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Effect of chemical composition, extraction methods, and therapeutic efficacy of garlic oil against various cancer cell lines

Ramadan M. Aboelhaggag[1*]; Hesham A. Eissa[1]; Mostafa T. Ramadan[1]; Mohamed K. Morsy[2] and Ahmed I. El-Desouky[2]

[1]Food industries and Nutrition research institute, National Research Centre, Giza, 12622 Egypt.
[2]Department of Food Technology, Faculty of Agriculture, Benha University, Toukh, 13736, Egypt

Abstract
Garlic oil is rich in organosulfur compounds (OSCs) that has significant anticancer effects. This study investigates the anticancer properties of garlic essential oil (GEO) at different concentration (0.5%, 0.25%, 0.125%, 0.0625%, 0.0312%, and 0.0156% V/V) and its bioactive components extracted through various methods, including hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assisted extraction (UEA). The chemical composition of garlic essential oils (GEOs) obtained using three extraction techniques has been studied by GC–MS analysis. GEO that was extracted by HD and SCF method contains almost the same percentage of the major constituents diallyl trisulfide and diallyl disulfide (63.16%) and (63.78) of the same components, respectively compared to GEO produced by UAE method. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed to study the effects of garlic oil on five human cancer cell lines (Caco-2, HepG2, A549, MCF7, and PC3) for 24 and 48 h. Results demonstrated that GEO exhibits significant cytotoxic activity against all tested cancer cell lines, with varying levels of efficacy depending on the extraction method used. Proliferation of all cells was obviously inhibited in the first 24 hours with the MTT assay for oils extracted by SCF and UEA methods. The inhibition effect was more significant after 48 h for all oils. The IC_{50} values demonstrate the potential of GEO as a natural therapeutic agent in cancer treatment especially oils extracted by SCF. This research highlights the importance of garlic-derived compounds in developing effective anticancer therapies and encourages further exploration into their mechanisms of action.

Keywords: garlic essential oil, MTT cytotoxicity, cancer cells, cell line

1. Introduction

Cancer is a prevailing contributor to global mortality, ranking as the second most common cause of death, behind cardiovascular disorders [1]. Initially, cancer arises from the distortion of a normal cell due to genetic abnormalities in DNA [2]. This aberrant cell reproduces asexually, disregarding signals associated with the control of cell development in its vicinity, therefore acquiring invasion property and inducing alterations in surrounding tissues [3]. Cancer is a significant health challenge in both developing and industrialized nations. Yearly, the global cancer incidence is 182 cases per 100,000 individuals, resulting in 102 deaths [4]. The World Health Organisation reports that there are 14 million cancer cases and 8 million cancer-related deaths per year globally [5]. For several decades, scientists have been investigating plants and vegetables to find naturally occurring chemo-preventive substances that are very successful in preventing cancer development and these compounds might be used with current pharmaceutical treatments [6-9]. Numerous research studies have investigated garlic's dietary anticarcinogenic properties using aged garlic extract (AGE), aqueous garlic extract, and dried garlic powder [10,11]. There is a crucial necessity for developing more effective strategies to mitigate morbidity and mortality, as well as the substantial economic impact linked to the disease [12]. Another crucial approach to mitigating this formidable public health hazard is the utilization of synthetic or natural substances to counteract, delay, or reverse the process of carcinogenesis [13]. More than 60% of the existing anticancer medications have been obtained from various mechanisms, from natural sources [14]. Furthermore, beyond their nutritional value, some natural components contribute to the maintenance of good health, therefore mitigating the likelihood of various illnesses, including cancer [15]. Phytochemicals have been extensively studied and have shown anticarcinogenic effects by modulating several pathways, such as cell proliferation, differentiation, apoptosis, angiogenesis, invasion, migration, and metastasis, so impairing cancer initiation, promotion, and progression [16-18].

Garlic (*Allium sativum* L)., is a long-standing plant that has been grown for its nutritional and therapeutic properties. The remarkable plant possesses a range of pharmacological properties including antibacterial, antiarthritic, antithrombotic, anticancer, hypoglycemic, and hypolipidemic effects. Among garlic's several advantageous pharmacological properties, anticancer action is likely the most extensively researched [19]. The anticancer impact is likely the most prominent among the various health advantages of garlic. Recent researches indicate that dietary garlic consumption offers significant protection against cancer risk. Prior research on garlic phytochemicals has predominantly concentrated on their cancer chemopreventive attributes [20,21]. Considering the multitargeted

*Corresponding author e-mail: rm.abdel-hafez@nrc.sci.eg (Ramadan Aboelhaggag).
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activities against carcinoma and the absence of significant toxicity, certain constituents of garlic are believed to be essential in the selective eradication of cancer cells [22]. This study provides a comprehensive analysis of the roles of garlic oil, its bioactive components in combating many forms of cancer. It also investigates the potential for using these chemical compounds in medications for cancer treatment.

