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Low temperature extraction of essential oil bearing plants by liquefied gases. 7. Seeds from cardamom (*Elettaria cardamomum* (L.) Maton)

ABSTRACT

The chemical composition of extract from the seeds of cardamom (*Elettaria cardamomum* (L.) Maton, obtained by extraction with tetrafluoroethane was analyzed using GC and GC/MS. The major compounds (concentration higher than 3%) of extract were: terpinyl acetate (36.8%), 1,8-cineole (29.2%), linalyl acetate (5.2%), sabinene (3.9%) and linalool (3.1%). The studied extract demonstrated antimicrobial activity against pathogenic species *Staphylococcus aureus*, *S. epidermidis*, *Salmonella abony* and was inactive against *Pseudomonas aeruginosa*. The extract possessed low antioxidant activity against DPPH radicals.

Key words: antimicrobial activity, antioxidant activity, cardamom, extraction with tetrafluoroethane

Introduction

Cardamom is produced from cultivated or wild plants in the mountainous regions of southern India, Sri Lanka, Indonesia, and Guatemala. It has been used in the traditional Chinese medicine and Indian Ayurvedic medicine for thousands of years, mainly for treating respiratory diseases, fevers and digestive complaints.

The cardamom essential oil is obtained by steam distillation from the ripe and dried seeds of the tropical grass *Elettaria cardamomum* (L.) Matori (*Zingiberaceae*). It is a colorless or very pale yellow liquid with an aromatic, penetrating, slightly camphoraceous odor and a persistent, pungent, strongly aromatic taste. The physico-chemical properties (ISO 4733:1981) were: relative density at 20/20°C: 0.191 - 0.936; refractive index at 20°C: 1.4620 - 1.4680; optical rotation at 20°C: range from +22 to +41; solubility: 1 vol. in max. 5 vol. 70% ethanol (Bauer et al., 1997; Georgiev & Stoyanova, 2006).

The major components of the cardamom oil are 1,8-cineole (21-41%) and α-terpinyl acetate (21-35%). The cardamom oil also contains the following components: α-terpineol (0.8-6.2%, and to 11.5% in oil from Pakistan), limonene (1.7-3.7%), sabinene+β-pinene (0.3-2.4%), borneol (0.1-1.2%), linalool (0.4-8.7%), linalyl acetate (1.6-2.4%), nerol (0.6-1.6%), geraniol (1.1-3.7%), neryl acetate (0.8-1.2%), farnesol (up to 12.5% from the total isomers), nerolidol (0.2-6.7%), isosafrole (3.8%) and other minor compounds. Trace constituents like unsaturated aliphatic aldehydes and α-terpinyl acetate may be important for the

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typical aroma of the oil (Bauer et al., 1997; Laurence, 2004; Georgiev & Stoyanova, 2006).

The oil possesses antioxidant activity (Misharina et al., 2009). It is used primarily for seasoning foods, alcoholic beverages, the applied doses in foods varied from 0.20 to 0.50 mg %, and the minimal notable doses varied in the range 0.04 - 0.05 mg % (Bauer et al., 1997; Georgiev & Stoyanova, 2006). In small dosages, the oil is also used in perfumery and cosmetics. For example, cardamom oil is a constituent of mouthwash to treat bad breath and in creams, because the oil has diuretic properties and may alleviate fluid retention and cellulite (Rose, 2002; Georgiev & Stoyanova, 2006).

Cardamom oil is mainly used in aromatherapy as a digestive remedy to alleviate flatulence, heartburn, nausea, indigestion and colic. It acts as a general tonic for the digestive system and speeds up sluggish digestion. In India, cardamom oil is believed to have aphrodisiac qualities and is used to reduce the feelings of stress and tension that may be inhibiting sexual fulfillment. Its restorative properties make it effective in treating physical and mental fatigue (Rose, 2002; Georgiev & Stoyanova, 2006).

Different extracts are produced from cardamom seeds by using various solvents such as diethyl ether (Ağaoğlu et al., 2005**;** Syed Abdul Rahman et al., 2010**)**, water (Suneetha & Krishnakantha, 2005), ethanol (Nanasombat & Lohasupthawee, 2005) and methanol (El-Segaey et al., 2007). Chemical compositions and antimicrobial properties of the extracts depend mainly on the type of the used solvent. The cardamom fruits can also be extracted with liquefied gases by supercritical carbon dioxide (Hamdan et al., 2008; Gopalakrishnan 1994; Marongui et al., 2004) and sub-critical propane (Hamdan et al., 2008) extractions. Propane was found to be more capable than carbon dioxide to recover seed oil at sub-critical condition with lower ratio of solvent/solid and better quality attributes (Hamdan et. al., 2008). The major constituents of both extracts were 1,8-cineole and α-terpinyl acetate and the content of these compounds in the final extract depend on the extraction conditions such as temperature, working pressure and solvent.

