EVALUATION OF CARDAMOM OIL ROLE AS ANTIMICROBIAL, ANTICARCENOGENIC AND ANTI- INFLAMMATORY AGENTS

EL-BASTAWESY, AMAL M.[1] AND RAGAA H. MOHAMED.[2]

[1] *Food Technology Res. Institute, Agric. Res. Center, Giza, Egypt.*
[2] *National Cancer Institute, Cairo Univ., Cairo, Egypt.*

(Manuscript received 4 December 2004)

Abstract

Separation and identification of cardamom volatile oil obtained by steam distillation were carried out by GC analysis and GC/MS. The fatty acids composition of cardamom fixed oil were also determined . The volatile and fixed oils were tested for their antimicrobial activity against four different genera of pathogenic bacteria namely, *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhimurium* and two strains of spoilage fungi namely, *Aspergillus flavus* and *Aspergillus ochraceus.* Cytotoxic and anticancer activities of the volatile oil were evaluated. As well as a comparative study of the anti- inflammatory activity of the cardamom volatile oil at a dose of 100 mg/kg body weight and the diclofenace at a dose of 3mg/kg against acute carrageenan which induced rat hind paw odema was also performed. The obtained results revealed that volatile oil represents about 5% of the seed contents and 24 constituents were identified in the free volatile fraction from 55 separated components of total fractions. The major fractions of cardamom volatile oil given as area % were 1,8 - cineole (37.28 %), terpinyl acetate (18.55 %) and endobornyl acetate (11.96 %) . It was also observed that volatile oil was rich in the oxygenated compounds and poor in terpene hydrocarbons. The volatile oil of cardamom seeds was more effective on the inhibition of the growth of the microbial species examined than fixed oil. The inhibitory effect of volatile oil against some pathogenic fungi was increase as volatile oil concentration increased and had highly inhibitory effect on the selected pathogenic bacteria. So, it could be concluded that this study has a very important economic reflex as it was proved to be applicable, practicable and available to produce cardamom volatile oil which could be utilized as antimicrobial agents. Moreover, it had a highly cytotoxic and anticarcenogenic activities against human tumor cell lines. Besides the cardamom volatile oil had a noticeable effect as anti-inflammatory agent .

INTRODUCTION

Recently, the interest in the utilization of natural substances and some questions concerning the safety of synthetic compounds have encouraged more detailed studies on natural plant resources . Therefore, there is an urgent need for other natural compounds instead of synthetic ones to act as antimicrobial, antioxidant and anticancer agents beside being safer for human consumption . Cardamom seeds (*Elettaria cardamomum*) are among popular spices in the Arabic world and which are usually used as a flavoring agents for bread, meat products and principally as an additive to Turkish coffee "Kahwa".

About 68 volatile compounds were identified in the free volatile fraction of cardamom oil. These compounds included aliphatic (23.7 %) aromatic (0.9 %) monoterpene (20.7 %) and sesquiterpene (32.0 %) compounds (Nirmala- Menon *et al.* 1999). Nirmala Menon (2000) and Anon (2002) found that the constituents of the steam distilled volatile oil of large cardamom were oxygenated monoterpenes (75.2 %) followed by monoterpene hydrocarbons (16.3 %) and sesquiterpenes (6.3 %). 1,8-cineole (61.3 %), α - terpieol, α and β -pinene and alloaromadendrene which were the predominant compounds .

Volatile oil of cardamom seeds was proved to be one of the most effective inhibitor of the microbial growth and also having a good antibacterial activity by direct contact (Kubo *et al.*, 1991; Elgayyar *et al.*, 2001 and Kalemba and Kunicka 2003). The extent of the inhibitory effect of the oils could be attributed to the presence of aromatic nucleus containing a polar functional groups. Moreover, the activity of these compounds is dependent mainly upon the aromatic groups, carbon chain length, number and location of carbonyl groups in their structure (Bowles and Juneja, 1998). Cardamom volatile oil had highly inhibitory effect on specified pathogenic and spoilage microorganisms, which could provide alternatives and supplements to conventional antimicrobial additives in foods (Elgayyar *et al.*, 2001; Leena *et al.*, 2003 and Ozkan *et al.,* 2003).

Volatile oils of many spices such as cardamom could also be used as medical drugs of gastrointestinal discords and as strong antitoxic , anticancer and anti-inflammatory agents (Al-Zuhair *et al.*, 1996 and Leal *et al.*, 2003).

A large and increasing number of patients all over the world have used medicinal plants and herbs for healthy purposes. Therefore, this study was carried out to extract the volatile oil from a popular spice namely, cardamom seeds . The

inhibitory effect of cardamom volatile oil against certain food- borne pathogenic fungi and bacteria was examined . Also, the cytotoxic and anticancer activities of cardamom volatile oil were studied .

MATERIALS AND METHODS

Materials

Green cardamom seeds (*Elletaria Cardamomum*) were obtained from a local retail spice market in Mansoura, Egypt. These seeds were imported from Guatemala.

Pathogenic stock cultures of bacteria , *Staphylococcus aureus* , *Bacillus cereus* NRRL B- 3711 as a Gram-positive and *Escherichia coli, Salmonella typhimurium* as a Gram-negative bacteria were obtained from Microbilogical Resources Center (MIRCEN), Fac. Agric., Ain Shams Univ., Egypt.

Fungal plant pathogens used in this study were *Aspergillus flavus* and *Aspergillus ochraceus* which were obtained from Plant Pathology Dept., Fac. Agric., Mansoura Univ., El-Mansoura . Egypt .

Human tumor cell lines were obtained from National Cancer Inst., Cairo Univ., Cairo, Egypt which purchased kindly from U.S.A.

Methods

Extraction of volatile oil

One hundred grams of ground seeds were suspended in 1.5L of distilled water . A continuous steam distillation extraction was performed for 3hr. and the oil was collected and stored at 4 °C until used .

Extraction of fixed oil.

Fixed oil was extracted with chloroform : methanol mixture (2:1 v/v) in a Soxhelt apparatus . The solvent was evaporated in a rotary evaporator under vacuum. The oily residue was considered as the total lipids and expressed as percentage of dry weight .

