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Anticancer Potential of Citrullus colocynthis Oil to enhanced suppression human glioblastoma AMGM-5 cell line

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ABSTRACT

With continuously rising morbidity and mortality, the scientists focus their efforts to outcome novel therapeutics due to improve cancer patients' survival. The major undesirable features of conventional therapies were low selectivity, high toxicity, drug resistance, and economic expenses. Opposite, natural compounds of medicinal plants play a crucial role in treatment of several diseases. Currently, many studies investigated the potential of secondary metabolites to fight cancer cells through enhanced apoptotic cell death or energy production pathway as a targeted and smart therapy strategies. The study aimed to evaluate and investigate of antitumor activity of Citrullus colocynthis fruit oil extraction against cancer and normal cell lines. AMGM-5 human glioblastoma and HBL-100 human breast epithelial cells were used to determination cytotoxic effect of C.colocynthis oil by MTT assay and detection of IC_{50} dose. Furthermore, this study evaluated apoptosis using fluorescent staining. In addition, we investigated of cell migration, invasion, and rapid rate proliferation of cancer cells by wound healing assay. The results showed significant impacts of essential oil accompanied by toxicity, apoptosis, and inhibition of cell proliferation, migration, and invasion against cancer cells, but, normal cells not affected. Our findings suggest natural compounds have possible cancer drugs without side effect on normal cells.

1. Introduction

Cancer isn't a single disease. Cancer is a group of diseases, and it's a major public health problem and the dangerous fatal disease in the world. [1]. Globally, there are 17 million people are diagnosed with cancer every year and this scenario is estimated to significantly increase in the near future [2]. It is a heterogenous disease characterized by uncontrolled of cell growth and rapid rate proliferation where the behavior of abnormal cells ravages and which spread to neighboring tissues and organs [3]. Although decades of research, mortality rates and recurrence still rising, and It has limited options for efficient therapies or strategies regarding cancer progression prevention. Cancer cells showcase

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messy, heterogeneous and highly differentiated structures, which is determinative to the lack of effective antitumor drugs [4,5].

Conventional therapy such as tradition chemotherapeutic agents that used to treat cancer patients have high toxicity, multidrug resistance, lack or low of selectivity, limiting their effectiveness, and economic expenses rising [6,7]. Thus, the discovery and development of new drugs play a crucial role in cancer avoiding and control [8]. Perfectly, any anticancer agent should practice a cytotoxic effect on malignant cells with a slim impact on normal cells [9]. Many drugs developed to fight tumor are of natural source origin or derived by them. The compounds selected from natural sources have a related role in developing new palliative therapies [10,11]. Furthermore, their anticancer activity, natural compounds have many advantage features owing to their low side effects, low cost, ease of availability, and high selectivity [12]. Medicinal plant compounds have been broadly utilized in pharmaceuticals for many decades, and they have aided in the development of novel therapies and have been widely used as an origin of natural compounds for the oncology therapy [13,14].

Recently, phytochemicals are consistently investigated for modern medicine [15]. These compounds represented as a major factor for generation and synthesis of various therapeutic drugs [16]. Phytochemicals have been reported to show various encouraging activities against human cancer in vitro or in vivo models [17]. Citrullus colocynthis (L.) is a plant belonging to the family Cucurbitaceae [18]. Several common names for this medicinal plant included in Cambridge English dictionary are bitter apple, and bitter gourd [19]. The Cucurbitaceae family is considered one of the most economically significant families. It has been 122 genus and 940 species, all found throughout the world's tropical and subtropical areas [20]. Due to their beneficial effects as dietary and therapeutic agents, many members of the Cucurbitaceae family are making significant contributions as domesticated species [21]. A lot genus of the family is tolerant of dry periods but intolerant of wet, frost-sensitive, and poorly drained soils [22]. Recently, mentioned that C. colocynthis had an elevated concentration of alkaloids, phenolics, glycosides, amino acids, tocopherol, flavonoids, volatile chemicals, proteins, and fatty acids [23].

Different C. colocynthis extractions secondary metabolites exhibited cytotoxic effect against human MCF-7 breast cancer cells [24], human colon HT-29 cells [25], human A375 melanoma cells [26], human lung cancer cells A549 [27], human liver carcinoma HepG2 [28]. The aim of the present study was extraction of oil from seeds of C. colocynthis by Soxhlet apparatus and purification, identification, and treatment both normal and cancer cell lines for several times with multi doses and evaluate the anticancer potential of our essential oil with cell cytotoxicity, and wound healing arrest.

2. Materials and Methods

2.1 Plant material collection

C. colocynthis bitter melon (common name) fresh seeds were collected from desert area of north and south Rumailah, Basrah government from June to September, 2022. The seeds were dried and grinded to prepare them for oil extraction.

2.2 Authentication of sample

The authentication of C.colocynthis plant material seeds were documented in Department of Biology, College of Education for Pure Science, University of Basrah.

2.3 Preparation of oil extraction and purification

The extraction of oil had done utilized a Soxhlet extraction apparatus and n-hexane as a non-polar solvent that does not contain double bonds to reduce the possibility of the solvent bonds interacting with the bonds of the oil. 50 grams of C.colocynthis powder put in filter paper with a diameter of 18 cm in 200 ml of hexane in a 500 ml beaker for 6-8 h. The resulting oil was incubated in an oven at 45 °C overnight to eliminate residual solvents by evaporation while ensuring that all

organic compounds were not denatured. The oil had filtered by used filter paper size 18 cm further sterilized with the 0.22 µm syringe filter (Membrane solution, USA) [29].

2.4 GC-MS analysis

In order to detection the bioactive compounds of C.colocynthis Gas chromatography/mass spectrometer method was used. An Agilent Technologies (Aanta Clara, CA, USA) 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI Signal detector was applied for the GC-MS analyses at the Basra Oil Company Laboratory. The HP-5ms 5% phenyl, 95% methyl siloxane (30m*250µm*0.25) was used. The oven temperature was set at 40 ºC hold for 5 min, then raised to 8 Cº/min to 300 ºC for 20 min. The helium carrier gas flow rate was 1 ml/min, and the purge flow was 3 ml/min. With an injection temperature of 290 ºC and a volume of 0.50 µl, the injection mode employed was pulsed split less. The mass spectrometer had an ion source with a temperature of 250 ºC, a mass range of 44-750 m/z, and a scan speed of 1562(N2).

