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COMPARISON THE COMPOSITION OF ESSENTIAL OIL EXTRACTED OF ELETTEARIA CARDAMOMUM L BY HYDRO-DISTILLATION AND MICROWAVE-ASSISTED HYDRO-DISTILLATION AND EVALUATION ITS ANTIMICROBIAL ACTIVITY

Annotation: *The essential oil has been extracted from Elettearia cardamomum L. seeds from Zingiberaceae by hydro-distillation method and using the microwave-assisted hydro-distillation. The composition, the qualitative and quantitative analysis of the essential oil was achieved and characterized by means of GC-MS . The biological effects of essential oil were studied on some pathogenic bacteria including: Staphylococcus aureus, Escherichia coli.*

Keywords: *Coriandrum sativum L, Essential oils, hydro-distillation, microwave, Staphylococcus aureus, Escherichia coli.*

Аннотация: *Эфирное масло было извлечено из семян кардамона L. из зингибера методом гидро-дистилляции и с использованием микроволновой гидро-дистилляции. Состав, качественный и количественный анализ эфирного масла был достигнут и охарактеризован с помощью ГХ-МС. Биологические эффекты эфирного масла были изучены на некоторых патогенных бактериях, в том числе: золотистый стафилококк, кишечная палочка.*

Ключевые слова: *Кориандрум сативум Л, Эфирные масла, гидро-перегонки, микроволновая печь, золотистый стафилококк, кишечная палочка.*

1. Introduction:

The family Zingiberaceae is well-known for its medicinal values The family Zingiberaceae is well-known for its medicinal values and it is distributed widely

throughout the tropics, particularly in Southeast Asia. Gingers are important natural resources, which provide many useful products for food, spices, medicines, dyes, perfume etc.[1,C.392] The ginger family consists of 53 genera and over 1200 species.[2,C.1682] The members of Zingiberaceae are annual or perennial rhizomatous herbs. The rhizome is sympodially branched and composed of distinct segments.[3,C.377].

The dried capsules, the essential oil, oleoresin and tinctures are extensively used in the formulation of compounded mixtures for liquors beverages baked goods, canned foods, meats, sauces and condiments. Cardamoms are stimulant, carminative and flavoring agent. Dried cardamom fruits are used as a masticatory and in medicine. They are used for flavoring curries, cakes, bread and other culinary purposes. Seeds used to treat eye inflammation, kidney and urinary disorder, infection of teeth, throat trouble, congestion of lung and pulmonary tuberculosis, asthma, heart disease, digestive disorder, cold, snake bite, scorpion bite, masticatory.[4,C.253] The volatile oil constituents of this species were extracted and identified. The major constituents are α-terpinyl acetate, 42.3%; 1,8-cineole, 21.4%; linalyl acetate, 8.2%; limonene, 5.6%; and linalool, 5.4%.[5,C.111].

2. Taxonomic description of Elettearia cardamomum L

The cardamom plant is a 2-4 m tall herbaceous perennial with branched subterranean rhizomes from which several leafy shoots arise, forming a clump. Leafy shoots have a limited life span; the first year is mainly for vegetative growth, the second year for reproductive growth (flowers and fruits), and the third year a senescence and death stage. New buds are formed from the base of the old shoots in the first and second year and thus, in a clump of old shoot. Young shoots and buds can be seen in varying numbers. Flowers are borne on erect, prostrate or semi-erect (flexuous) inflorescences depending on the variety.

The leaves are lanceolate in shape, and lamina tapers into a sharp tip, 25-90 cm long and 5-15 cm wide. Leaves are dark green and shiny on the upper surface and pale green on the lower surface. The lower surface of the leaf could be smooth (glabrous) or pubescent (hairy) depending on the variety. The inflorescence arises from the base

of the leafy shoots and is 45-120cm long. Flowers are borne in racemes, they are hermaphrodite, zygomorphic and about 4cm long and 1.5cm wide. The calyx is tubular green and shortly three-toothed and persistent. The corolla tube is as long as the calyx tube, with narrow spreading pale green lobes.[6][7,C.366].

