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Impact of Extraction Methods on The Chemical Composition and Biological Activity of Small Cardamom Essential Oils

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Abstract

Cardamom is considered one of the most famous spices used in Saudi Arabia; especially as a flavour in the preparation of a famous Saudi beverage; Arabic coffee. In this study, the essential oils of small cardamom collected from the local market were extracted by three different extraction methods: classical hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD), and n-hexane extraction (HX) followed by identifying the chemical and biological properties of the oils obtained. The results showed no significant difference between the oil yield of the two samples extracted by HD and MAHD while the yield by HX had a higher yield, which was attributable to the presence of fixed oil with the essential oil. In spite of the two methods, HD and MAHD gave nearly the same results from qualitative and quantitative analysis, but the extraction by microwave method (MAHD) saved time, energy, and was more rapid than HD. The oils showed variable in vitro anticancer, antibacterial, and antifungal activity. The authors recommend using the microwave method in the extraction of essential oil because it is energy and time-saving, in addition to being environmentally friendly. This result provides the basis for further and more in vitro and in vivo studies to evaluate the potential use of essential oils from cardamom as antimicrobial and anticancer agents in the hope of using these oils in the prevention and therapies of these diseases.

Keywords: Cardamom; essential oil; hydrodistillation; microwave-assisted hydrodistillation; n-hexane; anticancer; antimicrobial.

1. Introduction

Essential oils as an important phytochemical class have been recognized for many years as a great source of pharmaceutical agents and food additives, which has encouraged many scientists to study the essential oils from the chemical and pharmacological investigations to the therapeutic aspects [1-4].

There are many factors influencing the chemical composition and consequently the biological activity of the essential oils; climate, seasonal and geographic conditions, harvest period, and extraction techniques [5-7].

Cardamom (*Elettaria cardamomum*, Family Zingiberaceae) is considered one of the commercially significant spices worldwide. It is called the "Queen of spices". Although it is native to India and Sri Lanka, it is also grown and cultivated in many tropical and subtropical regions (e.g. Guatemala, Thailand, El Salvador, Cambodia, Tanzania, Indonesia, Nepal, Malaysia, and Central America). Guatemala is the largest producer of cardamom [8].

Cardamom has a number of biological actions; antispasmodic, stimulant, anthelmintic, anticipative, antimicrobial, anticancer, antiviral, antifilarial, aphrodisiac, anti-inflammatory, antipyretic, antinociceptive, antifungal, analgesic diuretic, alleviate flatulence, heartburn, nausea, indigestion, and carminative, as indicated by various reports. Searching and more studies on natural products especially essential oils increased day by day for exploring promising safer and cheaper and anticancer drugs to avoid the bad and side effects of using synthetic drugs [9-11].

In this study, the essential oils of small cardamom collected from the local market in Taif governorate, were extracted by three different extraction methods; classical hydrodistillation method; microwave-assisted hydrodistillation, and solvent extraction by n-hexane. The essential oils were analyzed using the

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gas chromatography-mass spectroscopy technique. In addition, the anticancer and antimicrobial activities of essential oils obtained were determined.

2. Experimental

2.1. Chemicals

All solvents, standards, and reagents were analytical and HPLC grade from Sigma-Aldrich Chemicals, USA.

2.2. Plant materials

The seeds of small cardamom were purchased from the herbal market in Taif governorate, KSA (January 2018). The seeds were crushed by an electric mill and become ready for essential oil extraction by the three mentioned methods.

2.3. Essential oil extraction

2.3.1. Hydrodistillation (HD)

150 grams of finely powdered seeds of cardamom were mixed with 1.5 L distilled water in a round flask and connected with the Clevenger apparatus, which is used as a condenser and oil collector. Heating mantle was operated at temperature 100 ^{o}C and the system was operated for 150 minutes until no more essential oil was obtained. The oil was obtained, dried over anhydrous sodium sulphate and filtered. The oil was stored a brown glass vial in freezer at -20 °C until biological and chemical investigation.

