Effects of essential oils of *Elettaria cardamomum* grown in India and Guatemala on *Pseudomonas aeruginosa*, *Escherichia coli,* and gastrointestinal disorders

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Abstract

The present study examined the volatile composition and antimicrobial and gastrointestinal activity of the essential oils of *Elettaria cardamomum* (L.) Maton harvested in India (EC-I) and Guatemala (EC-G). Monoterpene were present in higher concentration in EC-I (83.24%) than in EC-G (73.03%), whereas sesquiterpenes were present in higher concentration in EC-G (18.35%) than in EC-I (9.27%). Minimum inhibitory concentrations (MICs) of 0.5 and 0.1 mg/mL were demonstrated against *Pseudomonas aeruginosa* in EC-I and EC-G, respectively, whereas MICs of 0.125 and 1 mg/mL were demonstrated against *Escherichia coli* in EC-I and EC-G, respectively. The treatment with control had the highest kill-time potential, whereas the treatment with oils had shorter kill-time. EC-I was found to be more potent in the castor oil-induced diarrhoea model than EC-G. At 100 and 200 mg/kg, EC-I exhibited 40% and 80% protection, respectively, and EC-G exhibited 20% and 60% protection, respectively, in mice, whereas loperamide (positive control) exhibited 100% protection. In the in vitro experiments, EC-I inhibited both carbachol (CCh, 1 µM) and high K^+ (80 mM)-induced contractions at significantly lower concentrations than EC-G. Thus, EC-I significantly inhibited *P. aeruginosa* and *E. coli* and exhibited more potent antidiarrheal and antispasmodic effects than EC-G.

Keywords: *Elettaria cardamomum*, GC-MS, compositions, antibacterial, antidiarrhoeal, antispasmodic.

1. Introduction

E. cardamomum (L.) Maton*,* belonging to the *Zingiberaceae* family (local name: cardamom), is an expensive and commercially significant spice that is in demand worldwide. Although it is native to India and Sri Lanka, it is also grown in Guatemala, Thailand, El Salvador, Malay Archipelago, Vietnam, Papua New Guinea, Cambodia, Laos, and Tanzania, with Guatemala being the largest producer of *E. cardamomum* in the world [1-2].

Different species of the same plant may possess different activities and may have advantages over other species depending upon the variations in their yield due to differences in cultivation in their native areas [3]

E. cardamomum oil is known for its characteristic aroma and is widely used in the food and cosmetic industries as a flavouring and fragrance agent. It is an intestinal smooth muscle relaxant [4] and has exhibited antispasmodic, antidiarrhoeal, and antibacterial activities [5-7]. The antibacterial effect of essential oil of *E. cardamomum* against several Gram-negative bacteria [8] such as *Escherichia coli* and *Pseudomonas aeruginosa* has been reported. *E. coli* commonly resides in human colon [9], whereas *P. aeruginosa,* which is difficult to treat due to its innate resistance to several antibiotics [10], is transmitted along the food chain into the gut. Both these organisms could potentially cause diarrhoea [11]. Contradictory reports exist on the efficacy of *E. cardamomum* against both these bacteria. Few studies have reported its efficacy against *E. coli* but not against *P. aeruginosa* [12], whereas another study has reported its efficacy against *P. aeruginosa,* but not against *E. coli* [13].

These differences could be attributed to the variability or differences in solvent extraction. Therefore, comparative studies of different species must be conducted not only to understand their full potential as herbs but also to identify the most preferable species because cardamom growing in a specific region provides more health benefits than those growing in other regions. Additionally, a number of these activities have been tested in aqueous methanolic extracts rather than essential oils, which may exhibit different activities depending on the constituents eluted. Therefore, the present study attempted to compare the chemical composition and antimicrobial activity of the essential oil of Guatemalan and Indian *E. cardamomum* against *E. coli* and *P.*

aeruginosa and to explore the *in vivo* antidiarrheal effect and *in vitro* antispasmodic activity of the two oils to clarify differences in their medicinal properties.

2. Materials and methods

2.1 Fruits samples and chemicals

Capsules of Indian green cardamom (EC-I) (Emperor Akbar; 250 g) and Guatemalan green cardamom (EC-G) (Al-Othaim; 1 kg) were purchased in January 2019 from the Al-Kharj, Saudi Arabia. Samples were authenticated and kept in the herbarium (Indian: EC-Indian-01-PSAU/3/20 and Guatemala: EC-Guatemala-PSAU/2/20) of the Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia. Carbamylcholine (CCh), loperamide, and acetylcholine perchlorate (ACh) were obtained from Sigma Company, St. Louis, MO, USA. Potassium chloride (Sigma Co), calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, and sodium chloride (Merck, Germany) were used as reagents (salts) to prepare physiological buffer solution (Tyrode). All chemicals were of analytical grade, whereas castor oil was purchased from local pharmacy.

2.2 Isolation of essential oils

The capsules were ground, and the essential oil was extracted using a Clevenger apparatus. For 3 h, 100 gm of each sample powder was extracted, and the percentage yield was calculated after repeating the process thrice. The extracted essential oils were dried over anhydrous Na_2SO_4, transferred to an amber-coloured tight vial, labelled as EC-I or EC-G, and stored at 4°C for further analysis.

