Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(4): 3427 – 3457 (2025)
www.ejabf.journals.ekb.eg

Phytochemical Composition and *In Vitro* Anticancer Activities of the Marine Coastal Plant *Heliotropium curassavicum* L. Extract in Different Seasons

Menna A. Elbolaky[1]* , Ali H Ibrahim[1] , El Bous M.M[1], Mohamed M. Tawfik[2]
[1]Botany Department, Faculty of Science, Port Said University, Egypt
[2]Zoology Department, Faculty of Science, Port Said University, Egypt
*Corresponding Author: Alaamenna741@gmail.com

ARTICLE INFO

Article History:
Received: June 30, 2025
Accepted: Aug. 13, 2025
Online: Aug. 31, 2025

Keywords:
Heliotropium curassavicum,
Costal plant,
Cancer,
GC-MS,
qPCR,
MTT

ABSTRACT

Heliotropium curassavicum L. (Family Boraginaceae) is a marine coastal wild herb species, reported to have multiple therapeutic activities including anti-gout, ant-rheumatism, anti-arteriosclerotic and anti-cancer. The aim of the current study is assessing the seasonal changes in the chemical composition and anticancer activities of *H. curassavicum* collected from Port Said Governorate. The GC-MS analysis was performed to screen the seasonal variation in the phytochemical constituents of the hexane extract of the plant. GC-MS analysis of hexane extract of *H. curassavicum* identified the presence of thirty-two, fourteen, thirty-nine and forty-five compounds in winter, summer, autumn, and spring seasons, respectively. The extracts of *H. curassavicum* in all different seasons share three similar phytocompounds which were hexadecanoic acid, 9-octadecenoic acid (Z) in addition to 9,12-octadecadienoic acid (Z,Z). Erucic acid was the most common compound in autumn and spring, whereas the most prevalent compound in winter was eugenol, while summer extract was dominated by Z,Z. Quantitative phytochemical analysis revealed the seasonal variation in phytochemicals, where flavonoids and phenolic compounds were at a high level in the autumn and summer seasons, while alkaloid levels peaked during autumn and spring. The MTT assay demonstrated that the extract exhibited selective cytotoxic effects on cancer cell lines (HepG2 and MCF-7), with slight preference of autumn season. The underlying mechanism of cytotoxicity of IC_{50} of the autumn extract against targeted MCF-7 cells resulted in cell cycle arrest at the S phase, accompanied by a significant rise in the proportion of apoptotic cells, as indicated by flowcytometric analyses cell cycle analysis and annexin-V assay. Apoptotic activities of the autumn plant extract were further confirmed by detecting the expression levels of some related genes in MCF-7 cell lines treated with the extract by qPCR, which showed increased expression of pro-apoptotic genes such as *P53*, *BAX*, and caspase-8, along with a decrease in the expression of the anti-apoptotic gene *BCL-2* upon treatment. Therefore, the results highlight seasonal changes in the chemical composition of *H. curassavicum* extract, which are associated with differences in its anticancer activity.

INTRODUCTION

The Boraginaceae family, comprising approximately 154 genera and 2,000 species distributed globally, is among the largest groups of dicotyledonous flowering plants **(Cohen *et al.*, 2018)**. This plant family holds significant value in traditional medicine and is widely utilized in ethnobotanical practices and cosmetic applications

(Dresler *et al.*, 2017; ararenk *et al.*, 2025). Genus *Heliotropium* is a large member within the Boraginaceae family, commonly found across tropical and temperate regions of both hemispheres. Globally, it includes approximately 270 to 330 species, with 16 species recorded in Egypt **(Boulos, 2002)**. Traditionally, species within the *Heliotropium* genus have been employed in folk medicine to manage a variety of ailments, including gout, inflammation, skin disorders, menstrual issues, rheumatism, and bites from venomous animals **(Ghori *et al.*, 2016)**.

Heliotropium curassavicum L. (family Boraginaceae) is a widespread and troublesome weed that commonly invades newly reclaimed agricultural lands at Deltaic Mediterranean coast of Egypt. Its rapid growth and strong adaptation to saline and disturbed sandy soils enable it to establish and spread easily. Owing to its halophytic nature and ecological preferences, this species has become a significant invader of cultivated areas in this region **(Hegazy *et al.*, 1994)**. This species is glabrous, usually perennial herb, fleshy throughout a sand binder salt marsh. The stems and branches can reach lengths of up to 70cm, while the leaves measure between 15– 40mm in length and 4– 10mm in width, typically oblanceolate to linear-lanceolate in shape with an obtuse tip and nerves faint. The inflorescences are terminal or lateral, either simple or branched, scorpioid in form, and range from 3 to 6cm in length, with flowering occurring from March to August **(Boulos, 2002; Pothiraj *et al.*, 2021; Akbar *et al.*, 2023; Abutaha *et al.*, 2024)**. This halophytic plant contains many important phytochemicals such as alkaloids, phenols, tannins and steroid and protein **(Suthar & Solanki, 2021)**. *H. curassavicum* has a history of traditional use for treating wounds and diseases such as rheumatism, and it has also been investigated for its potential effects against liver cancer and diabetes **(Abutaha *et al.*, 2024)**. Although the biological activities of *H. curassavicum* are well recognized, the comprehensive therapeutic potential of its bioactive compounds, particularly concerning anticancer remains insufficiently investigated **(Sharma *et al.*, 2009)**.

Cancer remains a major cause of death globally. Various types of therapeutic strategies are involved in cancer treatment including radiotherapy and chemotherapy protocols **(Iqbal *et al.*, 2017; Tawfik *et al.*, 2023)**. These therapeutic approaches are effective in treating different cancers, but they have several limitations such as disease recurrence, limited bioavailability, toxicity, no specificity, fast clearance, restriction in metastasis and noncompliance owing to severe adverse effects including fatigue, pain, anemia, nausea and hair loss **(Bonam *et al.*, 2018)**. There is an increasing demand for new more safe and specific therapeutic agents to treat and control this life-threatening disease.

Medicinal plants are generally regarded as having lower toxicity and fewer side effects than conventional treatments like chemotherapy and radiotherapy. Several phytochemicals compounds such as flavonoids, alkaloids, phenolics, steroids glycosides, and tannins which have positive impact on health and cancer prevention are available in theses plants **(Azwanida, 2015)**. As a result, medicinal plants are gaining attention as valuable sources of alternative anticancer compounds, largely due to their ability to selectively target cancer cells while exhibiting minimal toxicity toward

normal cells **(Greenwell & Rahman, 2015; Lanchhana *et al.* 2023)**.

Phytochemicals are characterized by their countless medicinal benefits without causing any harm to human beings. They play an important role in the treatment of multiple conditions such as asthma, arthritis, cancer, and more **(Banu & Cathrine, 2012)**. These phytochemicals are chemicals that are naturally present in plants which possess antioxidant, antibacterial, anti-cancer inflammatory activities. Antioxidants may help in the prevention and treatment of cancer and other diseases by protecting cells from damage caused by free radicals (**Gamal *et al.,* 2023)**. Many plant-derived products have shown significant antitumor activity against various cancer cell lines in both animal models and humans **(Soomro *et al.,* 2018; Elfar *et al.,* 2023)**. Phytochemical content is influenced by various environmental factors, including altitude, soil characteristics, and seasonal variation. Changes in environmental conditions, particularly seasonal changes, have an effect on both the concentration and composition of these compounds. Exposure to fluctuating or extreme temperatures throughout different seasons can significantly impact the phytochemical compositions **(Gololo *et al.,* 2016; Boveiri Dehsheikh *et al.,* 2019)**. Environmental conditions affect not only plants growth but also their secondary metabolites. Consequently, availability and concentration of phytochemicals in plants are influenced by seasonal changes **(Mwamatope *et al.,* 2021)**.

Seasonal phytochemical studies of unrecognized species could lead to the discovery of new natural bioactive compounds. However, the effect of different seasons on chemicals composition and anticancer activity of *Helitropium curassavicum* was not apparent in the literature. Therefore, this work aimed to evaluate seasonal variations in phytochemicals composition, and the associated anticancer activities of *H. curassavicum* extract.

MATERIALS AND METHODS

Plant materials collection and extraction

Aerial parts of *Heliotropium curassavicum* were collected from west Port Said. 31º16`19.43N 32º 16` 4.96 E during the four seasons of 2021: autumn, winter, spring, and summer. The leaves were air-dried for approximately three weeks before being ground into a fine powder using an electric grinder. About 20g of the plant powder was added to 200mL of a solvent mixture (methanol & hexane), and the extraction was performed using soxhlet apparatus continuing the extraction process until the solvent became clear. The plant extract was subsequently filtered through Whatman filter paper, and the resulting filtrate was concentrated using a rotary evaporator at a controlled temperature of 45°C **(Gracelin *et al.,* 2013; Adetuyi *et al.,* 2020).**

Cell lines sources and types

Human cancer cell lines for breast (MCF-7) and liver (HepG2), along with normal monkey kidney cell lines, were obtained from VACSERA Company in Cairo, Egypt. The cells were cultured in RPMI-1640 medium supplemented with 10% heat-

inactivated fetal bovine serum (FBS) and 1% penicillin antibiotic, and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

GC-MS analysis

GC-MS analysis was conducted at Nawah Scientific Center using an instrument equipped with a TR-5 MS capillary column (30m × 0.32mm internal diameter, 0.25μm film thickness). Helium was used as the carrier gas at a steady flow rate of 1.0mL/ min, with a split injection ratio of 1:10. Program started at 60°C and was held for 1.0min, increased at a rate of 4.0°C/ min to 240°C, and was maintained at that temperature for an additional min. The injector and detector temperatures were maintained at 210°C. Each sample was diluted with hexane in a 1:10 (v/v) ratio, and 1.0 μL of the prepared solution was injected. Mass spectra were recorded using electron ionization (EI) at 70 eV, with a scan range of m/z 40–450 **(Ezhilan & Neelamegam, 2012; Joshi, 2020)**.