2.3.2. Microwave assisted hydrodistillation (MAHD)

Microwave-assisted hydrodistillation extraction of essential oil was carried out in a fully instrumented and controlled microwave system (Milestone NEOS-GR). 150 grams of finely powdered seeds were mixed with 0.75 L of distilled water in a two-liter cylinder Pyrex glass. The mixture was subjected to microwave treatment at 500 W, 100 ^{o}C for 40 minutes until no more essential oil was obtained. The oil was obtained, dried over anhydrous sodium sulphate and filtered. The oil was stored a brown glass vial in freezer at -20 °C until biological and chemical investigation.

2.3.3. n-Hexane extraction (HX)

For the preparation of n-hexane extract, 300 grams of ground seeds were soaked in 1.5 L n-hexane for one week at room temperature with shaking from time to time followed by filtration. The n-hexane was removed under vacuum yielding yellowish green oil. This extraction process was repeated three times. At the end of the experiment, the essential oil was collected, dried over anhydrous sodium sulphate and filtered. The oil was stored a brown glass vial in freezer at -20 °C until biological and chemical investigation.

2.4 Essential oil chemical analysis

The chemical composition of essential oils under study was obtained by the gas chromatography-mass spectrometry (GC-MS) technique. The analysis of the standards and samples were performed using a gas chromatography (GC, Model CP 3800, Varian, California, USA) coupled with a mass spectrometer (MS, Model Saturn 2200, Varian) and auto sampler (Model Combi Pal, Varian) system. The separation was done using a VF-5 fused silica capillary column (30 m x 0.25 i.d. mm, film thicknesses 0.25 µm, Varian). The optimum conditions for oven temperature were obtained after several trials to get good separation peaks for standards. The oven temperature was programmed for 2 min at 40 °C, raised gradually to 250 °C at 4 °C/min, and held for 5.5 min at 250 °C, solvent delay time 3 min, the total run time 60 min. For the MS detector, an electron impact (EI) ionization system with ionization energy of 70 eV was used. Trap temperature was set at 150 °C and axial modulation voltage at 4.0 volts. The ions were recorded with a mass range of 45-400 m/z. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperature were set at 200 and 170 °C respectively. Thirty-four standard compounds were prepared separately by dissolving known weight of each in n-hexane (1 mg/mL) and filtered using a membrane disc filter (0.45 µm). A mixture of standards was prepared containing 34 compounds (20 µg/mL for each standard) by collecting 20 µL of each previously prepared individual standard in a vial and completed it to 1 mL by n-hexane. For samples, solution (10 mg/mL) of oil was prepared in n-hexane and filtered using a membrane disc filter (0.45 µm). The injection volume for all samples and standards was 1 µL with a split ratio of 1:20 and carried out with the auto-sampler. N-alkane series mixture (C8-C20) was injected under the same conditions for samples and standards. Chromatograms of the standards and samples were analyzed using Varian MS Workstation software (Service Pack 1, Version 6.5). For samples, known peaks were identified by comparing their retention time (t_R), mass spectrum and retention indices, with standards. Unknown peaks were

identified by matching their mass patterns with Wiley and NIST electronic library and its retention indices with the review of literatures.

2.4 Biological studies

The essential oils obtained from cardamom by different extraction techniques were tested in vitro for their anticancer and antimicrobial activities.

2.4.1 Anticancer activity

The essential oils under study were investigated in vitro cytotoxicity assay against three human cell lines; colon cancer (HCT-116), lung cancer (A549), and breast cancer (MCF-7); in Applied Research Sector, Egyptian Company for Vaccine and Serum (VACSERA, Cairo, Egypt). The method used MTT assay according to [12-13].

2.4.2 Antimicrobial activity

Antimicrobial activity of the oils was determined in a microbiology unit, Micro-Analytical center, Faculty of Science, Cairo University, Egypt. The bacterial strains used were three Gram-positive; *Bacillus subtilis* (ATCC: 6051), *Streptococcus faecalis* (ATCC: 12600) and *Staphylococcus aureus* (ATCC: 19433); and three Gram- negative; *Escherichia coli* (ATCC: 11775), *Neisseria gonorrhoeae* (ATCC: 19424) and *Pseudomonas aeruginosa* (ATCC: 1014). And also, Fungal strains including *Candida albicans* and *Aspergillus flavus* tested in vitro antimicrobial activity of the tested samples was determined using the disc diffusion method [14-18].