2.5 Identification of phytochemical bioactive compounds

The NIST 2014, 2020 Library data set has been used as an extra resource to verify the authenticity of the chemicals. GC retention time was used to identify and characterize chemical components in different samples. The spectrum's mass have been compared by computer to standards found in the mass spectrum libraries at NIST. The sample constituents' percentage composition was reported as a percentage per peak area [30].

2.6 Cell lines and cell cultures

Two types of cell lines have been used in this study: glio-blastoma multiform (AMGM-5) as a cancer cells and (HBL-100) human breast epithelium as normal cells. These cell lines were obtained from cellular bank unit at tissue culture laboratory, Biology department, Collage of Education for pure science, University of Basrah, Basrah, Iraq. Cell lines were cultured in RPMI-1640 w/L glutamine, 25mM HEPES (USBiological life science, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA (Ethylene di-amine tetra acetic acid) when reached to 70-85% confluence twice per a week and incubated at 37 °C and 5% CO2. The falcon contains attached monolayer cells was de-attached with 1 ml of trypsin/versine to provide suspension of cells, then 10 ml added of prepared media to above solution. 150 µL of the cells were culture on clean sterile 96- well micro titer plate, then let the cells for make monolayer, then treated with extracted oil [31].

2.7 Cytotoxicity assay

To determine the cytotoxic effect, the MTT [3- (4,5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide] cell viability assay was conducted on 96-well plates. Cell lines were seeded at 1 × 104 cells/well [32]. After 24h or a confluent monolayer was achieved, cells were treated with several concentration of C.colocynthis oil (0.062, 0.125, 0.25, 0.50, and 0.75 %). Cell viability was measured after 24, 48, and 72 hrs. of treatment by removing the old medium. Then added 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 120 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [33]. The absorbency was determined on a microplate reader at 620 nm, and the assay was performed in triplicate. The number of inhibition cells was expressed as the percentage of control cells cultured in the extract medium free serum. The cell inhibitory is calculated using the following formula:

$$\text{Cytotoxicity rate\%} = [OD_{control} - OD_{treatment} / OD_{control}] \times 100\ \%.$$

Where, $OD_{control}$ Optical density of untreated cells (control); $OD_{treatment}$ Optical density of treated cells [34].

2.8 Determining of inhibited concentration

The IC_{50} values of C.colocynthis oil effect were calculated using the GraphPad Prism software version 10.1.2 (324). The AMGM-5 and HBL-100 cell line's IC_{50} was calculated using three replicate experiments of inhibition for each concentration [35].

2.9 Study of cell morphological changes

Once the cultures archived a monolayer density, the confluent cells plated in 6-tissue culture well plate containing sterilized coverslips inside each them at 7×10^5 cells per cover and incubated in humanified condition at 37 ºC to cell proliferation overnight. The cultured cells were treated with the IC_{50} concentrations 0.24 % of C.colocynthis seeds oil for 72 h to provide an appropriate time for the oil in order to increase the inhibited impacts or kill of the exposure cells in incubator. The treated and untreated cells were two replicates in this protocol, as a negative and positive groups. Finally, the old medium had dis-carded and the cultures of the cells were captured directly by light inverted microscopy [36].

2.10 Wound healing (scratch) assay

Cancer AMGM-5 and normal HBL-100 cells that was been exposure with the fresh medium or medium including oil for 72 h they seeded in 6 well cell culture plates and remain until reached to a confluent monolayer. The cells were then washed twice with PBS. A sterilized pipette tip 200 µL had been utilized to scratch a wounded on the midline of the culture well, and the cells were treated with IC_{50} dose of C.colocynthis oil for 72 h then incubate at 37˚C. The migration of cells had investigated by measuring the variance in area of the wounds at different times 0 and 72 h according to [37].

2.11 Statistical analyses

MTT assay was statistically analyzed using one-way analysis of variance test ANOVA by GraphPad Prism (GraphPad Software, Inc. San Diego, California, USA). Wound healing assay measured using ImageJ software v.154 and their statistics with GraphPad Prism. Data in graphs are shown as mean ± S. D [38].

Results and Discussion

3.1 Phytochemicals profile secerning by GC-MS test

The results of the current study outcome with Gas chromatography-Mass spectrometer test are illustrated in Table (1). The total of six components were detected, identification and nomenclature in purified oil solution during retention time 48 min shown in total ion chromatogram (TIC) of extracts of C. colocynthis only seeds heat oil fig (1) and fig (2). The higher peaks were kept track, 63.97421 % 11-Octadecenoic acid, (Z)- with C18H34O2 formula at 25.7113 min retention time fig (3A). Also 43.11012 % n-Hexadecanoic acid with formula C16H32O2 at 23.9681min retention time fig (3B). 24.39150 % Squalene and formula C30H50 at 31.0386 min retention time fig (3C). 20.90935 % Diethyl Phthalate with formula C12H14O4 at 20.0378 min retention time fig (3D). 14.80485 %. .beta.-Tocopherol with formula C28H48O2 at 33.0071 min retention time fig (3E). 9.25507 % Octacosane with formula C28H58 at 33.1754 min retention time fig (3F).

Table 1. Phytocompounds were detected in the seeds oil heat extraction of C. colocynthis analyzed with GC-MS.

Retention time	Compound name	CAS ID NO./NIST library	Molecular weight	Area (%)
20.0378	Diethyl Phthalate	84-66-2	222.2372	20.90935
23.9681	n-Hexadecanoic acid	57-10-3	256.4241	43.11012
25.7113	11-Octadecenoic acid, (Z)-	506-17-2	282.4614	63.97421
31.0386	Squalene	111-02-4	410.7180	24.39150
33.0071	.beta.-Tocopherol	148-03-8	416.6795	14.80485
33.1754	Octacosane	630-02-4	394.7601	9.25507