1. Materials and Methods

2.1. Raw materials and chemicals

Garlic (*Allium sativum* L) cultivars, namely, white-skin garlic bulbs, were purchased from (Horticulture institute, Agricultural Research Centre, Giza, Egypt). MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide), was obtained from Merck KGaA (Darmstadt, Germany). Ethanol 85%, sodium sulfate. MTT solution: 5mg/mL of MTT in 0.9% NaCl. Acidified isopropanol: 0.04 N HCl in absolute isopropanol. All other chemicals and reagents were of analytical grade

2.2. Cells and cell culture

Human epithelial colorectal adenocarcinoma (Caco-2), human epithelial-like hepatocellular carcinoma (HepG2), human epithelial lung carcinoma (A549), human epithelial mammary gland breast adenocarcinoma (MCF7) and human prostate carcinoma (PC3) cell lines, were all purchased from ATCC, USA. The RPMI-1640 medium was used for culturing the Caco-2, MCF7, and A549 cell lines. For the HepG2 cell line, cells were cultured in DMEM. All the media were supplemented with 10% fetal bovine serum (FBS), two mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/mL streptomycin sulphate, and 250 ng/mL amphotericin B. All from Lonza (Basel, Switzerland). Cells were maintained at sub-confluency at 37ºC in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%.

2.3. Extraction methods of GEO:

2.3.1. Hydrodistillation HD:

Garlic puree was blended for 3 min using a Lab Stomacher Blender 400-BA 7021 (Seward Medical, UK). The puree was combined with water (crushed garlic: water, 1:6; w/v) and put into 5-L round-bottom flasks, to which additional distilled water was added to facilitate hydro-distillation. Clevenger apparatus was used for the hydro-distillation of the essential oil for 3 h. Garlic essential oil (GEO), being thicker than water, was extracted from the side arm of the Clevenger apparatus, dehydrated using anhydrous sodium sulfate, and preserved in dark brown vials at 4°C until required [23].

2.3.2. Supercritical fluid (SCF) CO_2:

Garlic essential oil (GEO) was extracted using a laboratory-scale apparatus at the National Research Centre (Speed TM SFE-2/2, Applied Separations, in partnership with the USDA, USA). A stainless steel extraction cell with an estimated capacity of 20 mL was thermoregulated in the laboratory oven, and the system's backpressure was regulated using an LF-540 Pressure Tech valve (USDA1 - USA). For each extraction, 100 grams of finely powdered material were deposited in the extraction cell, with small amounts of glass wool positioned at both the top and bottom to avoid system clogging. Ethanol at 85% concentration was delivered by a Jasco PU2080 HPLC pump (Jasco Inc., Easton, PA) and mixed under high pressure with supercritical CO_2 as a solvent modifier. The combination of compressed CO2 and solvent modifier was sent into the heater before entering the extraction cell. Dynamic extractions were performed using high-purity CO_2 at a constant flow rate of 10 mL min^{-1} in conjunction with the solvent modifier at a flow rate of 0.5 mL min−1. The extraction cell was placed in the oven at a temperature of 40±1°C and a pressure of 100 psi under ideal circumstances. Ethanol was used to enhance the solubility of analytes with moderate polarity. The addition of a modest quantity of water (15%) was used to enhance the extraction effectiveness of polar chemicals, therefore minimizing significant alterations in the supercritical conditions of CO_2.. The extraction length for all experiments was 120 min [24].

2.3.3. Ultrasound-assisted extraction (UAE):

One hundred grams of garlic puree was introduced into a round-bottom flask containing a specific volume of distilled water. The resulting mixture of garlic puree and H2O underwent ultrasonic treatment using an Ultrasonic cell disrupter equipped with microtip probe (Ultrasonic Get 750, USA) at 40 kHz for 40 min, applying a power of 600 W at a temperature of 30±1°C, with a pulsing cycle of 10 seconds on-time and off-time. After the ultrasonic treatment, the resultant mixture was promptly subjected to hydro-distillation for garlic oil extraction using Clevenger-type equipment, as delineated in the standard hydro-distillation method [25].

2.4. GC/MS profile

The presence of garlic essential oil components was assessed using a modified approach from Liu *et al*. [26]. The garlic oil was analyzed using gas chromatography (Agilent 8890 GC System) combined with a mass spectrometer (Agilent 5977B GC/MSD) and fitted with an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The oven temperature was originally set at 50 °C, then increased from 50 to 220 °C at a rate of 5 °C/min, then from 220 °C to 280 °C at a rate of 15 °C/min, and finally maintained at 280 °C for 7 minutes. Helium served as the carrier gas, with a flow rate of 1.1 mL/min. The essential oil was solubilized in diethyl ether (30 µL essential oil per mL of diethyl ether), and thereafter, 1 µL of this solution was injected into the gas chromatograph with a split ratio of 1:50. The injection temperature was 230 °C. Mass spectra were acquired in electron impact mode (EI) at 70 eV, using a scan m/z range of 39 to 500 amu. The isolated peaks were detected by correlating them with data from the mass spectra database of the National Institute of Standards and Technology (NIST).

2.5. MTT Cytotoxicity

The MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) was procured from Merck KGaA, Darmstadt, Germany. It is used to evaluate the cytotoxic effects of the examined substances on various cell lines. The method relies on the capacity of active mitochondrial dehydrogenase enzymes in living cells to break the tetrazolium rings of yellow MTT, resulting in the formation of dark blue insoluble formazan crystals, which are directly proportional to the number of viable cells. In summary, cells ($1x10^4$ cells/well)

were inoculated in the appropriate media for each cell line inside a flat-bottom 96-well microplate and subjected to treatment with 20 µl of successive dilutions of the tested samples (100 µl of pure oil + 900 µl DMSO), commencing with 0.5% V/V and concluding at 0.0156% V/V. The experiment was performed for both 24 hours and 48 hours at 37 ºC in a humidified 5% CO_2 environment. Following incubation, the media were discarded, and 40 µL of MTT solution per well was added and incubated for an additional 4 h. The MTT crystals were solubilized by the addition of 180 µl of acidified isopropanol per well, and the plates were agitated at room temperature, followed by photometric measurement of absorbance at 570 nm using a microplate ELISA reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Ortenberg, Germany). Each concentration was subjected to three repetitions, and the average was computed [27]. The percentage of relative viability was calculated using the following equation:

$$\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \text{X } 100$$

Then the half-maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose-response curve.