Quantitative analysis of phytochemical constituents

Total alkaloids

Heliotropium curassavicum samples were used for the extraction and quantification of total alkaloids using titrimetric techniques **(Plummer, 1990; Chakraborty *et al.*, 2018)**. During this procedure, the total alkaloid content was calculated based on the following equivalent:

1.0 mL 0.1N HCl ≡ 0.0162 g alkaloid.

Total phenolics

The determination of total phenolic content was carried out following the method outlined by **Cumplido-Nájera *et al*. (2019)**. In this procedure, 0.1mL of the plant extract was mixed with 2.5mL of 0.1mol L^{-1} Folin-Ciocalteu reagent, followed by the addition of 2.0mL of saturated sodium carbonate (Na_2CO_3). The mixture was then allowed to stand for 1.0 hour, after which the absorbance was measured at 720nm, using water as a blank.

Total flavonoids

The total flavonoid content in the plant extract was measured using the aluminum chloride colorimetric technique. Exactly, 0.32mL portion of the extract was combined with 0.32mL of 2% (weight/volume) aluminum chloride ($AlCl_3$) solution and 3.36mL of analytical-grade ethanol. The mixture was left to react for 25min, after which absorbance readings were taken in triplicate at 413 and 427nm using a spectrophotometer. The blank consisted of 0.32mL of the extract solvent, 0.32mL of the $AlCl_3$ solution, and 3.36mL of ethanol **(Chakraborty *et al.*, 2018)**

DPPH scavenging assay

The antioxidant activity of the samples was assessed using the DPPH radical scavenging assay, following the method described by **Shoily *et al*. (2025)**. Briefly, 0.5g of plant material from both control and treated groups was collected and centrifuged. The samples were then homogenized in n-hexane at a concentration of 1.0mg/ mL. For the assay, 100μL of the prepared DPPH solution (0.004% in methanol) was mixed with 0.3mL of the sample extract. The mixture was thoroughly shaken, wrapped in aluminum foil, and incubated in the dark at 25°C for 30 to 60min. The color change

from deep violet to light yellow indicated radical scavenging activity. The absorbance was recorded at 515nm. For the control (A0), 2.7mL of DPPH solution was mixed with 0.3mL of the extract solvent **(Afroz Shoily *et al.*, 2025)**.

MTT assay (MCF-7 cell lines, HepG2 cell lines, and VERO cell lines)

The anticancer potential of *Heliotropium curassavicum* against HepG2, MCF-7, and VERO cell lines was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), which measures cell viability in the presence of the plant extract. As described by **Horiuchi *et al.* (1988)**, cells were seeded into 96-well plates at a density of 1.0×10^5 cells/mL (100 µL per well) and incubated at 37°C for 24 hours. After washing twice with phosphate-buffered saline (PBS), the cells were treated with the *H. curassavicum* extract for 48 hours. Following treatment, 20µL of MTT solution (prepared at 5 mg/mL in PBS) was added to each well, and the plate was centrifuged at 252xg for 5min to ensure thorough mixing. The cells were then incubated at 37°C with 5% CO_2 and 95% relative humidity. After incubation, the resulting formazan crystals were dissolved in 200µL of DMSO to ensure complete solubilization. Absorbance was measured at 560nm, using DMSO as the blank control **(Belakhdar *et al.* 2012; Senthilraja & Kathiresan, 2015)**.

Flow cytometry

To analyze the cell cycle, MCF-7 cells were seeded in 6-well plates at a density of 1.0×10^4 cells per well and incubated at 37°C with 5% CO_2 for 24 hours prior to treatment. Cells were then exposed to the IC_{50} concentration of *Heliotropium curassavicum* hexane extract for 48 hours. After the treatment, cells were harvested and centrifuged at 1007xg for 5min, after which the supernatant was gently removed to avoid disturbing the pellet. The cell pellets were fixed in 500µL of 70% cold ethanol and stored overnight at –20°C. After fixation, the cells were washed with 500µL of PBS and incubated at 4°C for 2 hours. Subsequently, the pellets were stained with a solution containing 50µg/ mL propidium iodide (PI), 0.1% Triton X-100, and 0.5mg/ mL RNase for 1.0 hour at 30°C in the dark. Flow cytometry was performed to analyze the stained cells using an excitation wavelength of 488nm. DNA content was quantified using a FACSCalibur flow cytometer (Becton Dickinson, USA) **(You *et al.*, 2018)**.

Assessment of apoptosis by annexin V–FIT

The percentage of apoptotic cells was determined through flow cytometry utilizing the annexin V-FITC Apoptosis Detection Kit, following the manufacturer's protocol. In brief, cells were seeded and exposed to the IC_{50} dose of *H. curassavicum* extract. After a 48-hour incubation period, cells were centrifuged at 112xg for 5min and were then resuspended in 500µL of annexin-V binding buffer. Next, 5µL of annexin-V-FITC and 5µL of propidium iodide (PI) were added to the suspension. The mixture was incubated in the dark at room temperature for 5min. Finally, apoptotic cell levels were assessed using a BD FACSCalibur flow cytometer **(Zhao *et al.*, 2015)**.

DNA fragmentation by diphenylamine assay (DPA)

Quantitative assessment of DNA fragmentation was estimated based on the method described by **Gibb *et al.* (1997)**. MCF-7 cells were treated with *H. curassavicum* extract for 48 hours and DNA fragmentation was evaluated using the diphenylamine (DPA) assay, which detects deoxyribose residues. Approximately 5×10^5 cells were placed in sterile 1.5mL microcentrifuge tubes and centrifuged at 448xg for 5min at 4°C. The resulting pellets and supernatants were carefully separated into individual tubes. The pellets were then resuspended in 0.5mL of lysis buffer. Exactly, 1.5mL of 10% trichloroacetic acid (TCA) was added to both the pellet (P) and supernatant (S) and incubated at 4°C for 10min. Subsequently, 2mL of DPA reagent was added to each fraction (the pellets and supernatant) and incubated at room temperature for 24 hours. Absorbance readings were taken at 600nm to quantify DNA content in both the supernatant (representing fragmented DNA) and the pellet (representing intact DNA), following the approach outlined by **Akolade *et al.* (2018)**. DNA fragmentation was calculated using the formula:

DNA fragmentation (%) = [S / (S + P)] × 100.

Assessment of DNA fragmentation by gel electrophoresis

Genomic DNA fragmentation was evaluated through agarose gel electrophoresis, following the procedure outlined by **Zhao *et al.* (2013)**. After a 48-hour treatment with *H. curassavicum* extract, DNA was isolated from MCF-7 cells. Electrophoresis was performed on an 8% agarose gel containing ethidium bromide. After separation, DNA bands were visualized under ultraviolet (UV) light and captured using a digital camera Canon (U.S.A).

Gene expression (qPCR)

MCF-7 cells were treated with *H. curassavicum* extract at its IC_{50} concentration (85.23µg) for 48 hours. The qPCR was then proceeded using the One-Step qPCR iScript kit with SYBR® Green, following the manufacturer's guidelines for reaction setup and thermal cycling conditions. The PCR master mix included 25µL of 2× SYBR® Green solution, 1.5µL each of forward and reverse primers (10µmol), 1.0µL of 50× iScript Reverse Transcriptase, and nuclease-free water to bring the final volume to 50µL. Prior to amplification, the reaction mixture was heated at 95°C for 5min to initiate denaturation. The amplification process consisted of 45 cycles, each involving denaturation, annealing, and extension phases **(Li *et al.*, 2022).** All used primers are listed in Table (1).

Table 1. Forward and reverse primers used in gene expression analysis

Gene	Forward	Reverse
BAX	5'-TCAGGATGCGTCCACCAAGAAG-3'	5'-TGTGTCCACGGCGGCAATCATC-3'
BCL-2	5'-ATCGCCCTGTGGATGACTGAGT-3'	5'-GCCAGGAGAAATCAAACAGAGGC-3'

P53	5'-CCTCAGCATCTTATCCGAGTGG -3'	5'-TGGATGGTGGTACAGTCAGAGC -3'
CASP8	5'-AGAAGAGGGTCATCCTGGGAGA-3'	5'-TCAGGACTTCCTTCAAGGCTGC-3'
GAPDH	5'-GTCTCCTCTGACTTCAACAGCG-3'	5'-ACCACCCTGTTGCTGTAGCCAA-3'

Hemolytic assay

The hemolytic activity of *H. curassavicum* extract was evaluated using a spectrophotometric method as described by **Yang *et al*. (2005)**. Fresh human blood samples were collected from healthy volunteers and transferred into EDTA tubes, followed by thorough mixing. The samples were centrifuged at 1007xg for 10min to separate the erythrocytes from the plasma. The supernatant was discarded, and the erythrocytes were washed multiple times with phosphate-buffered saline (PBS) until the plasma was completely removed. During each wash, the buffy coat was carefully removed and made suspension without hemolysis. A volume of 900µL of erythrocyte suspension was mixed with 100µL of *H. curassavicum* extract at varying concentrations (31.25, 62.5, 125, 250, 500, and 1000µg/ mL, prepared in PBS). These mixtures were incubated at 37°C for 1.0 hour. Following incubation, the samples were centrifuged at 11181xg for 5min at 4°C. The amount of free hemoglobin released into the supernatant was measured using a UV-Vis spectrophotometer at 570nm. Sterile PBS served as the negative control, while 0.1% Triton X-100 was used as the positive control. All experiments were performed in triplicate, and the average values were calculated **(Silva *et al.*, 2000; Zohra & Fawzia, 2014)**.