Identification of volatile compounds

Analysis of the volatile oil was carried out by gas chromatography (GC) and gas chromatography- mass spectroscopy (GC/MS) technique at the Central Laboratory of kato Aromatic Co., Cairo, Egypt. GC analysis was performed on a Hewlett- packed gas GC, model 5890 series II, equipped with a flame ionization detector (FID) and coupled to an electronic integrator. The chromatograph was fitted with methyl silicone column (20m x 0.2 mm, 0.33 mm film thickness). GC analytical conditions were the

carrier gas He with flow rate 1 ml/min, the injector and detector temp. were 200 °C and 250 °C, respectively. The oven temp. was programmed from 60 °C - 200 °C at a rate of 3 °C/min . Quantitative data were obtained by electronic integration of FID area data without the use of response factor correction .

GC/MS analysis was performed using a Hewlett-Packard MS 5970, equipped with TIC mass detector . The operation conditions were , carrier gas He, ionization voltage (70 eV), scanning speed 1s over 20 - 550 amu range and an electron multiplier voltage (1800 eV). The column and conditions were the same described before except injector temp. was 150 °C . Isolated peaks were identified by matching with data from the library of mass spectra and compared to those of standard compounds and published data (Adams, 1995). The quantitative determination was carried out depending upon peak area integration .

Fatty acids composition analysis

The determination of fatty acids composition of fixed oil was conducted using GC/MS technique at the Central Laboratory of Food Industries, Fac. Agric., Cairo Univ., Cairo. After saponification, sample was concentrated to about 0.25ml with stream of purified nitrogen gas and methylation by diazomethane . GC analysis were performed on a Hewelelt Packard model 6890, capillary column (5 me x 30 m, 0.30 mm film thickness). GC analytical conditions were carrier gas Helium at 0.80 cm/min, the injector and detector temp. were 250 and 300°C, respectively. The oven temp. was programmed from 80 to 230 °C for 15 min at a rate of 4°C/ min .

GC/Ms analysis were performed using a Hewelelt Packard model 5973 Mass selective detector and scanning mode was (m/z 70 to 650) . Separated compounds were identified using standard library (NIST Version 2.0)

Assessment of inhibition of bacterial growth

The respective bacterial strains (24 hrs. cultures) were diluted before testing in a 0.01M sterilized sodium phosphate buffer (pH 7.4$\pm$ 0.2 at 7°C) to give a population of approximately 2.8×10^5, 4.2×10^4, 2.1×10^5 and 2.5×10^5 cFu/ml for *E. coli, Salmonella typhimurium, Staphylococcus aureus* and *Bacillus cereus*, respectively as measured by their absorbance at 650 nm using Spekoll II colorimeter. Four specific media and four concentrations of the volatile and fixed oils (20, 30, 40 and 50 μ l, 30, 40, 50 and 70 μ l, respectively) were used. Fixed oil was dissolved in Tween 80 (1: 1 v/v) but volatile oil was added as it is. The measurement of growth inhibition was performed according to the method described by Deans and Ritchie (1987) using the

agar well technique. 1ml. of each culture was pipetted into separate sterile Petri dishes to which 15 ml. of specific media (45 °C) were added . Once set, wells of (4 mm) diameter were made in the center of each agar plate using a Carcoborar and the suggested oil concentrations were added . The plates were then left under cooling temperature for 10 min. to allow diffusion of the oil into the media and incubated inverted in the dark at 37 ± 0.1 °C for 48 hr. Following this, zones of growth inhibition were measured using Vernier calipers . All experiments were carried out in triplicate .

Assessment of inhibition of fungal growth

The plant pathogenic fungi was used to assess antimycotic activity by dry weight method in the liquid culture media (Czapek's medium). Volatile oil was dissolved (1:1 v/v) in a mixture of (absolute ethyl alcohol 95% and 5% tween 80) while fixed oil was dissolved (1:1 v/v) in tween 80 then added to the 20 ml of sterilized liquid media with different seven concentrations. Two discs (0.4 cm) of mycelial material taken from the edge of six-day- old fungal cultures were placed at the flask . The flasks with the inoculums were placed in the dark incubator under controlled temperature conditions of 23 ± 1°C for seven days. The measurement of growth inhibition was carried out according to the method described by Graven *et al.* (1992)

Cytotoxic and anti-tumor activities of cardamom volatile oil

Cardamom volatile oil was tested for any cytotoxic activity against the human tumor cell lines in Pharmacology Unit, Cancer Biology Dept., National Cancer Inst., Cairo Univ., Cairo. U251 (brain tumor cell line), MCF7 (breast carcinoma cell line) and Hepgz (liver carcinoma cell line) were used at volatile oil concentration between (1.00–10.00 μg/ml) using the Sulphodiamine-B assay (SRB) according to Skehan and Stroreng (1990). Anti-tumor activity of the (Earling Asictes Carcinoma) was also determined .

In vitro anticancer testing a set of sterile test tubes used, where 2.5 x 10^5 tumor cells per ml were suspended in phosphate buffer saline. Then 25, 50, 100 μg/ ml from drug were added to the suspension, kept at 37°C for 2 hours . Trypan blue dye exclusion test was then carried out to calculate the percentage of nonviable cells .

In vivo anticancer testing thirty adults female Swiss albino mices weighing 22 – 25 g, which were obtained from the experimental animals house of Ain Shams Univ., Cairo, Egypt, were injected with EAC (2.5 x 10^5 tumor cells). Then, mices were divided randomly into three groups (n=10). The first group was as negative control,

the second was injected by 5- Fluorauracil (5- Fu, 20 mg/kg body weight/day) positive control and the third was injected orally by cardamom volatile oil (100 mg/kg body weight) a dose – dependent inhibition of the growth in mice of Ehrlich ascites tumors carcinoma . The median survival tumor (MST) of the treated groups were compared with that of the control group using the following calculation:

$$\text{Increase in life span} = \frac{T - C}{C} x100$$

Where as : T = number of days the treated animals survived .

