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Antioxidant and antimicrobial activities of essential oil and various oleoresins of *Elettaria cardamomum* (seeds and pods)†

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Abstract

BACKGROUND: This paper describes the chemical analysis of the essential oil and various oleoresins of *Elettaria cardamomum* (seeds and pods) by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) techniques. It also compares the effects of the different extraction solvents used (chloroform, methanol, ethanol and diethyl ether) on the antioxidant and antimicrobial activities of the essential oil and oleoresins.

RESULTS: The essential oil was found to contain 71 compounds. The major components were α-terpinyl acetate (44.3%), 1,8-cineole (10.7%), α-terpineol (9.8%) and linalool (8.6%). The chloroform and methanol oleoresins both contained α-terpinyl acetate (21.8 and 25.9% respectively) as the main component, while 5-hydroxymethylfurfural (28.9%) was the most abundant compound in the ethanol oleoresin. However, very few components (total 0.61%) were found in the diethyl ether oleoresin. The antioxidant activities of the essential oil and oleoresins, studied in mustard oil by monitoring the peroxide value of the oil substrate, were comparable to those of the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at 0.02% concentration. The essential oil exhibited strong antibacterial activity against the micro-organisms *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhi* at 3000 ppm by the agar well diffusion method. Antifungal activity was tested against the food-borne fungi *Aspergillus terreus, Penicillium purpurogenum, Fusarium graminearum* and *Penicillium madriti*. The methanol and ethanol oleoresins gave the best results against *A. terreus* at 3000 ppm by the poison food method.

CONCLUSION: This study provides important information about the chemistry and antioxidant and antimicrobial properties of *E. cardamomum*.

Keywords: *Elettaria cardamomum*; essential oil; oleoresins; antioxidant activity; antimicrobial activity

INTRODUCTION

Food materials deteriorate during processing and storage as a result of oxidative processes. These chemical reactions are often catalysed by ions of metals such as iron and copper. The process of degradation affects the lipid, protein and carbohydrate content of stored food materials.[1] In this context, various synthetic antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been approved and are routinely used as food protection agents. However, these antioxidants suffer from the drawback that they are volatile and decompose easily at high temperature. Additionally, it is still unclear whether their long-term consumption can lead to health risks.[2]

Recently there has been growing interest in plant-derived food additives. Many herbs and spices commonly used to flavour foods contain phenolic compounds that are reported to show good antioxidant activity.[3,4] Consequently, identification of alternative natural and safe sources of food antioxidants is of great interest,[5] and the search for natural antioxidants, especially of plant origin, has increased markedly in recent years.

The seeds of cardamom (*Elettaria cardamomum*), a plant of the Zingiberaceae family, are widely used in culinary practice. Cardamom is a perennial shrub with thick, fleshy, lateral roots, which can grow to a height of about 2.5 m.[6] For use as a cooking spice, its darker seeds are removed from the pod and ground into a powder. Cardamom is primarily cultivated in

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southern India, Sri Lanka, Tanzania and Guatemala. Morocco is also a producer of cardamom for culinary use.[7] Historically known as the 'queen of all spices', cardamom has been used in India since ancient times.[7] As a spice, it is used in curries, coffee, cakes and breads and for flavouring sweet dishes and drinks.[8] The seeds and essential oil are used as flavouring components in a variety of foods, including alcoholic and non-alcoholic beverages, frozen desserts, candies, baked goods, puddings, condiments, relishes, gravies, meats and meat products. Cardamom is also used as a spice to make Moroccan tajine more tasty and is used generally in cooking, including the cooking of meats.

A recent survey has reported the importance of the use of spices in traditional healthcare.[9] However, herbs and spices usually contain essential oils which, in addition to their flavour characteristics, also show antioxidant activity.[10,11] The use of different solvents may lead to extracts with different chemical profiles and activities. Therefore we studied the chemical components and antimicrobial and antioxidant activities of the essential oil of *E. cardamomum* and oleoresins extracted using chloroform, ethanol, methanol and diethyl ether as solvents. There have already been a number of studies on the chemical and antimicrobial properties of the essential oil of *E. cardamomum*.[12–17] However, to the best of our knowledge, this is the first report on the antioxidant properties and bioactivities of different solvent extracts of *E. cardamomum*.

MATERIALS AND METHODS

Sample extraction

Elettaria is a small genus with three or four species found in East and Southeast Asia. Two botanical varieties were distinguished by earlier researchers, one for the wild taxon and one for the cultivated forms.[18] *Elettaria cardamomum* var. *major* Thwaites is a wild cardamom that is particularly common in Sri Lanka and southern India. For the present study, cardamom originating from Kerala, southern India was purchased from a local spice market in Gorakhpur, Uttar Pradesh, India during September 2005 and voucher specimens were kept at the Herbarium of the Faculty of Science, DDU Gorakhpur University. Powdered cardamom (250 g, 100-mesh particle size) was subjected to hydrodistillation in a Clevenger apparatus to obtain the essential oil in 3.1% yield.

Oleoresins were obtained by extracting 30 g of powdered spice with 300 mL of various solvents (chloroform, ethanol, methanol and diethyl ether) for 3 h in a Soxhlet extractor. Evaporation of the solvents at reduced pressure gave viscous extracts in 3.9–4.6% yields. The oleoresins and essential oil were stored in a freezer until further use.