2.3 Gas chromatography–mass spectrometry analysis

The gas chromatography–mass spectrometry (GC–MS) analysis of EC-I and EC-G essential oils was performed using the Shimadzu GC–MS system (TQ-8040, Tokyo, Japan) equipped with auto-sampler (AOC-20i). Analysis of the volatile composition was performed in the ionization mode (70 eV) with a scan time of 0.3 s and *m/z* range of 45–400 u. Both the injector and detector temperatures were set at 210°C. The Rxi-5 MS capillary column (0.25 mm inner diameter, 30 m × 0.25 μm) contained the stationary phase comprising 5% two-phenyl, and 95% two-methyl polysiloxane. The column temperature was programmed as follows: initial oven temperature

programmed at 40°C, held for 3 min; gradually raised to 90°C at 3°C/min, held for 4 min; raised to 115°C at 3°C/min, held for 10 min; and then increased to 140°C at 2°C/min and held for 8 min. Finally, the column temperature was increased to 210°C at 3°C/min and held for 5 min. The carrier gas was helium (99.995%) at a constant flow rate of 1 mL/min. The oil identification composition was based on a comparison of their mass spectra and retention time with data of libraries, NIST-14 and NIST-14s (National Institute of Standards and Technologies, Mass Spectra Libraries).

2.4 Antibacterial activity

2.4.1 Microorganisms and agar media

The antibacterial effect of the EC-I and EC-G oils was tested against two bacterial strains, namely *P. aeruginosa* (ATCC 27853*)* and *E. coli* (ATCC 35218).

2.4.2 Antibacterial Assay

The antibacterial activity of the essential oils was assayed using the disc diffusion method [14] with some modification, and each test was repeated thrice. Mueller–Hinton agar (MHA) was used for the antibacterial assay. *P. aeruginosa* and *E. coli* cultures in nutrient broth (HiMedia Biosciences) were separately inoculated and grown for 18 h at 37°C. The suspension of both the organisms was separately diluted with saline (phosphate buffer, pH 7.4) to obtain 1×10^6 colony forming units (CFU/ mL) of microbial suspension.

The bacterial inoculums were streaked onto an MHA plate by using a sterile swab. A 6-mm sterile disk was impregnated with 10 mg of EC-I and EC-G essential oil, and 2% dimethyl sulfoxide (DMSO) was used as the negative control. The plates were labelled, and the disks containing essential oil were placed onto the plates and incubated for 18–24 h at 37°C. The diameter of inhibition around the disk was measured and the mean of three tests was reported as the zone of inhibition. Different concentrations (4, 2, 1, 0.5, 0.25, and 0.125 mg/mL) of EC-I and EC-G essential oil were prepared in analytical grade 2% DMSO and used for the determination of minimum inhibitory concentration (MIC) through the broth dilution method.

2.5 Time-kill analysis

Time-kill kinetics of essential oil of EC-G and EC-I samples was performed using the method described by Kang et al. (2018) with slight modification. Two concentrations equivalent to $1 \times$

MIC (1 mg/mL and 0.5 mg/mL for *E. coli* and *P. aeruginosa,* respectively) and $2 \times$ MIC (2 mg/mL and 1 mg/mL for *E. coli* and *P. aeruginosa,* respectively) of the essential oil were prepared. An inoculum size of 1×10^6 CFU/mL was added and incubated at 37°C. A total of 1-mm inoculum of the medium was obtained at different time intervals of 0, 2, 4, 8, 12, 18, and 24 h. The colony forming unit (CFU) of the bacterial cells was determined. A negative control containing organisms and DMSO (without essential oil) was also evaluated. The assays were performed in triplicate, and time-kill graphs were constructed by calculating the log CFU/mL of mean colony count against time.

2.6 Gastrointestinal activities

Animals: Wistar rats (200–250 g) and Swiss albino mice (25–30 g) were purchased from the local animal vendors and housed in the animal house of the Barrett Hodgson University (BHU), Karachi, Pakistan. The rats were sacrificed under light anaesthesia (using thiopental sodium 70–90 mg/kg), followed by cervical dislocation. The guidelines detailed in the National Research Council [15] were followed for all animal-based experiments. The protocols were approved by the Ethical Committee of Research on Animals of the BHU bearing ERC number: BHU-ERC/Pharmacy-001/2020/PI-Dr. Amber Hanif Palla. All results were reported in accordance with the Animal Research: Report of In-vivo Experiments (ARRIVE) guidelines [16].

2.7 In vivo antidiarrheal study on mice

A total of 35 mice were arbitrarily allocated to 7 groups with equal numbers of mice in each group. Following 24 h of fasting, mice of the 1st and 2nd groups were exposed to oral gavage of saline (10 mL/kg) and labelled as sham control and negative control, respectively. After pilot screening for dose selection, the 3rd and 4th groups (test groups) were administered two increasing doses of EC-I (100 and 200 mg/kg), whereas the 5th and 6th groups were administered EC-G (100 and 200 mg/kg). The last group was administered loperamide (10 mg/kg) and labelled as the positive control. Separate cages were assigned to each animal with a blotting sheet on the floor of each cage to know the absence or presence of diarrhoea by a blinded observer. After 1 h, all mice except the sham controls were orally exposed to castor oil (10 mL/kg) by using a 1-ml syringe. After 4 h, blotting sheets in all individual cages were inspected for typical diarrhoeal droppings. Protection was noted in case of diarrhoeal drops, as previously reported by Rehman et al., [17].