3. Experimental Procedure:

3.1. Plant Material

Seeds of E. cardamomum L, were collected and dried in April 2018, from South-Est of Hama, Syria. The plant was authenticated by the Atomic Agent in Syria. A voucher specimen of plant was deposited in the laboratory of chemistry of natural products, Department of chemistry, Faculty of sciences, AL Baath University, Homs, Syria.

3.2. Essential oils analysis

The analysis of the essential oil was performed with Shimadzu Bruker Ultra Shield 400MHz gas chromatograph with a capillary column DB5 (30m × 0.25 μm) With an internal character (0.25μm). Temperature program was as follows: 3 min at 40°C, increased to 100°C at a rate of 5°C min, then, increased to 120°C at a rate of 5°C min and held at that temperature for 1 min, increased to 180°C at a rate of 6°C min, increased to 200°C at a rate of 20°C min, increased to 220°C at a rate of 30°C min, then increased to 280°C at a rate of 40°C min and held at that temperature for 1 min. Injection temperature was 230°C. Injection volume was 1.0 μL. Helium was used as a carrier gas (1 mL/min). the identification of the constituents was performed by comparing the spectra obtained with database of Wiley Spectral Library Collection and NSIT library database. Quantitative data were obtained from the electronic integration of the FID peak areas

3.3. Extraction the essential oil:

3.3.1. hydro-distillation (HD):

The E. cardamomum seeds (150 g) and 1,000 ml distilled water placed in a round bottom flask and connected to a Clevenger-type apparatus. Hydro-distillation was completed for 3h after boiling. Oil yield of the sample was calculated on a moisture

free basis. The oil was dried over anhydrous sodium sulphate and kept at 4 °C in the sealed brown vial until the analysis by GC-MS.

3.3.2. Microwave-assisted hydro-distillation (MAHD):

The oil was obtained from (150g) of the seeds of E. cardamomum by hydro-distillation for 30 min using a Clevenger type apparatus placed in a modified microwave oven (800 w). The oil was dried over anhydrous sodium sulphate and kept at 4°C in the sealed brown vial until the analysis by GC-MS.

4. Evaluation the biological activity:

The biological efficacy of the essential oil extracted from the seeds of E. cardamomum L, was studied in a against two bacteriostatic strains:Staphylococcus aureus, Escherichia coli. The lobster was highly effective on bacterium Effectiveness has been compared with gentamicin anti-inflammatory drug. Transfer (0.1) cm^3 from the diluted bacterial suspension to the center of nutritious Nutrient agar and spread on the surface of the center in a homogeneous manner and incubated for 30 minutes at a temperature of 37 °C for the purpose of sowing. In the meantime, the tablets were filled with oil extract and active ingredients. The discs were prepared from the filter paper with a perforation of the leaves and a diameter of 5 mm. These tablets were treated with different concentrations of the oil extract (100%, 50%, 25%). The steroid tablets containing the nutrient medium are then sterilized with sterile concentrates. At this time, the Gentamicin filter paper is coated with a concentration of 500 $\mu g/cm^3$. It is determined by the different concentrations of the oil extract and DMSO, all in one dish on the feeding medium and incubated at a temperature of (37) °C for a period of (16) hours.

5. Results and discussion:

The chemical composition of the essential oil obtained from E. cardamomum L in two methods (Microwave-assisted hydro-distillation and hydro-distillation) are represented together with the retention indices in Table 1, the oils yields of the plant was determined as1.5 % and 1 % w/w in MAHD and HD respectively. The GC–MS analyses of both samples revealed the presence of a total of 38 components.The compounds were identified in the hydro-distilled oil (Clevenger-type apparatus) which

accounted for 97.5 % of the total oil composition. This oil was dominated by monoterpenoids such as, 1,8-Cineole, α-Terpinyl Acetate and Linalyl acetate. In the same way, The compounds were identified from the microwave extracted oil which accounted for 97.7 % of the total oil composition. The MAHD EO was dominated by monoterpenoids such as 1,8-Cineole and α-Terpinyl Acetate.

