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Exploring the therapeutic potential of bioactive compounds derived from *Artemisia absinthium* against breast cancer cell line

Ibtisam Nadhim Al-assaf[1] Muthanna Jasim Mohammed[2] and Fawwaz Fadhil Ali[3]
[1]Department of Pharmacy Techniques, Mosul Technical Institute, Northern Technical University, Mosul, Iraq
[2]Department of Biology, College of Education for Pure Science, University of Mosul, Mosul, Iraq
[3]Department of Scientific Affairs, Northern Technical University, Mosul, Iraq

Abstract: *Artemisia absinthium*, renowned for its medicinal properties, boasts a wealth of biologically active compounds, rendering it indispensable for extracting chemicals from its aerial parts using Soxhlet extraction. Through diverse chromatography methods, fractions Ia and IIb were isolated, revealing numerous phenolics. XTT tests on cell cultures demonstrated that MCF-7 cancer cells treated with fatty acids exhibited significantly lower survival rates than the control group, with IC50 values of 43.24 and 347.2, respectively. Fraction Ia exhibited dose-dependent effects on cell viability, inhibiting MCF7 breast cancer cell proliferation by 76.4%, 67.08% and 48.98% at doses of 5, 10, and 20µg/mL, respectively, while exerting minimal impact on the healthy cell line WI38, with percentages of 97.82%, 95.49% and 91.52%, respectively. Similarly, fraction IIb significantly impeded MCF7 cell growth at doses of 5, 10 and 20µg/mL, with percentages of 66.12%, 47.05%, and 33.26%, respectively, yet demonstrated negligible effects on WI38 cells, with percentages of 98.80%, 96.73% and 95.55%, respectively. Notably, fraction IIb exhibited selective toxicity towards breast cancer cells, indicating the potential of A. absinthium plant extracts in breast cancer treatment.

Keywords: *Artemisia absinthium*, phenolic compounds, fatty acids, breast cancer.

INTRODUCTION

Medicinal plants play a significant role in the pharmaceutical industry, captivating the attention of researchers driven to explore the efficacy of diverse vital components or active substances derived from these plants within the medical and pharmaceutical sectors. The study and investigation of medicinal plants have become a compelling field, contributing to advancements in medicine and driving innovation in the pharmaceutical realm (Alaboo and Mohammed, 2023; Anwar *et al*., 2019; Máthé and Khan, 2022). *Artemisia absinthium* L., also known as wormwood, is a significant perennial shrubby medicinal plant naturally found in Asia, the Middle East, Europe and North Africa. It belongs to the Asteraceae family, which encompasses a vast range of species exceeding 500, categorized as annuals, perennials, Artemisia absinthium root is characterized by its perennial nature, featuring a sturdy and long-lasting woody and leafy stem biennials, or even miniature shrubs (Willcox, 2009). *A. absinthium* has garnered various vernacular names, including green ginger, absinthe, absinthium and wormwood in English, while it is known as Afsanteen in Arabic (Abad *et al*., 2012; Albermana and Hadi, 2012). The stem reaches a height of approximately 2 to 2.5 feet, displaying a white colouration and is adorned with delicate, silky hairs. The leaves on both sides of the stem exhibit a white hue. They are 3 inches long and 1.5 inches wide, showcasing slender and irregularly shaped segments. The leaf stalks possess slight wing-like protrusions along the edges, and on the flower stalks, the leaves may be reduced to three or even one linear subdivision. The flowering period occurs from early summer to early autumn. The flower heads are short and nearly circular, clustering together in an upright, leafy panicle. The small flowers hang downwards and possess a greenish-yellow hue. The leaves and flowers carry a highly bitter taste and a distinct aroma reminiscent of thujone (Batiha *et al*., 2020). *Artemisia absinthium* is a highly significant herb renowned for its diverse pharmacological properties. This herb has been proven to possess various medicinal activities, including antimicrobial, insecticidal, antiviral, hypoglycemic, hepatoprotective, wound healing, anti-inflammatory and cardiovascular disease-fighting properties. Furthermore, *A. absinthium* has also demonstrated a wide range of antioxidant and anticancer activities, making it a valuable natural resource in medicine and health (Ali *et al*., 2021; Khan *et al*., 2022). *A. absinthium* possesses oils with a ratio of approximately 1.5-2. Additionally, the plant harbours various phenolic compounds, including flavonoids such as quercetin, kaempferol, apigenin and artemethin. Moreover, several phenolic acids like chlorogenic, ferulic, gallic, caffeic, syringic and vanillic, along with caffeoylquinic acid derivatives, are also present in A. absinthium (Amat *et al*., 2010; Fiamegos *et al*., 2011; Beigh and Ganai, 2017).