C = number of days control animals survived .

Measurement of anti- inflammatory effect of cardamom volatile oil .

The anti-inflammatory activity of cardamom volatile oil was measured according to the method described by Winter *et al*. (1962).

Twenty four male albino rats (Sprage Dawely) weighing 120 – 150 gm which were supplied from the experimental animals house of Ain Shams Univ. Cairo, Egypt were divided randomly into three groups (n – 8) as follow:

G_1 : Rats injected orally with (2% tween 80) 1 hour before carragenass infection. (negative control)

G_2 : Rats injected orally with (3 ml/kg diclofenace) voltarin (positive control)

G_3 : Rats injected orally with (100 mg volatile oil/Kg body weight) 1 hour before carragenass infection.

All groups were injected with carrageenan in the region of the right hind paw of the rat . Finally, data were expressed as the difference between the final and the initial right hind paw volume .

Statistical analysis

. The data were subjected to statistical analysis using one way classification least significant differences method L.S.D. according to Steel and Torrie (1980). Significant differences were determined at the $P \leq 0.05$ level .

RESULTS AND DISCUSSION

Separation and identification of cardamom seed oils

The important constituents of cardamom seeds were determined and the data proved the main following points. Steam distilled volatile oil is considered to be one of the most the important component of cardamom seeds and was found to represent about 4.9740 %. The content of volatile oil in the cardamom seeds is strongly dependent on storage conditions but may be as high as 8 %. Also the seeds show a loss of about 40% of the volatile oil during storage for one year and reaches to 2 - 4% (Al-Zuhair *et al.,* 1996 and Anon, 2002). From the same table, it is obvious that fixed oil content of cardamom seeds was relatively high (15.4 %).

1- Volatile oil

The components of cardamom volatile oil fractions obtained by traditional steam distillation were separated by GC analysis and the produced peaks were identified by using GC/MS. Figure 1 shows the GC/MS chromatogram of the volatile components, meanwhile, the concentration of the cardamom volatile oil components is given in Table 1. From these data, it could be noticed that more than 55 components were separated and about 24 components were identified in volatile cardamom oil . Alcohols were the major groups identified followed by esters and hydrocarbons which represent in terms of peak area 47.14, 37.06 and 11.11 %, respectively. The predominant alcohol was 1,8- cineole which was also the major component of volatile fractions giving a value of about 37.28% and linalool was the major monoterpene alcohol 3.51 % (Table 1) . These results are in agreement with those of Nirmala- Menon (2000) and Anon (2002) who found that 1,8-cineole was the major component of cardamom volatile oil and it gives a harsh "eucalyptol" odor to the oil if present in high proportion .

Esters were the second major group identified in the cardamom volatile oil as seen in the same table. Six esters were separated with total area 37.07 %. The area of the predominant ester compound that identified was related to terpinyl acetate (18.55 %) followed by endobornyl acetate (11.96 %) and linalyl acetate, furfuryl -2-alcohol, 1,2 cyclohexanedimethanol, 3-(acetyloxy)-1,2 dimethyl-diacetate and propane, 1,1- sulfonylbis were also detected. Cardamom volatile oil was rich in oxygenated compounds and poor in terpene hydrocarbons .

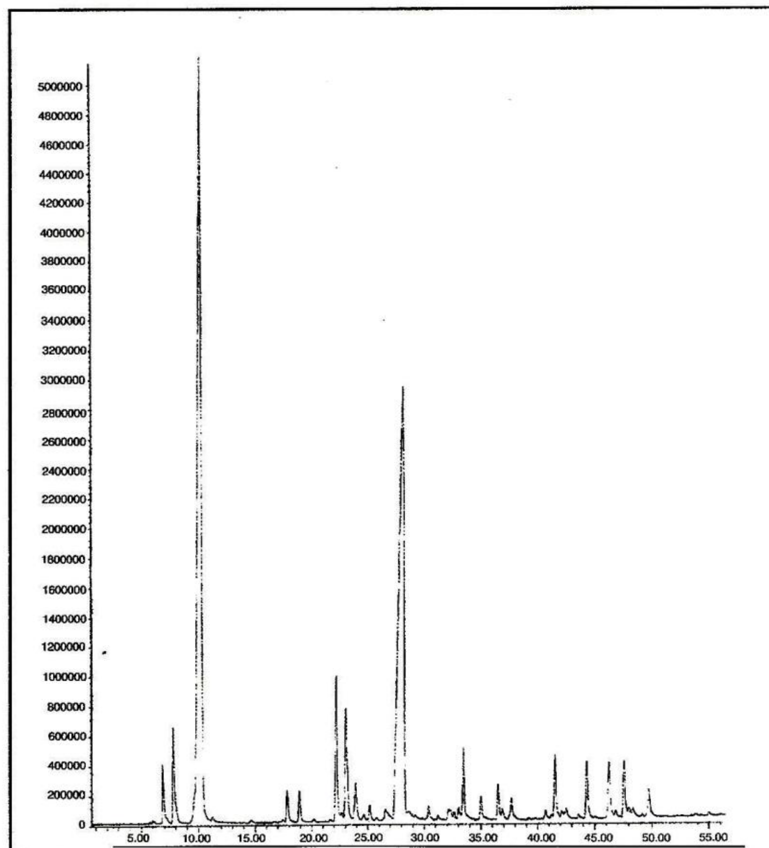

Figure 1. GC/MS chromatogram of volatile components of cardamom volatile oil

Table 1. Volatile constituents of the investigated cardamom seed oil.