Table 1. Chemical composition, retention time (RT)) and relative percent of cardamomum essential oils extracted by HD, MAHD

Peak number	Compound	RT		% Composition	
		HD	MAHD	HD	MAHD
1	n-nonane	4.20	4.20	0.2	0.1
2	α-Pinene	4.84	5.26	3.5	0.1
3	α-Thujene	5.53	5.53	0.3	0.1
4	Camphene	6.04	6.04	0.4	0.1
5	β-Pinene	6.74	6.74	0.5	0.1
6	Sabinene	7.14	7.14	2.5	0.1
7	Myrecene	8.06	8.06	1.2	0.5
8	n-octanal	8.35	8.36	0.1	0.3
9	α-phellandrene	8.73	8.54	1.8	1.2
10	α-Terpinene	9.09	9.09	0.4	0.1
11	Limonene	9.30	9.30	1.5	0.1
12	1,8-Cineole	10.21	10.28	37.2	51.2
13	cis-β-Ocimene	10.42	10.42	0.1	0.1
14	γ-Terpinene	10.65	10.65	1.2	0.5
15	trans-β-Ocimene	11.11	11.12	0.2	0.1
16	p-Cymene	11.39	11.39	0.2	0.2
17	Terpinolene	11.77	11.77	0.2	0.2
18	Octanal	11.97	11.97	0.1	0.3
19	Linalool	12.38	12.30	6.5	2.5
20	Linalyl acetate	12.64	12.73	1.8	2.8

21	trans p-2-Menthen-1-ol	12.78	12.76	0.3	0.3
22	Terpinen-4-ol	13.21	13.35	3	2.3
23	cis-p-Menth-2-en-1-ol	13.80	13.8	0.2	0.2
24	Neral	14.23	14.23	0.1	0.2
25	α-Terpinyl Acetate	14.58	14.50	25.8	25.9
26	Neryl acetate	14.86	14.86	0.4	0.4
27	β-Selinene	15.05	15.05	0.1	0.1
28	Geranial	15.51	15.52	0.5	1.5
29	Carvone	15.94	15.94	0.5	0.5
30	Geranylacetate	16.35	16.35	1.4	1.8
31	Nerol	16.74	16.75	0.3	0.3
32	trans-Carveol	17.96	18.0	0.6	0.1
33	Geraniol	18.28	18.33	2	1.5
34	p-Cymen-8-ol	18.71	18.71	0.2	0.2
35	cis-Carveol	19.50	19.50	0.2	0.2
36	trans-Nerolidol	20.18	20.18	1.3	1
37	Geranic acid	20.63	20.62	0.2	0.2
38	Hexadecanoic acid	21.11	21.05	0.5	0.3
	Yield (w/w)%			**1%**	**1.5%**
	Monoterpenes hydrocarbons			**14**	**3.6**
	Monoterpenes oxygenated			**83.5**	**94.1**
	Total			**97.5**	**97.7**