2.6. Statistical analysis

The data were analyzed using appropriate statistical tests (ANOVA o) to assess the significance of differences between treated and control groups. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using software such as GraphPad Prism and SPSS to ensure accurate calculations and visual representation of data [28].

3. Results and discussions

3.1. GC/MS profile

The volatile compounds in garlic oils produced by hydro-distillation, supercritical fluid, and ultrasound-assist extraction were analyzed using GC MS. **Figure (1-3)** and **Table (1)** illustrate qualitative and semi-quantitative variations in the volatile components extracted by hydro-distillation, supercritical fluid, and ultrasound-assisted extraction.

Although the predominant constituents of garlic oils derived during hydro-distillation, supercritical fluid, and ultrasound-assist remained the same, there were notable differences in the key compound proportions.

The major components of Garlic oil extraction by hydro-distillation were diallyl disulfide (DDS) and diallyl trisulfide (DTS). Garlic oil contains 36.79% DDS and 26.37% DTS. These two components together constitute 63.16% of the total composition of Garlic oil. On the other hand, the major component of garlic oil extracted by supercritical fluid was Diallyl trisulfide (DTS) 56.2%. Prior research has shown that DTS typically comprises 38.0–88.0% of garlic essential oil, but the DDS proportion may vary from 1.0 to 41.0% [29]. DTS is often the predominant component of Garlic oil, followed by DDS [30]. Nonetheless, garlic essential oils from various cultivars and locales exhibited 88.0–92.0% DDS and negligible quantities of DTS (0.0–0.1%) [31]. The fluctuation in the percentages of DTS and DDS may be attributed to genetic variety and regional origin.

Besides DTS and DDS in the current study, **Table 1** also revealed the presence of additional minor components such as diallyl sulfide, Allyl methyl disulfide, Dimethyl trisulfide, Allyl methyl trisulfide, Allyl methyl disulfide, and Diallyl tetrasulfide. The percentages of these compounds in garlic oil extracted by hydrodistillation and supercritical fluid were 32.56% and 42.04%, respectively.

Table (1). Chemical composition of the essential oil of *Allium Sativum* obtained by three different extraction techniques

Component name	Peak area%			
	RT	HD	SCF	UAE
Diallyl sulfide	4.728	7.71	7.45	2.83
Allyl methyl disulfide	5.935	12.04	8.95	0.43
Dimethyl trisulfide	7.240	0.88	nd	nd
Diallyl disulfide	10.152	36.79	7.58	2.36
Allyl methyl trisulfide	11.800	8.38	9.82	0.83
Diallyl trisulfide	16.281	26.37	56.2	5.22
Allyl methyl disulfide	13.878	0.78	nd	1.26
Diallyl tetrasulfide	18.404	2.77	8.24	0.34
β-Methylpyridine	10.730	0.43	nd	nd
Allyl (Z)-1-Propenyl disulfide	22.380	1.3	nd	0.3
Allyl (E)-1-Propenyl disulfide	18.512	1.6	nd	1.76
2-Vinyl-1,3-dithi-4-ene	13.934	0.5	nd	46.61
3-Vinyl-1,2-dithi-4-ene	13.172	nd	nd	21.3
2-Vinyl-1,3-dithi-4-ene	27.362	nd	nd	1.69
3-Isopropyl-4-methyl-1-decen-4-ol	32.837	nd	1.76	2.83

Hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).

Table (1) indicates that garlic oil obtained through hydrodistillation contains several minor components not found in garlic oil extracted via supercritical fluid, such as β-Methylpyridine (0.43%), Allyl (Z)-1-Propenyl disulfide (1.3%), Allyl (E)-1-Propenyl disulfide (1.6%), and 2-Vinyl-1,3-dithi-4-ene (0.5%). Likewise, the garlic oil extracted using supercritical fluid included a trace of 3-Isopropyl-4-methyl-1-decen-4-ol (1.76%), which was absent in the garlic oil extracted via hydrodistillation. The major components of GO extraction by ultrasound-assist extraction were 2-Vinyl-1,3-dithi-4-ene followed by 3-Vinyl-1,2-dithi-4-ene, which were 46.61% and 21.3%, respectively. These two components together constitute 67.91% of the total composition of GO. These compounds may be formed due to the mechanical and cavitation effects of ultrasound, which could promote chemical reactions or enhance the extraction of less volatile, thermolabile components [32]. However, traditional sulfur compounds like Diallyl disulfide and Diallyl trisulfide are present in much lower concentrations in garlic oil produced by ultrasound-assist extraction, potentially limiting its utility for applications that require these key components. The table further reveals the absence of certain components in specific methods, indicating the selectivity and limitations of each technique. For instance, Dimethyl trisulfide is only detected in HD, and β-Methylpyridine is exclusively found in hydrodistillation extraction at low levels, according to Bajer *et al* [33]. Conversely, ultrasound-assisted extraction produces compounds that are not detected in other methods of extraction, suggesting that it may extract a broader range of chemical structures. Supercritical fluid appears to prioritize certain dominant sulfur compounds at the expense of minor or unique constituents. According to El-Sayed et al. [17], dialyl disulfide (DDS) is the second most significant constituent of WGO, behind diallyl trisulfide (DTS). When the hydeodistillation method is employed for extraction, the components that make up 61.39% of the composition of WGO are 45.76% DTS and 15.63% DDS combined. According to earlier research, molecules with oxygen exhibit larger dipole moments than those without oxygen [34]. According to Kasamatsu et al. [35], garlic's primary volatile ingredients (nonoxygenated chemicals) were discovered to be polysulfides. Therefore, molecules containing sulfur that have higher polysulfide dipole moments than monosulfide compounds should be responsible for this occurrence. Garlic oils may contain more diallyl trisulfide due to their ionic and dipolar polarization when exposed to electromagnetic radiation. Garlic oils high in diallyl trisulfide may be used as natural ingredient candidates in nutraceutical and pharmaceutical products.