Numerous endeavours have been made to explore novel treatments, mainly derived from natural plant extracts, aimed at combating cancer. Cancer, a grave and fatal

**Corresponding author*: e-mail: dr.muthanna.j.m@uomosul.edu.iq

ailment poses a significant global health challenge affecting both developed and developing countries worldwide (Taylor *et al*., 2022). Among the various types of cancer that have been identified, breast cancer stands out as one of the most prevalent among women across the globe, closely followed by colorectal cancer. With the advancement in our understanding of the molecular aspects of existing therapies, there is a pressing need to develop innovative anti-cancer medications with high selectivity and specificity (Martínez-Aledo *et al.*, 2020; Koygun *et al.*, 2021). Genetic alterations are a common element in the development of cancer, including breast and colorectal cancer (Tor *et al*., 2015). The primary objective of the present study is to identify various natural compounds, with a particular focus on phenolic compounds, present in the flower extracts of wormwood and investigate the efficacy of these extracts in combating cancer. The findings of this study have the potential to shed light on novel therapeutic avenues and advance natural compounds' role in cancer treatment.

MATERIAL AND METHODS

Plant material

In April 2020, *Artemisia absinthium* arial part was carefully gathered from northern Iraq during their flowering season. The cultivated plants were explicitly raised in Mosul, a prominent city in northern Iraq. This specific geographical location was selected based on expert recommendations and a thorough review of existing literature. To maintain sterility, gloves were worn while collecting plant parts, which were then placed in sterile plastic bags for transportation to the laboratory. The collected plant materials were later identified and authenticated by the Ministry of Agriculture in Mosul, Iraq, personnel. Subsequently, the dried plant parts were meticulously crushed into an excellent powder or paste using a pre-chilled laboratory mortar and pestle. For all extraction, isolation, and analysis processes, only analytical-grade reagents, including hexane, ethyl acetate, ethanol, acetonitrile, water, phosphoric acid, gallic acid, salicylic acid, catechol, coumaric acid and resorcinol, were utilised, all of which were obtained from Sigma Aldrich in Baghdad, Iraq.

Extraction of by Soxhlet apparatus

100 g of pulverised *A. absinthium* aerial part was carefully placed in a thimble holder before being inserted into the Soxhlet apparatus. To initiate the extraction process, 1000 ml of hexane was added to a round-bottom flask. The extraction was conducted over 72 hours, maintaining a temperature range between 40 and 70°C. The completion of the extraction process was indicated by the clarity of the solution in the thimble, at which point the apparatus was switched off. Following the same conditions, the same plant sample underwent the extraction process again, using ethyl acetate and ethanol to increase polarity. Three different extracts were obtained: hexane to obtain fatty compounds and ethyl acetate and ethanol to obtain phenolic compounds. Each extraction was repeated three times for every solvent. To obtain a higher yield of extracts. Subsequently, a rotary evaporator was employed to evaporate the crude plant extract. Once the organic solvent had evaporated, the extract was filtered using Whatman filter paper No. 1 to eliminate unwanted materials. All the extracts were accurately weighed and stored in sterile, dark, airtight containers for further analysis (Alaboo and Mohammed, 2023).

Analysis of A. absinthium hexane extract by GC-MS

The hexane extract was subjected to GC-MS analysis using the GCMS-QP2010 Ultra instrument manufactured by Shimadzu Co., Japan. The instrument was equipped with a capillary column Rtx-5, measuring 0.25 m in film thickness, 0.25 mm in inner diameter and 30 m in length. The analysis was performed in electron impact mode with an ionization voltage of 70 eV. The injector temperature was set at 250°C and the detector temperature at 280°C. Helium, with a purity of 99.9%, served as the carrier gas at a flow rate of 1.2mL/min. Approximately 1mL of the sample was injected for analysis. The oven temperature was programmed to increase from an initial temperature of 35°C, held for 3 minutes, to 240°C at a rate of 5°C/min, followed by an increase from 240°C to 280°C at a rate of 3°C/min with a hold time of 4 minutes. The identification of compounds was based on the analysis of mass spectral data using the NIST and WILEY libraries as references (Mabuchi *et al*., 2018).

Fractionation and isolation of A. absinthium extract by column

Column chromatography (CC) was conducted to isolate and fractionate the extract of *A. absinthium*. Using the wet packaging technique, a chromatography column was prepared using 250g of silica gel (Sigma Aldrich, Baghdad, Iraq) with a mesh size of 60-120. A slurry was prepared by mixing the silica gel with a colourless liquid (hexane) and then poured into the column. After thoroughly mixing the extract with a small quantity of silica gel placed on top of the column, the extract was added. In order to increase polarity, various solvent mixtures, such as hexane, ethyl acetate, and ethanol, were employed as the mobile phase for column elution systems. The collected fractions from the purified CC were condensed to identify the fractions containing Artemisia. Subsequently, only the ethyl acetate and ethanol fractions were determined using HPLC, as the hexane fraction was identified using GC-MS (Mogana *et al*., 2020).

Analysis of phenols by High-Performance Liquid Chromatography (HPLC).