Peak No.	Identified constituents	Retention time	Area%
1	Unk.	2.310	0.0358
2	Unk.	2.663	0.1610
3	Unk.	2.803	0.0335
4	Alpha- Pinene	3.663	1.5851
5	Unk.	4.229	0.0768
6	1- beta – Pinene	4.880	3.0467
7	Unk.	5.085	0.0718
8	Unk.	6.291	0.1289
9	1-Limonene	6.733	2.4839
10	1.8-Cineole	7.172	37.2821
11	Benzene, 1-methyl-4-(1- methylethyl)	8.640	0.2106
12	Unk.	12.008	0.0783
13	Furfuryl – 2- Alcohol, Teterahydro- α ., α ., 5-Trimethyl-5- vinyl	14.620	0.7937
14	Unk.	15.595	0.0862
15	Linalool Oxide (2)	15.698	0.5843
16	Unk.	18.197	0.1107
17	Linalool	18.752	3.5132
18	Linalyl acetate	19.180	1.7063
19	Unk.	19.621	0.4420
20	Unk.	19.775	0.1408
21	Cyclohexanol, 1- methyl-4-(1- methylethenyl)	19.940	1.3539
22	1,3,6- Heptatriene , 2,5,5- trimethyl	20.518	0.9220
23	Unk.	21.290	0.1165
24	Unk.	21.490	0.1996
25	α -Phellandrene epoxide	22.640	0.5068
26	Unk.	23.411	0.5045
27	Unk.	23.626	0.0961
28	Endobornyl acetate	24.504	11.9571
29	Terpinyl acetate	24.641	18.5521
30	Unk.	25.610	0.1549
31	Cyclohexane,1,5-diethenyl-3-methyl-2- methylene –(1- α, 3. α, 5. α)	27.895	0.3991
32	Unk.	28.354	0.1059
33	Unk.	28.771	0.1442
34	Unk.	28.983	0.1382
35	Unk.	29.519	0.1821
36	Unk.	30.018	0.1881
37	Unk.	30.415	0.1854
38	Bicyclo (2.2.1) hept-2-en-7-ol	30.537	1.6903
39	1,4-cyclohexadiene -1-methanol, 4-(1-methylethyl)	32.136	0.4553
40	1,2-cyclohexanedimethanol,3-(acetyloxy)-1,2-dimethyl-diacetate.	33.845	1.1576
41	1H-cycloprop[e] azulen-4-ol, decahydro-1,1, 4,7-tetramethyl-,[1aR-(1a. α .,4. α .,4a,beta.,7. α ,7a.beta.,7b. α .)]	35.030	0.7288

Peak No.	Identified constituents	Retention time	Area%
42	Unk.	38.050	0.1728
43	Unk.	38.817	0.1188
44	2-methyl-6-methylene -3,7-octadiene -2-ol	39.095	1.5176
45	Unk.	39.501	0.1641
46	Unk.	40.167	0.1815
47	Unk.	41.199	0.0861
48	Unk.	41.829	0.1297
49	P-Mentha-Trans-2,8-Dien-1-ol	41.998	1.1992
50	Propane,1,1-sulfonylbis-	44.065	1.4037
51	Unk.	44.940	0.1428
52	2-cyclohexen-1-one,4-(2-oxopropyl)	45.545	1.4879
53	Unk.	46.060	0.2835
54	Unk.	47.503	0.0988
55	Limonene oxide	48.000	0.7710
Total Alcohols			47.1406
Total Esters			37.0584
Total Hydrocarbons			11.1093
Total Identified			95.3083
Total Unidentified			4.7594

Terpinyl acetate is the major component and the oil rich in esters like terpinyl acetate and linalyl acetate are known to give flowery smell (Nirmala-Menon *et al.* 1999 and Nirmala-Menon 2000) .

The oxygenated derivatives commonly named terpenoids are considered to be one of the important flavor compounds . Several natural terpenes such as α - pinene , β -myrcene and limonene are relatively cheap, produced in bulk quantities (Adams, 1995). On the other hand, the area of monoterpene hydrocarbons represented about (11.11 %) and 1- beta-pinene, 1- limonene and alpha-pinene were the most dominant hydrocarbon to be identified in volatile fractions (3.05, 2.48 and 1.59 %, respectively) as seen in Table 1. From this aforementioned table, it could be clearly observed that cardamom volatile oil is rich in oxygenated compounds and poor in terpene hydrocarbons . The major flavor compounds 1,8- cineole and terpinyl acetate were present in highly concentration.

2- Fixed oil

Cardamom fixed oil components were separated by GC analysis and identified using GC/MS and the results are shown in Figure 2 and Table 2. The identified groups of the tested fixed oil included alchols, aldehydes, esters, hydrocrbons and fatty acids . Alcohols and aldehydes areas were detected and constituted only 4.67 and 2.48 % of the total area peaks of the separated fixed oil, respectively. On the other hand, peak area of 1.8-cineole was considered to be the predominant alchol (2.55 %), while four esters were identified and represented about 16.46% of the total components. Octyl

ester of formic acid showed the highest area (9.90 %) if compared to other separated esters . Natural terpenes namely, α - terpinene , limonene , γ - terpinene, α - terpinolene and others were detected. The identified area of monoterpene hydrocarbons was 37.63 % and that of hydroxymethyl cyclododecane, pentane 2,3-dimethyl and hexane-2, chloro were 10.97, 9.25 and 6.69 %, respectively. The aforementioned hydrocarbons were the most dominant groups identified in fixed oil fractions.

Fatty acids were the first major group identified in cardamom fixed oil and nine fatty acids are detected with a total peak area of 38.75 % . A total five saturated fatty acids were identified in cardamom fixed oil which represnted about 13.69% . Palmitic acid $C_{16:0}$ was the major saturated one (7.80 %) followed by butyric acid $C_{4:0}$ (2.29 %) as seen in Table 2. From the same table, it was found that the unsaturated fatty acids of cardamom fixed oil were 25.06 % (as area %) of the total fatty acids . The unsaturated fatty acids namely, oleic, linoleic, palmitoleic and caproic acids were detected in descending order (Table 3).

It is worthy to mention that the relative area % of the unsaturated fatty acids was more than 64 % especially, essential fatty acids which reflect the medicinal value of the oil and play a very important role in incr ng the nutritional values of cardamom seed oil.