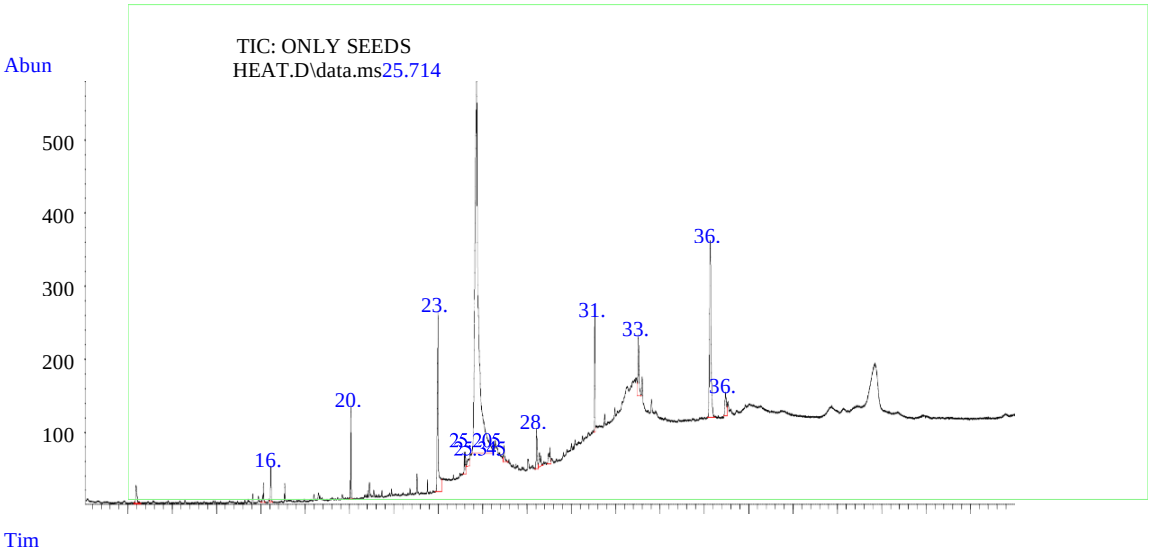


Fig. 1. Total ion chromatogram (TIC) of extracts of C. colocynthis only seeds heat oil.

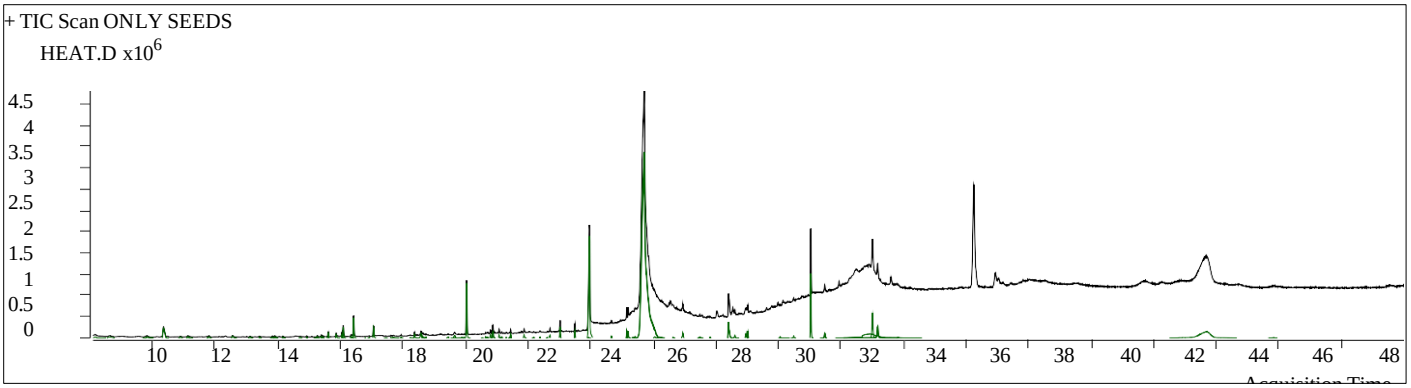
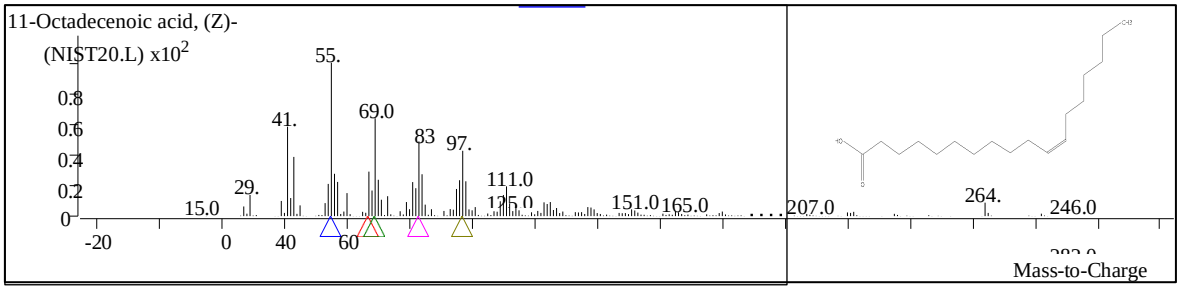


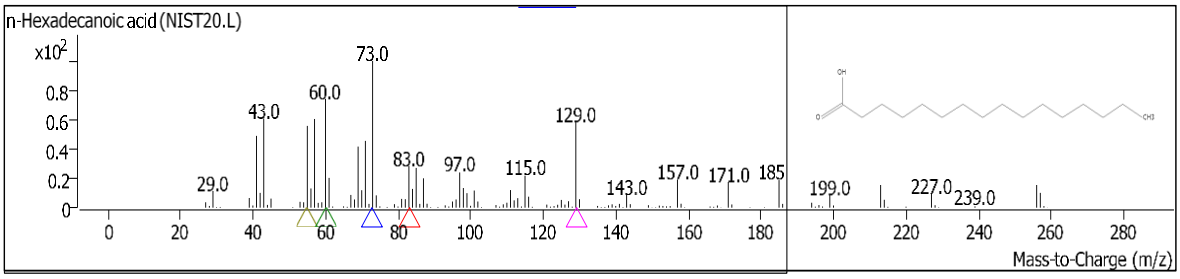
Fig. 2. GC-MS analysis of C.colocynthis only seeds heat oil extraction.

The medicinal plant is considered one of the medicinal plants that has therapeutic effects against many diseases [39]. Based on this principle, we chose this plant as a new therapeutic method. Through the results of the GC-MS technique, chemical compounds appeared in the extracted oil, and the reason for the selective toxicity to cancer cells may be attributed to one of these active compounds. Moreover, the results of this study revealed that these compounds are able to bind to other molecules within one of the cellular pathways, which hinders the rapid proliferation of tumor cells. And discourage it, and this is what was achieved. These compounds are also characterized by their biological activity due to

their stereoscopic structure, which belongs to chemical classes known for their antibacterial and anti-cancer properties [40].