Currently $C_2H_2F_4$ (1,1,1,2-tetrafluorethane) is prospective liquefied gas, which is licensed for producing of extracts for application in food and flavour industry. Unfortunately there are no publications for its application for extraction of cardamom seeds.

The aim of present study was to produce cardamom extract by 1,1,1,2-tetrafluorethane and to characterize the obtained product according to its chemical composition, antimicrobial and antioxidant properties.

Materials and Methods

Plant material

Cardamom seeds (*Elettaria cardamomum* (L.) Maton) were obtained from trade market, origin Guatemala harvest 2009, humidity 8% (Russian Pharmacopoeia, 1990).

Obtaining of extract

The air-seeds of cardamom were ground separately in an attrition mill to a size of 0.15-0.25 mm and the extract obtained by a 1 dm^3 volume $C_2H_2F_4$ (1,1,1,2-tetrafluorethane) laboratory-extractor (Nenov, 2006) under following conditions (continuous flow and evaporation of solvent): pressure 0.5 MPa; temperature 18-20^oC and extraction time 60 min. The physico-chemical properties were measured according to Russian Pharmacopoeia (1990).

Determination of chemical composition

GC analysis was performed using an Agilent 7890A gas chromatograph equipped with FID detector and HP-INNOWAX Polyethylene Glycol column (60 mm x 0.25 mm; film thickness 0.25 μm); temperature: 70°C - 10 min, 70-240°C - 5°C/min, 240°C - 5 min; 240-250°C - 10°C/min, 250°C - 15 min; carrier gas helium, 1 ml/min constant flow; injector split, 250°C, split ratio 50:1.

Gas Chromatography-Mass Spectrometry Analysis: GC/MS analysis was carried out on an Agilent 5975C gas chromatograph, carrier gas helium, column and temperature as for GC analysis, FID 280°C, MSD 280°C, transfer line.

Determination of antimicrobial activity

Antimicrobial activity of the cardamom extract was determined against pathogenic and spoilage bacteria from clinical and food isolates and also against reference microbial strains. The used test microorganisms and their origins are listed in Table 2. The strains are deposited in the Microbial Culture Collection at the Department of Biochemistry and Microbiology", Plovdiv University, Bulgaria. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of cardamom extract were determined by serial broth dilution method in accordance with CLSI reference method (CLSI Standards, 1990) A stock solution to be tested was prepared by diluting the respective cardamom extract sample in DMSO (Sigma-Aldrich Co.). Antimicrobial activity of the extract was determined in concentrations ranging from 0.00025 to 1.6% (w/v).

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Scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The radical scavenging capacity was determined according to the method described by Mensor et al. (2001). 1.0 ml from 0.3 mM alcohol solution of DDPH was added to 2.5 ml from the samples with different concentration of cardamom extract. The samples were kept at room temperature in dark and after 30 min the optical density was measured at 518 nm. The optical density of the working samples, the positive controls and the blank samples were measured in comparison with ethanol. The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of radical formation.

All experiments were done in triplicate and the results were statistically evaluated using a level of confidence γ=0.95.

Results and Discussion

The obtained extract is yellow mobile liquid with strong characteristic for the plant material odour and taste. The yield of cardamom extract is 2.7-3.0% (v/w).

Physicochemical properties were as follows: dry substance (105°C): 9.70%, refractive index (20°C): 1.4647, specific gravity (20°C): 0.9430, acid number: 3.6.

The chemical composition of the extract is presented in Table 1. Twenty-seven components representing 94.5% of the total content were identified. Ten of them were in concentrations over 1% and the rest 17 constituents were in concentrations under 1%. The major constituents (over 3%) were terpinyl acetate (36.8%), 1,8-cineole (29.2%), linalyl acetate (5.2%), sabinene (3.9%) and linalool (3.1%).

Results from the tests for antimicrobial activity are presented in Table 2.

Antioxidant activity of the cardamom extract is presented in Figure 1. As seen from the figure, 55.2% inhibition of DPPH radical was reached at concentration 100 mg/ml and the IC_{50} value was 63.3 mg/ml (correlation coefficient R^2=0.995).

According to physicochemical properties, the produced cardamom extract is almost equal to the cardamom essential oil. 90.7% of the identified substances in the extract belong to the group of monoterpenes, followed by phenyl propanoides (2.2%), sesquiterpenes (1.5%) and others compounds (0.1%). Oxygenated monoterpenes (80.0%) are the major group.

According to the content of major constituents, the produced freon extract of cardamom seeds is similar to the published in the literature (Hamdan et al., 2008). The qualitative differences in the rest of the constituents are due to the type of the used solvent and the process parameters.

