Article

Chemical Analysis of *Euphorbia denticulata* Lam. and Study of extracted oil on Cytotoxicity and anti-tumor potential on AMGM-5 and HBL 100 cell lines

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Abstract

Study have been conducted on the cytotoxicity of *Euphorbia denticulate* oil. In The normal human breast cell line HBL 100 and human brain cancer cell line AMGM-5 Depending on the MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay method Following the application of various oil concentrations (0.01, 0.05, 0.1, 0.5, 1, 5) µg \ ml to the cells, the current study was able to determine the The Half maximal inhibitory concentration(IC50) value in AMGM-5 to be 0.21505%, while the normal cell line HBL100 revealed an IC_{50} value of 0.18688%). the MTT assay test findings suggested a suppression of the proliferation of cancer cells. The AMGM-5cell lines changed cellularly as a result of the oil Following a 48-hour course of therapy, the degeneration showed varying degrees of atrophy, swelling, and breakdown. Ultimately, the cells completely disintegrated, resulting in the formation of necrotic regions devoid of cells. Cultures of cells: When the cells were exposed to high oil concentrations, these alterations worsened.

1. Introduction

Essential oils are defined as complex volatile secondary metabolites that are isolated from any part of plants .The French chemist Dumas (1833) performed the first systematic description of the constituents of essential oils and explained that the main characteristics of essential oils are volatile, aromatic, fluid, lipophilic, colored or colorless, generally of a density less than water, in addition to being rich in life-active components. They can be easily separated from other components of plants. Essential oils and their constituents are now gaining global attention due to their multi-purpose functional use [1] .Despite numerous plant oils have demonstrated efficacy in treating the most severe diseases, independent of the pathogens associated, many plants around the world are still being researched for their active components and potential role in disease prevention[2].The literature has addressed the various pharmacological characteristics of Essential oil (Eos), including their antioxidant, antibacterial, anti-diabetic, and cardiovascular preventative properties. [3]. However, there hasn't been much effort put into compiling an extensive study of the in vivo/in vitro anticancer applications of EOs, including drug transport methods and cell target selectivity. Furthermore, current data on the anticancer, antitumor, and anti-proliferative properties of essential oils (EOs), as well as their benefits for effective target-specific drug delivery (via nanoencapsulation or nanoemulsions) and combination use with traditional chemotherapy drugs, are not easily accessible in a single article. [4]. The challenge for researchers is also to develop safe therapeutic approaches that can distinguish between cancerous and healthy cells .The pharmaceutical industry has contributed to saving humanity from the scourge of diseases, especially the intractable ones, but it has become an obsession that haunts humanity, due to the disadvantages of these treatments, side effects and complications due to the cumulative action, in addition to the high cost of treatment. In this context, the goal of scientific experiments at the present time is to find alternative compounds to treat such diseases [5]. To achieve promising outcomes in the treatment of cancer, more thorough research is needed, including studies on target-oriented anticancer medication delivery. To further establish a commercial medication approach, it is important to clarify the molecular mechanisms behind the anticancer activities of EOs and their constituents. Thus, the goal of the current study was to determine whether the oil's constituents may inhibit cancer cells and advance them toward the stages of programmed death.

2. Materials and Methods

2.1 Chemical analysis of *Euphorbia denticulata* oil

The plant's dried leaves (20g)and 150ml of hexane as a solvent at 38 °C for eight hours were used to extract the oil using a sexolite Oil fractions were obtained by evaporating hexane at 68°C using a rotary evaporator. in the Marine Science Center's laboratories at Basrah University. The main compounds of ***Euphorbia denticulata*** oil 'were determined using a gas chromatography- mass spectrometer (GC-MS) (7890B) and a mass spectrometer (GC-MS) detector (5977A). Using helium gas, then the percentages of the different chemical compounds of oils were calculated [6] [7]

2.2 Maintenance of cell lines

The cell line was obtained from The IRAQ Biotech Cell Bank Unit in Basrah provided the human brain cancer (AMGM) and Normal human cancer (HBL-100) cell lines Ahmed Majeed Glioblastoma Multiform cancer cell line (AMGM-5)

The process of maintaining the cells was carried out in culture dishes of 25 ^{cm2} using the culture medium RPMI1640. Contains 10% fetal bovine serum (FBS) and 100 IU \ ml of penicillin and 100 µg \ ml of streptomycin It was placed in an incubator at 37℃ and 5% CO_2 When the cells grew and formed a monolayer by 80-90%, secondary cultures were done ,One of them is by using trypsin-fersin, where the culture medium is first poured and then the cells are washed twice with 1ml of buffer solution (phosphate buffer saline PBS) for each subsequent time , Add 1 ml of trypsin-fersin. The dishes are incubated in the incubator at a temperature of 37 and 5%. CO_2 The cells are examined. Then, using an inverted microscope, to ensure that the majority of the cells have separated from the dish, Pellet a small cell mass using centrifugation, then suspend it in 10% complete culture medium and divide it into Two plates and incubated in the incubator at a temperature of 37 °C and 5% CO_2 [8]