Chemical characterisation

Gas chromatography (GC)

A Hewlett Packard (HP) 5890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5 capillary column (5% phenyl polymethyldisiloxane, length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm) was employed. The injector and detector temperatures were maintained at 280 and 300 °C respectively. The injection volume was 1 μL and helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The column temperature was raised from 40 °C initially to 250 °C at 3 °C min^{-1} and held for 15 min.

Gas chromatography/mass spectrometry (GC/MS)

Analyses of the essential oil and oleoresins were run on an HP 6890 GC/MS system (Agilent Technologies) coupled to an HP 5973 mass-selective detector with an HP-5MS capillary column (5% phenyl polymethyldisiloxane, length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm). The injector and detector temperatures were maintained at 280 and 300 °C respectively. The injection volume was 1 μL (in split mode) and helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The column temperature was raised from 40 °C initially to 250 °C at 3 °C min^{-1} and held for 15 min.

Component identification

A hexane solution of C_8–C_{32} *n*-alkanes was previously separated under the above conditions, and their retention times were determined. Linear temperature-programmed retention index (LTPRI) values were calculated from the results of the separation of the essential oil and oleoresins:

$$\mathrm{LTPRI} = 100(t_x - t_n)/(t_{n+1} - t_n) + 100n \qquad (1)$$

where t_x, t_n and t_{n+1} are the retention times of component x and n-alkanes with n and $n+1$ carbon atoms respectively ($t_n < t_x < t_{n+1}$).[19] Following integration, the fraction of each component in the total ion current (TIC) was calculated. Components were identified with the aid of an automatic system, the PBM (probability-based matching) algorithm, for processing GC/MS data supplied by the NIST mass spectral library. Identification was considered reliable if the measured values of retention indices confirmed the results of a computer search of the mass spectral library (a home-made computer program was developed for identification; it is supplied with a database of LTPRI values for more than 5000 organic compounds).

Antioxidant activity in mustard oil

Sample preparation

The essential oil and oleoresins of cardamom were added individually to mustard oil at a level of 0.02% (v/v). The initial peroxide value of the mustard oil was 1.1 meq O_2 kg^{-1}. Synthetic antioxidants (BHA,

PG and BHT) were also tested at a level of 0.02% (w/v). The oxidation rate was assessed by periodic measurement of the peroxide value of the oil substrate.

Peroxide value

The peroxide values of all samples were measured[20] every 7 days by the Schaal oven method.[21] For this purpose a known weight of edible oil sample (3 g) was dissolved in glacial acetic acid (30 mL) and chloroform (20 mL). Then a saturated solution of potassium iodide (1 mL) was added and the mixture was kept in the dark for 15 min. After the addition of distilled water (50 mL) the mixture was titrated against sodium thiosulfate (0.01 mol L^{-1}) using starch as an indicator. A blank titration was done in parallel and the peroxide value (meq O_2 kg^{-1}) was calculated.

Antifungal assay

The essential oil and oleoresins were tested individually against the food-borne fungi *Aspergillus terreus* (MTCC 3374), *Fusarium graminearum* (MTCC 2088), *Penicillium purpurogenum* (MTCC 1786) and *Penicillium madriti* (MTCC 3003). Antifungal activity was assessed by two different methods, in both of which methanol was used for dilution of the essential oil and oleoresins.

Poison food method

This method is one of the most important bioassay methods[22] for evaluating antimicrobial activity. A calculated amount of diluted sample was added to molten Czapek dox agar (CDA) medium (~45 °C) to obtain the desired concentration. Control plates were prepared with and without the addition of test sample under the same conditions. The pathogen of interest from growing tips (punched in fungal mats grown on CDA medium in sterile Petri dishes) was placed at the centre of each plate and all plates were incubated at 26 °C. Radial growth of fungi in terms of diameter (mm) was measured after 5 days.

Inverted Petri dish method

This method[22] allows the determination of antifungal activity of compounds in the vapour phase. A calculated quantity of diluted sample was soaked on filter paper (Whatman No. 1, 10 mm diameter) and placed at the centre of the lid of an inverted Petri dish. Control agar plates were prepared under the same conditions except for the addition of test compound. Control plates were prepared with and without the addition of test sample under the same conditions. All plates were incubated at 26 °C. Mycelial growth of fungi in terms of diameter (mm) was measured after mycelia had reached the edges of the control plates.

Antibacterial assay

The food-borne bacteria *Staphylococcus aureus* (MTCC 3103), *Bacillus cereus* (MTCC 430), *Escherichia coli* (MTCC 1672) and *Salmonella typhi* (MTCC 733) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India for the present study. The agar well diffusion method was used to investigate the antimicrobial properties of the essential oil and oleoresins. The bacterial strains were activated on nutrient agar medium and stored at 4 °C, then diluted using Ringer's solution. Solutions of 1000 and 3000 ppm essential oil and oleoresins were prepared in absolute ethanol. Samples (30 μL) were placed in agar wells (9 mm diameter) and all plates were incubated at 26 °C. Amikacin and gentamycin were used as standards.