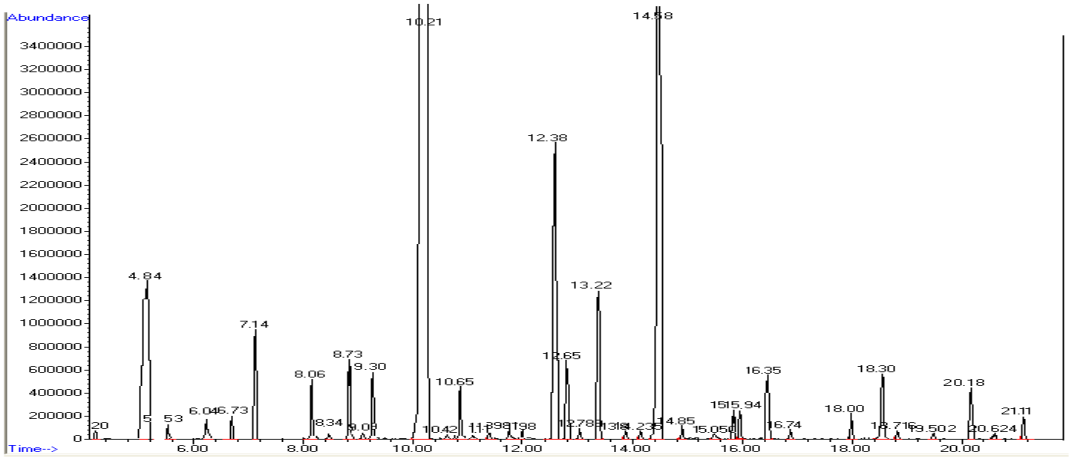


Figure 1: Chromatogram of oil obtained from cardamomum by HD method

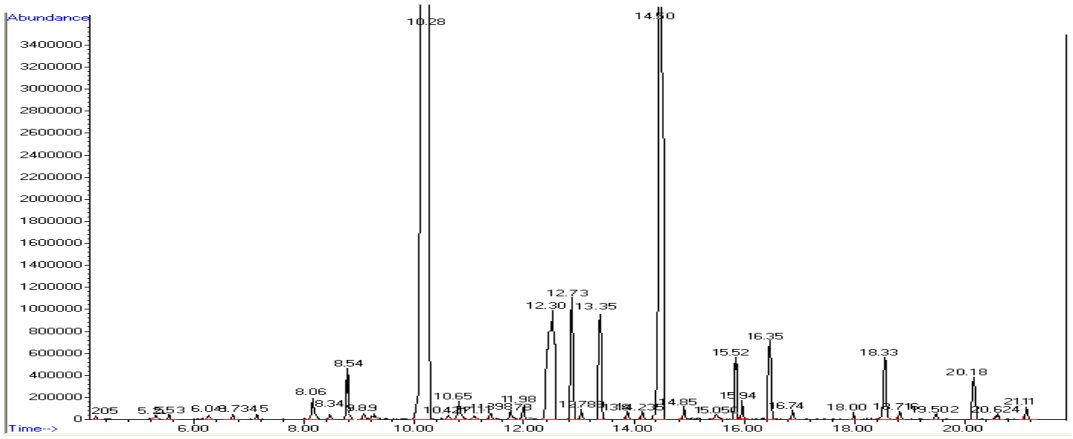
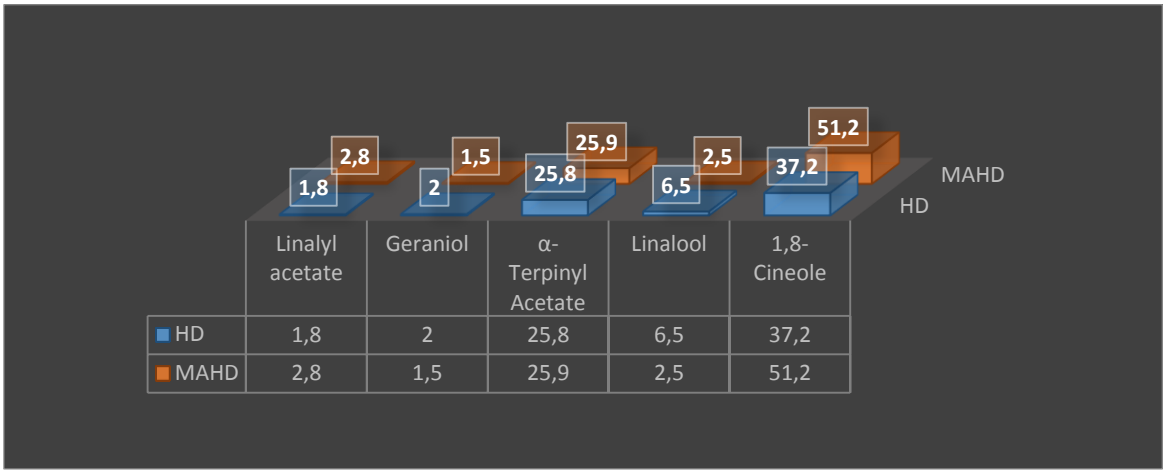