Statistical analysis

Data were statistically analyzed using SPSS software version 26.0. Results are presented as the mean ± standard error (SE). Group comparisons were performed using either Student's t-test or one-way ANOVA, followed by Tukey's post hoc analysis. A *P*-value ≤ 0.05 was considered statistically significant.

RESULTS

GC-MS analysis

GC-MS analysis was processed to identify and assess the temporal variation for phytochemicals that present in hexane extract of *H. curassavicum* in four different seasons. The GC-MS chromatogram analysis of the extract identified the presence of thirty-two, fourteen, thirty-nine and forty-five compounds in winter, summer, autumn, and spring seasons, respectively **(**Fig. 1**)**. The phytochemical constituents were identified based on their peak areas, retention times, and molecular formulas (Table 2). Erucic acid was the most prevalent compound at retention time of 34.98 with a peak area of 7.69%, and retention time (35) and a peak area of 5.38% in autumn and spring seasons, respectively. Moreover, the most common compounds in autumn

following erucic acid were benzene (5.10%), hexadecanoic acid (2.68%), 9,12-octadecadienoic acid "Z,Z" (2.67%), and tetratetracontane (1.70%). The prevailing compounds in spring season after erucic acid were benzene (5.85 %), "Z" (2.29%), and tetratriacontane (2.23%).

The most common compound in winter was eugenol at retention time 13.59 with peak area 40.54% followed by hexadecanol (7.83%), dodecanol (7.35 %), benzene, 1,2,4,5-tetramethyl- (6.95%), 9,10 dideutero (4.89%), hexadecanoicacid, (2.05%) and 9-octadecenoic acid "Z" (1.98%). The most dominant compound in summer season was Z,Z at retention time 30.68 with peak area (43.95 %) followed by palmitic acid, TMS derivative (19.91%), Z (6.83%), hexadecanoic acid (5.04%) ,phenol (4.07%) and cyclobutane (1.70%). Across all seasons, the extracts of *H. curassavicum* consistently contained three common phytocompounds: hexadecanoic acid, Z, and Z,Z.

The mass spectrum of *H. curassavicum* extract identified twenty-two compounds in winter such as eugenol with peak area (40.54 %), eicosapentaenoic acid (0.94%), limonen-6-ol,pivalate(0.29%), benzene, 1,2,4,5-tetramethyl(6.95%), p-cymene(0.21%), hydrocinnamic acid (0.37%), and methyl 2,4-tridecadiynoate(3.60%) that were not detected in the other seasons. It can be observed that four compounds were detected in the summer season and did not exist in the other seasons such as palmitic acid, TMS derivative (28.19%), phenol with peak area (2.51%), cholestan-3-ol, 2-methylene (1.15%) and tetraneurin-A-diol (1.27%). Additionally, there were three compounds that existed in autumn such as methyl stearate (1.33%), dimethylpropanoilhydrazono (0.20%) and docosane (0.80%) and two compounds in spring such as 17-Pentatriacontene(1.81%) and isomeric dodecylbenzene (0.37%) that were absent in the other seasons.

Table 2. Phytocomponents identified in the hexane extract of *H. curassavicum* during different seasons

Retention time	Compound name	M. Wt	Peak area %			
			W	S	a	S
4.15	Cyclobutane	426	0.	1.	N	N
5.17	7-Epi-Cis- Sesquisabinene Hydrate	222	0.	3.	N	N
5.15	Eicosapentaenoic Acid	302	0.	N	N	N
5.82	Limonen-6-Ol, Pivalate	236	0.	N	N	N
6.43	Benzene, 1-Methyl-4-(1-Methylethyl)	134	2.	N	N	N
7.30	Benzene, 1,2,4,5-Tetramethyl	134	6.	N	N	N
7.78	2,3-Epoxicaran, Trans	152	0.	N	N	N
7.78	P-Cymene	134	0.	N	N	N
9.19	Benzene, 1,3-Dimethyl-5-(1-Methyleth	148	2.	N	N	N
9.44	Benzaldehyde, 4-(1-Methylethyl)-	148	0.	N	N	N
10.50	N-Isobutylundeca-2(E)-En-8,10-Diyn	231	0.	N	N	N
10.88	Hydrocinnamic Acid	294	0.	N	N	N
11.93	Nonanoyl Chloride	176	0.	N	N	N
12.86	1,5,5-Trimethyl-6-Methylene-Cycloh	136	2.	N	N	N
13.44	Benzene, (1-Butylhexyl)-	218	N	N	1	2
13.59	Eugenol	164	4	N	N	N

13.60	Benzene, (1-Propylheptyl)-	218	3.	N	1	2
13.95	Benzene, (1-Ethyloctyl)-	218	N	N	1	2
13.98	Phenol	164	N	2.	N	N
14.59	Methyl 2,4-Tridecadiynoate	220	3.	N	N	N
14.66	Benzene, (1-Methylnonyl)-	218	N	N	2	3
15.19	1,3-Propanediol,	232	0.	N	N	N
15.25	Benzene, (1-Pentylhexyl)-	232	N	N	2	2
15.49	Octadecadiynoic Acid,	290	0.	2.	N	0
15.70	Benzene, (1-Methyldecyl)-	232	N	N	5	0
15.87	Benzene, (1-Ethylnonyl)-	232	N	N	4	0
16.10	Isomeric Dodecylbenzene	246	N	N	N	0
16.22	1-Dodecanol	186	7.	N	N	N
16.33	Tetraneurin - A - Diol	280	N	1.	N	N
16.44	Cholestan-3-Ol, 2-Methylene-	400	N	1.	N	N
16.55	Benzene, (1-Methyldecyl)-	232	N	N	5	0
16.84	3-Trifluoroacetoxypentadecane	324	0.	N	N	N
17.61	Dideutero Octadecanal	270	1.	NA	N	0
17.74	7-Methyl-Z-Tetradecen-1-Ol Acetate	268	N	1.18	N	0
18.61	1-Hexadecanol	242	7.	NA	N	0
18.76	Benzene, (1-Pentyloctyl)-	260	N	NA	5	5
20.44	Hexadecanoic Acid, Methyl Ester	270	N	NA	2	0
24.28	Hexadecanoic Acid	330	0.	5.04	1	1
20.67	Dioxo-11-À-Hydroxycona	341	1.	NA	N	N
21.47	Alanine, 3-(Benzyloxy)-, L-	195	0.	NA	N	N
23.56	Methyl Stearate	298	N	NA	1	N
25.97	Oxiraneoctanoic Acid, 3-Octyl-,	312	N	1.18	2	N
27.3	Pentatriacontane	492	N	NA	0	0
28.18	12-Methoxy-2-Trimethylsily	328	1.	NA	N	N
28.19	Palmitic Acid, Tms Derivative	328	N	28.1	N	N
28.65	Tetratetracontane	618	N	NA	0	0
29.30	9-(2',2'-Dimethylpropanoilhydrazono)	576	N	NA	0	N
29.90	Pentatriacontane	492	N	NA	0	0
	9-Octadecenoic Acid (Z)-	282	1.	2.52	0	2
30.68	9,12-Octadecadienoic Acid (Z,Z)	280	1.	43.9	2	2
31.12	Heptacosane	380	N	NA	0	0
31.12	Docosane	310	N	NA	0	N
32.29	Dotriacontane	450	N	NA	0	0
33.31	Nonacosane	408	N	NA	1	2
33.80	4h-1-Benzopyran-4-One,	610	N	NA	0	0
33.97	Isochiapin B	346	N	5.04	0	0
34.96	Hahnfett	0	N	NA	0	0
34.98	22-Tricosenoic Acid	352	N	NA	7	5
35.12	Oleic Acid	562	5.	NA	0	1
35.54	STIGMAST-5-EN-3-OL, (3á,24S)-	414	N	NA	0	1
35.34	17-Pentatriacontene	490	N	NA	N	1

Abbreviation : M.Wt: Molecular weight , win: winter , sum: summer , spr: spring,aut: autumn , NA: Not Applicable.

Quantitative analysis of phytochemical constituents

The phytochemical components of *H. curassavicum* extracts during different seasons are explored in Table (3). Data revealed that methanol-based extractions yielded a substantial presence of plant secondary metabolites. Exploring the phytochemical constituents of *H. curassavicum* extracts (Table 3) in different seasons showed that the extracts using methanol solvent contained a good amount of plant secondary metabolites. The result shows that all the seasons contain the phytochemicals in varied concentrations of phenols with the highest level occurring in autumn **23.2 (mg/g)**, followed by summer **21.64 (mg/g)**, spring 16.92 **(mg/g)** and the lowest level occurred in winter (**3.95mg/ g)**. *H. curassavicum* extract was found to have maximum amount of total alkaloids in autumn with **9.81**mgg^{-1} then in spring with **9.3**mgg^{-1} and in winter with a minimum amount of total alkaloids at **3.91**mgg^{-1}. While, the moderate amount was in summer with **6.13**mgg^{-1}. The total flavonoid content varied between **261.7** and **103.6**mg/ mL quercetin in summer and winter, respectively.