2.3 Cytotoxicity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, often known as the MTT reagent, The MTT test was used to study the *Euphorbia denticulata* oil cytotoxicity on the vitality of cells by culturing AMGM cancer and Normal human cancer (HBL-100) cell lines line and are in the 96-hole planting dishes with a ratio of ($1 * 10^4$) A cell for each hole and incubated in the incubator at 37 °C and 5% CO_2 [9] After 24 hours, the cells were exposed to a series of concentrations of oil(0.01, 0.05, 0.1, 0.5, 1, 5) µg \ ml of serum-free culture medium, at a rate of Four technical replicates for each concentration, and incubated in the incubator for 72 hours at a temperature of 37 °C and 5% CO_2 After the end of the incubation period, cell vitality was measured using MTT dye .This is after pouring the medium containing the concentrations oil and adding 100 µL of culture medium containing 10 µL of MTT dye solution (2 mg \ µL of PBS buffer solution) The dishes are incubated in the incubator for two hours at a Temperature 37°C and 5% CO_2 The dye solution is then poured out, and a crystalline substance remains in the dish pits, which is tetrazolium tetrazolium is dissolved by adding 100 µL of Dimethyl Sulphoxide (DMSO) and incubating at room temperature in dark conditions for 20 minutes)[10] Then the absorbance of the dissolved substance, which is formazan, is read at a wavelength 620 nm using a thermo dish reader device. The test was conducted in three replicates for each cell line .Four technical replicates for each concentration[11]

The cell Inhibition rate was calculated According to the equation below:

Cell vitality rate = treatment absorbance rate / control absorbance rate * 100
Inhibition % = (A control – A sample)/ A control

2.4 Detection of programmed cell death using the dye Acridine Orange/Ethidium Bromide (AO/EB)

The cells were separated from the surface of the culture vial by the trypsinization process and suspended in 10 ml of the culture medium containing the serum. Then the cells were planted on a cover glass slide and placed in a sterile dish measuring 3 cm, and incubated in a CO_2 incubator for 24 hours. After making sure that one cell layer was formed, the cells were treated. Cultivated with the concentration of IC50 for the study materials and incubated in the incubator for 48 hours Then it was stained with AO/EB [12] To determine the percentage of dead cells in cancer and normal cell lines treated with oil compared to untreated cells stained with AO/EB dye, use GraphPad Prism software to conduct a One Way Anova test.

3.Results and discussion

Naturally occurring chemicals with great pharmacological potential against a variety of tumor types make up the chemical components in essential oils.

The results of the chemical analysis of *Euphorbia dentculata* oil using the GC-mass device showed the presence of 93 chemical compounds within its composition, Shown in the table(1), the percentage of the compounds ranged between (0.138098-13.47946%,). This chemical compound mostly belongs to fatty acids, Triterpenoids and Monoterpenes Its effectiveness against cancer was tested and showed high efficiency in inhibiting cancer cells, which has been documented by many studies[13][14]Research is of particular importance to clarify the mechanism of action carried out by these chemical compounds to penetrate cancer cells and inhibit or destroy them.[15][16] The significance of fatty acid production for cancer cell proliferation and survival has been demonstrated by multiple research. Because the maintenance of cellular physiology depends on the regulation of lipid synthesis, the corresponding metabolism, as well as their uptake and degradation, disruption of these processes can have an impact on the development of cancer. Therefore, the presence of fatty acids outside the cancer cell causes confusion in the metabolism of fatty acids in it and thus inhibits it.[17] Triterpenoids were long believed to be physiologically inert; nevertheless, growing evidence on their broad spectrum of pharmacological activity and low toxicity profile has piqued interest once again with regard to human health and disease.[19][20][21][22]The table also shows some of the compounds that are included in Monoterpenes ,Monoterpenes, a class of secondary metabolites that makes up the majority in plants, contain hydrocarbons and are commonly found in essential oils. The synthesis of new physiologically active compounds often involves the use of monoterpenes and their derivatives .Certain monoterpenes have been shown in experimental research to have anticarcinogenic qualities, working at various cellular and molecular levels, utilizing animal cancer models as models of study. These findings suggest that monoterpenes may be a promising new class of anticancer medications. They are also effective and nontoxic dietary antitumorigenic agents.[23][24] While the quality of oil used medically is determined by the proportion of

monoterpenes with the required biological activity .The majority of chemotherapy treatments used in the treatment of cancer inhibit the reproduction of both healthy and cancerous cells, while essential oils and their natural components have multiple pharmacological activities to inhibit tumors without harming the body [25].