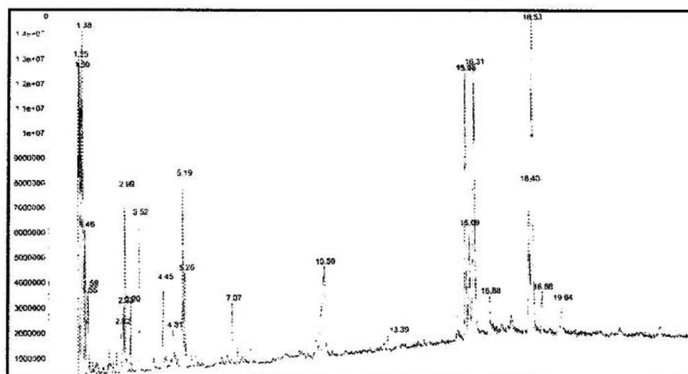

Figure 2. GC/MS chromatogram of the tested cardamom fixed oil.

Table 2. Identified constituents of the fixed oil extracted from cardamom seed.

Peak No.	Components	Retention time	Area %
	Alcohols		
9	1,8 – cineole	2.99	2.55
12	Atenolol	4.45	1.32
13	3-cyclohexene-1-methanol	4.81	0.80
	Total		**4.67**
	Aldehydes		
4	3-ethylheptanal	1.46	2.48
	Total		**2.48**
	Esters		
3	Octyl ester of formic acid	1.38	9.90
6	Hexyl octyl ether	1.59	1.09
15	α -terpinyl acetate	5.26	1.62
20	4-bromomethyl-2,2,5,5, tetramethyl	16.09	3.85
	Total		**16.46**
	Hydrocarbons		
1	Pentane 2,3-dimethyl	1.25	9.25
2	Hexane 2, chloro	1.30	6.69
7	α - terpinene	2.82	0.85
8	limonene	2.93	1.49
10	γ - terpinene	3.20	1.06
11	α - terpinolene	3.52	2.67
14	Adamantane	5.19	3.79
21	Hydroxymethyl cyclododecane	16.31	10.97
26	1- tetradecene	19.64	0.86
	Total		**37.63**
	Fatty acids		
5	Butanoic acid $C_{4:0}$ (Butyric)	1.55	2.29
16	3-hexanoic acid $C_{6:1}$ (Caproic)	7.07	1.35
17	Hexadecanoic acid methyl ester $C_{16:1}$ (Palmitoleic)	10.59	5.95
18	Decanoic acid methyl ester $C_{10:0}$ (Capric)	13.39	0.74
19	Methyl hexadecanoate $C_{16:0}$ (palmitic)	15.98	7.80
22	Heptadecanoic acid methyl ester $C_{17:0}$ (Margaric)	16.88	1.73
23	7,10- octadecadienoic acid methyl ester $C_{18:2}$ (linoleic)		4.43
24	9-Octadecenoic acid methyl ester $C_{18:1}$ (Oleic)	18.40	13.33
25	Octadecanoic acid methyl ester $C_{18:0}$ (stearic)	18.53	1.13
	Total	18.88	**38.75**
	Saturated		13.69
	Unsaturated		25.06

Antimicrobial activity of cardamom seed oils

1-Antifungal activity

The antimicrobial properties of cardamom seed oils (volatile and fixed) at different concentrations were tested using some selected pathogenic microorganisms. The antifungal activity of cardamom oils against two mycotoxic strains of fungi namely *Aspergillus flavus* and *Aspergillus achraceus* was evaluated and the data are shown in Table 3. There are wide variations in the antifungal activity between volatile and fixed oil effects since the volatile oil was found to be more active against the two studied strains than the fixed oil. From Table 3, the inhibitory effect of cardamom volatile oil increased with increasing the concentration of oil and indicating significant differences (P > 0.0001) for the two strains. Addition of 90 μ l of volatile oil inhibited approximately *A. flavus* and *A. achraceus* up to 99.13 and 98.0 %, respectively. These results indicate the strong effect of the volatile oil as anti fungal and so, coincide with those reported by Elgayyar *et al.* (2001), Leena *et al.* (2003) and Ozkan *et al.* (2003). This may be due to the presence of antimicrobial active components such as 1,8-cineole, terpinyl acetate and linalool. The effect of cardamom fixed oil on growth of pathogenic fungi is shown in the same table. The available data proved the presence of a slight growth inhibition of *A. flavus* at 30 μ l of fixed oil (7.65 %) then more activation growth occurred with increasing fixed oil concentration especially with *A. ochraceus* fungi than *A. flavus.* Increasing fixed oil concentration raised the growth activity of the spoilage fungus and the increment was highly significant . Clearly, the aforementioned results reveal that cardamom volatile oil has more greater effect as an antimycotic agent than the fixed oil .

2-Antibacterial activity

Both cardamom volatile and fixed oils were tested for their antibacterial activity against two strains of Gram negative bacteria (*E.coli* and *Salmonella typhimurium)* and two stains of Gram positive bacteria (*Staph.oureus* and *Bacillus cerues)* and the obtained results are given in Table 4. The obtained data indicate that a completely inhibition (100 %) was obtained when the lowest concentration of volatile oil was used against *Salmonella typhimurium.* The zones of growth inhibition increased gradually with raising volatile oil concentration and completely inhibition occurred at 40 μ L and 50 μ L of volatile oil against *Staph. aureus* and *Bacillus cereus,* respectively. In contrast, cardamom volatile oil had the lowest inhibition effect against *E. coli* (17.8 %) compared with other tested pathogens since zones of growth inhibitions were relatively small with the highest concentration of volatile oil 50 μ l. On such a base, *Salmonella typhimurium* was the highest sensitive bacteria to cardamom volatile oil

than the other strains (Table 4). These results are in agreement with those reported by Elgayyar *et al.* (2001) and Kalemba and Kunicka (2003) who found that *E.coli* was less sensitive to essential oils than the other strains tested. The action of volatile oils on microorganisms can be explained by their ability not only to attack the cytoplasmic membrane and destroying its permeability with the possibility of releasing intracellular constituents but also cause membrane function in respect of electron transport, nutrient uptake, nucleic acid synthesis and ATP-ase activity.