(A)



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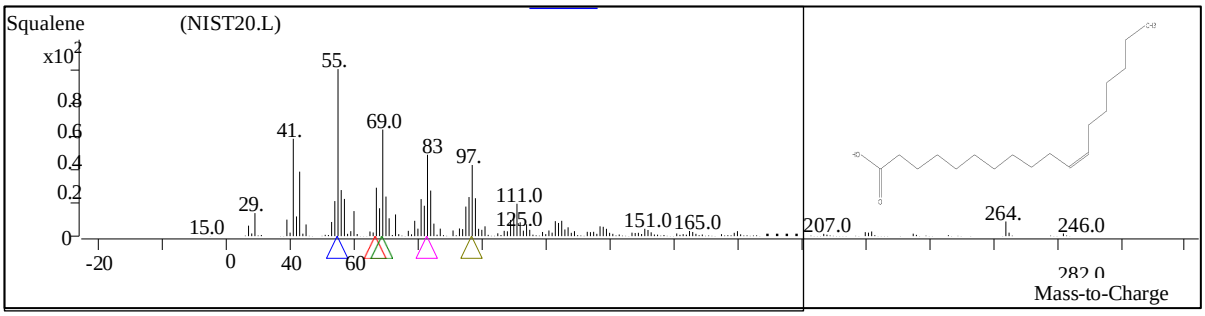

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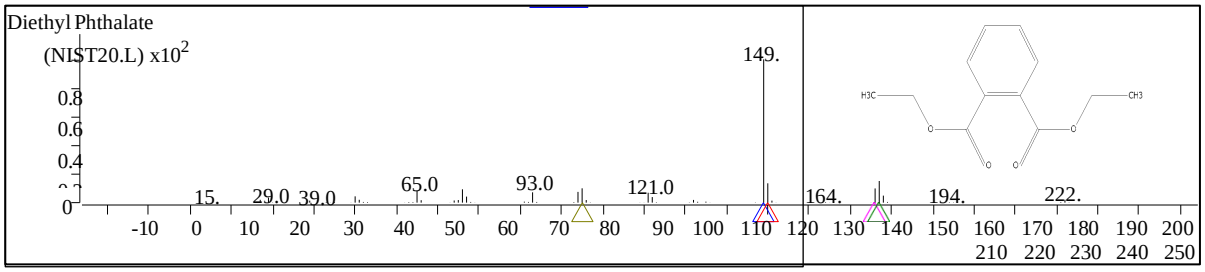
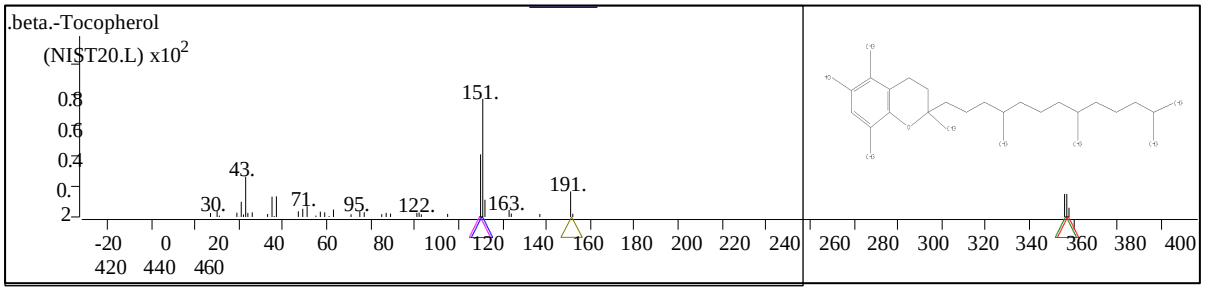
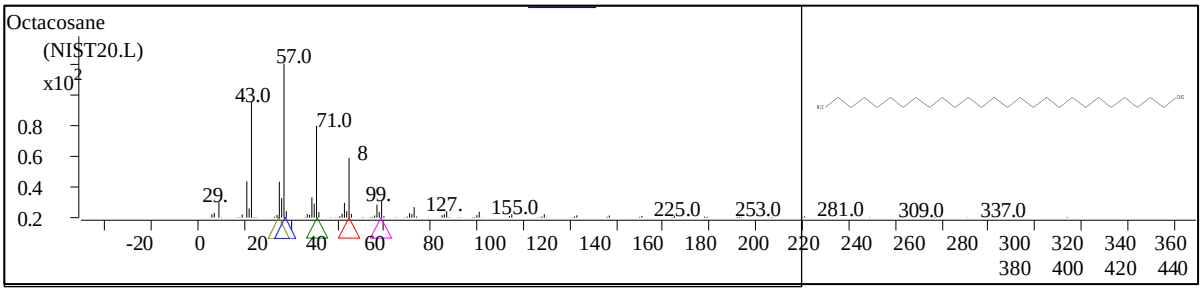


(D)



(E)



(F)

Fig. 3. The full time scan (48 min) of mass spectra to only seeds heat oil of C.colocynthis. The mass spectra illustrated based on the area percentage: (A) 11-Octadecenoic acid, (Z)-; (B) n-Hexadecanoic acid; (C) Squalene; (D) Diethyl Phthalate; (E) .beta. -Tocopherol; (F) Octacosane.

3.2 Enhanced cytotoxic effect and suppressed proliferation by C.colocynthis seeds oil extract against AMGM-5 cancer cells

To investigation the effect of C.olocynthis seeds heat oil on the inhibition or viability of AMGM-5 and HBL-100 cells, we used MTT assay protocol. The cytotoxic effect of C.olocynthis oil on above cell lines had illustrated by this assay. Cells seeded in a 96-tissue culture well plat at appropriate density and incubate in humidified conditions to grow in RPMI 1640 medium and left to achieved a monolayer. After the confluence reached to (75-90 %), the cells treated with (0.062, 0.125, 0.25, 0.50, and 0.75 %) for three separated duration times 24, 48, 72 h and four replicates for each concentration and triplicates for each experiment. The result indicates that the effect of C.olocynthis was increase with dose-dependent manner, due to as concentrations dose, so did the effect was rising. The first period in 24 h, noticeable the average of cytotoxicity in normal HBL-100 cells, as range of impact from 3.5 to 31.2 % at higher dose. However, the above concentrations evaluated slightly cytotoxicity against cancer AMGM-5 cells averaged from 4.9 to 53.5 % after 24 h of oil exposure, while the cytotoxicity assay analysis exhibit that IC50 value of C.olocynthis oil on cancer cells was 0.24 %, but, normal cells not achieved IC50 value as demonstrated in Table (2) and fig (4A and B).

Table 2. The results of MTT assay after treated cancer and normal cells for 24 h, showed the average of four replicates for each concentration and three replicates for this experiment. The percentage of inhibition rate and IC_{50} were obtained with GraphPad Prism software.