Statistical analysis

Three samples each of the essential oil and oleoresins were prepared for the assays of every antioxidant and antimicrobial attribute. Data are presented as mean ± standard deviation of three determinations. Statistical analyses were performed using one-way analysis of variance.[23] A probability value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Chemical component analysis

Chemical characterisation of cardamom essential oil resulted in the identification of 71 compounds, with α-terpinyl acetate (44.3%), 1,8-cineole (10.7%), α-terpineol (9.8%) and linalool (8.6%) being the major components found (Table 1). The chloroform and methanol oleoresins both contained α-terpinyl acetate (21.8 and 25.9% respectively) as the main component, while 5-hydroxymethylfurfural (28.9%) was the most abundant component in the ethanol oleoresin. However, very few components (total 0.61%) were found in the diethyl ether oleoresin. The major chemical constituents imparting a sweet flavour to cardamom oil are terpinyl acetate, linalyl acetate and linalool. 'Mysore' contains more terpinyl acetate than 'Malabar', while the latter contains more 1,8-cineole, which imparts a harsh camphor-like note to the oil.[24] Hussain *et al.*[25] reported that the essential oil from *E. cardamomum* fruits contained 1,8-cineole (74%) as the main constituent.[26] In another previous study (by JC Pieribattesti, J Smadja and JM Mondon), 1,8-cineole (54.4%) and α-terpinyl acetate (24%) were reported as the main constituents, while the main components of our essential oil were identified as α-terpinyl acetate (44.3%) and 1,8-cineole (10.7%).

Antioxidant activity in mustard oil

The addition of natural and synthetic antioxidants to mustard oil affected the peroxide value to different extents during accelerated oxidation at 60 °C for 28 days of storage (Fig. 1). Cardamom essential oil and oleoresins were able to reduce the oxidation rate of the mustard oil on heating at 60 °C in comparison with the control when assessed by changes in peroxide value. However, the synthetic antioxidants had a stronger effect than cardamom. The essential oil and

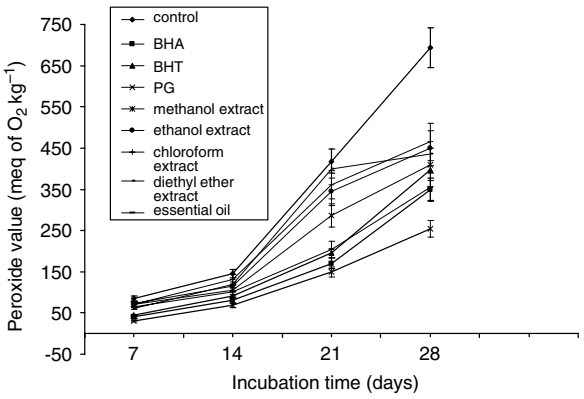

Figure 1. Antioxidant activities of cardamom essential oil and oleoresins in mustard oil in terms of peroxide value.

methanol oleoresin showed a better antioxidant effect during the final stage of the experiment. It has been stated that phenolic groups play an important role in antioxidant activity.[27] The presence of phenolic compounds such as thymol (0.08%) in the chloroform oleoresin and carvacrol (0.2%) in the chloroform and methanol oleoresins (Table 1) could be responsible for the strong antioxidant activity of cardamom. It has also been reported that natural antioxidant compounds often work synergistically to produce a broad spectrum of activities that creates an effective defence system against free radical attack.[28–30] A literature review showed that terpenoids play an important role in antioxidant activity.[29] GC and GC/MS analyses of cardamom essential oil oleoresins in the present study revealed that they contained a large number of terpenes which may contribute to antioxidant activity.

Antimicrobial studies

Antibacterial activities of cardamom essential oil and oleoresins are reported in Table 2. The essential oil

Table 1. Chemical composition of chloroform ($CHCl_3$), methanol (CH_3OH), ethanol (C_2H_5OH) and diethyl ether ($(C_2H_5)_2O$) oleoresins and essential oil (EO) of *Elettaria cardamomum*