Table 3. Effect of cardamom oil on growth of pathogenic fungi.

a-**Volatile oil

Pathogenic Fungus / Concentration μl.	*A. flavus* Mycilium dry w.(gm)	Inhibition* %	*A. ochraceus* Mycilium dry w.(gm)	Inhibition %
Zero	A 0.230 ± 0.03		A 0.150 ± 0.02	
20	B 0.143 ± 0.02	37.83	B 0.067 ± 0.02	55.33
30	C 0.077 ± 0.02	66.52	BC 0.0567 ± 0.01	62.20
40	CD 0.073 ± 0.01	68.26	BCD 0.050 ± 0.01	66.67
50	CD 0.053 ± 0.01	76.96	CD 0.043 ± 0.01	71.33
60	D 0.043 ± 0.01	81.31	D 0.030 ± 0.01	80.00
70	DE 0.011 ± 0.03	95.22	DE 0.009 ± 0.01	94.00
90	E 0.002 ± 0.01	99.13	E 0.003 ± 0.01	98.00
L.S.D	0.0001		0.0001	

b- **Fixed oil

Pathogenic Fungus / Concentration μl.	*A. flavus* Mycilium dry weight (gm)	Inhibition* %	*A. ochraceus* Mycilium dry weight (gm)	Inhibition %
Zero	A 0.170 ± 0.02		D 0.130 ± 0.01	
30	A 0.157 ± 0.12	+7.65	BC 0.170 ± 0.02	-30.77
40	A 0.187 ± 0.01	-10.00	0.160 ± 0.02	-23.08
50	A 0.173 ± 0.02	-1.76	0.137 ± 0.01	-5.38
70	A 0.207 ± 0.02	-21.76	B 0.183 ± 0.01	-40.77
90	A 0.233 ± 0.01	-37.06	A 0.237 ± 0.01	-82.31
L.S.D	0.5106		0.0001	

* Inhibition (%) expressed as relative control tasks .

**Each separated oil type was statistically analyzed with respect to individual pathogenic fungi.

Antibacterial activity of cardamom fixed oil against four selected- food borne pathogens are shown also in Table 4. The data indicate that , there is a slight inhibitory effect of cardamom fixed oil against all tested strains. The concentration of fixed oil has no great and non significant effects of its inhiborty activity for all tested strains which ranged from 11.28 to 22.0 mm. According to the aforementioned data, it could be concluded that food-borne pathogens were more less sensitive to cardamom fixed oil. On contrary, cardamom volatile oil was more potent against all tested food-borne pathogens and can be recommended as safe antibacterial agents to prevent the spoilage of food products .

Subsequently, it could be concluded that it is practicable and economic to produce cardamom volatile oil which could be utilized as anti microbial agents.

Table 4. Antibacterial activity of cardamom oil against some selected food- borne pathogens.

a- ***Volatile oil

Pathogenic bacteria / Concentration μl	*Escherichia coli*	*Salmonella typhi.*	*Staphylococcus aureus*	*Bacillus cereus*
	Zones of growth inhibition (mm)*			
20	C 7.50 ± 0.20	Clear zone**	C 23.77 ± 1.08	B 47.77 ± 6.53
30	B 10.87 ± 1.40	Clear zone**	B 77.83 ± 9.01	B 60.33 ± 11.55
40	B 13.23 ± 2.66	Clear zone**	A 90.00 ± 0.00	A 83.17 ± 11.41
50	A 17.80 ± 1.04	Clear zone**	A 90.00 ± 0.00	A 90.00 ± 0.00
L.S.D	0.0003	0.000	0.0001	0.0012

** clear Zone : completely inhibition 100% or ≥ 90 mm.

b- ***Fixed oil

Pathogenic bacteria / Concentration μl	*Escherichia coli*	*Salmonella typhi.*	*Staphylococcus aureus*	*Bacillus cereus*
	Zones of growth inhibition (mm)*			
30	B 15.00 ± 0.41	B 15.58 ± 3.25	C 11.28 ± 3.13	A 21.30 ± 3.26
40	B 16.25 ± 0.86	AB 18.20 ± 1.00	BC 13.75 ± 0.76	A 23.00 ± 1.53
50	A 19.63 ± 1.60	AB 18.88 ± 3.71	AB 16.20 ± 2.38	A 22.15 ± 2.41
70	A 19.88 ± 2.17	A 21.23 ± 1.75	A 18.98 ± 1.89	A 22.00 ± 8.83
L.S.D	0.0007	0.0730	0.0022	0.9693

* The values are the distance (mm) across the zone of inhibition and the agar well diameter (4mm). Plate diameter 90 mm.

***Each separated oil type was statistically analyzed with respect to individual pathogenic fungi.

Anti-cancer activity of cardamom volatile oil

Cancer is the most common cause of death in the world population, and the possibility that readily available now for using natural substances from plants, vegetables, herbs and spices may be beneficial for the prevention of cancer. So, the cardamom volatile oil was tested for cytotoxic activity against three human tumor cell lines namely, U251 (brain tumor cell line), MCF7 (breast carcinoma cell line) and Hepg2 (liver carcinoma cell line) and the results are shown in Table 5. The effect of cardamom volatile oil on growth of human tumor cells are shown in Figure 3. From these data the tested cardamom volatile oil was proven to have cytotoxic activity against tested human tumor cell lines. The doses of cardamom volatile oil which reduced the survival of the three human tumor cell lines to 50 % (50 % inhibition) were (0.37,0.54 and 0.44 μg/ml for U251, MCF7 and Hepg2, respectively). The same cardamom volatile oil was tested at a concentration varied between 1 - 10 μg/ml using the SRB assay to measure the potential of cytotoxicity as given in Figure 3. The cardamom volatile oil proved to have cytotoxic activity and its inhibitory effect was noticed to increase as volatile oil concentration increased to 10 μ g/ml for all of the tested human tumor cell lines.