Then there is an indication of the chemical compound Thiophene, whose effectiveness against tumors has been discussed in many scientific studies ,Thiophene is a heterocyclic scaffold that contains sulfur and has been extensively studied for its potential to generate a library of chemicals with anticancer characteristics. Reports state that thiophene analogs have been demonstrated to bind with a range of protein targets specific to cancer, depending on the kind and location of substitutions. Therefore, it has been proposed that the biological effects of thiophene analogs are caused by the blocking of numerous signaling pathways relevant to cancer. For different anticancer targets to work effectively, different structural attributes are required.[26] Research [27] demonstrate the potency of thiophene-2-acetic acid in inhibiting cancer cells by highlighting its involvement in the terminal enzyme microsomal prostaglandin E synthase-1 (mPGES-1) ,The thiophene moiety is used in cancer therapy to combat different types of cancer cells. Information from the several studies revealed the crucial function played by the thiophene moiety and its derivatives in the creation of the essential lead molecule. It has been discovered that tyrosine kinase, topoisomerase, and other essential anticancer processes [28] As mentioned [29] [30] [31] the derived cyclobutane compounds Which is found in low percentages in oil considered among the cancer inhibitors with anti-oxidative activity The results of this study are in line with others that have shown how *Euphorbia* species, and particularly *Euphorbia denticulate* with active compounds and oils, can combat various cancer types.[32][33][34][35][36]

Table (1) Chemical compounds of *Euphorbia denticulate* oil using GC-mass technology.

Name	Formula	Retention Time (RT)	Area	Score (Lib)	%
Toluene	C7H8	3.231	3569165	92.61	0.391392
Hexanoic acid	C6H12O2	6.459	2611672	89.12	0.286394
p-Cresol	C7H8O	7.654	1284682	90.35	0.140877
Nonane	C9H20	7.943	1867072	88.71	0.204742
Butanedioic acid, monomethyl ester	C5H8O4	7.966	3216667	86.87	0.352738
Dimethyl dl-malate	C6H10O5	8.178	1882469	92.13	0.20643
Dimethyl 3-hydroxy-3-methylpentane-1,5-	C8H14O5	9.039	1991151	92.2	0.2183

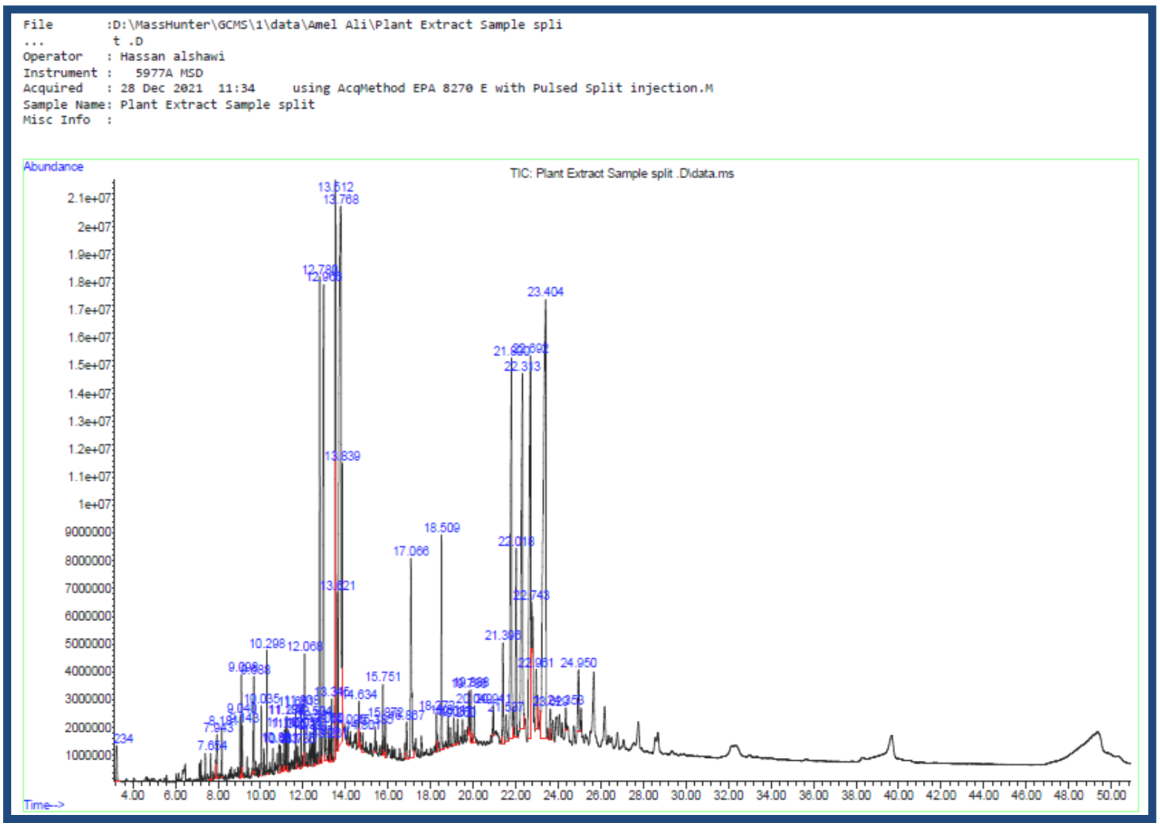
dioate					48
5-Hydroxymethylfurfural	C6H6O3	9.099	4981480	94.29	0.546266
Pivalic acid vinyl ester	C7H12O2	9.143	2722309	86.23	0.298527
Dimethyl 3-hydroxy-3-methylpentane-1,5-dioate	C8H14O5	9.685	7634208	69.15	0.837162
Benzaldehyde, 4-hydroxy-	C7H6O2	10.038	3204770	95.77	0.351433
Benzaldehyde, 3-hydroxy-4-methoxy-	C8H8O3	10.297	3957704	94.23	0.433999
Benzoic acid, 4-hydroxy-	C7H6O3	10.884	1992111	88.62	0.218454
Dodecanoic acid	C12H24O2	11.114	1948501	93.03	0.213671
3-Hydroxy-4-methoxybenzoic acid	C8H8O4	11.182	3118946	91.91	0.342021
Nonanedioic acid, monomethyl ester	C10H18O4	11.235	2094542	86.59	0.229686
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	C9H10O4	11.65	1943818	92.05	0.213158
Tridecanoic acid, 12-methyl-, methyl ester	C15H30O2	11.907	2378718	71.24	0.260849
Coniferyl aldehyde	C10H10O3	12.034	1340699	89.51	0.14702
Tetradecanoic acid	C14H28O2	12.067	3835688	90.33	0.420619
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	C9H10O5	12.33	1584302	80.15	0.173733
Pentadecanoic acid	C15H30O2	12.502	1636727	90.16	0.179482
Propionic acid, 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-	C7H10N2O3	12.581	1494178	74.79	0.163851
Hexadecanoic acid, methyl ester	C17H34O2	12.782	16876053	94.1	1.850616
Hexadecenoic acid, Z-11-	C16H30O2	12.853	1732910	82.14	0.19003
n-Hexadecanoic acid	C16H32O2	12.966	44203572	92.5	4.847334
Isopimara-9(11),15-diene	C20H32	13.052	1375249	81.43	0.150809
Heptadecanoic acid	C17H34O2	13.345	2536001	84.59	0.278096
9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	C19H34O2	13.51	34457295	83.75	3.778563
13-Octadecenoic acid, methyl ester	C19H36O2	13.534	16808807	66.92	1.843242