Changes in DPPH radical scavenging activity

The *H. curassavicum* extract demonstrated significant DPPH radical scavenging activity, which intensified with increasing concentrations, indicating a dose-dependent response (Table 3). The antioxidant activity was most notable during the summer season, reaching a peak value of 43.26 ± 0.005, whereas the lowest activity was recorded in the winter season (17.62±0.014).

Table 3. Changes in phytochemicals constituents and antioxidant activity of *H. curassavicum* during four different seasons. Values are mean ± standard error

Season	Alkaloid (mgg^{-1})	Flavonoids (mg/mL quercetin)	Phenols (mg/g gallic acid)	DDPH (%)
Winter	3.91 ± 0.015	103.6 ±0.008	3.95 ±0.008	17.62 ±0.014
Spring	9.3 ± 0.02	186.7 ±0.014	16.92 ±0.02	26.4 ±0.014
Summer	6.13 ± 0.012	261.7 ±0.017	21.64 ±0.008	43.26 ±0.005
Autumn	9.81 ±0.008	254.2 ±0.005	23.2 ±0.008	45.07 ± 0.286
$P \leq 0.05$	0.063	0.057	0.042	0.651

Cell proliferation by MTT assay

To evaluate the cytotoxic effects of *H. curassavicum* extracts obtained across four different seasons, human breast cancer (MCF-7), human liver cancer (HepG2), and African green monkey kidney (VERO) cell lines were exposed to varying extract concentrations (31.25– 1000µg/ mL). A significant, dose-dependent reduction in the proliferation of all three cell lines following treatment with the hexane extract for 48 and 72 hours. Cell viability was assessed using the MTT assay after 24 and 72 hours of

incubation. Several extracts exhibited cytotoxic effects in a dose-responsive manner, as shown in Fig. (1). Notably, the extracts from autumn and winter demonstrated the strongest cytotoxic activity against the MCF-7 cell line. The hexane extract of *H. curassavicum* in autumn season appeared to be the most robust against MCF-7 (IC_{50} = 85.23µg/ mL) followed by winter season (IC_{50} = 85.37 µg/mL) compared to spring and summer seasons with IC_{50}= 122.19 µg/mL) and IC_{50}=116.21 µg/mL, respectively. The highest cytotoxic activity of *H. curassavicum* extract against HepG2 was observed in winter with IC_{50}=70.59µg/ mL compared to the other seasons. Low or no cytotoxicity was detected in normal African green monkey kidney cell lines (VERO) after treatment with *H. curassavicum* extract in all seasons. *H. curassavicum* extract was found mostly selective toward cancer cells (Table 4).

These findings are noteworthy, as they indicate that the *H. curassavicum* extract exhibits greater toxicity toward cancer cells compared to normal cells. The results clearly demonstrate the increased efficacy of the hexane extract of *H. curassavicum* against cancer cell lines across all seasonal samples. It is clear that MCF-7 in autumn season was the most susceptible cancer cell line compared to all seasons and other cell lines. Therefore, cell cycle arrest and apoptotic analysis were carried out on this cell only. Obviously, after 48 hours of the treatment of MCF7 with *H. curassavicum* extract, during autumn season the highest selective index was obtained compared with other extracts. Accordingly, this autumn extract has been selected for further investigation against its target cell MCF7.

Table 4. IC_{50} values and selectivity index (SI) of *H. curassavicum* extract on MCF-7, HepG2, and VERO cell lines

Incubation period		IC_{50} (µg/mL)			Corresponding SI	
		MCF-7	HepG2	VERO	Vero/ MCF-7	Vero/ HepG2
48h	winter	85.37 ± 1.14	70.59 ± 3.26	279 ± 3.83	3.27	3.95
	spring	122.19 ± 1.82	110 ± 3.51	415.77 ± 2.71	3.4	3.78
	summer	116.21 ± 1.67	92.33 ± 1.33	376.21 ± 7.81	3.24	4.07
	autumn	85.23 ± 1.5	123.6 ± 2.84	409.63 ± 15.37	4.80	3.31
72h	winter	74.09 ± 4.65	142 ± 11.08	210 ± 4.3	2.83	1.05
	spring	113.75 ± 2.56	101.8 ± 0.59	354.16 ± 4.15	3.11	3.48
	summer	92.8 ± 0.77	118.19 ± 0.29	344.03 ± 2.22	3.7	2.91

	autumn	78.88 ± 2.97	118.18 ± 7.07	351.78 ±7.98	4.46	2.98

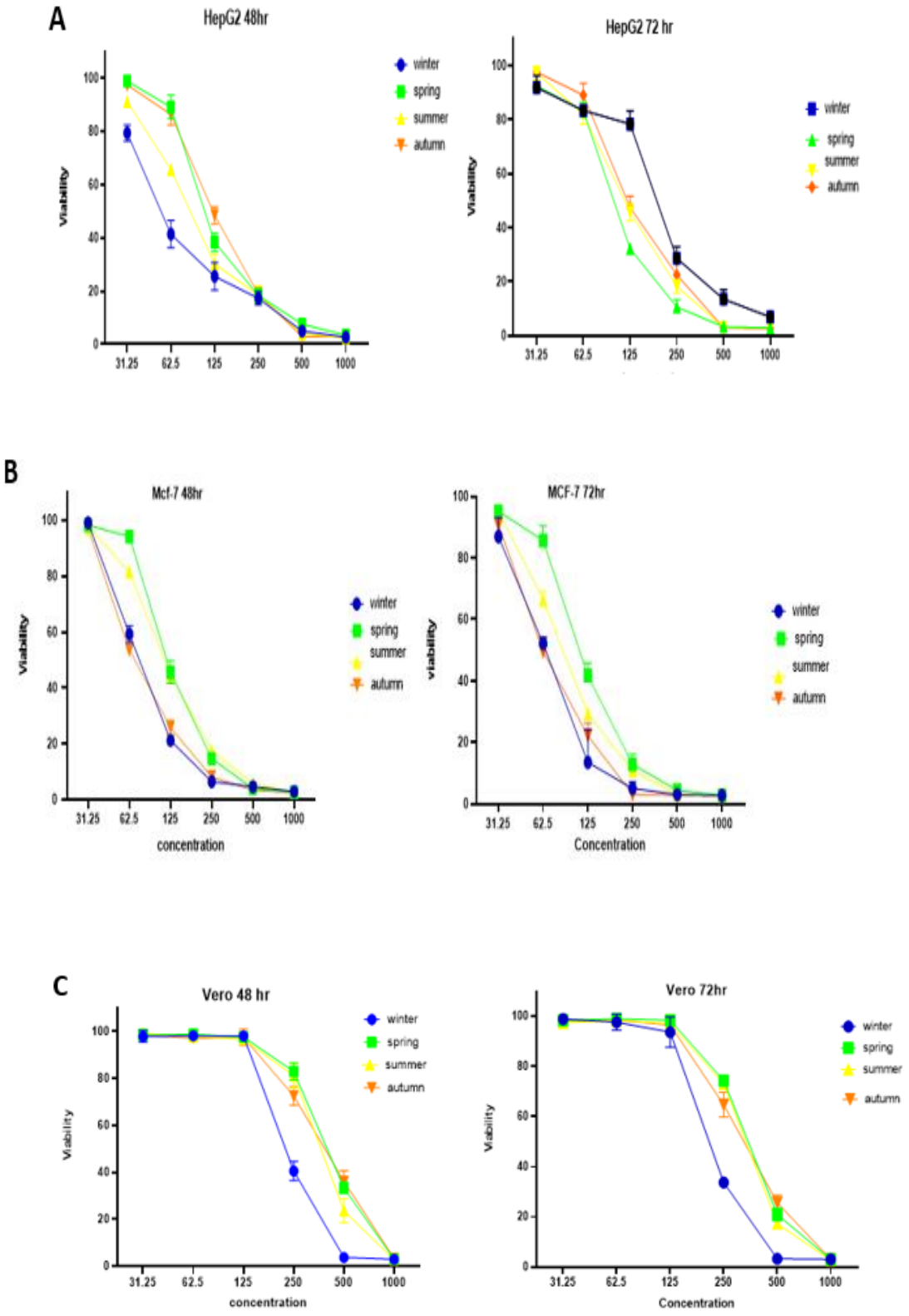

Fig. 1. Anticancer effects of the hexane extract of *H. curassavicum*, evaluated against two cancer cell lines (MCF-7 and HepG2) as well as a normal cell line (Vero).(A) The cell viability percentage of MCF-7 cell line, (B) The cell viability percentage of HepG2 cell line and (C) The cell viability percentage of the normal cell line Vero.

Flowcytometry

Cell cycle analysis was conducted using the hexane extract of *H. curassavicum* to quantitatively assess the degree of apoptosis. As shown in Fig. (2), treatment of MCF-7 breast cancer cells with the extract resulted in cell cycle arrest at the S phase. The treated cells exhibited a 35.63% population in the S phase, compared to 27.26% in the untreated control group.