HPLC was utilized to confirm the authenticity of the extracted phenolic components. The analytical HPLC system employed for this purpose was a reversed-phase

HPLC with a silica-based C18 column manufactured by Agilent Technologies in Santa Clara, CA, USA. The system comprised various components, including the SPD-10A UV-VIS detector, the VP pump LC-10AT, the auto-injector SIL-10AF and the system controller SCL-10A VP. The analytical column used was the Chiralcel® OD-RH, provided by Chiral Technologies Inc. in Exton, PA, USA, measuring approximately 150 mm in length and 4.6 mm in diameter, with 5 mm particle size. The mobile phase consisted of a mixture of acetonitrile, water, and phosphoric acid (in a ratio of 30:70:0.08, v/v/v) and was applied under isocratic conditions at the ambient temperature of 25±1◦C. A flow rate of 0.4 mL/min was maintained throughout the analysis. Each run lasted for 8 minutes, followed by a 15-minute clean-up period. The integrated SPD-10A UV-Vis detector detected the separated compounds, which operated at a wavelength of 288 nm (Chen *et al*., 2018). Individual compounds were confirmed by comparing the retention times of the peaks in the extract with those of the corresponding standards, as listed in table 1.

Cell culture

The MCF7 breast adenocarcinoma cell line (ATCC®HTB-22™) and the WI38 fetal lung fibroblasts cell line (ATCC®CCL-75.1™), representing non-cancerous cells, were procured from the American Type Culture Collection. MCF and WI-38 cell lines were at passage 6 when they were used for the designed experiments. MCF7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) from Sigma Aldrich; Merck USA, supplemented with 10% fetal bovine serum, 100 U/ml penicillin/streptomycin and L-Glutamine, also from Sigma Aldrich; Merck USA. The cells were maintained in a CO2 incubator at 37°C with 5% CO2 and the medium was refreshed every 24 hours. The cell's condition was assessed daily using an LED Inverted Fluorescence microscope (Model A16.0912-L, China) (Pamies *et al*., 2022).

Cell viability test

The study assessed the viability of MCF7 breast cancer and WI38 fetal lung fibroblast cell lines upon treatment with various fractions of A. absinthium plant extract (hexane, ethyl acetate, and ethanol) using an XTT assay kit (2,3-bis-(2-methoxy-4-nitro-5-sulfo phenyl)-2H-tetrazolium-5-carboxamide) obtained from RIBOBIO in China. This assay measured cell viability by evaluating the metabolic activity of the cells. Following the extraction process, the next step involves drying the extract and weighing the residual material to finalize the dilution procedure. Different concentrations of A. absinthium extract (5, 10 and 20µg/ml) were prepared in a 10% dimethyl sulfoxide (Sigma Aldrich, USA) solvent. Cell suspensions, consisting of 5000 cells per well, were created from actively growing cultures in the exponential phase. These cell suspensions were counted using the Trypan blue exclusion assay and added to individual wells of a 96-well plate from Genex in South Korea, followed by incubation at 37°C for 24 hours. Subsequently, the extract fractions were introduced into separate wells, and the plate was further incubated at 37°C for 6 hours. The absorbance was then measured at a wavelength of 630nm using an MB-530 Elisa Reader manufactured by Heales in China to determine cell viability values (Kamiloglu *et al*., 2020).

STATISTICAL ANALYSIS

The results were expressed as mean value ± standard deviation (n = 3). The statistical analysis was done using the Statistical Package for the Social Sciences (SPSS). Multiple comparisons were carried out by analysis of variance (ANOVA). The mean comparison was made using Tukey's test at a significance level (alpha = 0.05). Microsoft Excel (2007) and BioStat prepared the results.

RESULTS

GC-MS analysis

Numerous scientific investigations have been conducted to delve into the properties of fatty compounds, with a particular emphasis on the fatty acid compounds discovered in *A. absinthium* arial part. Chromatography techniques, specifically GC-MS, have played a pivotal role in these studies, owing to their ability to analyze and identify these compounds. This research has been driven by the promising medical efficacy exhibited by these compounds, particularly in the realm of antioxidants and cancer treatment, making them an area of significant interest and exploration. These were found in *A. absinthium* extracts through the utilization of PubChem and ChEBI databases and an extensive review of relevant literature. These compounds were discovered by employing GC-MS analysis. table 2 presents a comprehensive analysis of fatty acids obtained from *A. absinthium*. It enumerates six distinct fatty acids identified, furnishing their IUPAC names, standard designations, chemical nomenclature, retention times, and their corresponding proportions as a percentage of the total. This data is indispensable for characterizing the chemical composition of *A. absinthium* flowers and understanding the particular fatty acids contained within the plant. Furthermore, the presence and quantity of these fatty acids within the plant can hold significance for their potential applications in traditional medicine, dietary supplements, or other domains. The retention times and structural particulars play a pivotal role in verifying the identity of these fatty acids, distinguishing them from potential coexisting compounds in the sample. The area under the chromatographic curve for each fatty acid, presented as a proportion of the total area, measures the relative quantity or concentration of each fatty acid within the sample.

Table 1: Standards of phenolic compounds and their retention time.