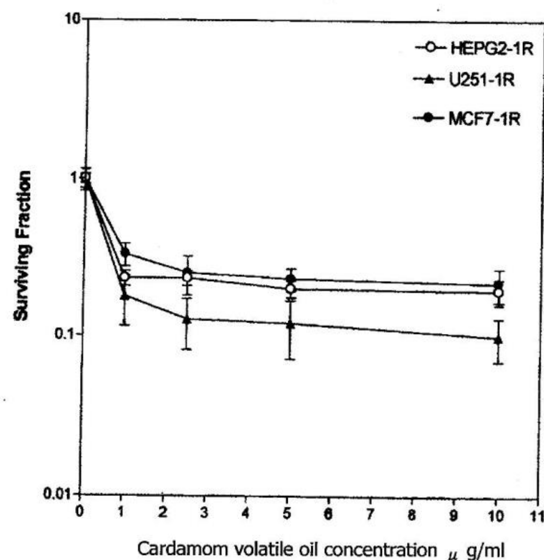

Figure 3. Cytotoxic activity of cardamom volatile oil against human brain tumor cell line (U251), human breast carcinoma cell line (MCF7) and human liver carcinoma cell line (Hepg2).

Table 5. Cytotoxic activity of cardamom volatile oil .

Cell line	IC_{50}
U251 (brain)	0.37 μ g/ ml
MCF7 (breast)	0.54 μ g/ ml
Hepg2 (liver)	0.44 μ g/ml

IC_{50} : Dose which reduces survival to 50%

The effect of cardamom volatile oil as antitumor activity of the Earling Asictes Carcinoma (EAC) is shown in Table 6. Cardamom volatile oil reduced asictes in mice induced by EAC cells and had a completely inhibition (100%) of cell viability when 25, 50 and 100 μ g/ml of volatile oil concentration were used. It had a highly anticarcenogenic effect on tumor cells. These may be due to the occurrence of photochemical that usually found in a variety of herbs and spices which have the ability to cure cancer both through necrosis and apoptosis as well as, many herbs and spices having free radical scavenging properties (Leal *et al.,* 2003). The effect of cardamom volatile oil on the survival of tumor-bearing mice is shown in Table 7. The median survival tumor (MST) for the control group was 21 $\pm$ 1.20 days, whereas it was 33 $\pm$ 1.20 days and 40 $\pm$ 2.10 days for the groups treated with cardamom volatile oil (100 mg/kg/day, p.o.) and 5-Fluorauracil (20 mg/kg/day, i.p.), respectively. The increase in the life span of tumor- bearing mice treated with cardamom volatile oil and 5-Fluorauracil was found to be 57.14 % and 90.47 %, respectively ($p \leq 0.01$) as compared to the control group. The effect of cardamom volatile oil on the peritoneal exudates cells of normal mice is an indirect method of evaluating its inhibitory effect on tumor cell growth. The available data found that the average number of peritoneal exudates cells per normal mouse was 5.8 $\pm$ 0.4 x 10^6 . Single treatment with cardamom volatile oil (100 mg/kg) enhanced peritoneal cells to 8.9 $\pm$ 0.9 x 10^6, while two consecutive treatments enhanced the number to 9.8 $\pm$ 1.1 x 10^6 ($p \leq 0.001$) . Moreover , the tumor volume of control animals was 2.96 $\pm$ 0.12ml. whereas for the group which treated with cardamom volatile oil was 1.54 $\pm$ 0.05 ml. The results show an antitumor effect of cardamom volatile oil against EAC in Swiss albino mice and a significant enhancement of MST and peritoneal cell count was observed. So, it could be concluded that cardamom volatile oil had a highly cytotoxic and anti tumor activities on carcinoma.

Table 6. Screening test for antitumor activity of cardamom volatile oil using (EAC) .

Oil concentration (μ g/ml)	Inhibition of cell viability %
25	100
50	100
100	100

Table 7. Effect of cardamom volatile oil on the survival of tumor – bearing mice .

Treatment	Median survival tumor (MST)	Increase in life span %
Tumor control	*21 ± 1.20	-
5- Fluorouracil (5-Fu 20 mg/kg, i.p.)	*40 ± 2.10	90.47
Cardamom volatile oil (100 mg/kg, p.o.)	*33 ± 1.20	57.14

* Values are expressed as mean ± SD, n = 10 /group, *p ≤ 0.01

P.O. : per /oral , i.p. : injection

Anti-inflammatory activity of cardamom volatile oil

A comparative study of the anti-inflammatory activity of the cardamom volatile oil at dose 100 mg/kg body weight and the diclofenace at a dose of 3mg/kg body weight against acute carrageenan inducing rat hind paw odema was studied and the results are shown in Table 8. Cardamom volatile oil exerts its anti- inflammatory action in inhibition value (32.63 %). It was also observed that the effect of cardamom volatile oil was approximately the similar diclofenance inhibition effect (35.67 %) through the comparative performed above. These results are in agreement with Al-Zuhair *et al.* (1996) who found that the cardamom volatile oil has highly effect as anti-inflammatory and as antispasmodic actions through muscarinic receptor plocking.

Table 8. Effect of cardamom volatile oil and the diclofenace alone on carrageenan – inducing rat hind paw odema .

Drugs and doses	Change in paw odema	
	Odema volume	% inhibition of control value
Tween 80 (negative control)	0.757± 0.09	-
Diclofenace 3mg/kg (positive control)	0.487± 0.34	35.67
Cardamom volatile oil (100 mg/kg)	0.510± 0.18	32.63
L.S.D	0.2805	-

Values are means ± SD, n= 8/group , P ≥ 0.05

REFERENCES

1. Adams, R. P. 1995. Identification of essential oil components by gas chromatography/mass spectrometry. Allured publishing carol stream Ilioins, USA.

2. Agha, Azza M. 1986. Pharmacological studies on certain aspects of the biological activity of some anti-inflammatory drugs as influenced by certain psychotropics. MS. Sci., Pharmaceutical Sci., Fac. Pharm., Cairo Univ., Egypt.

3. Al-Zuhair, H., B. El-Sayeh, H. Ameen and H. Al-Shoora. 1996. Pharmacological studies of cardamom oil in animals . Pharmacological Res., Vol. (34) No. 1/2: 79 - 82 (4).