1,8,11,14-Heptadecatetraene, (Z,Z,Z)-	C17H28	13.541	10537257	72.18	1.155508
Methyl stearate	C19H38O2	13.623	5482754	87.83	0.601235
1,1'-Bicyclobutyl	C8H14	13.756	52526207	59.42	5.759988
1-Heptanol, 2,4-diethyl-	C11H24O	13.784	35811716	65.43	3.927088
1,5-Cyclooctadiene, 3-(1-methyl-2-propenyl)-	C12H18	13.794	24999775	61.46	2.741458
Octadecanoic acid	C18H36O2	13.839	10676500	92.33	1.170778
Bicyclo[4.1.0]heptane, 7-pentyl-	C12H22	13.925	1359957	72.4	0.149132
13-Tetradecenal	C14H26O	14.632	2763723	84.26	0.303068
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	15.386	1704190	83.53	0.18688
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	15.748	3982537	88.85	0.436722
13-Oxabicyclo[10.1.0]tridecane	C12H22O	16.858	1722908	70.15	0.188933
Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	C19H34	17.065	18790118	82.73	2.060512
Undecane, 3,8-dimethyl-	C13H28	17.076	3759881	68.63	0.412306
Bitolylene diisocyanate	C16H12N2O2	17.09	1467781	53.66	0.160956
Albene	C12H18	17.125	2177234	69.21	0.238754
Hexadecanoic acid, 2-hydroxy-, methyl ester	C17H34O3	17.557	1259334	78.22	0.138098
Hexadecanoic acid, 2-hydroxy-, methyl ester	C17H34O3	18.271	2230829	77.62	0.244631
1-Hexadecanol	C16H34O	18.51	11165317	88.86	1.224381
1-Docosene	C22H44	18.808	1283830	93.28	0.140784
2(3H)-Benzofuranone, 6-ethenylhexahydro-6-methyl-3-methylene-7-(1-methylethenyl)-, [3aS-(3a.alpha.,6.alpha.,7.beta.,7a.beta.)]-	C15H20O2	19.09	1837071	70.13	0.201452
7-Oxabicyclo[4.1.0]heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-	C15H22O	19.263	1476603	69.42	0.161923
Nonadecane	C19H40	19.787	3266792	82.84	0.358234