Standards	R. T (min)	Concentration (ppm)	Area [1]
Gallic acid	2.019	25	6.77179 e4(0.01)
Resorcinol	2.686	25	5.09239e4(0.01)
Salicylic acid	2.405	25	3.03115 e4(0.01)
Catechol	2.715	25	7.75188 e4(0.01)
Coumaric acid	3.165	25	11615.83252(0.01)

Table 2: Fatty acids separated from *A. absinthium* flowers using GC-MS

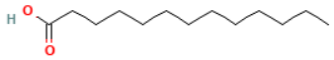
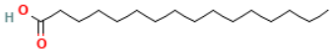
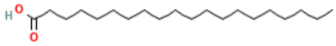
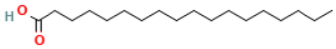
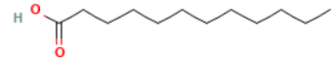
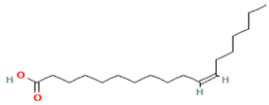
No.	IUPAC name	Common name	Chemical nomen-clature	R.T (min)	Area%	Area	structure formula
1	Tridecanoic acid	Tridecylic acid	C13:0	13.448	1.96	9147494	
2	Hexadecanoic acid	Palmitic acid	C 16:0	15.833	14.84	69128910	
3	Eicosanoic acid	Arachic acid	C20:0	16.732	0.61	2844620	
4	Octadecanoic acid	Stearic acid	C 18:0	17.856	4.55	21215759	
5	Dodecanoic acid	Lauric acid	C12:0	10.954	0.52	2414376	
6	cis-11-Octadecenoic acid	cis-Vaccenic acid	C18:1	17.683	7.08	32992590	

Table 3: Phenolic compounds in fractions (I^a and II^b) and their retention time.

Fractions	No. of Peak	R .T (min)	Conc. (ppm) [c]	Identified Compounds
I^a	1	2.072	0.366 ± 0.02	Gallic acid
	2	2.699	5.149 ± 0.1	Resorcinol
II^b	1	2.101	0.716 ± 0.05	Gallic acid
	2	2.415	0.446 ± 0.06	Salicylic acid
	3	2.704	4.009 ± 0.9	Catechol
	4	3.522	0.664 ± 0.09	Coumaric acid

[a] Fractions identified from ethyl acetate extraction; [b] Fractions identified from ethanol extraction; [c] Values represent mean and standard deviation (n = 3).

Table 4: shows the IC50 values for the three plant extracts, the cancer cell lines MCF7 and HT29, and the healthy line WI38

	A. absinthium		
Cell line	Fatty acids	Fraction I^a	Fraction II^b
MCF-7	43.24	19.41	11.09
HT-29	18.39	19.34	11.36
WI38	347.25	119.55	243.66

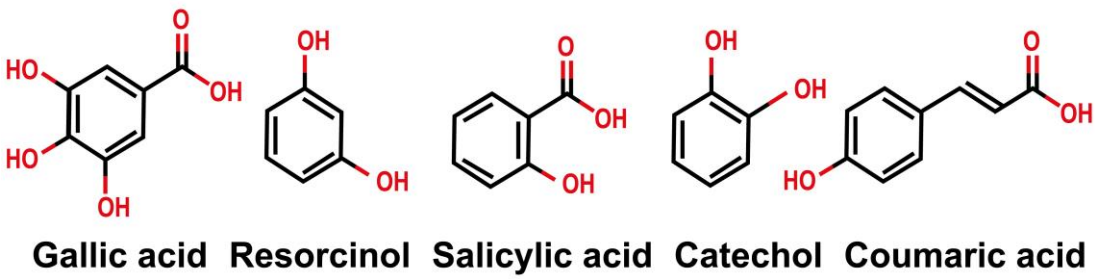


Fig. 1: 2D- chemical structure of phenolic compounds extraction from *A. absinthium*

This percentage holds particular significance because it reveals the proportional contribution of each fatty acid to the overall fatty acid content in the sample. For example, tridecanoic acid (C13:0) comprises a mere 1.96% of the total fatty acids identified. In contrast, hexadecanoic acid (C16:0) is the most prevalent, constituting 14.84%. On the other hand, eicosanoic acid (C20:0) constitutes a mere 0.61% of the total, while octadecanoic acid (C18:0) accounts for a notable 4.55%. Dodecanoic acid (C12:0) is relatively minor, representing just 0.52% of the total. cis-11-octadecenoic acid (C18:1) is a noteworthy contributor, making up 7.08% of the total composition.

Composition of phenolic compounds in a. absinthium fractions

After the extraction process, two distinct fractions were obtained. Fraction I^a was derived from ethyl acetate extraction, while Fraction II^b was obtained through ethanol extraction. A detailed analysis of the composition and content of phenolic compounds in these two fractions of *A. absinthium* was performed using HPLC analysis at a wavelength of 288nm, and the results are presented in Table 3. The phenolic compounds were identified by comparing their retention times and peak areas with the respective standards. fig. 1 illustrates the chemical composition of phenolic compounds obtained from *A. absinthium.* In Fraction I^a, the HPLC chromatographic profile revealed the presence of two major peaks, which were identified as gallic acid and resorcinol based on their standard counterparts. Conversely, Fraction II^b exhibited four significant peaks: gallic acid, salicylic acid, catechol, and Coumaric acid (fig. 2).