	Relative content in oleoresin or essential oil (%)[a]						Identification parameters	
Compound	$CHCl_3$	CH_3OH	C_2H_5OH	$(C_2H_5)_2O$	EO	CAS #	Target ions	M^{+}
1-Hepten-4-ol	Tr	–	–	–	–	3521-91-3	73, 43, 44, 55, 41	–
(*Z*)-2-Heptenal	–	–	–	–	Tr	57266-86-1	41, 83, 55	–
(*Z*)-2-Heptanal	–	–	–	Tr	–	57266-86-1	83, 55, 41, 70	112
Hexanal	–	–	–	–	0.01	66-25-1	44, 41, 56	–
1-Octanol	–	–	–	–	0.09	111-87-5	56, 55, 41	–
Heptanal	–	–	–	–	Tr	111-71-7	43, 44, 70	–
α-Thujene	–	–	–	Tr	0.02	2867-05-2	93, 91, 77, 136	136
α-Pinene	–	–	–	Tr	0.1	80-56-8	93, 91, 77	136
β-Pinene	–	–	–	–	0.06	127-91-3	93, 41, 69	136
Camphene	–	–	–	–	Tr	79-92-5	93, 121, 79	136
Sabinene	0.5	–	–	Tr	–	3387-41-5	93, 77, 91	136
2,3-Dehydro-1,8-cineole	–	0.5	–	–	0.07	92760-25-3	109, 43, 79, 94	166
Octanal	–	–	–	–	0.09	124-13-0	41, 43, 57	152
Myrcene	0.3	–	–	Tr	0.4	123-35-3	93, 41, 69	–
Pseudolimonene	Tr	–	–	–	–	499-97-8	93, 79, 136	136
m-Cymene	Tr	–	–	–	–	535-77-3	119, 91, 134, 105	136
α-Terpinene	0.06	–	–	Tr	–	99-86-5	121, 93, 136	134
γ-Terpinene	0.2	–	–	Tr	–	99-85-4	93, 91, 77, 136, 121	136
p-Cymene	0.7	0.2	–	Tr	–	99-87-6	119, 134, 91, 43	136
Limonene	3.1	1.0	–	0.1	–	–	68, 67, 93, 121	134
1,8-Cineole (eucalyptol)	0.6	0.4	–	0.07	10.7	470-82-6	43, 81, 108, 133	136
(*E*)-β-Ocimene	0.08	–	–	–	0.2	3779-61-1	93, 91, 79, 77	154
(*Z*)-β-Ocimene	0.1	–	–	–	0.1	3338-55-4	93, 91, 79, 77	136
Monoterpene alcohol	0.06	–	–	–	–	–	79, 93, 43, 94, 137	–
Linalool oxide	0.05	0.4	–	–	–	5989-33-3	59, 111, 68, 67, 43	152
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (alletone)	–	0.4	1.0	–	–	3658-77-3	43, 128, 57, 85	–
cis-Linalool oxide	–	–	–	–	0.1	5989-33-3	59, 111, 55	128
Furyl hydroxymethyl ketone	–	0.2	0.6	–	–	17678-19-2	95, 126, 67	170
2,5-Furandi carboxaldehyde	–	Tr	–	–	–	823-82-5	124, 123, 95	126
p-Cymenene	–	0.2	–	–	–	1195-32-0	117, 132, 91, 116	124

Table 1. Continued

Compound	Relative content in oleoresin or essential oil (%)[a]					CAS #	Identification parameters	
	$CHCl_3$	CH_3OH	C_2H_5OH	$(C_2H_5)_2O$	EO		Target ions	M^{+}
Hotrienol	–	Tr	–	–	–	29957-43-5	71, 82, 43	132
Sabinene hydrate	–	–	–	–	0.3	17699-16-0	71, 43, 93	–
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	–	4.0	–	–	–	28564-83-2	43, 144, 101, 44	154
cis-Sabinene hydrate	–	–	–	Tr	–	15826-82-1	71, 43, 93, 81	144
trans-Sabinene hydrate	–	–	–	0.07	–	15826-82-1	71, 43, 93, 81	154
Terpinolene	1.1	–	–	–	0.7	586-62-9	121, 136, 93	154
Mixture (terpinolene)	–	0.5	–	–	–	–	121, 93, 136, 128, 43	136
Linalool	0.6	1.0	–	0.2	8.6	78-70-6	71, 93, 43, 55	
trans-Carveol	–	0.3	–	–	–	1197-07-5	109, 84, 55, 137	154
Coumarin	–	0.5	–	–	–	496-16-2	120, 91, 119, 65, 63	152
cis-Limonene oxide	Tr	–	–	–	–	4680-24-4	67, 43, 93, 41, 94	120
trans-Limonene oxide	–	–	–	–	0.05	4959-35-7	43, 41, 67, 94	152
Perillene	–	–	–	–	0.1	539-52-9	69, 41, 81, 135	152
α-Phellandren-8-ol	–	0.4	–	–	0.1	1686-20-0	59, 81, 93, 43	150
4-Terpineol	0.3	–	–	0.1	3.2	562-74-3	71, 111, 93, 43, 136	152
p-Cymen-8-ol	0.2	–	–	–	–	1197-01-9	135, 43, 91	154
α-Terpineol	1.3	3.4	–	–	9.8	98-55-5	59, 93, 121, 136	150
n-Octyl acetate	–	–	–	–	0.03	112-14-1	43, 56, 70, 84	154
β-Citral	–	–	–	–	0.7	106-26-3	41, 69, 94, 109	–
α-Citral	–	–	–	–	1.8	141-27-5	41, 69, 123	152
Dihydro-α-terpinyl acetate	–	–	–	–	0.2	20777-40-6	43, 55, 93	152
α-Terpinyl acetate	21.8	25.9	0.4	–	44.3	80-26-2	43, 93, 121	–
Methyl (*E*)-2-decenoate	–	–	–	–	0.1	2482-39-5	87, 57, 41	–
Dodecen-5-enal	–	–	–	–	0.3	68820-33-7 (-34-8)	67, 68, 41	–
trans-Dihydrocarvone	0.1	–	–	–	–	5948-04-9	67, 95, 68, 81, 82	–
trans-Dihydrocarvone	–	0.2	–	–	–	7764-50-3	67, 95, 55	152
β-Elemene	–	–	–	–	0.1	515-13-9	93, 81, 107	152
γ-Elemene	–	–	–	–	0.07	29873-99-2	121, 93, 94	204
Methyl eugenol	–	–	–	–	0.03	93-15-2	178, 91, 41	204
Dodecanal	–	–	–	–	0.03	112-54-9	57, 41, 43, 55	178
(*E*)-β-Caryophyllene	–	–	–	–	0.07	87-44-5	41, 93, 133	–
α-Terpenyl propionate	–	–	–	–	0.4	80-27-3	121, 93, 136, 57	204
Epoxy-α-terpinyl acetate	–	–	–	–	0.1	–	43, 109, 137, 93	–
Isobornyl formate	1.2	–	–	–	–	1200-67-5	93, 121, 95, 67, 136	212
Geranyl acetone	–	–	–	–	0.05	689-67-8	43, 69, 107	–
(*E*)-8-Dodecenol	–	–	–	–	0.06	42513-42-8	67, 55, 68, 166	194
2-Dodecenal	–	–	–	–	0.02	4826-62-4	70, 41, 43, 55	–
Geranyl propionate	–	–	–	–	0.07	105-90-8	69, 57, 41	–
D-Germacrene	–	–	–	–	0.2	23986-74-5	161, 105, 91	210
Dendolasin	–	–	–	–	0.3	23262-34-2	69, 81, 41, 203	204
Geraniol	0.3	–	–	–	–	106-24-1	69, 41, 68, 129, 136	218
β-Germacrene	–	–	–	–	0.1	15423-57-1	121, 93, 105, 107	–
α-Selinene	–	–	–	–	0.2	473-13-2	189, 93, 204, 107	204
β-Selinene	–	–	–	–	0.4	17066-67-0	105, 93, 107	204
2-Tridecanone	–	–	–	–	0.05	593-08-8	58, 43, 71, 85	204
β-Cadinene	–	–	–	–	0.05	593-08-8	58, 43, 71, 85	198
γ-Cadinene	–	–	–	–	0.3	39029-41-9	161, 105, 204, 119	198
(liguloxide)	–	–	–	–	0.03	21764-22-7	207, 161, 105, 43	204
(*E*)-Nerolidol	–	3.1	–	–	3.3	7212-44-4	69, 93, 41, 107	222
Monoterpenoid	0.1	–	–	–	0.05	523-47-7	161, 204, 119, 134	222
Carvenone	0.2	–	–	–	–	499-74-1	110, 95, 152, 67, 41	204
Linalyl acetate	0.8	–	–	–	4.0	115-95-7	93, 69, 41, 43, 80	152
Viridiflorol	–	–	–	–	0.09	552-02-3	43, 161, 105	–