1-Hexadecanol	C16H34O	19.829	1915387	90.58	0.21004
Stigmasta-3,5-diene	C29H48	19.891	3541716	90.06	0.388382
dl-.alpha.-Tocopherol	C29H50O2	20.052	2337582	90.32	0.256338
Ergost-5-en-3-ol, (3.beta.)-	C28H48O	20.944	3476669	85.45	0.381249
Lanosterol	C30H50O	21.394	8250224	75.19	0.904714
4.alpha.,14-Dimethyl-5.alpha.-ergosta-8,24(28)-dien-3.beta.-ol	C30H50O	21.537	2806726	83.7	0.307784
.gamma.-Sitosterol	C29H50O	21.799	57939749	93.89	6.353633
Lanosta-8,24-dien-3-one	C30H48O	21.872	1519690	43.3	0.166648
Stigmastanol	C29H52O	21.888	1897027	63.75	0.208027
Lanosterol	C30H50O	22.016	18494369	80.05	2.02808
Obtusifoliol	C30H50O	22.315	61917600	77.41	6.789842
Caparratriene	C15H26	22.395	1286097	52.07	0.141033
Lup-20(29)-en-3-one	C30H48O	22.451	1417153	48.66	0.155404
9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	C30H50O	22.692	67268986	93.74	7.376671
17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopent(a)phenanthren-3-ol	C36H54OS	22.741	9384764	64.65	1.029127
Lupeol	C30H50O	22.796	13814467	86.18	1.514885
1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	C20H32	22.96	8459279	75.92	0.927639
24-Methylenecycloartan-3-one	C31H50O	23.025	4357936	64.87	0.477888
Silane,dimethyl(4-(2-phenylprop-2-yl)phenoxy)tridecyloxy-	C30H48O2Si	23.251	2091869	49.26	0.229393
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	C31H52O	23.402	122921264	89.73	13.47946
9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	C30H50O	23.625	2608342	69.87	0.286029
9,19-Cycloergost-24(28)-en-3-ol, 4,14-	C32H52O2	23.745	1860926	64.83	0.2040

dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-					68
17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopent(a)phenanthren-3-ol	C36H54OS	23.889	1606340	38.44	0.17615
Eremophila ketone	C15H24O	23.939	2297588	56.19	0.251952
9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-	C31H52O	24.049	2694793	64.59	0.295509
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	C31H52O	24.352	4413141	85.14	0.483942
9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	C32H54O2	24.722	1609923	50.85	0.176543
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	C32H52O2	24.95	10574987	71.89	1.159646
9,19-Cyclo-27-norlanostan-25-one, 3-(acetyloxy)-24-methyl-, (3.beta.,24R)-	C32H52O3	25.067	4340793	73.64	0.476008
1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	C20H32	25.664	11535977	70.39	1.265027
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	C32H52O2	26.176	5805732	68.63	0.636653
Rhodium, [(1,2,5,6-.eta.)-1,5-cyclooctadiene](1,1,1,5,5,5-hexafluoro-2,4-pentanedionato-O,O')-	C13H13F6O2Rh	26.356	1477930	45.87	0.162069
9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	C32H54O2	26.777	2258535	69.66	0.247669
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	C31H52O	27.062	1237124	63.52	0.135662
Dihydrotachysterol	C28H46O	27.761	4410481	56.62	0.48365
5-Azulenemethanol, 1,2,3,3a,4,5,6,7-octahydro-.alpha.,.alpha.,3,8-tetramethyl-, [3S-(3.alpha.,3a.beta.,5.alpha.)]-	C15H26O	28.558	2166866	52.45	0.237617
Docosa-8,14-diyn-1,22-diol, (Z)-, 2TMS derivative	C28H54O2Si2	28.685	3428940	53.71	0.376015
Pentyl linoleate	C23H42O2	32.346	5996927	52.08	0.657619
1,3-Dimethoxypropan-2-yl palmitate	C21H42O4	39.663	7740837	49.26	0.848855
Cyclobutaneacetonitrile, 1-methyl-2-(1-methylethylidene)-	C10H15N	49.335	34052915	43.13	3.734219
Thiophene-2-acetic acid, 6-chlorohexyl	C12H17ClO2	49.365	2413378	40.62	2.6464

ester	S		1		94



Figer(1) It shows the peaks of 66 compounds in the oil. The compounds were identified by matching the mass spectrum with the library prepared in the device.

The highest peaks were represented by the chemical compound **9,19-Cyclolanost-24- en-3-ol, (3.beta.) with RT** 22.692 and the lowest by the compound **Hexanoic acid with RT** 6.459 t_m Both have proven effectiveness against cancer cells

3.2 Cytotoxicity of *Euphorbia denticulata* oil in cell lines

Based on MTT technology, The current study's findings demonstrated that the cancer cell line was suppressed by the *Euphorbia denticulate* oil. (AMGM-5) after being treated for 72 hours at various doses (0.01, 0.05, 0.1, 0.5, 1, 5%), with the rate of inhibition increasing with As the concentration was increased, cell cultures treated with concentrations of 0.5, 1, and 5 showed the highest rates of inhibition, reaching more than 80%. As shown in Figure (2), the lowest rates of inhibition, however, were at concentrations of 0.01, 0.05, and 0.1 since they did not surpass 20%. At the probability level of $P \leq 0.05$, the statistical analysis employing one way anova revealed that there were significant differences between the various concentrations. When the extracted oil was applied to the normal cell

line (HBL-100) at various doses (0.01, 0.05, 0.1, 0.5, 1, 5%) over the course of 72 hours, it also demonstrated an inhibitory impact on the cell line. As concentration increased, correspondingly the rate of inhibition increased. The treated cell cultures showed the highest rates of inhibition. With all concentrations, over 60% beginning with the low dose of 0.5. At the probability level of P≤0.05,

statistical analysis utilizing one way anova revealed significant differences between the various concentrations.