3. Result and Discussion

3.1. Extraction time and yield

The essential oils yield obtained from the seeds of cardamom by three different methods; HD, MAHD, and HX were 7.88, 7.22, and 11.98 %, respectively. The obtained data showed that there was no great difference between the oil yield of classical HD and MAHD. Our results were in agreement with other studies using these techniques for different plants [19-22], while other studies showed that there was a difference in yield between HD and MAHD techniques in which the authors revealed the high yield by MAHD to the quickly rupture effect of microwaves on the glandular walls, resulting in high extraction efficiency in a shorter time [23]. For oil extracted by n-hexane, it has appeared that the high yield compared to the other two methods could be attributed to the fact that n-hexane not only extracted essential oil of the seeds but also extracted other materials including fixed oil that in turn increased the total oil yield of extract. The obvious advantages that appeared in this step of the study were time and energy savings. The extraction time for the complete HD method was about 150 minutes, while for MAHD it was 40 minutes. On the other hand, the extraction by HX took one week. The greater extraction time for the HD method requires more energy than the lower time for MAHD. The HD method takes approximately 35 minutes to reach the boiling point of water and appear the first droplet of essential oil in the collector, whereas the microwave method appears the first droplet of essential oil in the collector after 10 minutes. the microwave techniques used in this study were rapid and energy saving, in addition to reducing the amount of water used. The extraction by HX takes more time and cost more, but this study, used this technique as a comparison study hoping to extract different materials not extracted by the other two methods.

3.2 Essential oils chemical compositions and impact of extraction methods

The chemical compositions of the essential oils extracted from cardamom by HD, MAHD, and HX were identified using GC-MS analysis. To the best of our knowledge, this is the first study compares between the three different methods. Figure (1) showed the total ion chromatograms of essential oils obtained from HD, MAHD, and HX respectively. All chromatograms showed well separated peaks, signal-noise was good and drift was absent from the horizontal baseline. Table (1) showed the chemical composition, retention time (t_R), retention indices (RI), and relative percentage of identified compounds in each oil.

Our study revealed that HD oil contained mainly monoterpenes and sesquiterpenes. The monoterpene group constitutes the majority of the oil (98.4 %) while the sesquiterpene group constitutes the minor percentage (1.42 %). The detailed entire classification of the monoterpene group showed that the two classes; monocyclic monoterpene cyclic ether and monocyclic monoterpene ester constitute the higher percent (41.41 and 37.3 % respectively) whereas the other classes showed < 10 %. The main components of the monoterpene group were 1,8 cineol (41.41 %) followed by α-terpinyl acetate (37.12 %). The other monoterpene compounds that showed percent > 1 were sabinene (5.38 %), D-limonene (3.54 %), myrcene (2.25), α-pinene (1.86 %), terpinen-4-ol (1.24 %) and α-terpineol (1.16 %). The aroma differences in different sources of cardamom are attributed to the proportion of the esters and 1,8 cineol. The high level of α-terpinyl acetate compared

to 1,8-cineol gives a superior odor quality of cardamom essential oil. In addition to α-terpinyl acetate, other terpenoids such as α-terpineol, linalool, linalyl acetate, and geraniol impart a sweet flavour that counterbalances the camphorated-sharp touch of 1,8-cineol. The chemical composition of small cardamom seeds essential oil extracted by HD in this study was found to be in good accordance with previous studies in which the 1,8-cineol and α-terpinyl acetate were the major components [24-25].