Table 1. Continued

Compound	Relative content in oleoresin or essential oil (%)[a]					CAS #	Identification parameters	
	$CHCl_3$	CH_3OH	C_2H_5OH	$(C_2H_5)_2O$	EO		Target ions	M^{+}
n-Hexadecane	–	–	–	–	0.03	554-76-3	57, 43, 71, 85	222
n-Heptadecane	–	–	–	–	0.03	629-78-7	57, 43, 71, 85	226
Alloaromadenderene oxide	–	–	–	–	0.03	–	69, 41, 95, 123	–
(*Z*,*E*)-Farnesal	–	–	–	–	0.2	4380-32-9	69, 41, 81, 84	220
(*E*,*E*)-Farnesal	–	–	–	–	0.3	502-67-0	69, 84, 41	220
Geranyl caproate	–	–	–	–	0.01	10032-02-7	69, 68, 41, 43	220
Carvocryl chloride	0.1	–	–	–	–	4395-79-3	153, 117, 133, 119	–
(*E*)-Anethole	0.2	–	–	–	–	104-46-1	148, 147, 133	168
Carvacrol	0.2	0.2	–	–	–	499-75-2	135, 150, 91	148
Thymol	0.08	–	–	–	–	89-83-8	135, 150, 91, 41	150
Terpene alcohol acetate	0.1	–	–	–	–	–	43, 93, 136, 59, 79	150
Terpene alcohol acetate	0.2	–	–	–	–	–	119, 43, 93, 91, 59	–
exo-2-Hydroxycineole	3.3		–	–	–	92999-78-5	71, 108, 43, 93, 135	–
Hydroxy-α-terpineol acetate	–	1.1	0.7	–	–	–	109,43,137,152	170
$C_{12}H_{18}O_2$	1.1	–	–	–	–	–	135,93,67,41,68	–
Not identified	0.7	–	–	–	–	–	93, 135, 41, 67, 188	170
Neryl acetate	0.1	–	–	–	–	1421-12-8	69, 68, 41, 43, 121	–
Not identified	0.6	–	–	–	–	–	135, 43, 93, 119	–
α-Copaene	0.08	–	–	–	0.04	3856-25-5	161, 69, 119, 105	170
Geranyl acetate	0.5	–	–	–	3.8	16408-44-2	69, 41, 43, 93, 121	204
8-Hydroxy carvotanacetone	0.1	0.2	–	–	–	7712-46-1	59, 110, 95, 150	–
α-Terpenyl butyrate	0.1	–	–	–	–	2153-28-8	121, 93, 136, 57	–
(*E*)-β-Farnesene	0.5	–	–	–	–	18794-84-8	69, 93, 41, 133, 161	–
α-Terpineol Propionate Propionate	–	0.1	–	–	–	80-27-3	121, 93, 136, 57	204
γ-Murolene	0.1	0.3	–	–	0.07	30021-74-0	161, 105, 119, 93	–
1-Methyl-4-(1-acetoxy-1-methylethyl) cyclohex-2-ene	–	0.2	–	–	–	–	43, 94, 137, 59	204
β-Guaiene	0.6	–	–	–	–	88-84-6	161, 105, 132, 91	–
Sesquiterpene	0.12	–	–	–	0.07	–	119, 161, 93, 105, 91	204
α-(*E*,*E*)-Farnesene	0.4	–	–	–	–	502-61-4	41, 93, 107, 79	204
δ-Cadinene	1.1	0.4	–	–	0.1	438-76-1	161, 71, 119, 134	204
Calacorene	0.4	–	–	–	–	21391-99-1	157, 142, 43, 200	204
S-Carvone acetate	1.8	2.2	1.4	–	–	86421-35-4	43, 150, 108, 107	200
Not identified	0.4	–	–	–	–	–	43, 101, 93, 121, 135	–
Dill apiole	0.2	0.1	–	–	–	484-31-1	222, 207, 177	–
Not identified	0.7	–	–	–	–	–	43, 101, 59, 137, 203	222
(*E*,*E*)-Farnesol	0.3	0.6	–	–	0.5	106-28-5	69, 41, 81, 93, 161	218
(*E*,*E*)-Farnesyl acetate	0.3	–	–	–	0.1	4128-17-0	69, 43, 41, 93	222
n-Hexadecanoic acid	1.5	–	0.9	–	–	57-10-3	73, 60, 43, 129	264
Diterpenoid	0.3	–	–	–	–	–	69, 81, 93, 107, 272	256
cis-9-Retinol	0.2	–	–	–	–	514-85-2	147, 284, 81	290
Retionoic acid	–	0.6	–	–	–	302-79-4	123, 69, 165	284
Oleic acid	0.8	–	–	–	–	–	55, 83, 69	300
Linoleic acid	–	0.8	–	–	–	60-33-3	67, 81, 95, 55	282
Palmitic acid	–	2.1	–	–	–	57-10-3	73, 60, 43, 129	280
(*Z*)-Oleic acid	–	2.7	0.5	–	–	112-80-1	55, 83, 69	256
(*E*)-Oleic acid	–	0.2	–	–	–	112-79-8	55, 83, 69	282
n-Octadecanoic acid	0.1	–	–	–	–	57-11-4	73, 60, 43, 129	282
δ-Tocopherol	–	0.2	–	–	–	119-13-1	402, 137, 177, 43	284
n-Tricosane	0.3	–	–	–	0.01	638-67-4	57, 71, 43	402
n-Tetracosane	0.1	–	–	–	–	646-31-1	57, 71, 43	324
(*Z*)-12-Pentacosene	0.1	–	–	–	–	–	97, 88, 57, 55, 41	338