The Half maximal inhibitory concentration (IC_{50}) for *Euphorbia denticulate* oil was determined by processing the inhibition percentage data collected in the Graphpad Prism program. Its value in the malignant cell line AMGM-5 reached (0.21505%), whereas its value in the normal cell line HBL100 reached (0.18688). %). Figure (2)

The effect of programmed cell death-inducing oil on cell proliferation and density was studied and differences in cell vitality were studied using Crystal Violet dye. Cells treated with oils for cell lines (AMGM-5, HBL-100) separated from the surface of tissue culture dishes as a result of their death. Therefore, the violet dye appears in a light color due to the lack of live pigmented cells, and this was more evident in the treated cancer cells than in normal cells, compared to the color of the control group, which appeared in a dark violet color, which indicates the vitality of the cells,

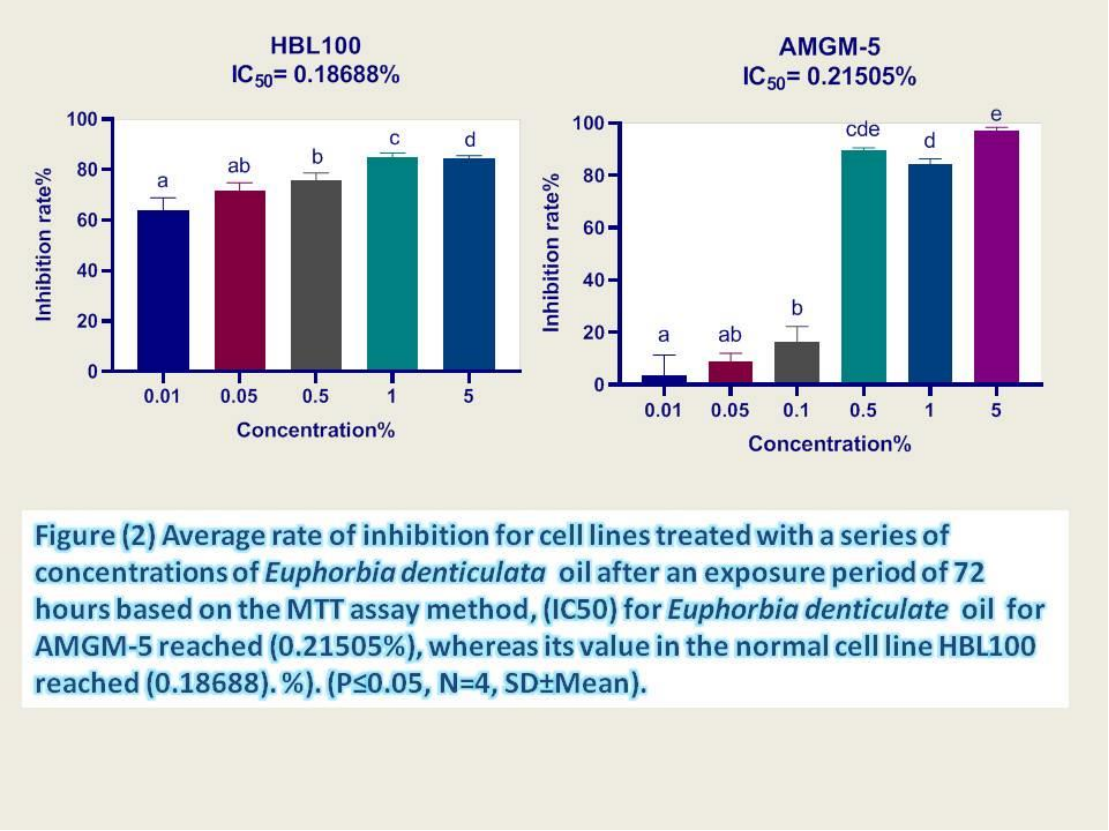


Figure (2) Average rate of inhibition for cell lines treated with a series of concentrations of *Euphorbia denticulata* oil after an exposure period of 72 hours based on the MTT assay method, (IC50) for *Euphorbia denticulate* oil for AMGM-5 reached (0.21505%), whereas its value in the normal cell line HBL100 reached (0.18688). %). (P≤0.05, N=4, SD±Mean).

3-4 Detection of programmed cell death using the dye Acridine Orange/Ethidium Bromide (AO/EB)

The results of fluorescent microscope examination of normal and cancerous cell lines HBL100 and AMGM-5 showed stained for 48 hours using AO/EB. AO dye gave untreated cells a green stain, signifying their safety and lack of damage. Unaffected Certain cells treated with IC_{50} oil were colored yellow, signifying their early stage of programmed cell death. On the other hand, other cells treated with EB dye were colored red, showing their advanced level of programmed cell death. The results of the statistical analysis of dead cells in normal and cancerous cell lines showed that there were significant differences between the cells treated with the oil compared to the control group at a probability level of $P \leq 0.05$.