Regarding MAHD, monoterpenes constitute the main components of the oil with relative percent (98.22 %) followed by minor sesquiterpenes (1.52 %) and aliphatic aldehydes (0.26 %). The oxygenated monoterpenes subgroup had the major relative percent (86.41 %) whereas non-oxygenated or monoterpene hydrocarbon had the lowest relative percent (11.82 %).The detailed entire classification of monoterpene group showed that the two classes; monocyclic monoterpene cyclic ether (43.45 %) and monocyclic monoterpene ester (38.6%) whereas the other classes showed < 10 %. The main components of the monoterpene group were 1,8 cineol (43.45 %) followed by α-terpinyl acetate (38.41%). The other monoterpene compounds that showed percent > 1 were sabinene (4.20 %), D-limonene (2.49 %), myrcene (1.90 %), α-pinene (1.28 %) and terpinen-4-ol (1.27 %).

The HX oil was found to contain mainly monoterpenes with relative percent (97.32 %) followed by sesquiterpenes (2.37 %) and aliphatic aldehydes (0.26 %). The oxygenated monoterpenes subgroup had the major relative percent (86.55 %) whereas non-oxygenated or monoterpene hydrocarbon had relative percent (10.77 %). The detailed entire classification of monoterpene group showed that the two classes; monocyclic monoterpene cyclic ether (35.72 %) and monocyclic monoterpene ester (45.94 %) whereas the other classes showed < 10 % as tabulated in table 1. The main components of monoterpene group were 1,8 Cineol (35.72 %) followed by α-terpinyl acetate (45.59 %). The other monoterpene compounds that showed percent > 1 were sabinene (4.17 %), D-limonene (2.41 %), myrcene (1.81 %), α-pinene (1.37 %) sabinene hydrate acetate (1.63 %), and linalool acetate (1.09 %).

By the naked eye, it can be seen the high similarity between the three chromatograms from the point of qualitative analysis (figure 1). Through comprehensive data analysis of the GC-MS analysis, it has appeared that the qualitative chemical composition of the three oils was the same.

Table (1) showed the comparison of the chemical composition of the three essential oils extracted by HD, MAHD, and HX, respectively. The first three columns showed the relative percentage between different components in the same oil. It was obvious that the qualitative chemical composition of the oils extracted by three methods was nearly the same. The three oils were found containing major monoterpenes and minor sesquiterpenes with very little aliphatic aldehyde. The monoterpene group constitutes the majority of the oils (98.4, 98.22, and 97.32 % for HD, MAHD and HX respectively) while sesquiterpene group found with minor percent (1.42, 1.52 and 2.37 % for HD, MAHD and HX respectively). Also, it was obvious that the MAHD method increases the extraction of sesquiterpene compounds than the classical HD and this was in good accordance with other studies [26-28]. As previously reported, the basic and major components of small cardamom essential oil were α-terpinyl acetate and 1,8-cineol [29]. In this study these two compounds also constitute the major compounds of the three essential oils with relative percentage for each oil; 41.41, 43.45, 35.72 for 1,8-cineol and 37.12, 38.41, 45.59 for α-terpinyl acetate respectively. It was noticed that the high similar percentage and ratio of the two compounds in HD and MAHD were reversed in oil extracted by HX. In addition to the appearance of some ester compounds only with HX extraction that could be attributed to the effect of the solvent used or the hydrolysis of some esters during HD and MAHD.

The data analysis from the relative percent in the first three columns of table (1) did not give the actual effect of extraction methods on the extraction of different components between the three oils. The second three columns of table (1) resolve this problem by calculating the actual relative percent of the same compound within the three oils. It has appeared that the low percentage of compounds found in HX could be regarded to that the oil extracted by n-hexane not extracted only essential oil but also other non-polar compounds. Then the amount taken for analysis contains other compounds than aroma compounds and this column and thermal program may not suitable for separation and identification for these compounds. By comparing the percentage of the major components in the two other methods; 1,8 cineol and α-terpinyl acetate had a higher amount in MAHD than HD (43.37 and 37.41 for 1,8 cineol and 39.77 and 34.78 for α-terpinyl acetate respectively).