Diallyl disulfide is less effective than diallyl trisulfide at releasing hydrogen gas (H2S) [36]. Therefore, there has been a lot of interest in extracting garlic oil that is rich in diallyl trisulfide and incorporating the resulting oils into medical products to treat the chronic diseases stated above [37].

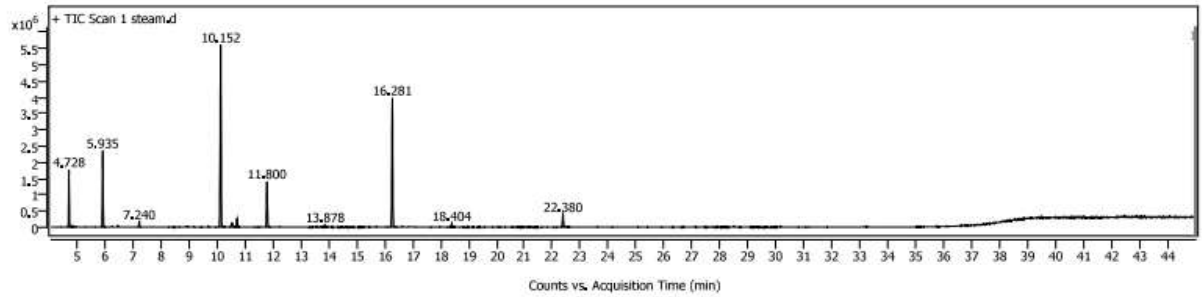

Fig (1): Chromatographic analysis for garlic essential oil was obtained by hydrodistillation extraction techniques.

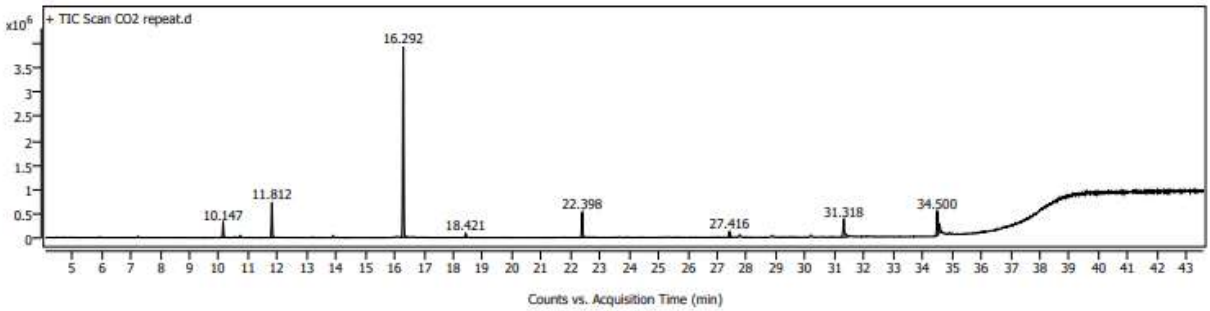

Fig (2): Chromatographic analysis for garlic essential oil was obtained by supercritical fluid extraction techniques.

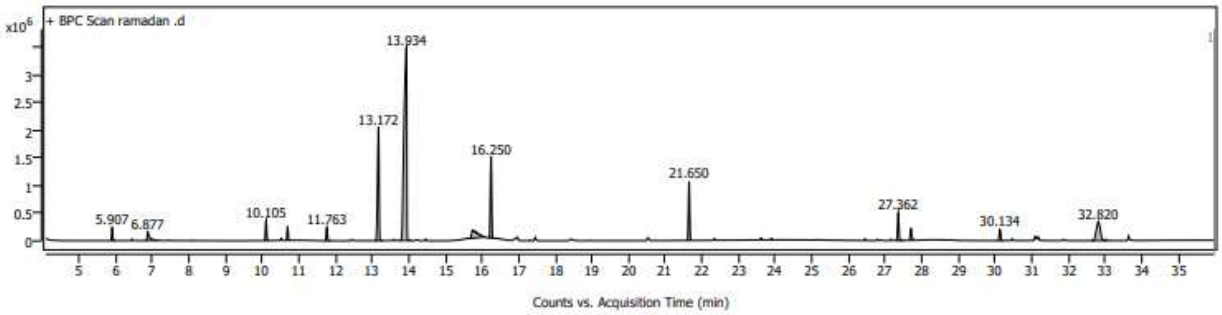

Fig (3): Chromatographic analysis for garlic essential oil was obtained by ultrasound-assist extraction techniques.