2.8 In vitro antispasmodic activity on isolated rat ileum

The method described by Shah et al., [18] was followed to sacrifice rats and to isolate the ileum, the last part of the small intestine. Briefly, rats were anaesthesised with thiopental sodium (70 mg/kg, given intraperitoneally) and then cervically dislocated by a blow on the head. Following isolation, required segments of the ileum (2–3 cm length) were cleaned from adjacent tissues and faecal material and mounted in a tissue bath (volume 20 mL) that was attached with an isotonic transducer coupled to a digital PowerLab (ML-845) data acquisition system (AD Instruments; Sydney, Australia) and a computer using lab chart software (version 5.3). A fresh tyrode was filled in 20-mL tissue baths gassed with carbogen, and temperature was set at 37°C. The composition of Tyrode's solution (mM) was as follows: KCl, 2.68; NaCl, 136.9; $MgCl_2$, 1.05; $NaHCO_3$, 11.90; NaH_2PO_4, 0.42; $CaCl_2$, 1.8; and glucose, 5.55; pH 7.4). Tension of 1 g was applied by rotating the transducer knob clockwise, and the tissues were left for stabilisation for 30 min with multiple exposures to acetylcholine (0.3 µM). After obtaining the stable band in the spontaneous ileal contractions, test samples were added to the bath solution in increasing concentrations, which resulted in the inhibition of the CCh and high K^+-induced contractions.

2.9 Statistics

Results of the antibacterial assay were expressed as mean of three repeated experiments. Protection from diarrhoea was statistically evaluated by comparing all the groups with the saline control group by using Chi square (χ^2) test. A P value of <0.05 was considered statistically significant. Results of the antispasmodic activity assay are expressed as mean ± standard error of mean (SEM). The statistical parameters applied were Student's t-test or two-way ANOVA followed by Bonferroni's post-test for multiple comparisons of concentration-response curves (CRCs) with control. Graph Pad prism (version 4) was used for regression analysis of CRCs.

3. Results

Table 1 presents the compositions of the essential oils of EC-I and EC-G capsules identified through GC–MS. About 64 and 75 constituents were identified in the essential oils obtained from EC-I and EC-G, respectively. Total ion–current chromatograms of the typical essential oil (EC-I and EC-G) are shown in Figure 1.

Table 1: Metabolite identified in the essential oils of Guatemala and Indian *E. cardamomum* using GC-MS

No	RT	% Area *E. cardamomum*		Compositions
		EC-G	EC-I	
Monoterpenes hydrocarbons (MTH)				
1	5.436	0.43	0.72	α-Phellandrene
2	5.859	0.09	0.13	Camphene
3	6.296	2.22	3.74	β-Phellandrene
4	6.365	0.66	1.03	Sabinene
5	6.543	2.13	3.46	β-Pinene
6	6.995	1.4	0.06	(+)-2-Carene
7	7.216	1.36	1.77	DL-Limonene
8	7.515	2.01	2.95	β-cis-Ocimene
9	7.738	1.23	1.51	γ-Terpinen
10	8.172	1.22	2.80	α-Terpinene
11	8.595	0.2	-	β-Myrcene
12	10.686	3.21	2.89	β-trans-Ocimene
Monoterpenes oxygenated (MTO)				
13	7.478	10.59	14.03	1,8-Cineole
14	7.918	0.81	-	Dihydrocarveol
15	7.921	-	1.25	trans-Sabinenhydrate
16	8.407	5.51	2.63	Linalool
17	8.798	0.79	0.94	α-Terpinenol
18	9.166	0.2	-	(S)-cis-verbenol
19	9.58	0.96	-	α-Phellandren-8-ol
20	9.737	3.45	3.53	Terpinen-4-ol
21	9.978	3.45	5.11	β-Fenchyl alcohol
22	10.054	0.21	0.49	n-Nonyl acetate
23	10.121	-	0.08	trans-Pipertiol
24	10.158	0.88	-	(D)-Verbenone
25	10.335	1.88	0.31	Z-Citral
26	10.859	4.00	3.55	cis-Geraniol (Nerol)
27	11.218	0.35	0.23	Borneyl acetate
28	11.357	0.65	0.12	4-Terpinenyl acetate
29	11.501	-	0.2	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, (1.alpha.,3.beta.,4.beta.,6.alpha.)-