In general, the results of this study showed no great difference between the yield and the qualitative and quantitative composition of cardamom essential oil extracted by HD and MAHD. But The oil extracted by HX had a higher yield that was attributed to the presence of fixed oil with essential oil. The qualitative composition of HX essential oil is similar to that of HD and MAHD with some quantitative differences in some compounds. The microwave method was more rapid, saving time and energy compared to the other two methods. This study is considered to be the first study to compare the chemical compositions of cardamom essential oil under different extraction techniques.

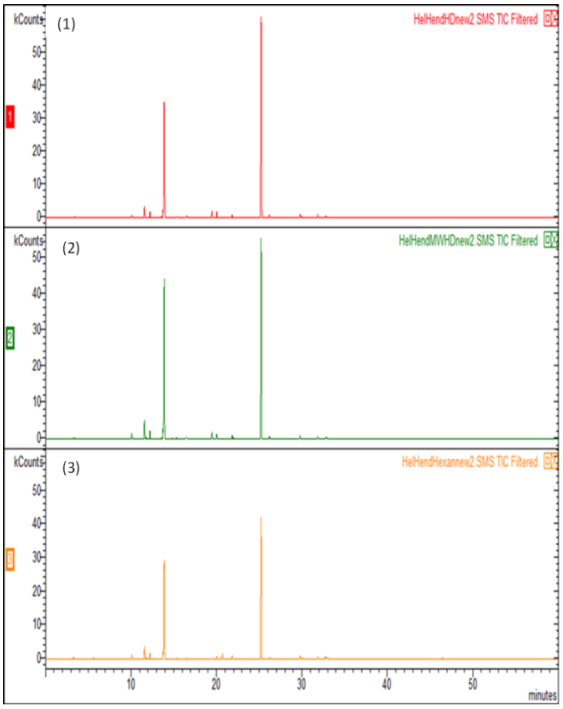

Fig. 1. Collective GC-MS total ion chromatograms of cardamom essential oils extracted by HD (1), MAHD (2) and HX (3).

Table (1): Chemical composition, relative percentage (%) between different compounds within the same method, and relative percentage (%) of the same compound within different methods of cardamom essential oils extracted by HD, MAHD and HX.

No.	Name	t_R	RI	Chemical class	% Between different compounds within the same method			% Between the same compound within different methods		
					HD	MAHD	HX	HD	MAHD	HX
1	α-Thujene	9.82	926	Bicyclic monoterpene	0.20	0.17	0.17	40.57	39.00	20.43
2	α-Pinene	10.09	933	Bicyclic monoterpene	1.86	1.28	1.37	45.49	34.52	19.99
3	Sabinene	11.59	973	Bicyclic monoterpene	5.38	4.20	4.17	43.05	37.07	19.88
4	β-Pinene	11.77	978	Bicyclic monoterpene	0.41	0.35	0.33	41.32	38.60	20.08
5	Myrcene	12.21	992	Acyclic monoterpene	2.25	1.90	1.81	41.43	38.64	19.93
6	α-Terpinene	13.27	1017	Monocyclic monoterpene	0.40	0.28	-	56.62	43.38	-
7	p-Cymene	13.58	1026	Alkylbenzene monoterpene	0.13	0.08	0.06	50.68	35.24	14.08
8	D-Limonene	13.76	1030	Monocyclic monoterpene	3.54	2.49	2.41	45.86	35.57	18.57
9	1,8 Cineol	13.90	1034	Monoterpene cyclic ether	41.41	43.45	35.72	37.41	43.37	19.22
10	β-Trans-ocimene	14.41	1047	Acyclic monoterpene	0.05	0.04	-	53.84	46.16	-
11	γ-Terpinene	14.86	1059	Monocyclic monoterpene	0.70	0.53	0.17	50.89	41.98	7.13
12	Sabinene hydrate cis	15.37	1073	Bicyclic monoterpene	0.18	0.40	0.27	23.04	56.21	20.75
13	α-Terpinolene	15.89	1086	Monocyclic monoterpene	0.33	0.18	0.06	58.33	35.58	6.09
14	β-Linalool	16.46	1101	Acyclic monoterpene alcohol	0.53	0.41	0.17	49.05	41.91	9.04
15	Nonanal	16.54	1104	Acyclic aliphatic aldehyde	0.18	0.26	0.26	28.65	46.69	24.66
16	Trans thujone	16.92	1114	Bicyclic monoterpene ketone	0.12	0.11	0.11	37.88	40.79	21.33
17	Terpinen-4-ol	19.48	1184	Monocyclic monoterpene alcohol	1.24	1.27	0.31	43.84	49.67	6.49
18	α-Terpineol	20.03	1198	Monocyclic monoterpene alcohol	1.16	0.86	0.66	46.49	37.86	15.65
19	Sabinene hydrate acetate <cis->	20.71	1218	Bicyclic monoterpene ester	-	-	1.63	-	-	100
20	Linalool acetate	21.85	1250	Acyclic monoterpene ester	0.35	0.65	1.09	20.24	41.79	37.97