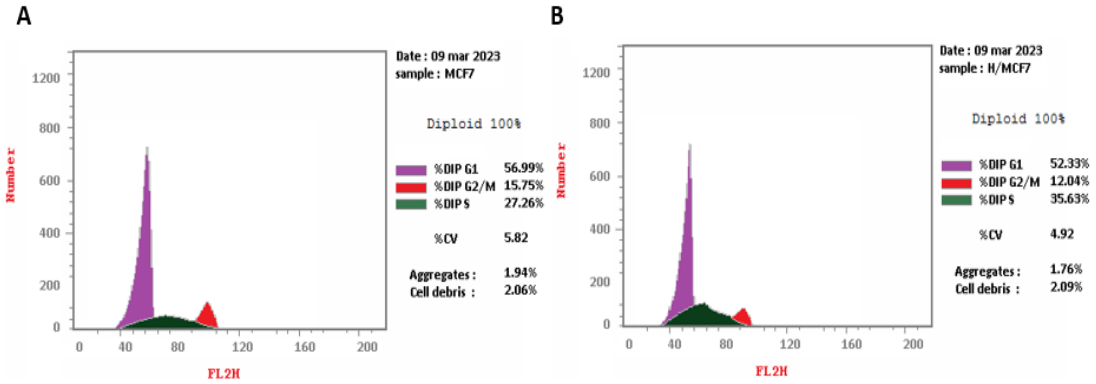

Fig. 2. The cell cycle phase distribution in MCF-7 cells after exposure to the hexane extract of *H. curassavicum* was analyzed following treatment at the IC_{50} concentration for 48 hours. Panel (A) illustrates the cell cycle profile of untreated control cells, whereas panel (B) presents the profile of MCF-7 cells treated with the extract.

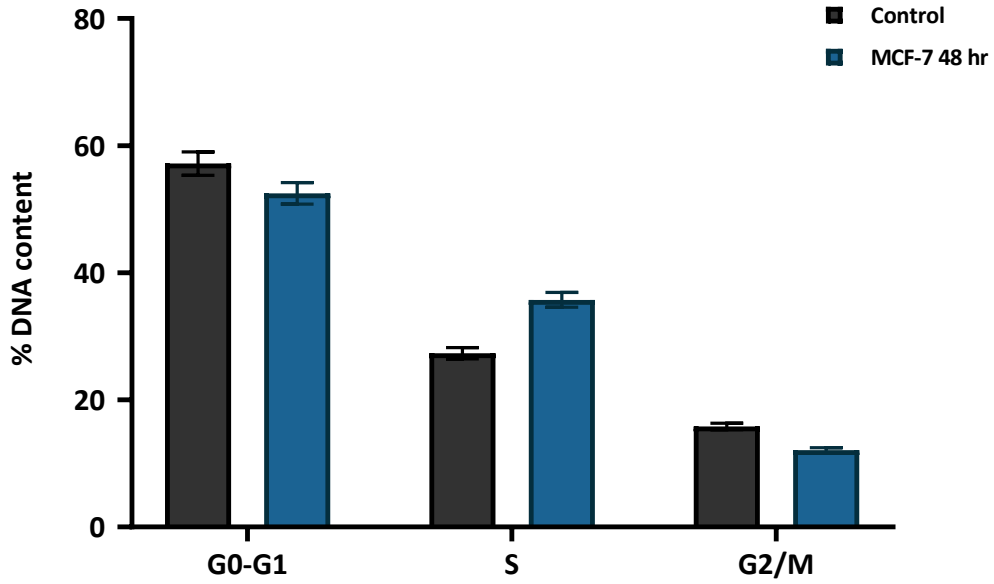

Fig. 3. The proportion of cells distributed across the G0-G1, S, and G2/M phases of the cell cycle

Apoptotic cell death analysis

Apoptosis was evaluated using annexin-V-FITC/PI staining, which showed that treatment with the IC_{50} concentration of *H. curassavicum* extract (85.23) significantly increased the total apoptotic rate in MCF-7 cells to (31.29%) compared to the control group (2.28%) (Fig. 4).

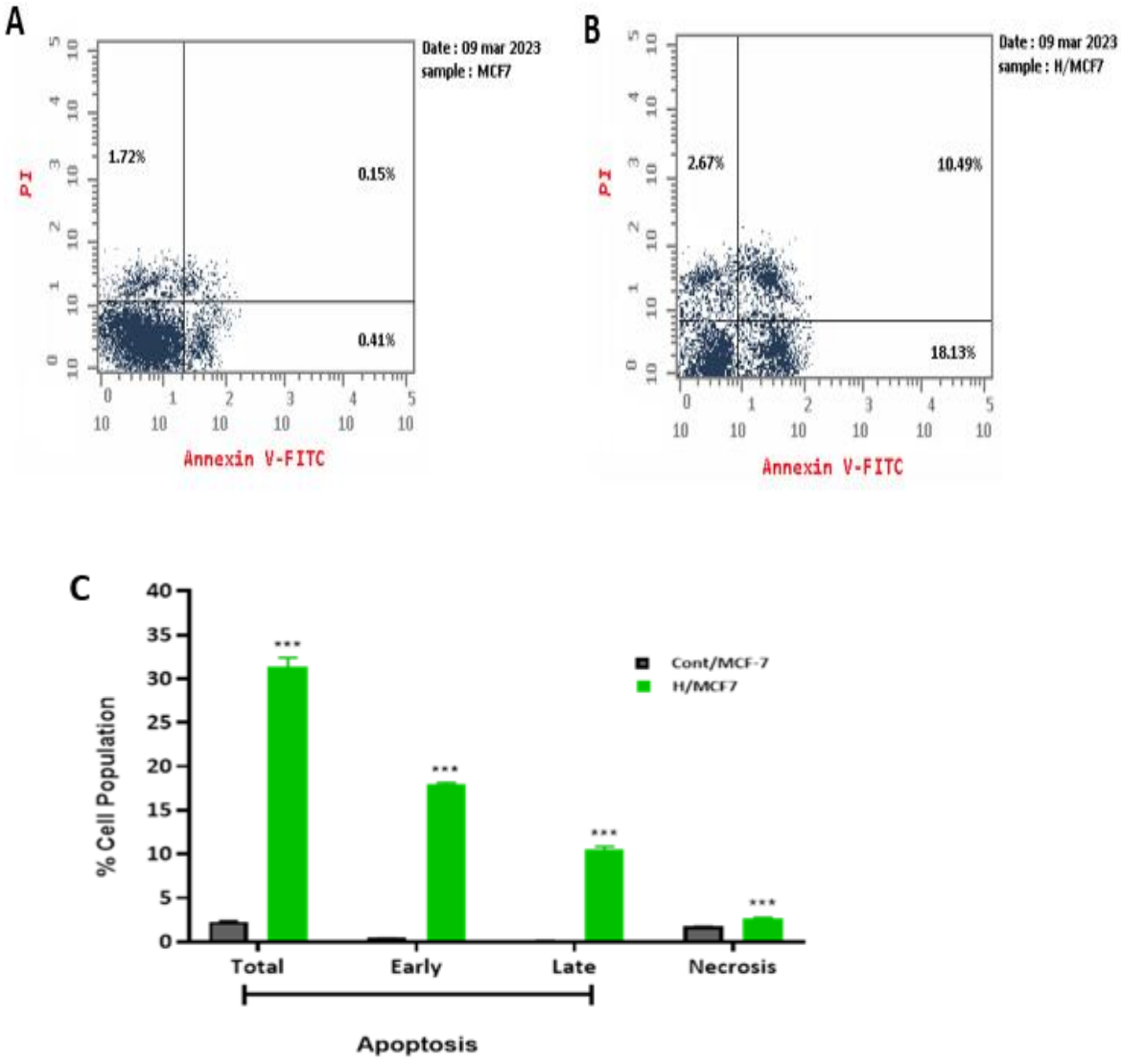

Fig. 4. Apoptosis in MCF-7 cancer cells induced by the hexane extract of *H. curassavicum* was evaluated using Annexin V-FITC/PI staining. Cells were treated with the IC_{50} concentration for 48 hours. The lower-left quadrant indicates early apoptotic cells, while the upper-left quadrant represents late apoptotic cells. Panel (A) shows the control MCF-7 cells, panel (B) displays cells treated with *H. curassavicum* extract, and panel (C) provides a quantification graph of the Annexin V/PI double-staining assay, analyzed using a BD FACSCalibur flow cytometer. Data are presented

as mean ± SEM. *** indicates a statistically significant difference ($p < 0.001$) compared to the control.

DNA fragmentation

Quantification results of DNA fragmentation in MCF-7 cells following treatment with the IC_{50} concentration (85.23) of *H. curassavicum* extract as measured by the DPA assay are shown in Fig. (5). Compared to the control group, which showed 4.58% ± 0.24 DNA fragmentation, the treated cells exhibited a significantly higher fragmentation level of 27.31% ± 1.43. These results suggest that the plant extract induced considerable DNA damage.

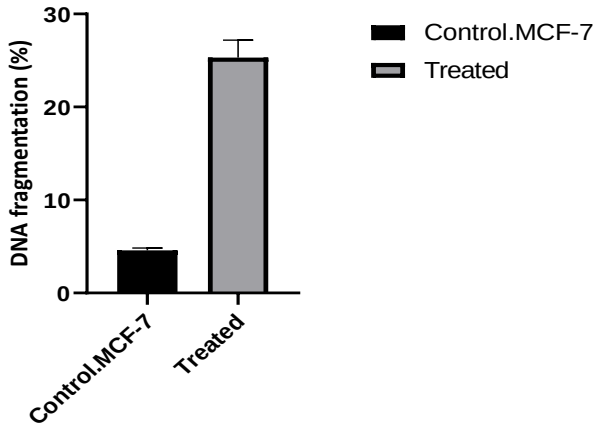

Fig. 5. DNA fragmentation levels in treated MCF-7 cells as determined by the diphenylamine (DPA) method

DNA agarose gel electrophoresis

The DNA gel pattern of untreated MCF-7 control cells displayed distinct bands indicating intact DNA. In contrast, treatment with the IC_{50} concentration of *H. curassavicum* extract (85.23) led to increased DNA smearing and the appearance of a ladder-like fragmentation pattern, which intensified with higher extract concentrations compared to the control group (Fig. 6).

