h] | 28.3 ± 0.6[h] | 21.3 ± 0.6[h] | 26.3 ± 1.5[h] |
| MIC (µg/mL) | | 0.39[c] | 0.78[s] | 1.56[s] | 1.56[s] |

Data are expressed as means ± SD (n= 3), asterisks indicated statistically significant differences in inhibition zones or MIC values (µL/mL oil v/v = mg/mL w/v) due to activity of EOs compared to the positive control (p<0.05). The mean inhibition zones with similar letters are not significantly different from each other based on the *post hoc* Tukey HSD test. MIC: minimum inhibitory concentration where there was no visible growth, -: not active at tested concentration, s: biostatic, c: bactericidal.

**Table 4.** Cytotoxic activity of *E. cardamomum* and *C. verum* essential oils against breast cancer MDA-MB-231 cell line.

| Cell line | Sample | % inhibition ± SD | | | | | IC$_{50}$ ± SD (µL/mL) | SI |
|---|---|---|---|---|---|---|---|---|
| **Fibroblast** | (µL/mL) | **0.1** | **0.3** | **0.5** | **1** | **3** | | |
| | *E. cardamomum* | 18.3 ± 1.1 | 23.0 ± 2.3 | 26.8 ± 1.7 | 29.6 ± 3.1 | 34 ± 1.6 | 37.7 ± 6.6[*‡] | - |
| | *C. verum* | 24.6 ± 3.3[a] | 34.8 ± 6.1[a,b] | 46.2 ± 2.1[b] | 51.1 ± 1.6[b] | 61.9 ± 4.1[b] | 0.92 ± 0.07[*] | - |
| | **µg/mL** | | | | | | **µg/mL** | |
| | | **0.1** | **0.2** | **0.3** | **0.5** | **1** | | |
| | Doxorubicin | 30.3 ± 1.3[c] | 56.7 ± 5.1[d] | 73.0 ± 3.6[d,e] | 91.6 ± 1.6[f] | 95.1 ± 1.5[f] | 0.16 ± 0.00 | - |
| **MDA-MB-231** | (µL/mL) | **0.1** | **0.3** | **0.5** | **1** | **3** | | |
| | *E. cardamomum* | 1.8 ± 0.3[g] | 61.8 ± 3.1[h] | 67.9 ± 0.8[h] | 79.1 ± 0.6[h,i] | 84.9 ± 0.9[i] | 0.46 ± 0.02[*] | 81.5[*] |
| | *C. verum* | 46.0 ± 1.4[j] | 53.0 ± 5.1[j,k] | 71.0 ± 3.7[k] | 75.0 ± 4.4[k] | 79.0 ± 1.1[k] | 0.14 ± 0.01[*] | 6.6[*] |
| | **µg/mL** | | | | | | **µg/mL** | |
| | (µL/mL) | **0.1** | **0.2** | **0.3** | **0.5** | **1** | | |
| | Doxorubicin | 16.7 ± 5.7[l] | 42.9 ± 4.6[m] | 60.7 ± 8.3[m,n] | 88.3 ± 1.2[n] | 90.1 ± 0.7[n] | 0.22 ± 0.01 | 0.7 |

Results are expressed as means ± SD (n= 3), SI: selectivity index, asterisks indicated statistically significant differences in IC$_{50}$ values (µL/mL oil v/v = mg/mL w/v) or selectivity index of EOs compared to positive control (p<0.05). The mean % inhibition with similar letters is not significantly different from each other based on the *post hoc* Tukey HSD test. ‡ IC$_{50}$ value of *E. cardamomum* EO was estimated based on a dose-response curve that was appraised using PROBIT regression analysis with a 95 % confidence limit.

Captivatingly, EOs of both spices showed potent cytotoxic activity against the MDA-MB-231 breast cancer cell line. EO of *C. verum* was more effective than *E. cardamomum* EO in inhibiting tumor cell proliferation with IC$_{50}$ 0.14 µL/mL compared to 0.46 µL/mL, respectively (Table 4). *E. cardamomum* and *C. verum* EOs were significantly more selective than the standard drug (DOX) in inhibiting the MDA-MB-231 breast cancer cells proliferation with SI of 81.5 and 6.6 compared to 0.7, respectively (p<0.05). Worth noting, the ability of *E. cardamomum* volatile constituents to up-regulate the antioxidant enzymes, bind to various cellular growth factors, and modulate several cellular signaling in survival/apoptosis pathways of the cell could be the profound mechanisms through which it exhibits the anticancer competence (Elguindy et al., 2016). Furthermore, it was documented that *E. cardamomum* EO has a chemoprotective effect on chemically induced hepatocarcinoma and skin papilloma in mice-model; it down-regulated and deactivated the expression of the proinflammatory factors, such as the nuclear factor NF-κB, the tumor necrosis factor TNF-*a*, and the cyclooxygenase COX-2 (Das et al., 2012; Elguindy et al., 2016). Therefore, the oil might inhibit cancer cell proliferation, angiogenesis, invasion, metastasis, and suppress cellular apoptosis.