3.2. MTT Cytotoxicity assay

Garlic is recognized for its potential anticancer properties due to the presence of specific sulfur and organic compounds. These bioactive chemicals influence several biological pathways, including cell cycle disruption, inhibition of signaling pathways, and activation of apoptosis, autophagy, and antioxidant activity. They interact with various stages of cancer cell development, encompassing genesis, proliferation, growth, invasion, migration, and metastasis. The sulfur compounds in garlic, whether water-soluble or fat-soluble, exhibit anticancer effects by reducing oxidative stress, inhibiting carcinogen metabolism, and enhancing immune function [38]. Furthermore, Nkrumah *et al*. [39] identified two primary categories of compounds with demonstrated anticancer effects: lipid-soluble allyl sulfur compounds such as Diallyl disulfide (DADS) and Diallyl trisulfide (DATS), and water-soluble compounds like S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SAMC). To evaluate the impact of garlic essential oil on various cancer cells, we tested different concentrations of the oil (0.5%, 0.25%, 0.125%, 0.0625%, 0.0312%, and 0.0156% V/V). The results indicated a significant effect of the oil at both lower and higher concentrations (**Figure 4-8),** demonstrating that garlic essential oil effectively reduces cell viability in cancer cells.

Table (2) presents IC_{50} values, a measure of concentration needed to inhibit 50% of cell viability, for various extraction methods, Hydro distillation, Supercritical CO_2, and Ultrasound-assist extraction, across different cancer cell lines at two-time intervals (24 and 48 hours). The cell lines include Caco2 (colorectal carcinoma), HepG2 (liver carcinoma), A-549 (lung carcinoma), MCF7 (breast adenocarcinoma), and PC3 (prostate carcinoma), representing a range of cancer types. Lower IC_{50} values indicate higher toxicity, meaning the extract is more effective at inhibiting cell viability at lower concentrations. Hydro-distillation shows varying toxicity depending on the cell line and exposure time. It is ineffective, or data is unavailable for certain cell lines, such as Caco2 and HepG2 at 24 h, and shows minimal toxicity in MCF7, and PC3 cells at 48 h, as indicated by "Not toxic" labels. However, the oil was so potent on the A-549 cell line that even the lowest concentration of 0.0156% was still under the 50% cytotoxicity. In contrast, hydro distillation appears to be more toxic to Caco2 and PC3 cells at 48 hours, suggesting that this method's efficacy may increase with time in certain lines but lacks broad toxicity across all tested lines. Supercritical CO_2 extraction generally yields lower IC_{50} values than hydro distillation, implying a stronger anticancer effect across most cell lines and time points. It shows stable and high toxicity in Caco2, HepG2, and PC3 cells at both 24 and 48 h, demonstrating consistent effectiveness over time.

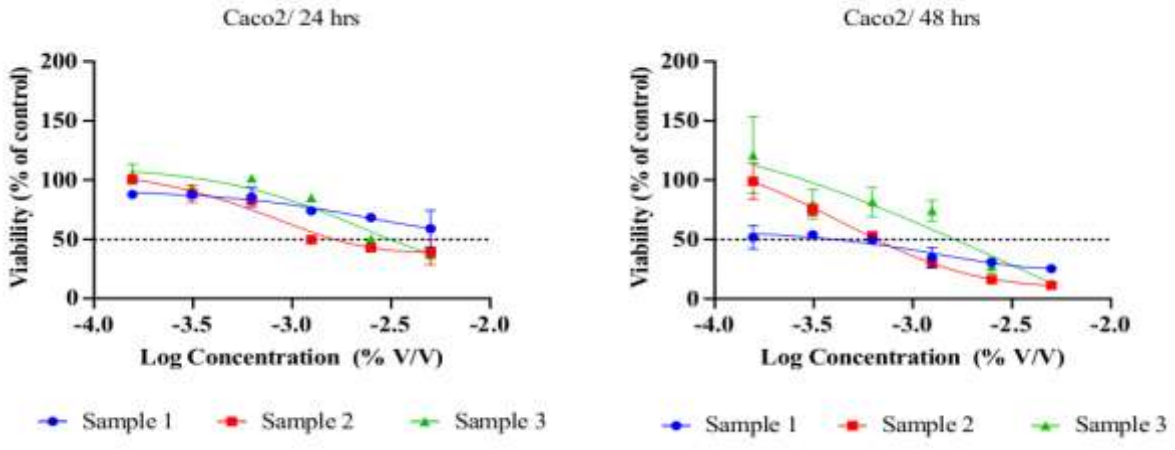

Fig (4): Evaluation of cell viability percentage of colorectal adenocarcinoma patient cancer cell line (Caco2) posttreatment with garlic essential oil: (sample 1) Hydrodistillation HD, (sample 2), supercritical fluid SCF, and (sample 3)ultrasound-assist extraction (UAE).