Table 1. Continued

Compound	Relative content in oleoresin or essential oil (%)[a]					CAS #	Identification parameters	
	$CHCl_3$	CH_3OH	C_2H_5OH	$(C_2H_5)_2O$	EO		Target ions	M^{+}
2,4-Bis-(1-phenylethyl)phenol	0.2	–	–	–	–	–	287, 302, 105, 209	350
n-Pentacosane	0.7	–	–	–	0.02	629-99-2	57, 71, 43	302
Not identified	0.2	–	–	–	–	–	123, 69, 165	352
Octyl palmitate	0.2	–	–	–	–	–	257, 57, 112	300
n-Hexacosane	0.2	–	–	–	0.01	630-01-3	57, 71, 43	368
Heptacosene isomer 2	0.4	–	–	–	–	–	97, 83, 57, 55	366
n-Heptacosane	0.4	–	–	–	–	593-49-7	57, 71, 43	378
Not identified	0.3	–	–	–	–	–	138, 68, 69, 96, 82, 81	380
n-Octacosane	0.4	–	–	–	0.03	630-02-4	57, 71, 43	–
(*Z*)-14-Nonacosene isomer 1	0.5	–	–	–	–	–	97, 83, 57, 55	–
14-Nonacosene isomer 2	0.4	–	–	–	–	–	97, 83, 57, 55	406
n-Nonacosane	0.6	–	–	–	–	630-03-5	57, 71, 43	406
Farnesyl-β-D-mannofuranoside	1.0	–	–	–	–	–	69, 41, 93, 121, 136	–
n-Triacontane	0.5	–	–	–	–	–	57, 71, 43	–
Not identified	0.1	–	–	–	–	–	391, 406, 105	422
Not identified	0.2	–	–	–	–	–	391, 406, 105, 301	406
7-Dehydrodiosgenin	0.2	0.2	–	–	–	85706-84-9	43, 394, 135	406
n-Hentriacontane	0.2	–	–	–	–	630-04-6	57, 71, 43	394
n-Dotriacontane	0.7	–	–	–	–	–	57, 71, 43	–
n-Tritriacontane	0.6	–	–	–	–	630-05-7	57, 71, 43	450
β-Sitosterol acetate	Tr	–	–	–	–	915-05-9	396, 43, 381	–
Steroid (stigmasta-3,5-dien-7-one)	0.2	–	–	–	–	(2034-72-2)	174, 410, 69	396
n-Tetratriacontane	0.2	–	–	–	–	–	57, 71, 43	410
5-Acetoxymethyl-2-furaldehyde	–	0.3	–	–	–	10551-58-3	126, 79, 109, 43, 97	–
Steroid (stigmast-4-en-3-one)	0.5	–	–	–	–	(1058-61-3)	124, 229, 412, 289, 370, 43	168
n-Pentatriacontane	0.2	–	–	–	–	–	57, 71, 43	412
Pyruvaldehyde	–	0.3	–	–	–	78-98-8	43, 45	–
Furfuryl alcohol	–	0.6	2.0	–	–	–	–	–
Vanillic acid	–	–	Tr	–	–	121-34-6	168, 153, 97, 125	
5-Methyl-2(3*H*)-furanone	–	Tr	Tr	–	–	591-12-8	55, 98, 43, 70	168
p-Benzoquinone	–	–	–	0.07	–	106-51-4	108, 54, 82, 80	98
Furfural	–	1.4	1.9	–	–	98-01-1	96, 95, 67, 43	108
5-Hydroxy methylfurfural	–	21.5	28.9	–	–	67-47-0	97, 41, 69	96
2-Cyclopentene-1,4-dione	–	0.1	–	–	–	930-60-9	96, 42, 68, 54	126
Methyl pyruvate	–	1.3	Tr	–	–	600-22-6	43, 102, 59	96
4-Vinylguaicol	–	0.3	–	–	–	7786-61-0	150, 135, 77, 107	102
trans-3-Caren-2-ol acetate	–	0.9	–	–	–	–	119, 93, 43, 91, 134	150
Eugenol	–	0.1	–	–	–	97-53-0	164, 149, 131, 103	–
Pseudothiohydantoin	–	0.4	–	–	–	556-90-1	116, 69	164
Acetylfuran	–	0.05	–	–	–	1192-62-7	95, 110, 65, 43	116
γ-Butyrolactone	–	0.2	1.0	–	–	96-48-0	42, 86, 56	110
γ-Crotonolactone	–	0.1	–	–	–	497-23-4	55, 84	86
1,2-Cyclopentanedione	–	0.7	2.8	–	–	3008-40-0	98, 55, 42, 41, 69	84
5-Methylfurfural	–	0.5	0.9	–	–	620-02-0	110, 109, 53, 43	98
2,4-Dihydroxy-2,5-dimethyl-3(*H*)-furanone	–	0.4	1.1	–	–	10230-62-3	101, 43, 73, 55	110