30	11.607	0.85	0.58	(E) Ocimenyl acetate
31	11.671	-	1.96	(Z)-Geranic acid,
32	11.743	1.40	-	Thymol
33	12.389	18.71	24.65	α-terpinyl acetate
34	12.53	1.93	2.52	cis-Geranyl acetate
35	22.385	0.25	-	Myrtanol
Sesquiterpenes hydrocarbons (STH)				
36	12.769	0.44	0.29	E)- β-Eelemene
37	13.335	0.96	0.21	α-Muurolene
38	13.933	0.99	1.58	D-Germacrene
39	14.015	1.28	0.11	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-
40	14.06	3.16	1.89	δ-Guaiene
41	14.131	-	1.19	α-selinene
42	14.42	-	0.07	α-Panasinsanene
43	14.906	-	0.78	α-caryophyllene
44	15.253	0.91	0.26	γ-Gurjunene
45	17.03	0.37	-	Ledene
66	19.043	0.87	-	Alloaromadendrene
Oxygenated Sesquiterpenes (OST)				
47	13.4	0.49	0.19	trans-Caryophyllene oxide
48	13.972	0.79	-	γ-Eudesmol, 10-epi-
49	14.821	5.00	2.29	D-Nerolidol
50	15.684	0.25	-	β-Spathulenol
51	16.01	1.15	-	α-Cadinol
52	16.31	0.41	-	Longifolenaldehyde
53	16.452	0.15	0.06	Aromadendrene oxide-(1)
54	16.526	-	0.12	(Z,E)-Farnesal
55	16.823	0.3	0.09	(Z,Z)-Farnesal
56	17.822	0.29	0.14	Farnesyl acetate
57	18.219	0.13	-	Isoaromadendrene epoxide
58	19.381	0.41	-	Costunolide
Diterpenes (DT)				
59	19.951	0.19	-	Cembrene C
60	23.244	0.43	1.08	α-Springene
Oxygenated diterpenes (ODT)				
61	15.108	0.27	-	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-
62	21.797	0.14	-	Thunbergol
Non-terpenes				
63	6.42	0.18	0.03	[a] 6-Methyl-5-hepten-2-one

64	6.775	0.31	0.29	[(b)] Octanal
65	8.222	0.14	0.14	[(g)] Benzene, 2-ethenyl-1,3-dimethyl-
66	8.709	t	0.09	[(c)] Octanoic acid, methyl ester
67	9.132	-	0.13	[(c)] Pentanoic acid, 2-hydroxy-4-methyl-, methyl ester
68	9.32	0.16	0.16	[(a)] Sabine ketone
69	9.393	0.13	0.47	[(f)] 1,2-Dimethyl-3,5-divinylcyclohexane
70	9.842	-	0.25	[(b)] cis-4-Decenal
71	11.265	-	0.09	[(a)] 2-Undecanone
72	12.674	0.18	-	[(f)] Cyclodecene
73	12.679	-	1.01	[(b)] 9,17-Octadecadienal
74	13.01	0.12	0.23	[(c)] 1-Decanol acetate
75	15.088	-	0.31	[(e)] 5,7-Dodecadiene, (Z,Z
76	15.534	0.42	-	[(h)] 7-Heptadecyne, 1-chloro-
77	15.81	0.24	-	[(f)] (1'RS,2'RS,3'SR)-3-(2',3'-epoxy-2',6',6'-trimethylcyclohexyl)-1-methyl-1-cyclobutene
78	17.51	0.08	-	[(i)] β-Ionol
79	17.663	0.62	0.09	[(f)] 2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one
80	20.153	0.27	0.44	[(j)] Tetracosamethyl-cyclododecasiloxane
81	20.469	t	-	[(h)] Cholest-5-ene, 3-bromo-, (3.beta.)-
82	20.827	0.91	0.48	[(f)] Cyclodeca-cyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-
83	21.235	0.1	-	[(h)] 6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-,
84	21.403	0.13	0.09	[(j)] Cyclononasiloxane, octadecamethyl-
85	22.022	0.1	t	[(i)] 13-Octadecenal
86	22.242	0.08	0.06	[(d)] Heptadecane, 2,6,10,15-tetramethyl -
87	22.557	1.94	1.69	[(j)] Eicosamethylcyclodecasiloxane
88	23.884	0.3	-	[(h)] Octadecane, 1-chloro-

RT:

Retention time (min) and percentage area based on available libraries (e.g., NIST or Wiley).

Others compounds a: Ketone, b: Aldehyde, c: Ester, d: Alkane, e: Alkene, f: Cycloalkane, g: Benzene, h: Halogen, i: nor isoprenoids, j: siloxane, (-): not identified, (t): trace.
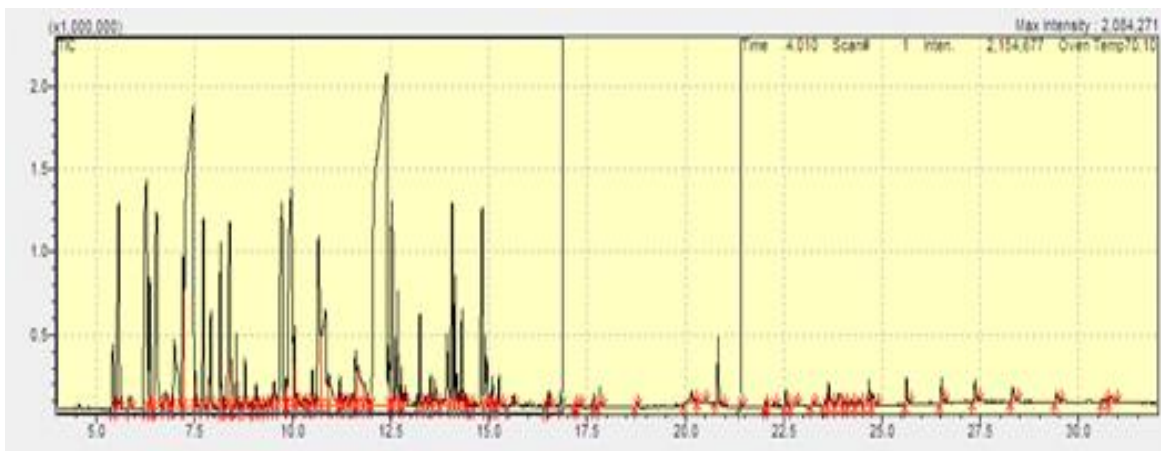
A