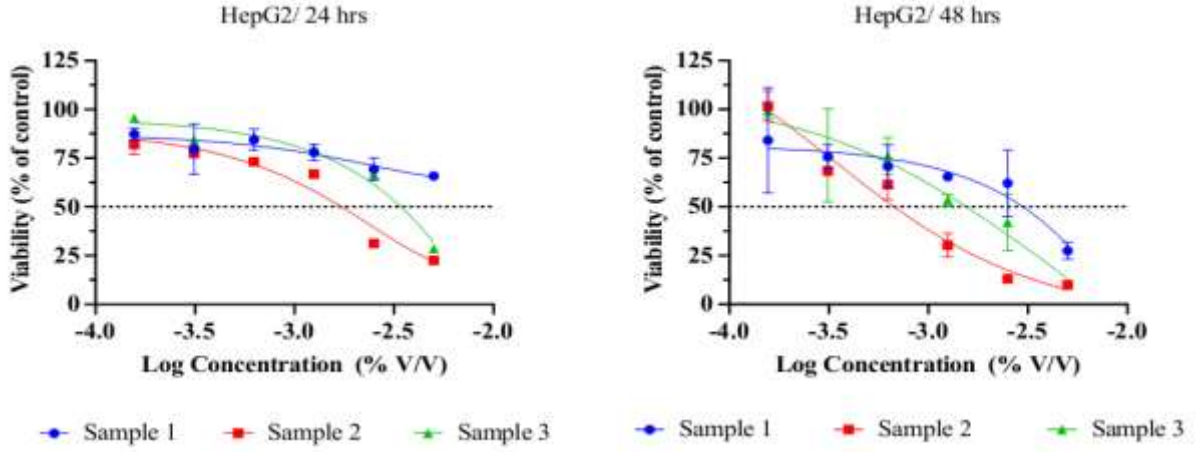

Fig (5): Evaluation of cell viability percentage of liver cancer cell line (HepG2) posttreatment with garlic essential oil: (sample 1) Hydrodistillation HD, (sample 2), supercritical fluid SCF, and (sample 3) ultrasound-assist extraction UAE.

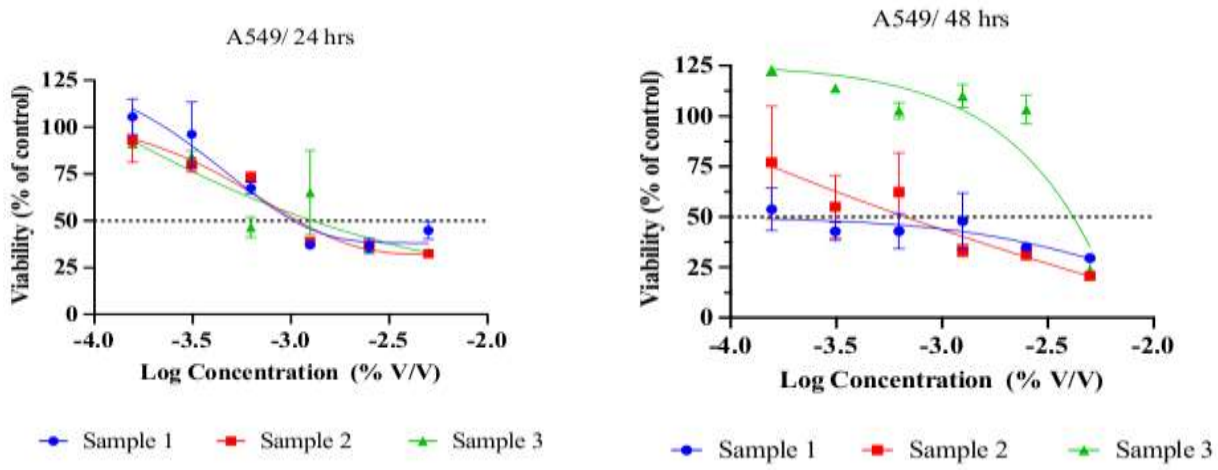

Fig (6): Evaluation of cell viability percentage of lung cancer cell line (A-549) posttreatment with garlic essential oil: (sample 1) Hydrodistillation HD, (sample 2), supercritical fluid SCF, and (sample 3) ultrasound-assist extraction UAE.