However, the promising anti-breast cancer proliferation activity of *C. verum* EO, noticed herein, is relatively in accordance with the approved cytotoxic activity of *C. zeylanicum* against ras active fibroblast-like 5RP7 cells (IC$_{50}$ 20 µg/mL) (Unlu et al., 2010), breast cancer cell lines (MCF-7 and MDA-MB-231), neuroblastoma cells SH-SY5Y, and erythroleukemia cells K562 (IC$_{50}$ 6-20 ppm) (Najar et al., 2020); an activity that might refer to the *E*-cinnamaldehyde, *O*-methoxycinnamaldehyde, and eugenol content. *E*-cinnamaldehyde was reported to induce G1 cell cycle arrest in melanoma cells and G2/M cellular arrest in lymphomas and colon cancer (Nagle et al., 2012). Meanwhile, *O*-methoxycinnamaldehyde has anti-angiogenesis and inhibited colon cancer cell proliferation; eugenol induced the pro-apoptotic and cycle regulator proteins or suppressed the pro-inflammatory, tumor progression, and tumor metastasis nuclear factor NF-κB (Al-Sharif et al., 2013). These volatile constituents exert their effect, due to their hydrophobicity, by passing through the cell wall and membranes and accumulating in the lipid bilayer; distorting the lipid-protein interaction, altering the pH gradient across membranes leading to reduced ATP pool, and causing loss of mitochondrial transmembrane potential leading to cell death by apoptosis and necrosis (Angelini et al., 2018).

Altogether, the variation in chemical composition and dominant constituents in EOs from each of the two spices were ascertained to what have previously been reported, variation in some biological activities was also noticed. EOs containing mainly aromatic phenols or aldehydes have been reported to exhibit substantial antimicrobial activity compared to those containing terpene derivatives (Choi et al., 2016). Furthermore, the lower susceptibility of Gram-negative

bacteria to EOs than Gram-positive bacteria could be attributed to the presence of the outer membrane and the lipopolysaccharide barrier for the hydrophobic compounds that deteriorate their bioactivities. In several reports, oxygenated monoterpenes in essential oil demonstrated antioxidant activity (Tangjitjaroenkun et al., 2020), but the fail of the ABTS assay to detect the antioxidant activity of *E. cardamomum* might be attributed to the fact that EO is a hydrophobic extract, which was insoluble in ABTS aqueous solution in contrast to DPPH method that predicts the antioxidant capacity for hydrophobic antioxidants. Moreover, EOs with high content of phenolic compounds and also some monoterpenes and sesquiterpenes possessed potential cytotoxic activity against various cancer cell lines (Kallel et al., 2019).

Remarkably, in the current study, the dosage of EOs required to inhibit the growth of 50% of tested cancer cell line (0.14-0.46 µL/mL, mg/mL) was more than the criteria set by the National Cancer Institute for natural substances considered cytotoxic ($IC_{50} < 30$ µg/mL) (Pérez-González et al., 2019) indicating their potential biosafety as crude EOs.

## CONCLUSION

*E. cardamomum* EO has oxygenated monoterpenes as the dominant constituents. It exhibited weak activity against tested Gram-positive bacteria and promising antioxidant activity. However, the EO of *C. verum* constituted mainly of phenylpropanoids compounds and exhibited potent antibacterial activity against both tested Gram-positive and Gram-negative bacteria. Furthermore, EOs of both spice plants showed antiproliferative activity against the breast adenocarcinoma (MDA-MB-231).

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ACKNOWLEDGMENTS

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|---|---|---|---|---|---|---|
| Concepts or ideas | x | x | x | | | x |
| Design | x | x | x | | | x |
| Definition of intellectual content | x | x | x | | | |
| Literature search | | | x | x | x | |
| Experimental studies | x | x | | x | x | x |
| Data acquisition | x | x | | x | x | |
| Data analysis | x | x | x | x | x | |
| Statistical analysis | x | | | x | | |
| Manuscript preparation | x | | | | x | |
| Manuscript editing | x | x | x | | | x |
| Manuscript review | x | x | x | x | x | x |