B

Figure 2. GC-MS chromatograms of *Elettaria cardamomum* of A (Indian, EC-I) and Guatemala, EC-G) essential oils

The percentage yield of essential oil extracted from EC-I capsules (4.8%) was higher than EC-G (3.9%) capsules. The oxygenated monoterpene and α-terpinyl acetate were the main constituents of the essential oils of EC-I (24.65%) and EC-G (18.71%), respectively, whereas 1,8 cineole was the identified as the second main volatile constituent, with percentages of 14.03% and 10.59%,

respectively, in EC-I and EC-G essential oils. Phellandrene, β-pinene, limonene, α-terpinene, ocimene, linalool, terpinen-4-ol, β-fenchyl alcohol, cis-geranyl acetate, guaiene, and nerolidol were identified as other components common to both the samples.

Volatile components were divided into monoterpenes (hydrocarbons and oxygenated), sesquiterpenes (hydrocarbons and oxygenated), diterpenes (hydrocarbons and oxygenated), and non-terpenes, on the basis of their functional groups (Table 2). Out of the total components, approximately 73.03% and 83.24% monoterpenes were identified in the essential oil of EC-G and EC-I capsules, respectively.

Table 2: Class of terpene identified in the essential oils obtained from Guatemala and Indian *E. cardamomum*

Terpenes	EC-G		EC-I	
	no. of compounds	% Area	no. of compounds	% Area
Monoterpenes Hydrocarbons	12	16.16	11	21.06
Oxygenated monoterpenes	19	56.87	17	62.18
Total Monoterpenes	**31**	**73.03%**	**28**	**83.24%**
Sesquiterpenes Hydrocarbons	8	8.98	9	6.38
Oxygenated Sesquiterpenes	11	9.37	6	2.89
Total Sesquiterpenes	**19**	**18.35%**	**15**	**9.27%**
Diterpenes hydrocarbons	2	0.62	1	1.08
Oxygenated diterpenes	2	0.41	0	0
Total Diterpenes	**4**	**1.03%**	**1**	**1.08%**
Others				
Ketone a	2	0.34	3	0.28
Aldehyde b	1	0.31	3	1.55
Ester [c]	2	0.16	3	0.45
Alkane d	1	0.08	1	0.06
Alkene e	0	0	1	0.31
Cycloalkane f	5	2.08	3	1.04
Benzene g	1	0.14	1	0.14
Halogen h	4	0.86	0	0
13-Octadecenal i	1	0.1	1	0.04
Silioxane j	3	2.34	3	2.22
Total (88)	74	98.82	63	99.68

Among monoterpenes, approximately 56.87% and 62.18% oxygenated monoterpenes were identified in the EC-G and EC-I oils, respectively, whereas 16.16% and 21.06% monoterpene hydrocarbons were identified in the EC-G and EC-I essential oils, respectively. This analysis represented the chemical difference in the EC-G and EC-I samples.

3.1 Antimicrobial activity

The antibacterial activity of EC-I and EC-G is presented in terms of zone of inhibitions (ZOI) and MIC in Table 3.

Table 3: Antimicrobial activity of the essential oils obtained from EC-G and EC-I.

Microorganism	EC-G		EC-I	
	ZOI (mm)	MIC (mg/mg)	ZOI (mm)	MIC (%)
P. aeruginosa	17.33 ± 0.47	0.5	16.66 ± 0.47	0.5
E. coli	10.13 ± 0.23	1	14.4 ± 0.1	1

The ZOI differed marginally with different capsules and microorganisms used in the assay. Both the samples were found to be inhibitory to *P. aeruginosa* and *E. coli,* and the EC-G oil was found to be the most active agent. The MIC of EC-G oil was found to be 0.5%–1%, whereas that of EC-I was 1% against both the bacteria. Thus, the EC-G oil was more active against both the gram negative bacteria.

3.2 Time-kill kinetic assay

Time-kill assays were performed to explore the cell viability (kill-time) of EC-G and EC-I essential oil, and the results were articulated as a logarithm of viable counts (Figures 1 and 2). Non-treated *E. coli* exhibited growth from 5.24 to 8.32 $\log_{10}$ CFU/mL and moved into the static phase after 8 h. After treatment with EC-G, *E. coli* growth decreased dramatically in the first 8 h and retained steadily at approximately $3.45 \times \log_{10}$ CFU/mL, whereas EC-I treatment decreased the growth in the first 8 h and retained steadily at approximately $2.99 \times \log_{10}$ CFU/ml, suggesting a stronger EC-I killing efficacy against *E.coli*.