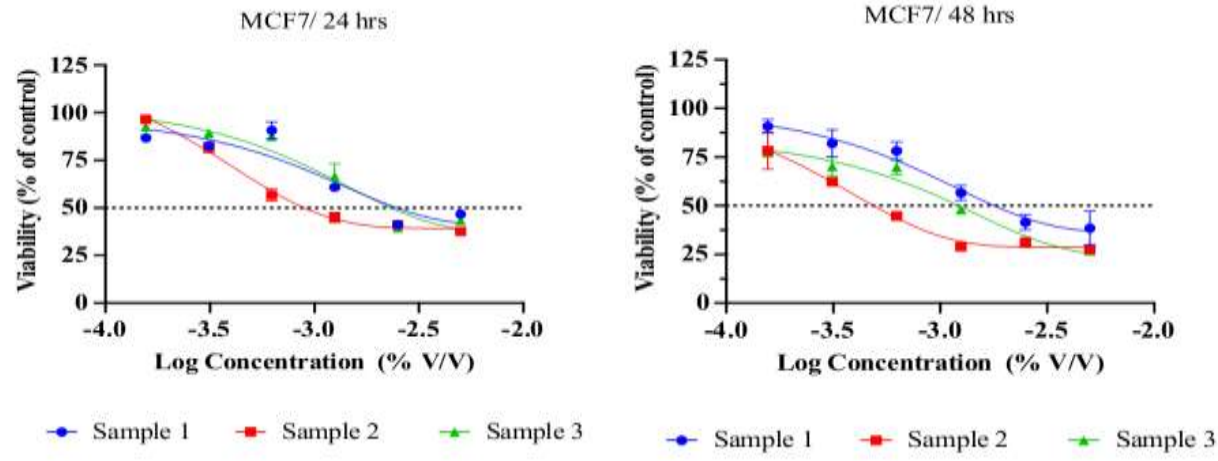

Fig (7): Evaluation of cell viability percentage of breast cancer cell line (MCF-7) posttreatment with garlic essential oil: (sample 1) Hydrodistillation HD, (sample 2), supercritical fluid SCF, and (sample 3) ultrasound-assist extraction UAE.

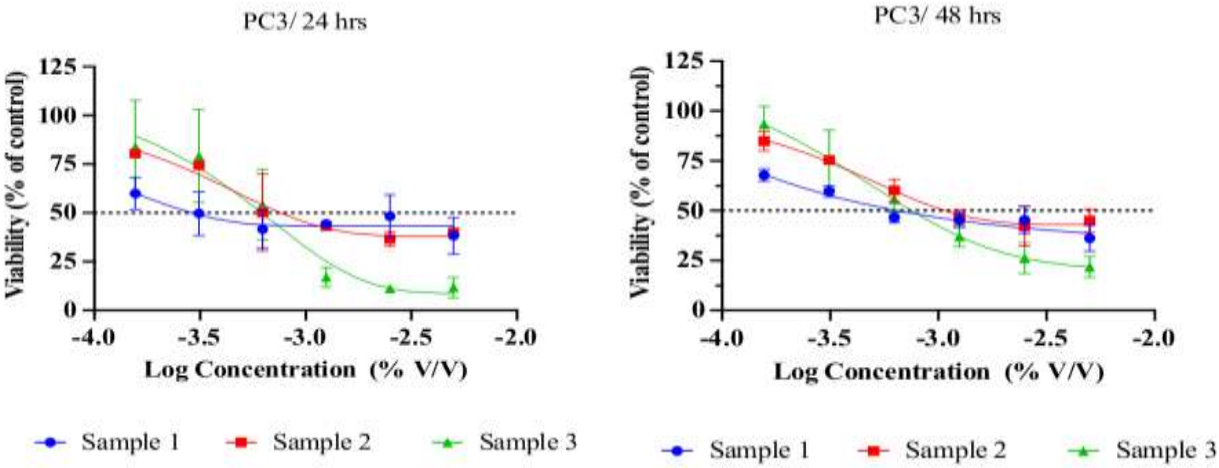

Fig (8): Evaluation of cell viability percentage of prostate cancer cell line(PC-3) posttreatment with garlic essential oil: (sample 1) Hydrodistillation HD, (sample 2), supercritical fluid SCF, and (sample 3) ultrasound-assist extraction UAE

The ultrasound-assist method produces higher IC_{50} values compared to supercritical CO_2, suggesting it is less potent for most cell lines. It demonstrates effectiveness in several lines at 24 hours but loses some potency by 48 hours, as seen with A-549 and PC3, where higher IC_{50} values are recorded. This pattern suggests that ultrasound-assisted extraction may be less stable over time in terms of maintaining its toxic effects on certain cancer cells. Garlic essential oil can induce apoptosis in all studied cell lines by reducing the mitochondrial membrane potential, hence activating caspase-3, caspase-8, and caspase-9. The obtained results were in agreement with those noticed by Zou *et al*. [40], where, in liver cancer cells, allicin has been shown to diminish the mitochondrial membrane potential and activate caspase-3.

Overall, the data illustrates that each extraction method exhibits unique efficacy across different cancer cell lines and time points, underscoring the importance of selecting appropriate extraction techniques in anticancer studies. Furthermore, the variability in response across cell lines highlights the complexity of cancer treatment and the potential need for tailored therapeutic approaches. The comparison with some extraction methods might yield compounds with synergistic effects, enhancing toxicity against cancer cells.

Table (2) The IC_{50} (% v/v) of each sample at each cell line at its respective time interval

Extraction	HD	SCF CO2	USE
Cell line		**mg/100 mL**	
Caco2/ 24 h	NT	164 ± 0.033[b]	312 ± 0.015[a]
Caco2/ 48 h	40 ± 0.011[c]	65± 0.006[b]	163 ± 0.026[a]
HepG2/ 24 h	NT	171± 0.004[b]	342 ± 0.003[a]
HepG2/ 48 h	298 ± 0.022[a]	67 ± 0.009[c]	155 ± 0.043[b]
A-549/ 24 h	103± 0.012[c]	108 ± 0.003[b]	128 ± 0.065[a]
A-549/ 48 h	NT	67± 0.048[b]	417 ± 0.015[a]
MCF7/ 24 h	247 ± 0.0001[a]	89 ± 0.002[c]	232 ± 0.022[b]
MCF7/ 48 h	179 ± 0.013[a]	48 ± 0.003[c]	122 ± 0.006[b]
PC3/ 24 h	29± 0.001[c]	76.1 ± 0.005[a]	61 ± 0.003[b]
PC3/ 48 h	64 ± 0.005[c]	107 ± 0.009[a]	75 ± 0.005[b]