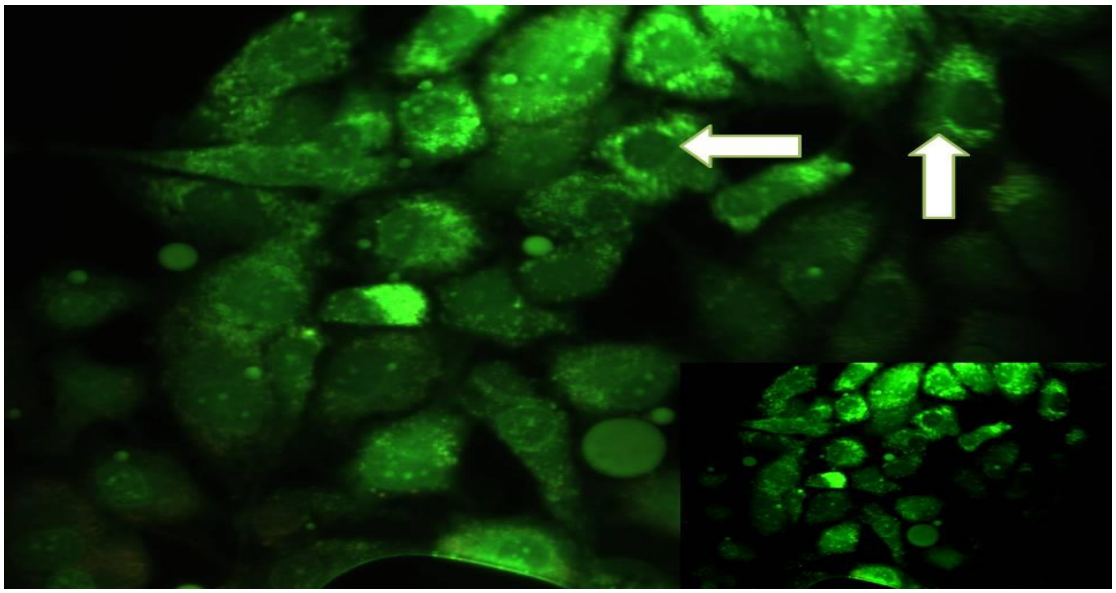
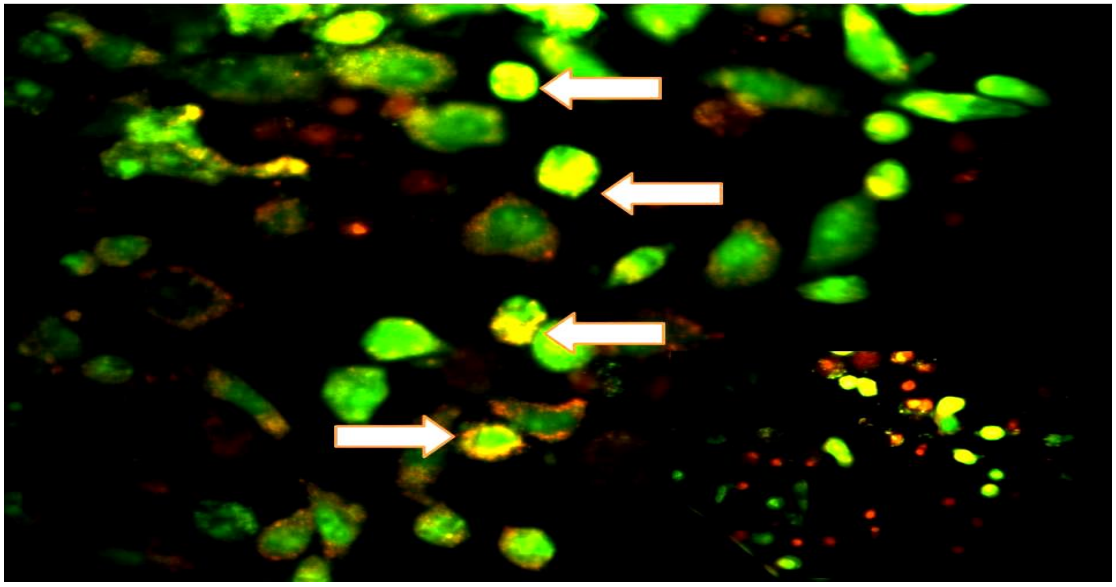
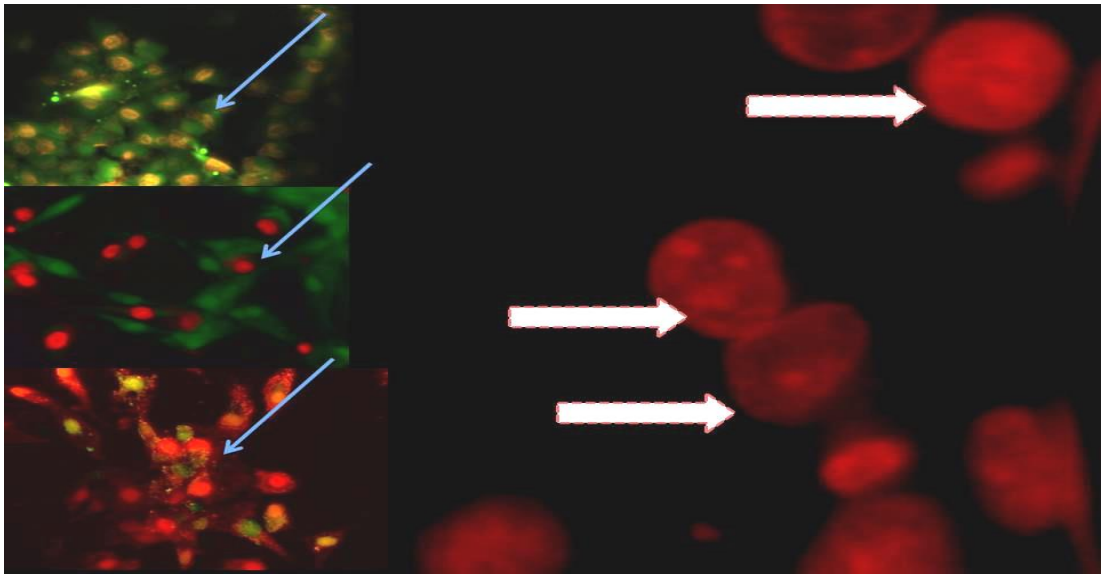