Similarly, non-treated *P. aeruginosa* exhibited growth from 5.17 to 8.17 $\log_{10}$ CFU/mL and moved after 8 h into the static phase. After treatment with EC-G, *P. aeruginosa* growth decreased dramatically in the first 4 h and retained steadily at approximately $2.94 \times \log_{10}$ CFU/mL. After

treatment with EC-I, *P. aeruginosa* growth decreased in the first 4 h and was retained steadily at approximately $2.04 \times \log_{10}$ CFU/mL, suggesting a stronger EC-I killing efficacy against *P. aeruginosa*. The plot of both the samples assessed at the $2 \times$ MIC level was almost similar to that at $1 \times$ MIC. The results indicated that EC-G exhibits a lethal effect on *P. aeruginosa* and *E. coli* after 4 h and 8 h, respectively.
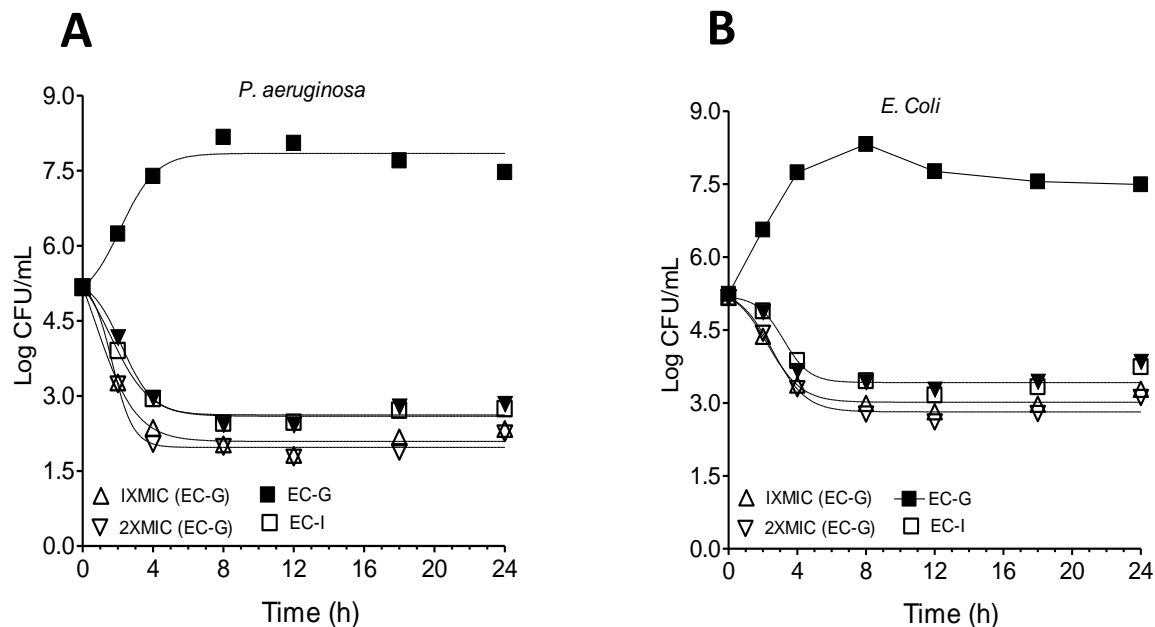

Figure 1: Time-kill analysis of *P. aeruginosa* and *E. coli*.

Similarly, EC-I exhibited a lethal effect on the growth of both *P. aeruginosa* and *E. coli* after 8 h of incubation. The plot of both samples measured at the 2-MIC stage was approximately identical to that at 1-MIC. EC-I exhibited a rapid killing effect on *P. aeruginosa* development, with a lethal effect after 4 h of incubation and after 8 h on *E. coli*. The effects of EC-I on *P. aeruginosa* and *E. coli* growth were destroyed after 8 h of incubation.

3.3 Gastrointestinal activity

3.3.1 In vivo antidiarrhoeal study on mice

Protection in castor oil-provoked diarrhoea: Both orally administered samples of EC-I and EC-G exhibited dose-dependent protection of mice, whereas the saline group did not exhibit any effect. At the lower tested dose of EC.I (100 mg/kg), two out of five mice exhibited protection, indicating

40% protection. A higher dose of 200 mg/kg exhibited 80% protection, whereas 20% and 60% protection was observed at lower (100 mg/kg) and higher doses (200 mg/kg), respectively. No diarrhoeal spot was observed in any mice treated with loperamide (100% protection) (Table 4).

Table 4: Comparative antidiarrheal activities of the extracted essential oil of Elettaria cardamomum of Indian (EC-I) and Guatemala (EC-G) on castor oil (10 mL/kg)-induced diarrhea in mice.

Treatment (p.o.), dose (mg/kg)	No. of mice with diarrhea	% Protection
Saline (10 mL/kg) + Castor oil	5/5	0
EC-I + Castor oil		
100 (mg/kg) + 10 (mL/kg)	3*/5	40
200 (mg/kg) + 10 (mL/kg)	1*/5	80
EC-G + Castor oil		
100 (mg/kg) + 10 (mL/kg)	3/5	20
200 (mg/kg) + 10 (mL/kg)	1*/5	60
Loperamide (10 mg/kg) + Castor oil	0**/5	100

* $P < 0.05$ and ** $P < 0.01$ vs. Saline + Castor oil treated group (χ^2-test).