Cell viability of treated cancer cells

The XTT test results on cell cultures revealed a noteworthy reduction in the viability of MCF-7 cancer cells when treated with the fatty acids extract derived from A. absinthium, compared to the control cell line. This effect demonstrated a dose-dependent relationship, with a substantial increase in potency as the extract concentration increased ($P \leq 0.001$). In contrast, no significant impact was observed on the WI38 normal cell line (fig. 3).

The outcomes of the XTT test, as illustrated in Fig. 4, conducted on cell cultures, demonstrated that the fraction I^a extract had varying effects on the percentage of cell viability at concentrations of 5, 10 and 20µg/mL about the breast cancer cell line MCF7. Specifically, these concentrations resulted in viability percentages of 76.4%, 67.08% and 48.98%, respectively. Conversely, the impact of the extract on the healthy cell line WI38 was relatively mild, with viability percentages of 97.82%, 95.49% and 91.52%, respectively, at the same concentrations.

In the examination of cell cultures, the fraction II^b extract exhibited remarkable efficacy at concentrations of 5%, 10%, and 20% in inhibiting the growth of the breast cancer cell line MCF7, resulting in viability percentages of 66.12%, 47.05% and 33.26%, respectively. Conversely, the impact of this extract on the healthy cell line WI38 was notably milder, with viability percentages of 98.80%, 96.73%, and 95.55%, respectively, at the same concentrations (fig. 5).

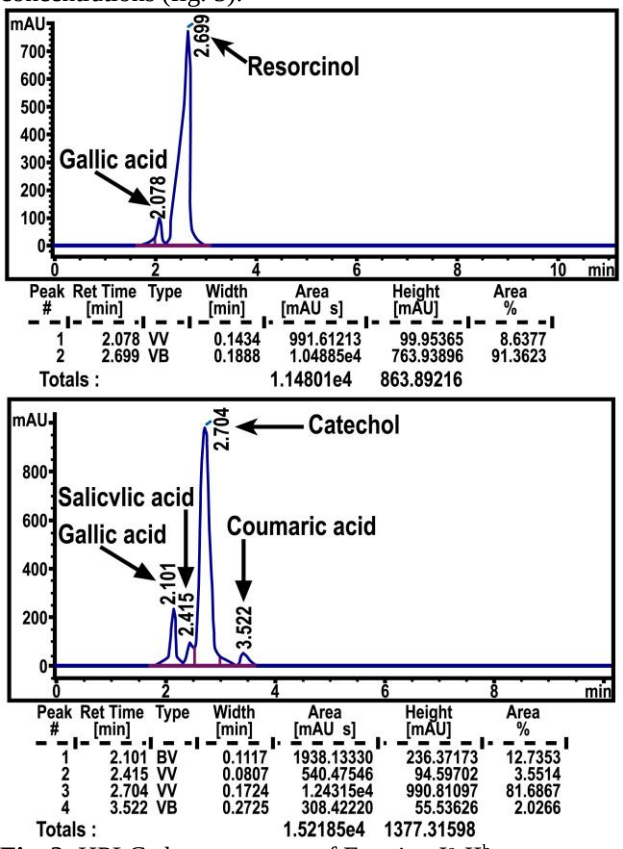


Fig. 2: HPLC chromatogram of Fraction I^a-II^b

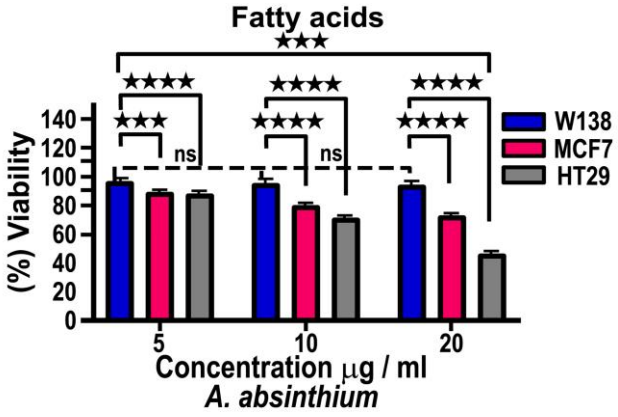


Fig. 3: Cell viability test of breast cancer cells MCF7 and WI38 non-cancerous cells treated with *A. absinthium* fatty acids extracts analyzed by XTT. The plant extract selectively inhibits the viability of cancer cells breast cancer cells MCF7 and WI38 non-cancerous cells as analyzed by XTT, and the data were analyzed statistically according to a two-way ANOVA comparing test, and the data were expressed using Graph Pad Prism 9. The columns represent the average cell viability. significance ****$p \leq 0.0001$ and ***$P \leq 0.001$. ns is non-significant.