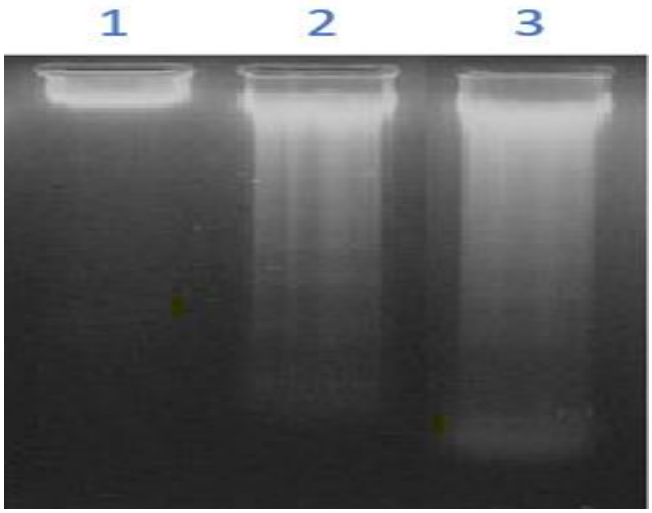

Fig. 6. DNA fragmentation patterns from MCF-7 cells analyzed using 8% agarose gel electrophoresis. Lane (1) shows intact DNA from untreated MCF-7 cells, while lane (2&3) displays fragmented DNA from MCF-7 cells exposed to 85.23 µg/mL of *H. curassavicum* extract.

qPCR analysis

The qPCR analysis of MCF-7 cells treated with *H. curassavicum* extract revealed an upregulation in the expression of *P53*, *BAX*, and caspase-8 by 3.2674-fold, 3.1886-fold, and 4.6837-fold, respectively. In contrast, a downregulation of *BCL-2* expression was observed in the treated cells. An increased expression of caspase 8, *BAX*, and *P53* was observed in treated cells compared to the untreated control cells (Fig. 7).

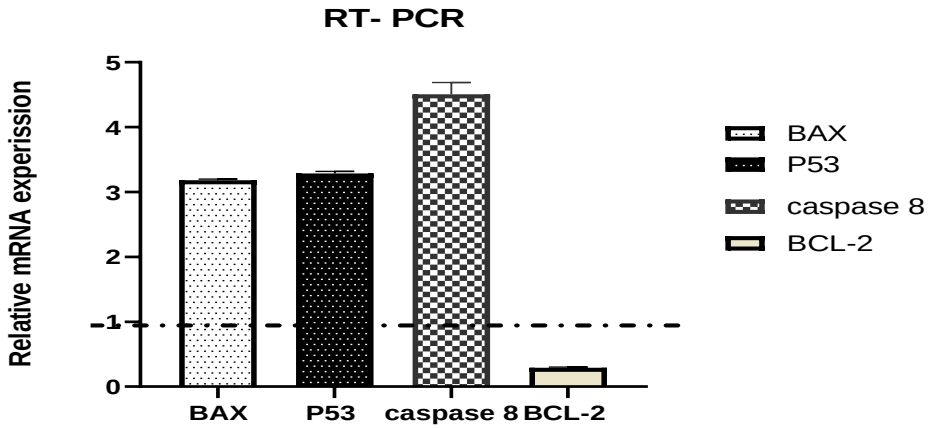

Fig. 7. qPCR was conducted to evaluate the expression of apoptosis-related genes in both untreated MCF-7 cells and those exposed to the IC_{50} concentration of the hexane extract of *H. curassavicum*. The dashed line represents the control group, set at a fold change of 1.0.

Hemolytic assay

The *H. curassavicum* extract demonstrated a mild hemolytic effect on human red blood cells across all four seasons. The plant extract of *H. curassavicum* through four different seasons displayed low or no hemolytic activity on erythrocytes (Figure 8), as compared to negative and positive controls.

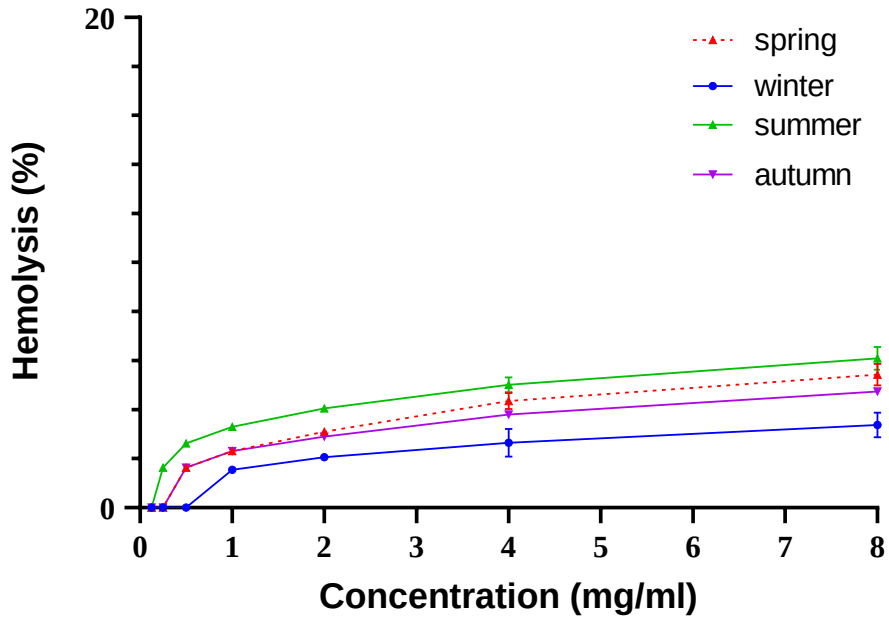

Fig. 8. Hemolytic activity of hexane extract of *H. curassavicum* against human erythrocytes through four different seasons

DISCUSSION

Due to the increasing prevalence of chemotherapeutic drugs resistance and their related toxic side effects, medicinal plant-based anticancer therapies may have potent activities with less toxic side effects **(Housman *et al.*, 2014; Sultana *et al.*, 2014)**. Recently, the medicinal plants have received great attention due to the presence of high levels of the bioactive phytochemicals with anticancer potentials **(Katalinic *et al.*, 2006; Nyamai, 2016; Huang *et al*, 2025)**. It is well established that both spatial and temporal variation have significantly influenced the phytochemical composition of plants **(Ogwu *et al.*, 2025)**.

Species of *Heliotropium* contain various bioactive compounds, including alkaloids, terpenoids, phenolics, pyrrolizidines, and quinones, which are well known for their strong antioxidant and antimicrobial activities **(Ghori *et al.*, 2016)**. These antioxidants help reduce oxidative stress induced by reactive oxygen species (ROS), which is associated with the development of diseases such as cancer, arteriosclerosis, rheumatoid arthritis, and the aging process **(Liguori *et al.* , 2018)**.

H. curassavicum is an important medicinal herb, containing various vital bioactive phytochemicals like alkaloids, glycosides, phenols, flavonoids, saponins etc. It is found in seashores and marshy areas near or not far from the sea. It can be readily identified by its glabrous, fleshy stems and leaves, along with its terminal scorpioid cymes **(Charilaou, 2018)**.

GC-MS analysis of the hexane extract of *H. curassavicum* demonstrated that seasonal changes affect its phytochemical composition. Eugenol was identified as the predominant compound during the winter season. Similar findings have been reported elucidating the presence of eugenol in the essential oil of the same plant of *H. curassavicum.* **(Joshi, 2020)**. Z,Z was the most dominant compound in summer season. These findings agree with **(El-Kamali *et al.*, 2019)** which have found Z,Z in the n-hexane extract of *H. sudanicum.* GC-MS analysis revealed the presence of the fatty acid compound erucic acid as one of the most common components in autumn and spring seasons. This fatty acid compound was detected throughout the extract of the entire plant of *Anchusa azurea* belonging to the Boraginaceae family **(Engel *et al.*, 2016)**.

Our findings revealed the hexane extract of *H. curassavicum* showed the highest antiproliferative activity against MCF-7 in autumn season followed by winter season compared to spring and summer seasons, whereas the highest cytotoxic activity of *H. curassavicum* extract against HepG2 was observed in winter season. In contrast, the Vero normal cell line showed no significant response to the same concentrations of the plant hexane extract, suggesting that its cytotoxic activity is selectively directed toward cancer cell lines. Comparable selective inhibitory effects of this plant have been reported on the growth and proliferation of HeLa, SiHa, and C33A cells, showing a dose-dependent response when compared to untreated controls **(Paul *et al.*, 2018)**. However to our knowledge, the existence of erucic acid, eugenol and Z,Z may explain the variation in the anti- proliferative activity of *H. curassavicum.* The anticancer of these mentioned constituents is well established in a variety of reports **(Jaganathan & Supriyanto, 2012; Altinoz *et al.*, 2021; Diab, Donia & Saad-Allah, 2021)**.The hexane extract of this plant contains unique compounds such as eugenol, Z,Z and erucic acid, which have a potential anti-proliferative effect against a variety of human tumor cell lines. In earlier studies, eugenol have been reported to exhibit antimutagenic, antioxidant, anti-inflammatory, antigenotoxic, and anticancer properties **(Jaganathan & Supriyanto, 2012).** Z,Z has been previously shown to possess anticancer activity. Similarly, the presence of Z,Z in the extract of Boraginaceae species; *Cordia sebestena* L. also has multiple biological uses with antimicrobial, anticancer and hepatoprotective effects **(Diab *et al.*, 2021)**. *Boragno officinalis* (family: Boraginaceae) is one of the richest species in erucic acid with a potent anticancer activity **(Galanty *et al.*, 2023)**. In addition to these findings, the hexane extract of *H. curassavicum* exhibited notably selective cytotoxic effects when compared to other Boraginaceae species, including *Heliotropium ciliatum* and *Heliotropium subulatum* **(Singh *et al.*, 2002; Ilonga, 2012)**.