Concentration %	Cytotoxicity % in HBL-100 cells	Cytotoxicity % in AMGM-5 cells	IC_{50} in HBL-100 and AMGM-5 cells
0.062	3.5	4.9	- / -
0.125	8.8	18.3	- / -
0.25	17.2	29.5	- / -
0.50	25.9	39.4	- / -
0.75	31.2	53.5	- / +

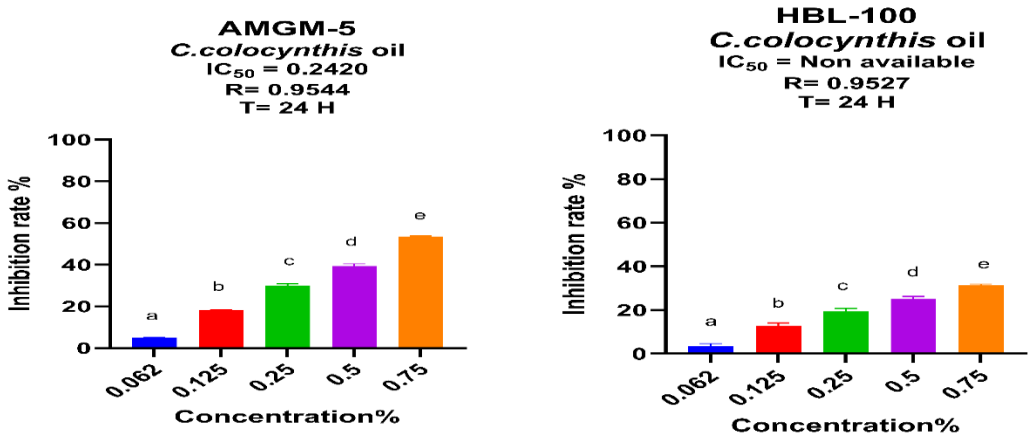


Fig. 4. The effect of C.colocynthis oil based on cytotoxicity assay after treated cells with several concentration for 24 h, demonstrated a slightly toxic impact on cancer cells, but, not in normal cells at (0.75 %) higher dose.

Furthermore, after treated cells for 48 h the cytotoxicity effect of oil against AMGM-5 cells was increase by increase duration time, thus the impact exhibit two side as dose-dependent and time-dependent manner, the range was from 9.4 to 60.1 % in cancer cells. Opposite, at the same time C.colocynthis seeds oil don't shown significant cytotoxic impact against normal cells while the ranged from 5.7 to 35.1 % as showed in Table 3 and fig (5A and B).

Table 3. The results of MTT assay after treated cancer and normal cells for 48 h, showed the average of four replicates for each concentration and three replicates for this experiment. The percentage of inhibition rate and IC_{50} were obtained with GraphPad Prism software.

Concentration %	Cytotoxicity % in HBL-100 cells	Cytotoxicity % in AMGM-5 cells	IC_{50} in HBL-100 and AMGM-5 cells
0.062	5.7	9.4	- / -
0.125	11.6	24.2	- / -
0.25	18.3	36.8	- / -
0.50	26.9	52.5	- / +
0.75	35.1	60.1	- / +

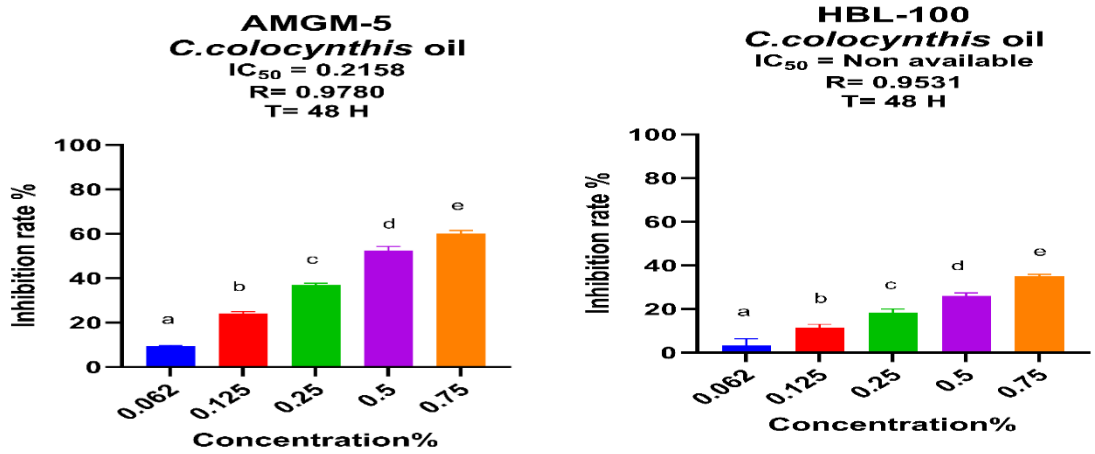


Fig. 5. The effect of C.colocynthis oil based on cytotoxicity assay after treated cells with several concentration for 48 h, demonstrated a slightly toxic impact on cancer cells, but, not in normal cells at (0.75 %) higher dose.

In the last duration time 72 h, the cytotoxicity had increased in cancer cells with compared in 24 and 48 h, the effect was exhibiting significant difference and the cytotoxic ranged reach from 12.6 to 67.8 %. In contrast, normal cells not affected with C.colocynthis oil treated cells and the average of cytotoxicity from 7.4 to 36.4 % as illustrated in Table (4) and fig (6A and B).

Table 4. The results of MTT assay after treated cancer and normal cells for 72 h, showed the average of four replicates for each concentration and three replicates for this experiment. The percentage of inhibition rate and IC_{50} were obtained with GraphPad Prism software.

Concentration %	Cytotoxicity % in HBL-100 cells	Cytotoxicity % in AMGM-5 cells	IC_{50} in HBL-100 and AMGM-5 cells
0.062	7.4	12.6	- / -
0.125	14.6	28.1	- / -
0.25	21.7	51.4	- / +
0.50	30.8	57.5	- / +
0.75	36.4	67.8	- / +

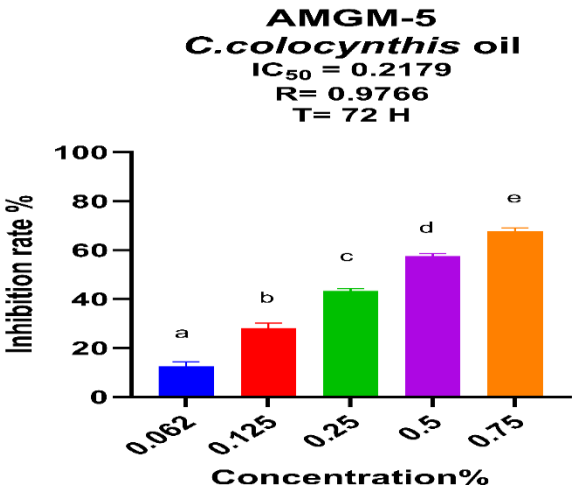


Fig. 6. The effect of C.colocynthis oil based on cytotoxicity assay after treated cells with several concentration for 72 h, demonstrated a slightly toxic impact on cancer cells, but, not in normal cells at (0.75 %) higher dose.