3.3.2 Gut inhibitory effects

When tested against CCh and high K^+-mediated spasm in rat ileum preparations, EC-I and EC-G caused dose-dependent (0.01–5 mg/mL) complete inhibition. In CCh-mediated contractions, EC-I exhibited inhibition with resultant EC_{50} values of 0.76 mg/mL [0.54–0.92, 95% confidence interval (CI), n = 4], whereas EC-G exhibited inhibition with higher EC_{50} value of 4.22 mg/mL (3.86–4.12, 95% CI, n = 4) (Figure 1A). EC-I and EC-G exhibited inhibition against high K^+-mediated contractions with EC_{50} values of 0.08 mg/mL (0.06–0.09, 95% CI, n = 4) and 0.24 mg/mL (0.18–0.28, 95% CI, n = 4), respectively (Figure 1B).
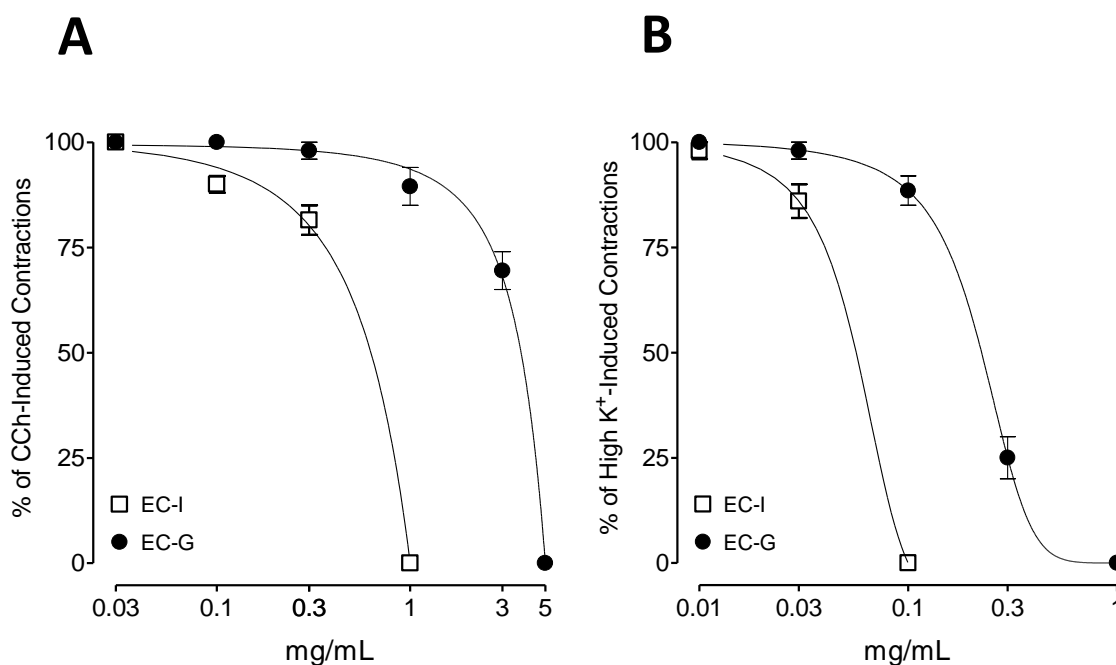
Figure 3. Concentration-response curves showing comparison of the extracted essential oil of *Elettaria cardamomum* of Indian (EC-I) and Guatemala (EC-G) for the inhibitory effect against **(A)** carbachol (CCh, 1 μM) and **(B)** high K$^+$-induced contractions in isolated rat ileum preparations. Values shown are mean ± SEM, n=4-5.

4. Discussion

Studies have reported that for better fragrances, α-terpinyl acetate is always present in higher amount than 1,8 cineole, which may also be an indicator of high-quality *E. cardamomum* essential oils; findings of the present study are concurrent with earlier reports [19-20].

In the present study, monoterpene components such as β-phellandrene, β-pinene, DL-limonene, β-cis-ocimene, γ-terpinen, α-terpinene, sabinene, α-phellandrene, camphene, β-fenchyl alcohol, terpinen-4-ol, α-terpinyl acetate, cis-geranyl acetate, and D-germacrene were found to be in higher concentrations in the EC-I essential oil. The content of constituents such as β-trans-ocimene and (+)-2-carene, linalool, Z-citral, trans-geraniol, and (E)-ocimenyl acetate was higher in the EC-G essential oil. These components have also been reported by several investigators [20-23]. *Trans-*

sabinenhydrate and (Z)-geranic acid were the two major oxygenated monoterpenes identified only in the EC-I sample, whereas thymol, α-phellandren-8-ol, (D)-verbenone, and dihydrocarveol were other major oxygenated monoterpene identified only in EC-G.

The concentration of sesquiterpene D-germacrene was higher in the essential oils of EC-I, whereas β-elemene, α-murolene, bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-, δ-guaiene, γ-gurjunene, D-germacrene, D-nerolidol, (Z,Z)-farnesal, trans-caryophyllene oxide, aromadendrene oxide-(1), and farnesyl acetate were in higher concentrations in the essential oil of EC-G capsules. Sesquiterpenes such as α-selinene, α-caryophyllene, and (Z,E)-farnesal were identified only in the essential oil of EC-I, whereas ledene, alloaromadendrene, α-cadinol, γ-eudesmol, 10-epi, β-spathulenol, longifolenaldehyde, costunolide, and isoaromadendrene epoxide were identified only in EC-G. Diterpene and α-springene were observed in both the samples (0.43% and 1.08% in EC-G and EC-I oils, respectively), whereas cembrene, kauran-18-al, 17-(acetyloxy)-, (4.beta), and thunbergol were detected only in the essential oil of EC-G. Majority of these components were not reported previously.