21	Geraniol	21.98	1254	Acyclic monoterpene alcohol	0.24	0.36	0.05	35.38	60.61	4.01
22	Carvacrol	23.49	1297	Monoterpene phenol	-	-	0.09	-	-	100
23	Terpinen-4-ol acetate	24.09	1315	Monocyclic monoterpene ester	0.12	0.12	0.18	32.03	37.86	30.11
24	Methyl Geranate	24.34	1323	Acyclic monoterpene ester	0.09	0.12	0.11	32.08	45.11	22.81
25	Terpinyl acetate	25.23	1350	Monocyclic monoterpene ester	37.12	38.41	45.59	34.78	39.77	25.45
26	Geranyl acetate	26.21	1379	Acyclic monoterpene ester	0.53	0.49	0.61	36.96	37.64	25.40
27	α-Terpinyl propionate	27.99	1436	Monocyclic monoterpene ester	0.06	0.07	0.17	24.40	31.64	43.96
28	Germacrene D	29.51	1485	Monocyclic sesquiterpene	0.05	0.06	0.06	30.74	43.56	25.70
29	Valencene	29.78	1493	Bicyclic sesquiterpene	0.51	0.50	0.94	31.58	34.05	34.37
30	α-Selinene	29.99	1500	Bicyclic sesquiterpene	0.24	0.20	0.42	34.12	31.17	34.71
31	δ-Cadinene	30.49	1517	Bicyclic sesquiterpene	0.10	0.09	0.17	27.78	25.00	42.22
32	Isocaryophyllene	31.87	1564	Bicyclic sesquiterpene	0.52	0.67	0.78	30.04	43.22	26.74
33	Spathulenol	32.19	1574	Tricyclic sesquiterpene alcohol	-	-	0.05	-	-	100
Monoterpene hydrocarbons					15.3	11.82	10.77	44.19	37.61	18.20
Oxygenated monoterpenes					83.1	86.40	86.55	36.10	41.5	22.40
Total monoterpenes					98.4	98.22	97.32	37.15	40.97	21.88
Sesquiterpene hydrocarbons					1.42	1.52	2.37	31.41	37.29	31.3
Oxygenated sesquiterpenes					-	-	0.05	-	-	100
Total Sesquiterpene					1.42	1.52	2.42	31.23	37.07	31.7
Total aliphatic hydrocarbons					0.18	0.26	0.26	28.7	46.7	24.6
Extraction time					150 min.	40 min.	1 week			
Yield %					7.88	7.22	11.98			