3.3 Induced apoptotic cell death with enhance C.colocynthis oil to antitumor activity

The results of the light inverted microscopy evaluated that the cancer AMGM-5 and normal HBL-100 untreated cells (positive group) after 72 h were have been elongated and fusiform shape, the nucleus of them appear like ovum shape, furthermore, the normal cells that exposure with IC_{50} of oil fig (7A and B). The treated AMGM-5 cancer cells occur area of clear cells as atrophy and damage of cell membrane and nucleus [42], however, normal cells appeared as a monolayer and elongated after treatment for 72 h with C.colocynthis seeds oil fig (7C and D). The hallmarks of cancer considered as a fingerprint characteristic that include resisting of apoptosis signals, immortal and rapid rate proliferative, metastasis, angiogenesis, and avoiding growth suppressors [43,44]. Thus, approach treatment strategies aimed to inducing apoptotic cell death and cell cycle arrest are remain clear significance issue. The behavior of C.colocynthis oil was exhibited induce both mitochondria dependent apoptosis pathway (the intrinsic) and death receptor-dependent apoptosis pathway (the extrinsic) which finding in the present study.

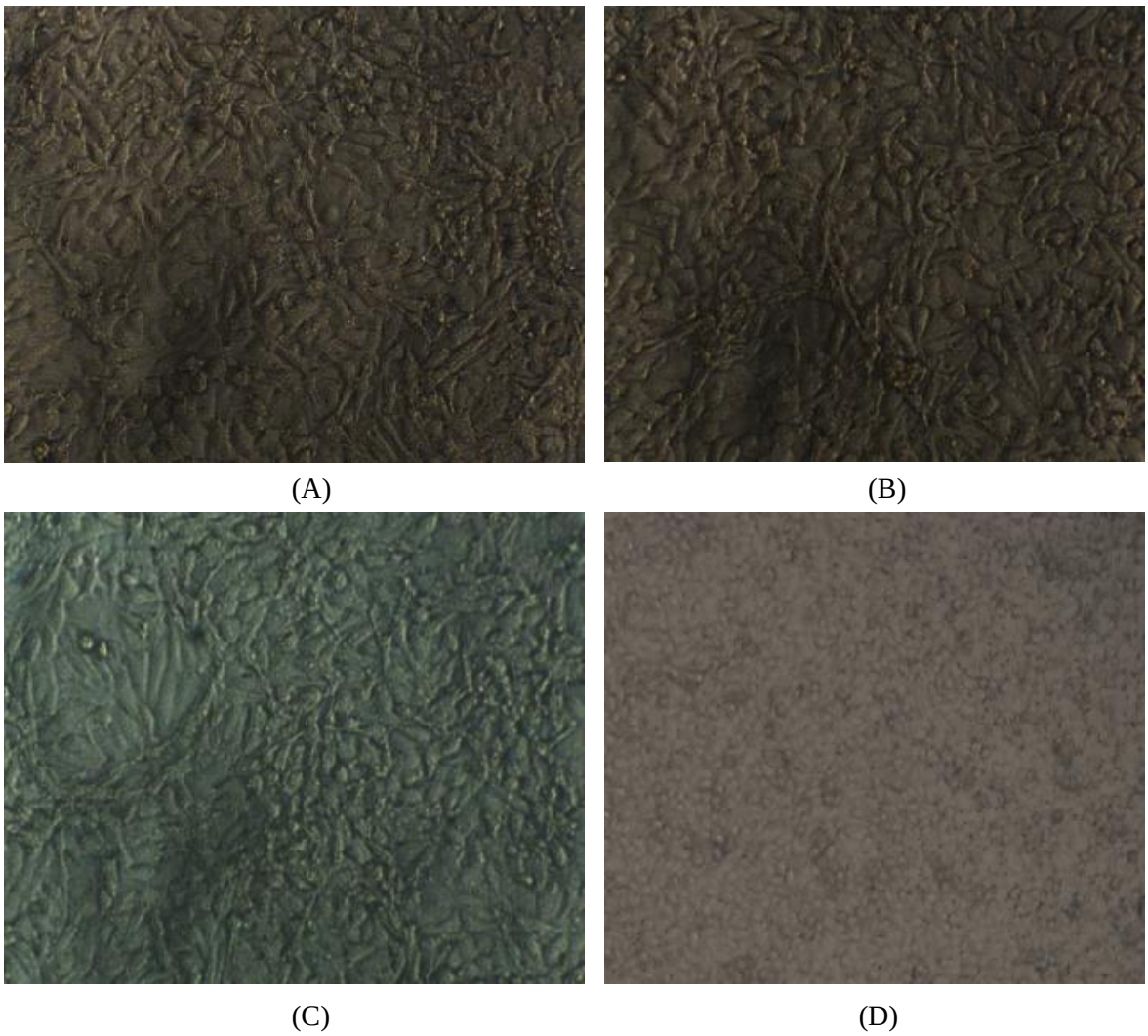
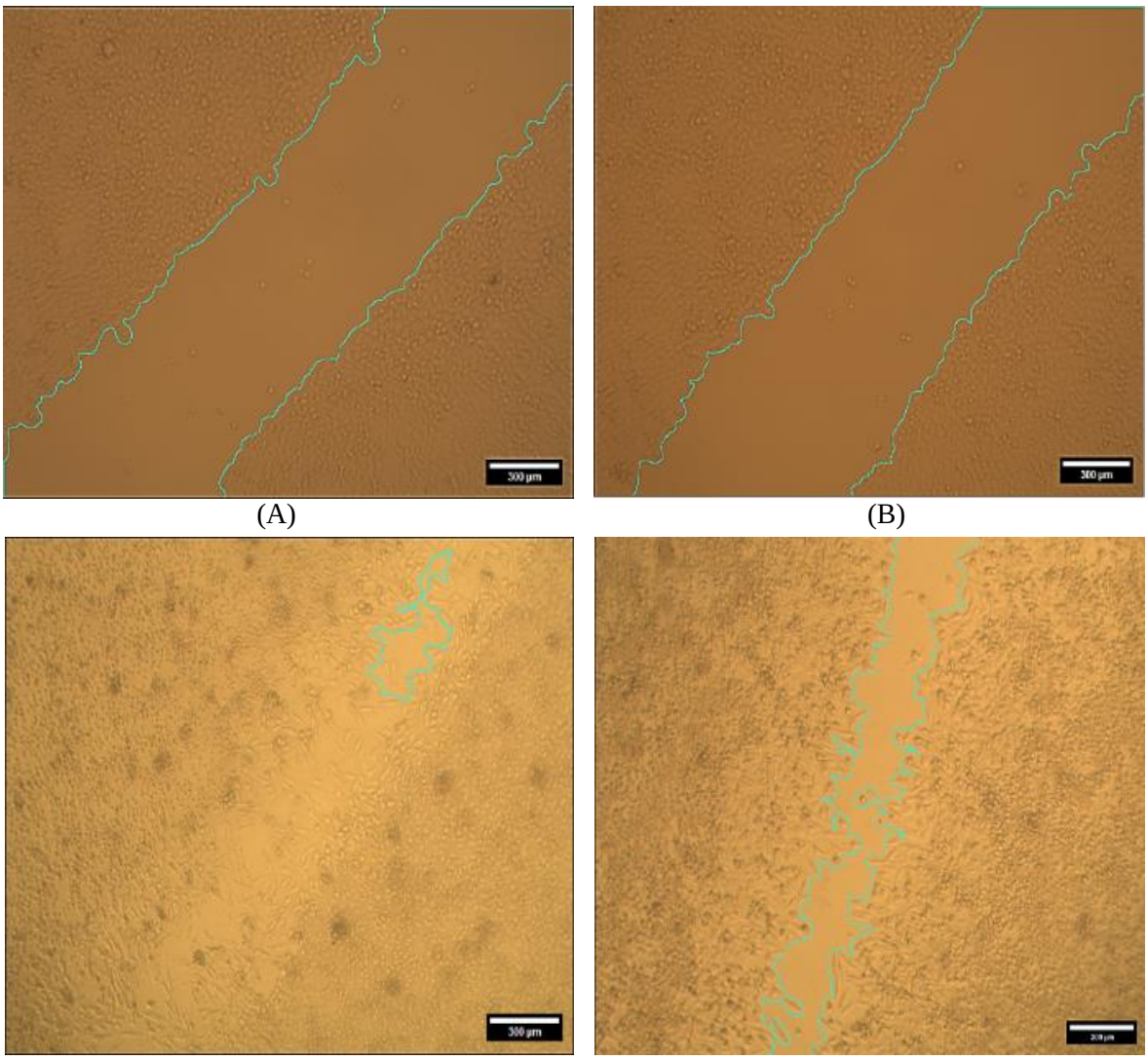