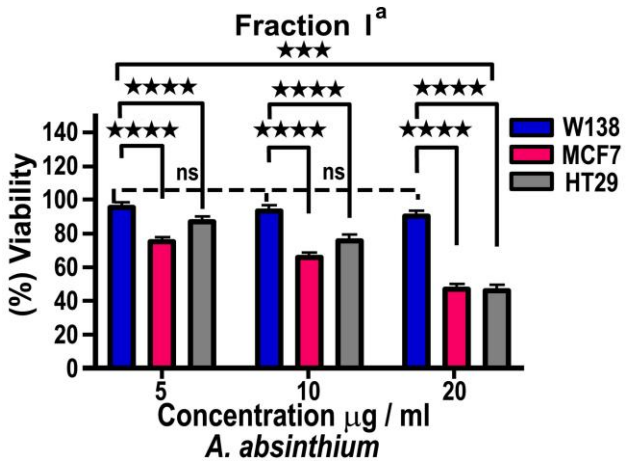


Fig. 4: Cell viability test of breast cancer cells MCF7 and WI38 non-cancerous cells treated with *A. absinthium* fraction I[a] extracts analyzed by XTT. Selectively inhibit the viability of cancer cells breast cancer cells MCF7 and WI38 non-cancerous cells as analyzed by XTT; Data were analyzed statistically according to a two-way ANOVA comparing test and the data were expressed using Graph Pad Prism 9. The columns represent the average cell viability. significance ****p≤0.0001 and ***P≤0.001. ns is non-significant.

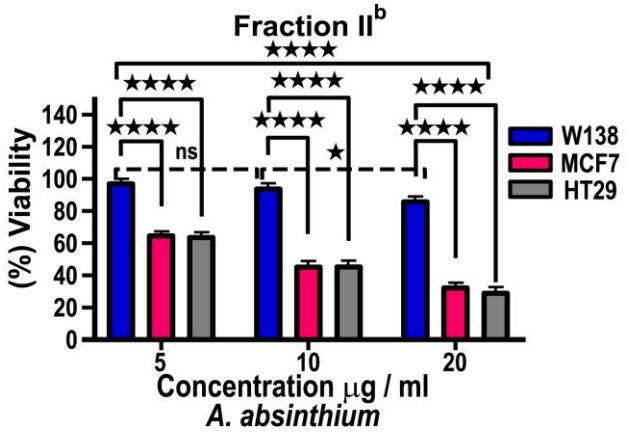


Fig. 5: Cell viability test of breast cancer cells MCF7 and WI38 non-cancerous cells treated with *A. absinthium* fraction II[b] extracts and analyzed by XTT. *A. absinthium* ethanol extracts selectively inhibit viability of cancer cells breast cancer cells MCF7 and WI38 non-cancerous cell as analyzed by XTT and the data were analyzed statistically according to a two-way ANOVA comparing test, and the data were expressed using Graph Pad Prism 9. The columns represent the average cell viability. significance ****p≤0.0001 and *P≤0.05. ns is non-significant.

Table 4 presents the IC50 values of three extracts from Artemisia absinthium against cancer cell lines MCF-7 and HT-29 and the healthy cell line WI38. For Artemisia absinthium, the extracts analyzed were Fatty acids, Fraction I, and Fraction II. The IC_{50} values indicate the concentration at which 50% inhibition of cell proliferation occurred, with lower values indicating higher cytotoxicity against the respective cell lines. In MCF-7 cells, Fatty acids exhibited an IC50 of 43.24, Fraction I of 19.41, and Fraction II of 11.09. Similarly, in HT-29 cells, the IC50 values were 18.39, 19.34, and 11.36 for Fatty acids, Fraction I, and Fraction II, respectively. In contrast, WI38 cells showed significantly higher IC_{50} values, indicating lower cytotoxicity than cancer cells, with 347.25, 119.55, and 243.66 values for Fatty acids, Fraction I, and Fraction II, respectively.

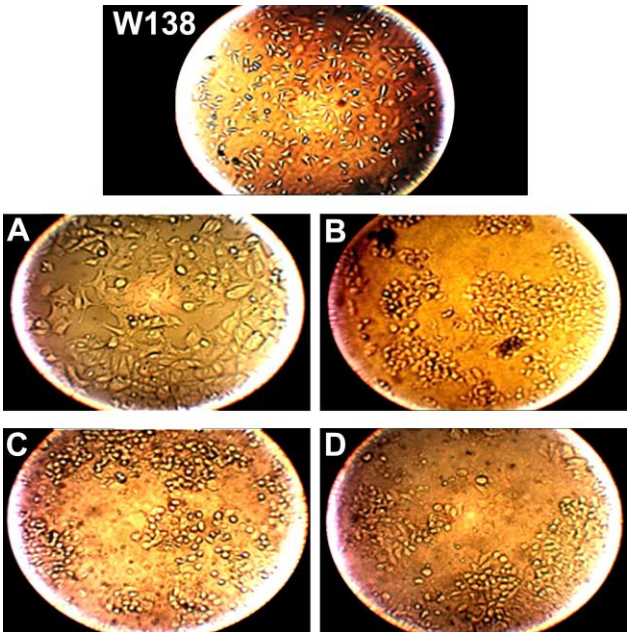


Fig. 6: breast cancer cell line MCF7treated with plant extracts(A) Untreated (B) fatty acids, (C) fraction I[a] and (D) fraction II[b], (WI38) normal with a concentration of 20µg/ml after 72h of treatment showing the change in cell shape, detachment and death viewed under an inverted light microscope (×100)