Table 1. Continued

Compound	Relative content in oleoresin or essential oil (%)[a]					CAS #	Identification parameters	
	$CHCl_3$	CH_3OH	C_2H_5OH	$(C_2H_5)_2O$	EO		Target ions	M^{+}
Glutaconic aldehyde	–	0.5	1.0	–	–	5926-95-4	112, 55, 84, 54	144
Geraniol acetate	–	0.2	–	–	–	16409-44-2	69, 43, 41, 93, 136	112
(*E*,*E*)-2,6-Dimethyl-1,3,5,7-octatetraene	–	0.2	–	–	–	460-01-5	91, 119, 77, 105	–
β-Pyronene	–	Tr	–	–	–	514-96-5	121, 93, 136, 91	134
Methyl acetate	–	–	1.3	–	–	79-20-9	43, 74, 59	136
4-Methyl-5*H*-furan-2-one	–	–	Tr	–	–	6124-79-4	98, 69, 41	74
4-Ethylcyclohexanone	–	Tr	–	–	–	5441-51-0	55, 126, 70, 97	98
Methyl acetoxyacetate	–	0.09	–	–	–	5837-80-9	43, 102, 56, 73	126
Total	60.3	84.9	47.1	0.61	97.6			

[a] Tr, trace; – , not present.

Table 2. Antibacterial activities of cardamom essential oil and oleoresins by agar well diffusion method

Sample	Dose (ppm)[a]	Inhibition zone (mm)[b]			
		Gram-positive bacteria		Gram-negative bacteria	
		SA	BC	EC	ST
Methanol oleoresin	1000	30 ± 1.5	44.6 ± 0.5	64 ± 0.5	12 ± 0.3
	3000	38 ± 1.1	64.0 ± 1.1	81 ± 0.3	22 ± 0.5
Ethanol oleoresin	1000	40 ± 0.5	44.6 ± 0.5	56 ± 1.1	–
	3000	56 ± 1.2	64.0 ± 1.1	84 ± 1.2	32 ± 0.6
Chloroform oleoresin	1000	26 ± 0.3	44.6 ± 0.5	64 ± 0.5	42 ± 1.3
	3000	42 ± 1.1	64 ± 1.1	81 ± 0.3	66 ± 0.5
Diethyl ether oleoresin	1000	18 ± 0.5	13 ± 1.5	36 ± 0.5	–
	3000	22 ± 0.5	24 ± 0.3	48 ± 0.2	13 ± 0.2
Essential oil	1000	72 ± 0.6	72 ± 0.5	56 ± 1.5	33 ± 0.2
	3000	88 ± 1.5	85 ± 0.5	79 ± 1.1	66 ± 0.5
Standards					
Amikacin	10	20.6 ± 0.8	–	–	18.8 ± 1.3
Gentamycin	30	18.6 ± 0.7	25.0 ± 0.2	17.9 ± 0.6	21.1 ± 0.7

[a] Samples were diluted in methanol.
[b] Values are mean ± standard deviation of three replicates. SA, *Staphylococcus aureus*; BC, *Bacillus cereus*; EC, *Escherichia coli*; ST, *Salmonella typhi*.

exhibited strong inhibition against all tested micro-organisms at 3000 ppm. The methanol, ethanol and chloroform oleoresins showed strong inhibition against *E. coli* at 3000 ppm. The oleoresins showed moderate to good activity against other tested bacterial strains.