Fig. 7. The morphological changes of cell lines after treatment with C.colocynthis seeds oil for 72 h. 40X magnification (A) Normal HBL-100 cells appeared as active and fusiform shape; (B) Cancer AMGM-5 cells appeared as active and elongated shape; (C) Normal cells were not affected and no sings any histopathological changes; (D) Cancer AMGM-5 cells appeared as damaged of cell membrane and bubbles in cytosol indicates to apoptotic cell death.

3.4 The effect of C.colocynthis seeds oil on the in vitro cells invasion and migration

The monolayer wound healing assessment had evaluated and investigated to observed the effect of C.colocynthis oil on both normal HBL-100 and cancer AMGM-5 cell lines. After the cells formed a confluency monolayer, cells treated with IC_{50} dose of oil in fresh RPMI-1640 medium supplemented with 10 % serum and incubated in 37 ºC and 5 % CO2 for 72 h. The cultures photographed directly after cells wounded in 0 h for both treated and untreated cells, further, captured at 72 h. Our outcomes based on cell migration assay, showed that the area of wounded normal cells was de-creased once 72 h fig (8A, B, C, and D).

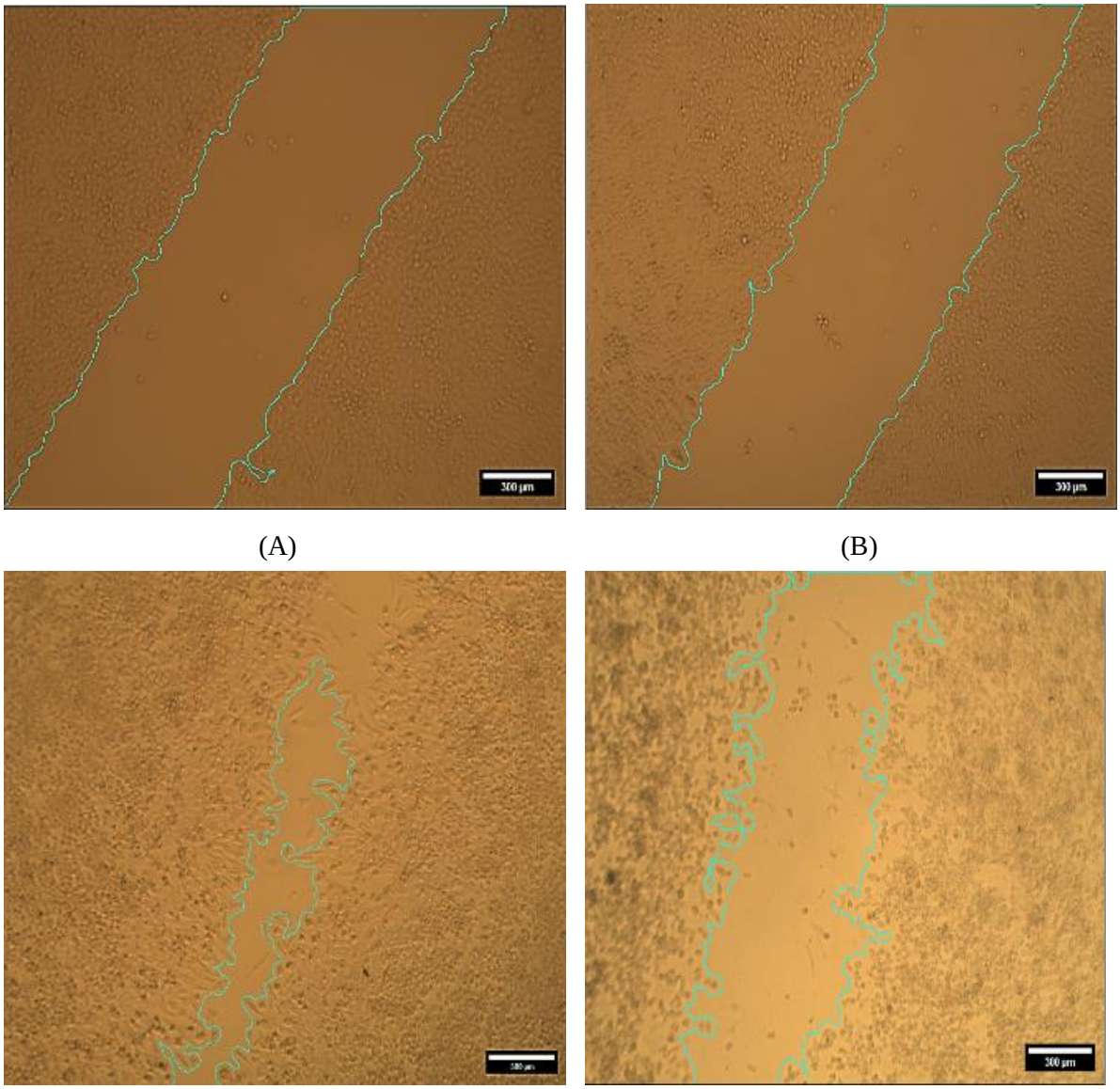


(A) (B)

(C) (D)

Fig. 8. Wound healing evaluation the effect of C.colocynthis seeds oil against normal HBL-100 cell line (A) The control (untreated) cells in 0 h; (B) The treated normal cells with IC_{50} concentration of oil; (C) HBL-100 untreated cells in 72 h; (D) HBL-100 cells treated with IC_{50} concentration of oil.

However, the area of wounded cancer cells was still at a same size, due to the cells lost their ability to proliferation and arrested of cell cycle Fig. 9A, B, C, and D. The migration of normal cells was highly than cancer cells after treatment. The area indicate to the impact of C.colocynthis seeds oil. In the AMGM-5 cells the invasion had inhibited and more upregulated with compare of control cells. The essential oil may be breakdown of the cancer energy production pathway (glycolysis) and leading to ATP depletion, thus the cell cycle arrested and rapid rate proliferation defects, on the other hand, healthy cells not affected and their proliferation was contentious. The area had analyzed with Image J soft-ware and measured according to [37].



(A) (B)

(C) (D)

Fig. 9. Wound healing evaluation the effect of C.colocynthis seeds oil against cancer AMGM-5 cell line (A) The control (untreated) cells in 0 h; (B) The treated AMGM-5 cancer cells with IC_{50} concentration of oil; (C) AMGM-5 untreated cells in 72 h; (D) AMGM-5 cells treated with IC_{50} concentration of oil.

3. Conclusion