Image (1) HBL-100 cell line after staining with AO/EB(The green arrow indicates Which shows clear nuclei in the cells) (control) (400X magnification power.)



Image(2) AMGM-5 Cells treated with IC_{50} concentration of *Euphorbia denticulate* oil and The arrow indicates cells suffering from Early apoptosis signifying their stage of programmed cell death(400X magnification).



Image(3) Cells treated with IC_{50} concentration of oil are colored red The arrow indicates cells suffering from late apoptosis, The blue arrows are the stages through which treated cancer cells undergo programmed death(400X magnification).

The use of plant products has recently witnessed a remarkable growth in various fields of life and their applications, which has prompted researchers to pay attention to studying their effectiveness in life [37] Exposing cells to toxic compounds causes various effects on them called Cytotoxicity , and this term has an important role in the discovery and development of pharmaceutical industries [38] It appears that one of the causes that stimulate programmed cell death in cancer cells treated with oil is the depletion of energy due to the oil, which leads to mitochondrial dysfunction and changes in pH [39] Energy depletion can lead to many other disorders, including a decrease in the sodium pump, thus releasing the calcium ion Ca+2 into the cytoplasm and increasing its level, leading to the activation of proteins and hydrolytic enzymes in the cell [40][41] The inhibitory effectiveness of the oil on cancer cells greatly exceeded its effectiveness on normal cells, with a clear significant difference The probable cause of this could be attributed to the essential characteristics of cancer cells, which require a significantly higher quantity of energy than normal cells [42] Moreover, cancer cells have a weak oxygen sensor, so they primarily get their energy from the breakdown of glucose, which produces lactate even in the presence of oxygen [43] Certain cellular signaling pathways, such as the Ras-ERK and AKT-PI3K pathways, are known to have mutations in cancer cells that lead to cell proliferation [44] In contrast to normal cells, cancer cells exhibit pronounced metabolic alterations as a result of these pathways being active Because the oil induces dysfunction in the mitochondria, it lowers the amounts of glucose-dependent energy production in cancer cells, which explains why the pace of inhibition between cancerous and normal cells differs. [45][46] One of the various methods for assessing cytotoxicity was the MTT assay, which was used in the current

investigation to assess the cytotoxicity of the oil in malignant and normal cell lines (AMGM-5 and HBL-100). Which depends on reducing the yellow substance Tetrazolium bromide to purple Formazan crystals in living cells [47] The results of the study showed a clear decrease in cell vitality depending on the concentration This indicates that the oil has a cell-inhibitory activity and has cytotoxicity on treated cancer cell lines when compared to untreated cells. Through necrosis, halting the cell cycle, programmed cell death, and interference with organelle functioning, the oil can induce the death of cancer cells. This is accomplished by making the cell membrane more permeable. Reducing the production of Adenosine Triphosphate (ATP), changing the pH gradient, and losing the ability of mitochondria, and these are the main causes of cell death [48][49][50]

Conclusion study that the oil is one of the most promising oils in combating and reducing cancer cells. We hope to conduct subsequent studies on other types of cancer. It is also possible to isolate the active ingredients in this oil and invest it as a medicinal drug against cancer. Conducting a complementary study that clarifies the pathways and mechanisms by which oil attacks cancer cells, whether through the cell membrane or their metabolic pathways.

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