The essential oil showed 100% mycelial zone inhibition against *P. purpurogenum* at 3000 ppm by the poison food method (Table 3). The diethyl ether oleoresin exhibited strong inhibition against all fungal isolates at 3000 ppm. The methanol and ethanol oleoresins gave the best results against *A. terreus* at 3000 ppm by the inverted Petri dish method (Table 4). The essential oil and oleoresins showed moderate to strong antifungal activity against other tested fungal isolates by both methods.

Most of the antimicrobial activity in essential oils of spices and culinary herbs is thought to be derived from phenolic compounds,[31] while other constituents are believed to contribute little to antimicrobial effects.[32] It is clear that cardamom essential oil and oleoresins contain phenolic compounds such as thymol, carvacrol and methyl eugenol (Table 1) and hence their antimicrobial activity could be due to these compounds. The lower efficacy of the essential oil and oleoresins against some micro-organisms in the present study might be due to the low activity of their main constituents against particular fungi or bacteria. It is likely that the antifungal effects of essential oils and oleoresins result from synergistic action of all their components.[33] Such synergistic or antagonistic action probably occurred with cardamom essential oil and oleoresins. The strength of inhibition and the spectrum of antimicrobial activity of the essential oil and oleoresins suggest that complex interactions between individual components led to the overall activity. It is not completely clear why Gram-negative bacteria should be less susceptible, but this may be associated with the outer membrane of Gram-negative bacteria, which endows

Table 3. Antifungal effects of cardamom essential oil and oleoresins (at doses of 1000 and 3000 ppm, diluted in methanol) against various food-borne pathogenic fungi by poison food method

	Mycelial zone inhibition (%)[a]							
	PP		FG		AT		PM	
Sample	1000	3000	1000	3000	1000	3000	1000	3000
Methanol oleoresin	–	12 ± 1.1	10 ± 0.5	15 ± 1.1	25 ± 1.2	32 ± 0.6	13 ± 1.3	28 ± 2.2
Ethanol oleoresin	36 ± 0.1	48 ± 0.3	18 ± 0.6	29 ± 2.2	11 ± 2.2	30 ± 1.6	27 ± 1.1	52 ± 0.6
Chloroform oleoresin	42 ± 0.3	56 ± 0.9	12 ± 1.3	20 ± 0.6	9.0 ± 0.1	18 ± 1.1	17 ± 2.3	46 ± 0.2
Diethyl ether oleoresin	62 ± 0.5	72 ± 0.9	50 ± 0.6	62 ± 0.4	32 ± 1.1	40 ± 1.2	24 ± 0.1	59 ± 0.6
Essential oil	92 ± 0.6	100	42 ± 0.3	60 ± 0.2	41 ± 0.3	55 ± 0.3	61 ± 0.7	83 ± 0.1
Standard								
Carbendazim	–	–	8.5 ± 0.6	20 ± 2.2	–	19.4 ± 1.2	23 ± 0.2	36.2 ± 0.1

[a] Values are mean ± standard deviation of three replicates. PP, *Penicillium purpurogenum*; FG, *Fusarium graminearum*; AT, *Aspergillus terreus*; PM, *Penicillium madriti*.

Table 4. Antifungal effects of cardamom essential oil and oleoresins (at doses of 1000 and 3000 ppm, diluted in methanol) against various food-borne pathogenic fungi by inverted Petri dish method

	Mycelial zone inhibition (%)[a]							
	PP		FG		AT		PM	
Sample	1000	3000	1000	3000	1000	3000	1000	3000
Methanol oleoresin	13 ± 2.0	36 ± 0.1	21 ± 0.2	40 ± 0.1	38 ± 0.6	74 ± 0.9	16 ± 1.1	32 ± 1.2
Ethanol oleoresin	22 ± 0.6	54 ± 0.1	30 ± 0.2	62 ± 2.4	56 ± 2.3.	93 ± 2.2	22 ± 2.1	59 ± 0.3
Chloroform oleoresin	20 ± 1.1	48 ± 1.2	12 ± 0.2	36 ± 0.3	15 ± 1.2	25 ± 1.3	62 ± 1.4	88 ± 1.8
Diethyl ether oleoresin	18 ± 1.3	42 ± 1.5	10 ± 1.7	16 ± 0.1	35 ± 0.2	57 ± 2.1	20 ± 2.0	54 ± 1.9
Essential oil	56 ± 1.1	72 ± 0.3	65 ± 0.9	90 ± 1.3	77 ± 1.3	89 ± 0.2	66 ± 1.3	94 ± 0.2
Standard								
Carbendazim	–	–	8.5 ± 0.6	20 ± 2.2	–	19.4 ± 1.2	23 ± 0.2	36.2 ± 0.1

[a] Values are mean ± standard deviation of three replicates. PP, *Penicillium purpurogenum*; FG, *Fusarium graminearum*; AT, *Aspergillus terreus*; PM, *Penicillium madriti*.

the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier.[33]

CONCLUSION

The results of this research indicate that cardamom essential oil and oleoresins possess considerable antioxidant capacity. The essential oil and oleoresins also showed a broad spectrum of antimicrobial activity against the various fungal and bacterial isolates tested. However, further investigation of individual components *in vivo* and of the antioxidant activity mechanism is warranted. The present study can serve as a useful starting point for further applications of cardamom essential oil and oleoresins and their constituents as food additives.

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