The application of three distinct plant extracts induced discernible phenotypic alterations within the cultures of the MCF7 cancer cell line. These changes were characterized by cell separation, a transformation in cell morphology into a circular configuration, cytoplasmic vacuoles and a noticeable reduction in cell count and vitality. These observations collectively point towards the suppression and inhibition of cancer when utilizing the specified concentrations. Notably, a distinctive feature distinguishing dead and dying cells was the breakdown of cellular and nuclear membranes, as illustrated in Fig. 6. In contrast, the healthy cell line WI38 displayed no notable changes in appearance, maintaining its characteristic monolayer structure.

DISCUSSION

Plant oils and their constituents are gaining increased attention due to their perceived safety, widespread

consumer acceptance and potential medicinal applications against various diseases. A prior study revealed that *Artemisia* species have a notable fat content, ranging from 3.31 to 17.78 milligrams of total fatty acids (TFA) per gram of fresh weight. These findings emphasize the substantial presence of fatty acids in wormwood compared to other plants like spinach (1.7mg/g), lettuce (0.6mg/g), purslane (8.5mg/g) and mustard (1.1mg/g) (Carvalho *et al*., 2011).

Subsequent research confirmed these findings, identifying eleven fatty acids in the aerial parts oil. Saturated fatty acids (SFA) comprised 41.00±5.10% of TFA, with palmitic, stearic and behenic acids contributing 32.95±4.80%, 3.06±0.78% and 3.05±1.85% of TFA, respectively. Interestingly, the same plant from a different region had the highest SFA levels at 44.46 6.23% of TFA. This was primarily due to the high levels of palmitic (33.91 2.10% of TFA), behenic (4.53 1.94% of TFA) and stearic (4.22 0.26% of TFA) acids (Xiang *et al*., 2005). Other studies explored the fat content of *Artemisia* plants from various Tunisian regions. In the Kasserine region, SFA reached its peak at 44.46±6.23% of total fatty acids (TFA), primarily due to abundant palmitic acid (33.91±2.10% of TFA), behenic acid (4.53±1.94% of TFA), and stearic acid (4.22±0.26% of TFA). Conversely, monounsaturated fatty acids (MUFA) exhibited similar proportions, constituting approximately 21.91±3.21% and 21.67±3.11% of TFA in the Boukarnin and Kef regions. In the Kairouan region, the study revealed the highest MUFA content at 24.06±1.56% of TFA, primarily attributed to oleic acid, which contributed 19.82 ± 0.65% of TFA (Msaada *et al*., 2015). Furthermore, research on *Artemisia annua* flowers and leaves showed that palmitic acid was the predominant compound (24.2%), followed by linoleic (14.3%), myristic (7.5%), linolenic (6.3%) and stearic (5.4%) acids. Interestingly, the composition of detected fatty acids in *Artemisia herba-alba* aerial parts exhibited significant variation (P< 0.001)(Msaada *et al*., 2009).

Plants possess a wide range of bioactive compounds with diverse biological activities, with particular emphasis on their antioxidant and anti-cancer properties. These beneficial effects are often attributed to polyphenols, including phenolic compounds, flavanols and flavonoids (Działo *et al*., 2016). In a recent study, researchers aimed to explore the phenolic compound profile of the methanol extract derived from the above-ground portion of *A. absinthium*. This investigation revealed a significant correlation between the total phenolic content and the observed anti-cancer activity, suggesting that the combined action of various phenolic compounds could potentially enhance the effectiveness of anti-cancer treatments even further (Sengul *et al*., 2011). Further analysis of three distinct extracts obtained from the leaves of *A. absinthium* highlighted noticeable variations in the levels of phenolic compounds present. Among these extracts, the water extract displayed the highest concentration, measuring 134.47mg per 100g of dry weight (DW), closely followed by the methanol extract at 131.18 mg per 100g DW. In contrast, the ethyl acetate extract exhibited a relatively lower quantity of phenolic compounds, measuring 51.49mg per 100g DW (Lee., 2013). Moreover, the study demonstrated the significant impact of different extracts on the overall phenolic content of *A. absinthium*. Methanol was found to have a positive and direct influence on the total phenolic content and emerged as a crucial factor in the recovery of phenolic compounds. (Şahin., 2013).

Wild plants harbour an array of natural products, such as lignin, flavonoids, and oils, which have demonstrated superior properties for cancer treatment. These compounds undergo rigorous laboratory and animal studies to evaluate their potential biological activities, including anti-tumour effects. Before these investigations, numerous studies have explored the cytotoxic actions of various plant extracts in vitro, gauging their anticancer potential across various human cancer cell lines. Building on findings from other plants, numerous studies have validated Artemisia species' cytotoxic and anti-cancer properties. These species have exhibited toxicity towards cancer cells in vitro and in vivo, likely attributable to one or more essential compounds within the plants (Aghajanpour *et al*., 2017; Ali *et al*., 2021; Koyuncu, 2018).