DNA fragmentation assay was performed by seeding of MCF-7 with the hexane extract of *H. curassavicum* for 24 hours and showed a typical ladder formation

for incubated cells. DNA damage is characteristic of apoptotic cell death. The assay was conducted to assess the genotoxic potential of the extract leading to cell death. Extensive nuclear DNA damage serves as an internal signal to the mitochondria, triggering the release of cytochrome c and shifting the balance toward cell death instead of repair and survival. The assay was performed to evaluate genotoxicity potential of the extracts **(Al Ssadh & Madar, 2018; Tawfik *et al.*, 2021)**. Similar fragmentation of DNA was observed for the same plant extracts in previous studies **(Paul *et al.*, 2015; Sarkar *et al.*, 2021)**. Interestingly, eugenol, erucic acid and octadecadienoic acid induces apoptosis and DNA damage **(Dewey *et al.*, 2007; Shin *et al.*, 2007; Manivannan *et al.*, 2017)**.

Notably, *H. curassavicum* showed a powerful capacity to trigger apoptosis and to cause cell cycle arrest in MCF-7 cancer cells. Cell cycle analysis indicated that the hexane extract caused growth arrest at the S phase, potentially as a result of DNA damage **(Ye *et al.*, 2003)**. These results are consistent with previous studies showing that members of the same genus, such as *Heliotropium curassavicum*, can trigger apoptosis in various cancer cell lines by inhibiting the G1/S transition, as observed in HeLa, C33A, and SiHa cells **(Paul *et al.*, 2018)**. It also caused a G1/S phase cell cycle arrest in cervical cancer cells. Similar results were observed in other Boraginaceae species, such as *Echium arabicum* extracts, which caused S-phase arrest in HepG2 liver and colon cancer cells **(Abutaha *et al.*, 2022)**.

Furthermore, flow cytometry analysis of MCF-7 cells treated with *H. curassavicum* extract revealed a notable increase in both early and late apoptotic cell populations. The increase in Annexin V-FITC stained cells clearly indicates the induction of apoptosis in the treated cells. Previous studies have reported that other species extracts of the same family induce apoptosis rather than necrosis on different cancer cell lines such as *Heliotropium ramosissimum* and *Onosma bracteata*. on colorectal carcinoma and human osteosarcoma cell lines, respectively **(Fayed*et al.*, 2022; Kumar *et al.*, 2022)**. Mitochondria play a key role in regulating apoptosis by releasing cytochrome c and other apoptosis-inducing factors into the cytoplasm, as well as decreasing the mitochondrial membrane potential (**Kumar *et al.*, 2022)**. The ability to induce apoptosis is a critical parameter for assessing the anticancer potential of plant extracts **(Engel *et al.*, 2016)**.

Apoptosis plays a vital role in maintaining and supporting life by regulating cell numbers during growth and development, as well as eliminating damaged cells that could disrupt homeostasis. Triggering apoptosis in cancerous cells through natural phytochemicals presents a promising approach for cancer therapy using chemotherapeutic agents. Apoptosis is initiated through two primary pathways: the mitochondrial pathway and the death receptor pathway. Caspase-9 is involved in the mitochondrial pathway, whereas caspase-8 plays a key role in the death receptor pathway. Both pathways converge on the activation of the effector enzyme caspase-3, ultimately leading to cell death **(Ranneh *et al.*, 2023)**. Additionally, apoptosis induction in MCF-7 cells treated with *H. curassavicum* extract was confirmed by the increased

expression of pro-apoptotic genes such as *P53*, *BAX*, as well as the activation of caspase-8, which collectively play a role in promoting apoptotic cell death. This was accompanied by a reduction in the expression of the anti-apoptotic gene *BCL-2*. Similarly, treatment with root extracts of *Lithospermum erythrorhizon* Sieb (a member of the Boraginaceae family), in studies addressing human hepatocellular carcinoma cells, has demonstrated an increase in pro-apoptotic markers such as *P53*, *BAX*, and caspases-3, -8, and -9, along with a decrease in the expression of the anti-apoptotic protein *BCL-2* **(Wu *et al.*, 2012; Wang *et al.*, 2018)**. Similarly, treatment of cervical cancer cell lines with the methanolic extract of *H. curassavicum* resulted in reduced *BCL-2* levels and elevated expression of pro-apoptotic proteins *BAX* and *P53* **(Paul *et al.*, 2018).**

The *P53* tumor suppressor protein is crucial in initiating the process of apoptosis and its inactivation can lead to uncontrolled cell growth and genomic instability. Due to its critical role in controlling apoptosis, cell cycle arrest, and senescence, *P53* has become a compelling target in the development of cancer therapies. Studies have reported increased expression of *P53*, caspase-3, and caspase-9, along with a reduction in *BCL-2* levels, as indicative markers of apoptosis **(Kumar *et al*., 2022)**. Moreover, *P53* can activate genes responsible for apoptosis and cell cycle regulation. In response to apoptotic stress, *P53* moves to the mitochondria, where it binds to the anti-apoptotic protein *BCL-2*, releasing the pro-apoptotic protein *BAX*. This interaction promotes the release of cytochrome c into the cytosol, thereby initiating the apoptotic process **(Cao *et al*., 2022)**.

In the present study, the *H. curassavicum* extract demonstrated minimal hemolytic activity against human red blood cells, suggesting its potential as a candidate for developing novel anticancer treatments. Several medicinal plants with strong anticancer properties, including *Nonea vesicaria* from the Boraginaceae family, have also been reported to exhibit low or negligible hemolytic effects **(Mouffouk *et al.*, 2020)**.

The findings also suggest that this plant contains elevated levels of various phytochemicals. Specifically, flavonoids and phenols were found in higher concentrations during the autumn and summer seasons, while alkaloid accumulation peaked in autumn and spring. These results are consistent with observations reported in earlier studies. In this regard, five species of *Heliotropium* (family Boraginaceae), *H. bacciferum, H. longiflorum , H. zeylanicum H. jizanense* and *H. pterocarpum* have been reported to exhibit varying pigment yields, with significant differences in phenolic and flavonoid content observed among the species **(Radwan, *et al.*, 2020)**.

CONCLUSION

The results of this study disclosed the anti-proliferative properties of *H. curassavicum* extract and demonstrated its ability to induce apoptosis *in vitro*. These findings suggested a promising potential for the use of *H. curassavicum* in the development of therapeutic strategies, particularly for breast and liver cancers. Due to its selective antiproliferative activity, this plant extract may serve as a valuable source

for anticancer drug discovery. The data further indicated that the extract suppresses growth and proliferation of MCF-7 cells by triggering apoptosis and causing cell cycle arrest at the S phase. These anticancer effects highlight the potential for further investigation into this plant, aiming to isolate the specific bioactive compounds responsible for the observed biological activities.

REFERENCES

Abutaha, N.; AL-Mekhlafi, F.A.; Almutairi, B.O. and Wadaan, M.A. (2022). S-phase cell cycle arrest, and apoptotic potential of *Echium arabicum* phenolic fraction in hepatocellular carcinoma HepG2 cells. *Journal of King Saud University - Science*, 34(1): p.101735. Available at: https://doi.org/10.1016/j.jksus.2021.101735.

Abutaha, N.; Alghamdi, R.; Alshahrani, O. and Al-Wadaan, M. (2024). Heliotropium curassavicum extract: Potential therapeutic agent for liver cancer through cytotoxicity, apoptosis, and molecular docking analysis. *Arabian Journal of Chemistry*, *17*(10): p.105986.

Adetuyi, B.O.; Adebisi, O.A.; Awoyelu, E.H.; Adetuyi, O.A. and Ogunlana, O.O. (2020). Phytochemical and Toxicological Effect of Ethanol Extract of Heliotropium Indicum on Liver of Male Albino Rats. *Letters in Applied NanoBioScience*, 10: pp.2085–2095. Available at: https://doi.org/10.1016/j.jksus.2021.101735.

Akolade, O.R.; Chinwe, A.S.; Olalekan, B.T.; Halima, A.T.; Fatima, A.A.; Emuejevoke, T.T. and Herbert, C.A. (2018). Haematological and genotoxicity evaluations of phytochemical compounds from n-hexane extract of Uvaria chamae stem on selected organs in mice. *Annals of Science and Technology*, 3(1): pp. 28–34. Available at: :https://doi.org/10.2478/ast-2018-0012.

Ssadh, H.A.; Srinivasan, S.; Meshram, Y.; Madar, I.H. and Tayubi, I.A. (2018). Apoptotic induction by Cassia fistula leaf extracts against human hepatocarcinoma cell lines. *Int J Sci Innovs*, *1*(5): pp.92-101.

Akbar, W.A.S.; Arokiarajan, M.S.; Christopher, J.J.; Ahmed, N.Z. and Meena, R. (2023). Evaluation of bioactive compounds as antimicrobial and antidiabetic agent from the crude extract of Heliotropium curassavicum L. *Biocatalysis and Agricultural Biotechnology*, *50*: p.102745.