Figure 2:Chromatogram of oil obtained from cardamomum by MAHD method

According to results in current study, the major compound in both EO was 1,8-Cineole which its amount in HD and MAHD was 37.2% and 51.2%, respectively. Significant difference was observed in the 1,8-Cineole content of MAHD EO and HD EO. The total amount of monoterpene hydrocarbons in HD EO differ significantly with the amount in MAHD EO (14 % compare to 3.6 %). Moreover there were a difference between oxygenated monoterpenes content in MAHD EO and HD EO (94.1 % compare to 83.5 % respectively). The content of α-Terpinyl Acetate in MAHD EO (25.9 %) increased compared to HD EO (25.8%), significantly. A critical observation of the oil compositions revealed that higher amounts of oxygenated monoterpenes are present in the essentiall oil isolated by MAHD (94.1 %) in comparison with the oil extracted by HD (83.5 %). MAHD method was important in terms of saving energy and extraction time (30 min compared to 180 min in HD method) and the amount of

oxygenated monoterpenes which play the great role in the essential oil properties increased, although the oil yield and total composition decrease by using this method.

The previous studies showed that 1,8-Cineole and α-Terpinyl Acetate were the main compounds on the EO cardamomum [8,C.287] [9,C.366] , which agrees with our research that 1,8-Cineole and α-Terpinyl Acetate were major components in the MAHD and HD EO . In the same way the EO chemical composition of cardamomum that the major compounds were 1,8-Cineole (25.6 %) and α-Terpinyl Acetate (40.7%) [10,C.42][11,C.1079].



	Linalyl acetate	Geraniol	α-Terpinyl Acetate	Linalool	1,8-Cineole
HD	1,8	2	25,8	6,5	37,2
MAHD	2,8	1,5	25,9	2,5	51,2

Figure 3: Comparison of the major components of the essential oil of cardamomum by HD and MAHD

The essential oil exhibit significant activity against the pathogenic bacteria including: Staphylococcus aureus, Escherichia coli.in comparing to the gentamicin. The damping area is measured by a.

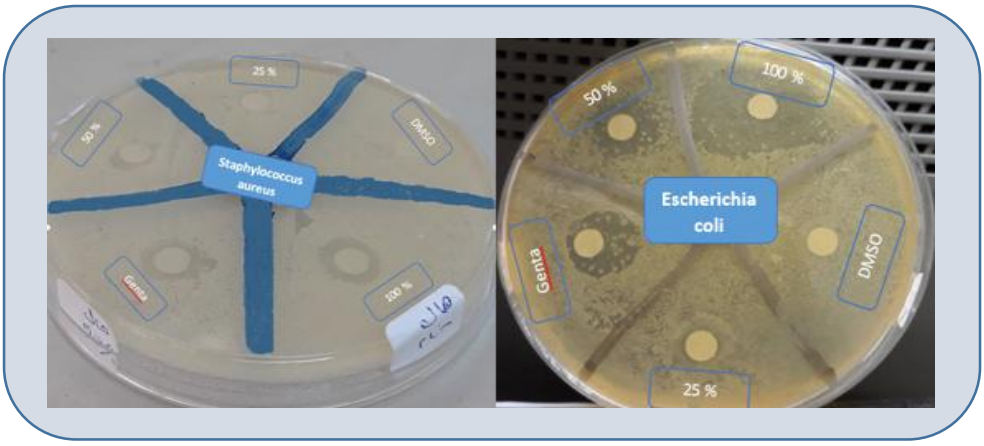


Figure 4: The zones of inhibition by essential oil of cardamomum against to the growing of Staphylococcus aureus and Escherichia coli. in different concentration

Table 2. The activity of essential oil of E. cardamomum L, it is calculated by measuring the diameter of the inhibition zone (mm).

Org.	Essential oil Con.				
	100%	**50%**	**25%**	**Gentamicin**	**DMSO**
E. coli	28.00 ±1.00	17.33 ±1.20	1.5 ±0.99	15.5	-
Staph. aureus	5.00 ±0.50	3.5 ±0.5	1.00 ±0.2	7.5	-

6. Conclusions:

In MAHD method, time extraction is significantly shorter than HD method. The MAHD with lower energy consumption than HD is offered instead traditional methods. Therefore, considering the operation cost MAHD could be carried out using half of the expenses required by HD. The MAHD yielded less percentage of γ-terpinene, α-pinene and Sabinene compared to HD, therefore, MAHD is more efficient method for obtaining essential oil of Coriander.

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