† The data is represented with the standard deviation.
*Means (±SD) followed by different superscripts (within columns) is significantly different ($p \leq 0.05$). hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).
NT, Not toxic

The anticancer properties of garlic and its bioactive compounds have been extensively studied, revealing significant anti-proliferative effects across various cancer cell lines. Lan *et al.* [41] demonstrated that garlic oil exerts a dose- and time-dependent antiproliferative effect, particularly notable in PANC-1 cells. In their study, lower concentrations (2.5, 10, 25, and 50 µM/L) exhibited a significant inhibitory effect after 24 hours. Conversely, in AsPC-1 cell lines, the 48-hour exposure resulted in pronounced inhibition across all tested concentrations (2.5, 10, 25, 50, 100, and 200 µM/L). For Mia PaCa-2 and PANC-1 cells, significant inhibitory effects were observed only at higher doses (100 and 200 µM/L for PANC-1; 25, 50, 100, and 200 µM/L for Mia PaCa-2). These findings suggest that garlic oil may promote apoptosis or induce cellular damage in pancreatic cancer cells with statistical significance ($P < 0.05$). Rosas *et al*. [42] further explored the effects of allicin on breast cancer cell viability in luminal MCF-7 and triple-negative HCC-70 cell lines. Their results indicated that allicin's impact was most pronounced at lower concentrations. In MCF-7 cells, stimulation with 20 and 45 µM of allicin for 24 and 48 h resulted in cell viability reductions to approximately 30% and 25% after 24 h and around 20% and 25% after 48 h. Notably, these reductions surpassed those observed with the positive control (ETO), which showed viability percentages of approximately 60% and 37% at the same time points. Allicin also significantly reduced the viability of triple-negative HCC-70 cells at concentrations of 12 and 20 µM, indicating that these cells are more sensitive to allicin compared to MCF-7.

Miron *et al.* [43] assessed the proliferation rate of exponentially growing HL60 cells in the presence of allicin at varying concentrations (0–10 µM) over periods extending up to 72 hours. Their findings revealed that allicin inhibited cell growth in a concentration-dependent manner; specifically, HL60 cells experienced approximately 50% inhibition of proliferation after just 22 hours at a concentration of 5 µM, while a concentration of 10 µM resulted in an impressive 80% inhibition rate within the same timeframe. The impact on cell viability and proliferation is associated with G1 and G2/M cell cycle arrest in various tumour cells [44]. Wang *et al*. [45] emphasized the significance of allicin's effects on cell viability and proliferation, noting that tumours rely on continuous cell division to expand their numbers and establish an optimal microenvironment for growth. Supporting this notion, Pourzand et al. [46] found that high garlic intake correlates with a remarkable reduction up to 50% in breast cancer incidence among participants compared to controls. This relationship was further corroborated by case-control studies involving French women that indicated an inverse association between garlic consumption and breast cancer risk [47]. In prostate cancer research, Ijayababu *et al*. [48] reported that diallyl disulfide

significantly decreased the proliferation of prostate cancer cells in a dose-dependent manner relative to control groups. Notably, diallyl disulfide induced DNA damage in PC-3 cells at doses of both 50 µM and 100 µM, leading to the conclusion that this compound inhibits prostate cancer cell proliferation through apoptosis induction.

Chung *et al*. [49] further elucidated garlic's effects on prostatic health by showing that garlic treatment reduced relative prostate weight ratios, inhibited mRNA expression levels of androgen receptor (AR), lowered serum dihydrotestosterone (DHT) levels, and controlled prostatic tissue development in rats induced with benign prostatic hyperplasia (BPH). Additionally, garlic supplementation was associated with decreased levels of inflammatory proteins such as iNOS and COX-2 within prostatic tissue. Iciek *et al*. [50] investigated the effects of various sulfur compounds—Diallyl sulfide (DAS), Diallyl disulfide (DADS), and Diallyl trisulfide (DATS)—on HepG2 cell viability at concentrations up to 100 mM. They found that all compounds reduced cell viability at this concentration, with DATS exhibiting the strongest inhibition. Importantly, DATS significantly inhibited cell proliferation as measured by BrdU incorporation and markedly increased caspase-3 activity, indicating a pro-apoptotic effect. Furthermore, DATS elevated hydrogen peroxide levels to 117% at 100 mM and to an impressive 160% at 200 mM, demonstrating a unique pro-oxidant action.

Overall, the collective evidence supports the hypothesis that garlic-derived compounds possess potent anticancer properties through multiple mechanisms, including apoptosis induction, cell cycle arrest, and modulation of oxidative stress pathways. These findings underscore the potential for incorporating garlic or its bioactive constituents into therapeutic strategies against various cancers.

4. Conclusion

The findings from this study underscore the potential role of garlic essential oil (GEO) as a promising anticancer agent. The varying cytotoxic effects observed across different cancer cell lines suggest that the bioactive components within GEO can selectively target malignant cells while sparing normal cells. Notably, the extraction method significantly influences the chemical composition and efficacy of the oil, indicating that optimization of extraction techniques could enhance its therapeutic potential. Future research should focus on elucidating the specific mechanisms through which garlic compounds exert their anticancer effects and evaluating their efficacy in clinical settings. By integrating natural products like GEO into cancer treatment regimens, we may improve therapeutic outcomes and reduce reliance on conventional chemotherapeutics with adverse side effects.

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