Table 1. *Chemical composition of the cardamom extract.*

Components	%	RI
MONOTERPENES		
Hydrocarbons		
Sabinene	3.9	973
d-Limonene	2.3	1026
Myrcene	1.9	990
α-Pinene	1.9	939
β-Pinene	0.4	981
γ-Terpinene	0.3	1059
Oxygenated monoterpenes		
Terpinyl acetate	36.8	1340
1,8-Cineole	29.2	1032
Linalyl acetate	5.2	1254
Linalool	3.1	1093
α-Terpineol	1.6	1167
Geraniol	0.9	1240
Geranial	0.8	1225
Terpinen-4-ol	0.6	1182
Sabinene hydrate	0.3	1054
Fenchone	0.6	1090
Neral	0.3	1253
Geranyl acetate	0.3	1378
Carvyl acetate	0.2	1362
Terpinyl propionate	0.1	1430
SESQUITERPENES		
Hydrocarbons		
β-Selinene	0.3	1458
γ-Cadinene	0.2	1522
Germacrene	0.1	1468
Oxygenated sesquiterpenes		
Nerolidole	0.9	1534
PHENYL PROPANOIDS		
Anethole	2.1	1269
p-Cymene	0.1	1020
OTHERS		
Decenal	0.1	1260

The cardamom extract demonstrated antimicrobial activity against Gram-positive and Gram-negative bacteria, belonging to species *S. epidermidis, S. aureus, E. coli* and *S. abony*. The extract was inactive against both strains of *P. aeruginosa*, which belong to the group of the most resistible bacterial strains. The ability of *P*. aeruginosa to produce extracellular polysaccharides increased antimicrobial resistance of these bacteria mainly through permeability barrier.

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Table 2. *Antimicrobial activity of extract from cardamom..*

№	Test microorganisms	Origin	MIC, % (v/v)	MBC, % (v/v)
1	*Staphylococcus epidermidis*	Clinical isolate	0.4	0.8
2	*Staphylococcus aureus*	ATCC 6538	0.4	0.8
3	*Escherichia coli*	Food isolate	0.8	0.8
4	*Escherichia coli*	ATCC 8739	0.8	0.8
5	*Salmonella abony*	Clinical isolate	0.8	0.8
6	*Salmonella abony*	ATCC 6017	0.8	0.8
7	*Pseudomonas aeruginosa*	Food isolate	inactive	
8	*Pseudomonas aeruginosa*	ATCC 9627	inactive	

These strains also produced two types of soluble pigments, pyoverdin and pyocyanin, which probably participate in cell defense against antimicrobials.

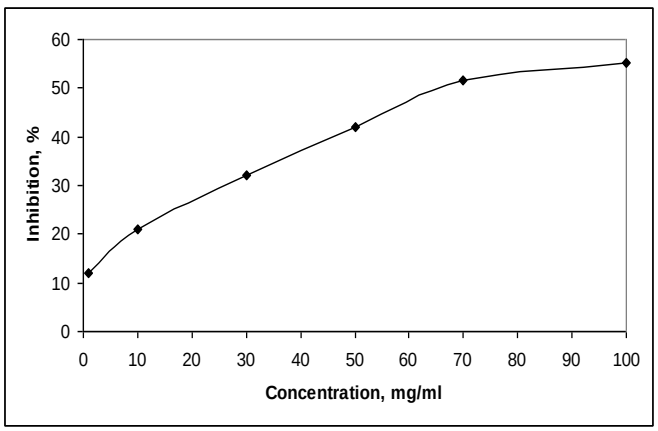

Figure 1. *Antiradical activity of cardamom extract against DPPH.*

In comparison with strong antioxidants such as ascorbic acid (4.20 μg/cm^3), rutin (14.65 μg/cm^3), BHT (1.12 μg/cm^3) and BHA (4.41 μg/cm^3), which are traditionally used in cosmetics and food industry, the produced cardamom extract possesses considerably lower antioxidant activity. In comparison with other extracts produced by low temperature extraction with 1,1,1,2-tetrafluorethane from anise fruirts (IC_{50}=8.32 mg/ml – Atanasova, 2007), coriander fruits (IC_{50}=17.74 mg/ml – Atanasova et al., 2010) and cinnamon barks (IC_{50}=0.38 mg/ml – Nenov et al., 2011), the cardamom extract also demonstrates lower antioxidant activity.

Conclusion

The extract from cardamom (*Elettaria cardamomum* (L.) Maton) seeds produced by low temperature extraction with tetrafluoroethane characterized with higher content of terpinyl acetate (36.8%), 1,8-cineole (29.2%), linalyl acetate (5.2%), sabinene (3.9%) and linalool (3.1%) with characteristic odour and taste. The produced extract demonstrated antimicrobial activity against some of the most widely spread pathogenic and spoilage bacteria in foods and characterized with low antioxidant activity in comparison with other extracts produced by low temperature extraction. Currently, the experiments for application of the produced cardamom extract in cosmetic and food products are in progress.

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