Moreover, a growing body of research has consistently demonstrated the inhibitory effects of fatty acids on the proliferation of breast and prostate cancer cell lines in vitro and their potential to reduce the risk and progression of these tumours in animal models. Many studies have shown that these fatty acids exert their influence by inhibiting cyclooxygenase-2 and the oxidative metabolism of arachidonic acid to PGE2. Additionally, EPA and DHA have been found to inhibit lipoxygenase, which is responsible for metabolizing arachidonic acid into hydroxyl eicosatetraenoic acids and leukotrienes, molecules that suppress apoptosis, promote angiogenesis, and stimulate tumour cell division. These mechanisms suggest the potential of n-3 PUFAs to impact carcinogenesis in specific ways, as elucidated by Mozolewska *et al*. (2020).

Furthermore, Ansari *et al*. (2021) have provided substantial evidence in experimental and human studies, highlighting the protective role of omega-3 PUFAs and monounsaturated fatty acids against breast, colon, and prostate cancers. In a melanoma cancer cell experiment using the WM793 cell line, Domagała *et al*. (2021) observed a significant reduction in proliferation ($p \leq 0.05$) ranging from 21.29% to 30.54% when exposed to a mixture of fatty acids at concentrations of 0.35, 0.50 and

0.70mg/mL of the culture medium, with no discernible impact on normal cells. Notably, progressive inhibition of proliferation was noted within 72 hours at concentrations of 0.50 and 0.70mg/mL. The mixture of fatty acids within the tested concentration ranges did not exhibit significant effects on the proliferation of cancer cells. Additionally, Jóźwiak *et al*. (2020) have illuminated the role of fatty acids in inducing apoptosis in colon cancer cells of the SW480 line, as evidenced by an increase in the annexin V signal, a key marker of apoptosis. Activation of caspase 3, a particular caspase known for irreversibly driving cell death, occurs through both mitochondrial and receptor-mediated apoptosis pathways.

In another investigation, dichloromethane, methanol, ethyl acetate, and n-hexane extracts obtained from the upper parts of various *Artemisia* species have displayed potent antiproliferative characteristics, suggesting their potential as promising chemotherapeutic agents in cancer treatment. Further studies have confirmed that ethanolic extracts of Artemisia are rich in essential compounds, including flavonoids and phenolic acids, known for their robust antioxidant activity and cytoprotective effects against oxidative damage in fibroblast cells. These findings underscore *A. absinthium* as a promising candidate for treating skin disorders. Moreover, in the context of human muscle cancer cells, extracts derived from Artemisia have demonstrated a profound impact, resulting in the inhibition of 88-93% of cancer cells, thereby validating the anticancer potential of this plant extract, as reported by Adewumi *et al*. (2020). Research has shown that the active constituents of *Artemisia* species play a pivotal role in apoptosis, a programmed cell death process initiated through cell cycle arrest. Activation of caspases, mitochondrial membrane depolarization, or the downregulation of Bcl-2 gene expression can induce apoptosis in cells (Abraham *et al*., 2010). For instance, the smoke and water extracts of *Artemisia* princeps were found to induce apoptosis in human breast cancer MCF-7 cells through the mitochondrial pathway, offering a promising avenue for breast cancer treatment (Sarath *et al*., 2007).

Furthermore, the methanol extract of the AR (*Artemisia absinthium* L.) plant was examined for its antioxidant activity based on phenolic and flavonoid content using the ABTS and CUPRAC methods. The results indicated that the AR plant exhibits antioxidant activity, suggesting its potential as a natural antioxidant source with free radical scavenging effects. (Lee *et al*., 2013; Araújo *et al*., 2011). In the context of the anti-cancer properties of *A. absinthium* L. (AR) plant, Shafi *et al*. conducted a study examining the cytotoxic and apoptotic effects of the ethanolic extract of the AR plant on MCF-7 and MDA-MB-231 breast cancer cell lines. The study's findings indicated that the AR plant exerts a cytotoxic and apoptotic effect on breast cancer cell lines, potentially through its impact on the Bcl-2 family proteins and the MEK/ERK pathway in breast cancer cells (Shafi *et al*., 2012).

CONCLUSIONS

This study aims to get phenolic compounds and fatty acids from the flowering part of Artemisia absinthium and use different chromatography methods to test their possible anticancer effects on MCF7 breast cancer cells. Among the compounds identified, two phenolic compounds, gallic acid and resorcinol, were found in Part Ia. In comparison, Part IIb contained four phenolic compounds: gallic acid, salicylic acid, catechol and coumaric acid. Fatty acids demonstrated significantly lower survival rates than the control group, 43.24 and 347.2, respectively. Fraction Ia inhibited MCF7 breast cancer cell proliferation by 76.4%, 67.08% and 48.98%, while fraction IIb exhibited percentages of 66.12%, 47.05% and 33.26%, respectively.

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