3.3. Antimicrobial and anticancer activities of cardamom essential oils

3.3.1. Antimicrobial activity

The oils of cardamom; HD, MAHD, and HX; were examined for antibacterial activity against three gram-positive bacteria; B. subtilis, S. aureus and S. faecalis, and three gram-negative bacteria; E. coli, N. gonorrhoeae, and P. aeruginosa. Also, the three oils were investigated against two fungi; A. flavus, and C. albicans. The agar disc diffusion method was used. Table (2) showed the susceptibility of tested oil against the selected microbes expressed by the diameter of the inhibition zone (IZ) (mm) after treatment with 10 µg/ mL of the three essential oils. The HD and MAHD showed antimicrobial activity toward all selected bacteria and fungi while HX showed effect toward some microbes. The positive actions of the oils showed moderate antimicrobial activity in comparison to standards like Ampicillin and Amphotericin B. The HD and MAHD oils showed nearly a similar effect against the different microbes. The inhibition zones produced by the effect of HD and MAHD toward gram-positive bacteria; B. subtilis, S. aureus, and S. faecalis; were 12, 11 and 11 mm for each HD and MAHD whereas HX showed only activity toward S. faecalis with IZ 12 mm. For gram-negative bacteria, HD and MAHD showed activity against E. coli, N. gonorrhoeae, and P. aeruginosa with IZ 11, 15, 12 for HD and 11, 14, 12 for MAHD, whereas HX oil showed only activity toward N. gonorrhoeae with IZ 14 mm. The antifungal activity results showed that HX oil has no effect on the two selected fungi; A. flavus, and C. albicans; while HD and MAHD showed activity with IZ 10 and 13 for HD and 11 and 12 for MAHD. Our results in this

study were in a good agreement with many reports that revealed the presence of antimicrobial properties of cardamom oil [30-31]. study were in good agreement with many reports that revealed the presence of antimicrobial properties of cardamom oil [30-31].

Table (2): Inhibition zone diameter (mm) caused by (10 µg/mL) of cardamom essential oils extracted by HD, MAHD and HX against six bacterial and two fungal strains by diffusion disc method.

Samples	Inhibition zone diameter (mm)							
	Bacteria						Fungi	
	G^+			G^-				
	B. subtilis	*S. aureus*	*S. faecalis*	*E. coli*	*N. gonorrhoeae*	*P. aeruginosa*	*A. flavus*	*C. albicans*
HD	12	11	11	11	15	12	10	13
MAHD	12	11	11	11	14	12	11	12
HX	0	0	12	0	14	0	0	0
Ampicillin	26	25	28	26	21	27	-	-
Amphotericin B	-	-	-	-	-	-	16	21

3.3.2. Anticancer activity

The essential oils from cardamom obtained by HD, MAHD, and HX showed variable anticancer activity toward the three human tumor cell lines; HCT-116, A549, and MCF-7 (table 3). The three oils showed high activity toward HCT-116 and MCF-7 with half-maximal inhibitory concentration (IC50) between 10.02-0.44 µg/mL whereas the effect of oil toward A549 was moderate with IC50 between 86-40.8 µg/mL. It was obvious from the result that the n-hexane oil had higher activity toward the three cell cancer lines. This observation gave us the indication that the activity was not attributed only to the essential oil content but that there were other compounds that were responsible for this activity also. The result of the oils toward the two cell lines; HCT-116 and MCF-7 gave them a characteristic of promising anticancer because the results fall within the range of the American National Cancer Institute (NCI) criteria which considered the agent is promising when its IC50< 20 µg/mL [32].

Table (3): Anticancer activities expressed by half-maximal inhibitory concentration (IC_{50}) of cardamom essential oils; toward three different carcinoma cell lines.

Samples	IC_{50} (µg/mL)		
	A549	MCF-7	HCT-116
HD	86	10.02	5.76
MAHD	53.99	8.57	4.61
HX	40.8	0.87	0.44

3.4 Conclusion and Recommendation

Extraction methods are considered major factors that affect the essential oils, chemical composition and, consequently, their biological activity. Recently, the use of microwaves for natural product extraction from plant material has shown tremendous research interest and potential. To the best of our knowledge, this is the first study that demonstrated the effects of extraction methods; HD, MAHD, and HX on the essential oil composition, yield, and time of cardamom. The results showed that the usage of the microwave method in the extraction of essential oil is recommended due to energy and time saving, in addition to being environmentally friendly. Also, these results provide the basis for further and more in vitro and in vivo studies to evaluate the potential use of essential oils from cardamom as antimicrobial and anticancer agents in the hope of using these oils in the prevention and therapy of these diseases.

4. Conflicts of interest

The Authors have declared that no conflicting or competing interests exist. This work earned no particular grants from federal, private or non-profit funding agencies

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