In the present study, monoterpenes were in higher concentrations in EC-I (83.24%) than in EC-G (73.03%), whereas sesquiterpenes were in higher concentrations in EC-G (18.35%) than in EC-I (9.27%). However, no significant differences in diterpenes (1.03% and 1.08% in the EC-G and EC-I, respectively) were reported between EC-I and EC-G. Gradinaru et al. reported 84.54% oxygenated monoterpenes and 8.27% monoterpene hydrocarbons [24], whereas Kumar et al reported approximately 87% oxygenated monoterpenes and 8.24% monoterpene hydrocarbons in the essential oil of different cardamom samples [25]. Noumi et al. reported the presence of approximately 88.7% oxygenated monoterpenes and 7% monoterpene hydrocarbons in cardamom essential oils [23].

In the present study, two gram negative bacterial strains, *P. aeruginosa* and *E. coli,* were chosen for measuring the antibacterial activity because these bacteria are becoming resistant to various drugs and scientists are exploring new molecules to combat these resistant strains. In the present study, both samples exhibited antibacterial effects against both selected gram negative bacteria, where the MIC of EC-I was lower than that of EC-G oil. The antimicrobial activity of essential oils depends on the percentage of the active constituents α-terpinyl acetate and 1,8 cineole. The monoterpene hydrocarbons and oxygenated monoterpenes in the essential oil of different plants

possess major antimicrobial, antifungal, and antiviral activities [26]. Our results indicating antibacterial activity against *E. coli* and *P. aeruginosa* are concurrent with those of other studies [12-13]. The cardamom oil was probably active against *P. aeruginosa* and *E. coli* due to the presence of 1,8 cineole and α-terpinyl acetate, which is supported by several investigations [7, 23]. Time-kill kinetic studies indicated that essential oil of *E. cardamomum* exhibits bacteriostatic activities against *P. aeruginosa* and *E. coli,* which may be due to the presence of 1,8 cineole, α-terpinyl acetate and other active antimicrobial volatile agents [27-29].

Keeping in view the medicinal use of *E. cardamomum* in multiple gut-related disorders, the essential oils of EC-I (India) and EC-G (Guatemala) were evaluated and compared for their antidiarrhoeal and gut inhibitory activities through in vivo and in vitro assays. A castor oil-induced diarrhoea model was used to study the antidiarrhoeal effect, whereas isolated rat ileum preparations were used in the in vitro experiments for elucidation of the detailed mechanism [30]. Diarrhoea was induced in normal mice by using castor oil, which after hydrolysis into ricinoleic acid, led to evoked spasms in the gut [31]. Pre-administration of both EC-I and EC-G protected the mice from diarrhoea in a dose-dependent manner; however, higher potency was observed with EC-I. After observing the antidiarrheal response, the method described by Palla et al. was followed to test and compare both the samples for antispasmodic effect in vitro in the isolated rat ileum [32]. For this purpose, EC-I and EC-G cumulative concentrations were added to organ bath after inducing sustained contractions with CCh and high K^+. Interestingly, both samples demonstrated dose-dependent complete inhibition of both types of contraction. A critical analysis of the pattern of the inhibitory CRCs of EC-I and EC-G against CCh and high K^+-induced contractions indicated that EC-I produces relaxation with significantly higher ($p < 0.05$) potency than EC-G. The mechanism supposed to be involved in the antispasmodic effect might be the inhibition of PDE enzyme [6] and voltage-dependent Ca^{++} channels because both these mechanisms are involved in smooth muscles relaxation [33-34]. The antidiarrheal effect of EC-I is related to the inhibition of smooth muscle contraction and may be due to the presence of high concentration of the major compound α-terpinyl acetate and 1,8 cineole in this essential oil [35]. The present study elucidates an additional antispasmodic mechanism of cardamom not reported so far, namely the PDE enzyme inhibition. Gilani et al. reported Ca^{++} channel blocking-like mechanisms; however, they did not test it against CCh-induced contractions, which is used to decipher the PDE inhibitory and/or cholinergic mechanisms (REF). Gilani et al. used aqueous methanolic extract, whereas we used

essential oil of cardamom [6]. However, our results are also concurrent with those reported by Gilani et al. because they reported that the petroleum ether fraction of cardamom is the most potent in the CCB activity (inhibitory effect at 0.1 mg/mL). We explored the antispasmodic and antidiarrhoeal effects of cardamom essential oils for the first time, and our findings indicate that the activity of oils varies mainly due to the presence of 1,8 cineole.

5. Conclusion

GC–MS analysis revealed that α-terpinyl acetate and 1,8 cineole are the major components and present in high concentrations in EC-I. Monoterpenes (MTH and MTO) were identified as the major components in both the essential oils; however, EC-I was found to have higher percentage of monoterpenes than EC-G. Both EC-G and EC-I oils possessed significant antibacterial activity, with EC-I processing more active components than EC-G essential oils. In addition to the antibacterial activity, essential oil of *E. cardamomum* also exhibited antidiarrhoeal effects along with the antispasmodic activity. Overall, these differences may be due to the presence of different percentages of active and other constituents in the EC-G and EC-I samples. Thus, EC-I exerts more potent antidiarrheal and antispasmodic effects than EC-G.

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