The aim of present study in cancer therapies is widely recognized to be to reduce the cytotoxicity of chemotherapeutic medicines while increasing the probability and degree of therapeutic responses. Our findings showed that C.colocynthis oil might increase cytotoxicity by blocking the energy production and triggering apoptosis, which in effect could reduce the growth and proliferation of the AMGM-5 cancer cell line. The current study discovered that phytochemicals at low concentrations induced apoptosis in glioblastoma AMGM-5 cells.

On normal cells, this concentration of the bioactive compounds showed less cytotoxicity. These effects were associated with particular limitations in the essential oil high rate of proliferation, invasion, migration, and metastasis. In the near future, novel data from our

research will help create innovative, well-targeted therapeutic approaches for the treatment of cancers of the head and nick and other tumors.

Declaration of competing interest

The authors declares that they have no competing interests.

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إمكانات زيت نبات الكولوسينثيس المضادة للسرطان في تعزيز قمع خلايا الورم الأرومي الدبقي البشري من سلالة AMGM-5

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الكلمات المفتاحية

السمية الخلوية، الهجرة الخلوية، سرطان الخلايا الدبقية البشري، الخطوط الخلوية السرطانية، الموت الخلوي المبرمج، تقنية التآم الجروح، النباتات الطبية، العلاج المضاد للسرطان، قدرة الخلايا السرطانية على الغزو و الأنتشار.

الملخص

مع الارتفاع المستمر في معدلات الإصابة بالمرض والوفيات، يركز العلماء جهودهم على التوصل إلى علاجات جديدة لتحسين بقاء مرضى السرطان على قيد الحياة. وكانت السمات الرئيسية غير المرغوب فيها للعلاجات التقليدية هي الانتقائية المنخفضة، والسمية العالية، ومقاومة الأدوية، والنفقات الاقتصادية. وفي المقابل، تلعب المركبات الطبيعية للنباتات الطبية دوراً حاسماً في علاج العديد من الأمراض. حاليًا، بحثت العديد من الدراسات في إمكانات المستقلبات الثانوية لمحاربة الخلايا السرطانية من خلال تعزيز موت الخلايا المبرمج أو مسار إنتاج الطاقة كاستراتيجيات علاجية مستهدفة وذكية. هدفت الدراسة إلى تقييم ودراسة النشاط المضاد للأورام لمستخلص زيت ثمار نبات Citrullus colocynthis ضد السرطان وخطوط الخلايا الطبيعية. تم استخدام الورم الأرومي الدبقي البشري AMGM-5 والخلايا الظهارية للثدي البشري HBL-100 لتحديد التأثير السام للخلايا لزيت C.colocynthis عن طريق فحص MTT والكشف عن جرعة IC50. وعلاوة على ذلك، نقوم بتقييم موت الخلايا المبرمج عن طريق التصبيغ المتفلور. بالإضافة إلى ذلك، قمنا بالتحقيق في هجرة الخلايا، والغزو، وانتشار الخلايا السرطانية بمعدل سريع عن طريق فحص التئام الجروح. أظهرت النتائج تأثيرات كبيرة للزيت العطري مصحوبة بالتسمم وموت الخلايا المبرمج وتثبيط تكاثر الخلايا وهجرتها وغزوها ضد الخلايا السرطانية، ولكن الخلايا الطبيعية لم تتأثر. تشير النتائج التي توصلنا إليها إلى أن المركبات الطبيعية تحتوي على أدوية محتملة للسرطان دون آثار جانبية على الخلايا الطبيعية.

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