4. Anon. 2002. Himalaya Herbal Health Care, Herb Finder, Herbal monograph, Elettaria cardamomum. The Himalaya Drug Co.

5. Bowles, B. L. and V. K. Juneja. 1998. Inhibition of food- borne bacterial pathogens by naturally occurring food additives. J. Food Safety, 18: 101 - 112.

6. Deans S. G. and G. Ritchie. 1987. Antibacterial properties of plant essential oils. International J. of Food Microbiology, 5: 165 – 180 .

7. Elgayyar, M., F. S. Draughon, D. A. Golden and J. R. Mount. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms . J. of Food Protection, 64 (7): 1014 - 1024.

8. Graven, H., G. Deans, P. Svoboda, S. Mavi and G. Gundidza. 1992. Antimicrobial and antioxidative properties of the volatile (essential) oil of Artemisia afra Jacq. J. Flavour and Fragrance, 7: 121 - 123.

9. Kalemba, D. and A. Kunicka. 2003. Antibacterial and antifungal properties of essential oils. Current- Medicinal- Chemistry., 10 (10): 813 - 829.

10. Kubo, I., M. Himejima, and H. Muroi. 1991. Antimicrobial activity of flavor components of cardamom (*Elattaria cardamomum*) seed. J. Agric. And Food Chem., 39 (11): 1984 -1986.

11. Leal, P. F., M. E. Braga, D. N. Sato, J. E. Carvalho, Marques, M. O. and M. A. Meireles. 2003. Functional properties of spice extracts obtained via supercritical fluid extraction. J. of Agric. and Food Chem., 51 (9): 2520 - 2525.

12. Leena Daswani, Bohra A. and L. Daswani. 2003. Toxic effect of Elettaria cadamomum on the growth of *Salmonella typphi*. Advances in Plant Sci., 16 (1): 87 - 89.

13. Nirmala- Menon, A. 2000. Studies on the volatile of cardamom (Elleteria cardamomum). J. Food Sci. and Tech., India., 37 (4): 406 - 408.

14. Nirmola-Menon, A., S. Chacko and C. S. Narayanan. 1999. Free and glycosidically bound volatiles of cardamom (Eletteria cardamomum Maton var. minisculla Burkill). Flavour and Fragrance J., 14 (1): 65 - 68.

15. Ozkan, G., O. Sagdic and M. Ozcan. 2003. Inhibition of pathogenic bacteria by essential oils at different concentration. Food Sci. and Tech. International., 9 (2): 85 - 88.

16. Skehan, P. and R. Storeng 1990. New coloremetric cytotoxicity assay for anti-cancer drug screening. J. National Cancer Inst., 82: 1107 - 1112.

17. Steel, R. G. and T. H. Torrie. 1980. Principles and procedures of statistics. Abiometrical Approach. McGraw Hill Book Comp., Inc., New York, USA.

18. Winter, C. A., E. A. Risely and G. W. Nuss. 1962. Proc. Soc. Exp. Biol. Med., 111, 544 [C. F. Agha, Azza, M. (1986)].

تقييم دور زيت الحبهان كمضاد للميكروبات وللسرطان وللإلتهابات

أمل محمد البسطويسى[1] ، رجاء حسنى محمد[2]

[1] معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – الجيزة – مصر.
[2] المعهد القومي للأورام – جامعة القاهرة – القاهرة – مصر.

في هذه الدراسة تم فصل الزيت الطيار من بذور الحبهان بواسطة التقطير بالبخار، مع التعرف على مكونات هذا الزيت بواسطة جهاز GC/MS بالإضافة إلى تقدير الأحماض الدهنية في الزيت الثابت. كما تم اختبار كفاءة كل من الزيت العطري والزيت الثابت من حيث تأثيرهما كمضادات للميكروبات ، وذلك ضد أربعة سلالات من البكتريا الممرضة وهى *Staphylococcus aureus*، *Bacillus cereus*، *Escherichia coli* and *Salmonella typhimurium* وسلالة من الفطريات *Aspergillus flavus* and *Aspergillus ochraceus* المنتجة للسموم ، بالإضافة إلى دراسة النشاط البيولوجى للزيت العطري وخاصة التأثير السام والمضاد للسرطان وللالتهابات له . وقد أثبتت النتائج المتحصل عليها أن الزيت العطري يمثل حوالي ٥% من المحتوى الكلى لبذور الحبهان ، وقد تم التعرف على أربعة وعشرين مركب عطري من ضمن خمسة وخمسين مركب تم فصلهم من بذور الحبهان بواسطة جهاز GC/MS أما المركبات الأساسية التي تم فصلها من الزيت العطري فقد كانت 1,8- cineole, terpinyl acetate and endobornyl acetate بمساحة نسبتها (٣٧,٢٨ ، ١٨,٥٥ ، ١١,٩٦ % على التوالي) وقد تبين من النتائج المتحصل عليها أن الزيت الطيار أغنى في محتواه من المركبات الأكسجينية عن المركبات الهيدروكربونية ، وأن الزيت العطري له تأثير أقوى في تثبيط نشاط جميع الميكروبات محل الدراسة عن الزيت الثابت . كما وجد أن التأثير المثبط للزيت الطيار ضد الفطريات المنتجة للسموم يزداد بزيادة تركيز الزيت ، بينما وقف تماماً نشاط جميع الميكروبات الممرضة عند تركيز ٥٠ ميكروليتر من الزيت العطري . نخلص من هذا البحث بصفة عامة أن الزيت العطري لبذور الحبهان يمكن استخدامه بكفاءة عالية كمضاد للميكروبات وخاصة المرضية منها والمفرزة للسموم ، بالإضافة إلى أنه له تأثيرات قوية في وقف نشاط الخلايا السرطانية وكمضاد للسرطان وللالتهابات ، وبذلك يتضح أن لهذه الدراسة أهمية اقتصادية كبرى في فصل تلك الزيوت التي لها تطبيقات عملية تبين جدوى هذه الدراسة وارتباطها بالتطبيق العملي والفعلي للبحث .