Altinoz, M.A.; Elmaci, I.; Hacimuftuoglu, A.; Ozpinar, A.; Hacker, E. and Ozpinar, A. (2021) . PPARδ and its ligand erucic acid may act anti-tumoral, neuroprotective, and myelin protective in neuroblastoma, glioblastoma, and Parkinson's disease. *Molecular Aspects of Medicine*, 78: p. 100871. Available at: https://doi.org/10.1016/j.mam.2020.100871.

Arndt, S.K.; Irawan, A. and Sanders, G.J. (2015). Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. *Physiologia Plantarum*, 155(4): pp.355–368. Available at: https://doi.org/10.1111/ppl.12380.

Azeez, S.O.; Ayo-Lawal, R.A.; Olawuni, I.J. and Abraham, O.G. (2020). Evaluation of the bio-toxicity and cytogenetic effects of methanolic leaf extract of Heliotropium indicum L. *International Journal of Biological and Chemical Sciences*, 14: pp.1–10. Available at: https://doi.org/10.4314/ijbcs.v14i1.1.

Azwanida, N.N. (2015) . A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants*, 4(196): pp. 2167–0412.

Banu, K.S. and Cathrine, D.L.(2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, *2*(4): pp.25-32.

Belakhdar, G.; Benjouad, A. and Abdennebi, E.H. (2015). Determination of some bioactive chemical constituents from Thesium humile Vahl. Sci, 6(10): pp.2778-2783.

Bonam, S.R.; Wu, Y.S.; Tunki, L.; Chellian, R.; Halmuthur, M.S.K.; Muller, S. and Pandy, V. (2018). What has come out from phytomedicines and herbal edibles for the treatment of cancer?. *ChemMedChem*, 13(18): pp.1854–1872. Available at: https://doi.org/10.1002/cmdc.201800343.

Boulos L (2000) Flora of Egypt, Volume 2 (Geraniaceae–Boraginaceae); Al-Hadara Publishing: Cairo, Egypt.

Boveiri Dehsheikh, A.; Mahmoodi Sourestani, M.; Boveiri Dehsheikh, P.; Vitalini, S.; Iriti, M. and Mottaghipisheh, J. (2019). A comparative study of essential oil constituents and phenolic compounds of Arabian Lilac (Vitex trifolia var. Purpurea): An evidence of season effects. *Foods*, *8*(2): p.52.. Available at: https://doi.org/10.3390/foods8020052.

Cao, Z.; Guo, C.; Chen, G.; Liu, J.; Ni, H.; Liu, F.; Xiong, G.; Liao, X. and Lu, H. (2022). Shikonin Inhibits Fin Regeneration in Zebrafish Larvae. *Cells*, *11*(20): p.3187. Available at: https://doi.org/10.3390/cells11203187.

Chakraborty, G.; Manna, K.; Debnath, B.; Somraj, S.W. and Goswami, S. (2018). Phytochemical analysis, anti-oxidant and cytotoxic activity of seed coat of Macrotyloma uniflorum in different solvents. *Nat Prod Chem Res*, *6*(335): p.2. Available at: https://doi.org/10.4172/2329-6836.1000335.

Charilaou, P. (2018). Heliotropium curassavicum L.(Boraginaceae), a new alien species collected in Cyprus. *Cypricola*, *10*: pp.1-4.

Cohen, J.I. (2025). Phylogenomics Of Boraginaceae and Boraginales Using Lineage-Specific and Angiosperms353 Loci. *Systematic Botany*, *49*(4): pp.749-767.

Cumplido-Nájera, C.F.; González-Morales, S.; Ortega-Ortíz, H.; Cadenas-Pliego, G. ; Benavides-Mendoza, A. and Juárez-Maldonado, A. (2019). The application of copper nanoparticles and potassium silicate stimulate the tolerance to Clavibacter michiganensis in tomato plants. *Scientia horticulturae*, *245*: pp.82-89. Available at: https://doi.org/10.4172/2329-6836.1000335.

Dresler, S.; Szymczak, G., and Wójcik, M. (2017). Comparison of some secondary metabolite content in the seventeen species of the Boraginaceae family. *Pharmaceutical biology*, *55*(1): 691-695.

Dewey, A.; Baughan, C.; Dean, T.P.; Higgins, B. and Johnson, I. (2007). Eicosapentaenoic acid (EPA, an omega-3 fatty acid from fish oils for the treatment of cancer cachexia. *Cochrane database of systematic reviews*, (1). Available at: https://doi.org/10.1002/14651858.CD004597.pub2.

Diab, T.A.; Donia, T. and Saad-Allah, K.M. (2021). Characterization, antioxidant, and cytotoxic effects of some Egyptian wild plant extracts. *Beni-Suef University Journal of Basic and Applied Sciences*, 10(1): p. 13. Available at: https://doi.org/10.1186/s43088-021-00103-0.

Elfar, S.A., Bahgat, I.M., Shebl, M.A., Lihoreau, M. and Tawfik, M.M. (2023). Intraspecific variability in proteomic profiles and biological activities of the honey bee hemolymph. *Insects*, *14*(4): p.365.

El-Kamali, H.H.; A. Tawfig, A. and Kordofani, M.A.Y. (2019). Comparison of the Chemical Compositions of Two Different Non-polar Extracts of Heliotropium sudanicum Aerial Parts from Sudan. *Annual Research & Review in Biology*, *32*(5): pp. 1–6. Available at: https://doi.org/10.9734/arrb/2019/v32i530099.

Engel, N.; Ali, I.; Adamus, A.; Frank, M.; Dad, A.; Ali, S.; Nebe, B.; Atif, M.; Ismail, M.; Langer, P. and Ahmad, V.U. (2016) .Antitumor evaluation of two selected Pakistani plant extracts on human bone and breast cancer cell lines. *BMC complementary and alternative medicine*, *16*: pp.1-18.. Available at: https://doi.org/10.1186/s12906-016-1215-9.

Ezhilan, B.P. and Neelamegam, R. (2012) .GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L. *Pharmacognosy Research*, 4(1): pp.11–14. Available at: https://doi.org/10.4103/0974-8490.91028.

Fayed, M.; Abouelela, M. and Al Refaey, M. (2022). Heliotropium ramosissimum metabolic profiling, in silico and *in vitro* evaluation with potent selective

cytotoxicity against colorectal carcinoma. *Scientific Reports*, 12: p. 12539. Available at: https://doi.org/10.1038/s41598-022-16552-1.

Galanty, A.; Grudzińska, M.; Paździora, W. and Paśko, P. (2023) Erucic acid—Both sides of the story: A concise review on its beneficial and toxic properties. *Molecules*, 28(4): p. 1924. Available at: https://doi.org/10.3390/molecules28041924.

Gamal, E., Hassan, A.K., Abdel-Rahman, M.A., Hassan, M.K. and Tawfik, M.M.(2023). Immune responses and bioactive peptides of insect hemolymph. *Egyptian Academic Journal of Biological Sciences. A, Entomology*, *16*(3): pp.13-25.

Ghori, MK.; Ghaffari, MA.; Hussain, S.N.; Manzoor, M.; Aziz, M. and Sarwer,W. (2016) Ethnopharmacological, phytochemical and pharmacognostic potential of genus Heliotropium L. Turk J Pharm Sci, *13*(1): pp.143-168. Available at:https://doi.org/10. 5505/tjps.2016.20591 5.

Ghosh, P.; Das, P.; Das, C.; Mahapatra, S. and Chatterjee, S. (2018). Morphological characteristics and phytopharmacological detailing of hatishur (Heliotropium indicum Linn.): a concise review. *Journal of Pharmacognosy and Phytochemistry*, *7*(5): pp.1900-1907.

Gibbs, M.; Dawson, D.A.; McCamley, C.; Wardle, A.F.; Burke, T. and Armour, J.A.L., (1997). Chicken microsatellite markers isolated from libraries enriched for simple tandem repeats. *Animal Genetics*, *28*(6): pp.401-417.

Gololo, S.S.; Shai, L.J.; Agyei, N.M. and Mogale, M.A. (2016). Effect of seasonal changes on the quantity of phytochemicals in the leaves of three medicinal plants from Limpopo province, South Africa. *Journal of Pharmacognosy and Phytotherapy*, *8*(9): pp.168-172. Available at: https://doi.org/10.5897/JPP2016.0408.

Gracelin, D.H.S.; Britto, A. and Kumar, B.J.R.P. (2013) .Qualitative and quantitative analysis of phytochemicals in five Pteris species. *Int J Pharm Pharm Sci*, *5*(1): pp.105-107.

Greenwell, M. and Rahman, P.K.S.M. (2015). Medicinal Plants: Their Use in Anticancer Treatment. *International journal of pharmaceutical sciences and research*, 6(10): pp. 4103–4112. Available at: https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12.

Hegazy, A.K.; Soliman, M.I. and Mashaly, I.A. (1994). Perspectives on the Biology of Heliotropium curassavicum in the Deltaic Mediterranean Coast of Egypt. *Arab Gulf Journal of Scientific Research*, *12*(3):pp.525-545.

Horiuchi, N.; Nakagawa, K.; Sasaki, Y.; Minato, K.; Fujiwara, Y.; Nezu, K.; Ohe, Y. and Saijo, N., (1988). In vitro antitumor activity of mitomycin C derivative (RM-49) and new anticancer antibiotics (FK973) against lung cancer cell lines determined by tetrazolium dye (MTT) assay. *Cancer chemotherapy and pharmacology*, *22*(3): pp.246-250.

Housman, G.; Byler, S.; Heerboth, S.; Lapinska, K.; Longacre, M.; Snyder, N. and Sarkar, S. (2014). Drug resistance in cancer: an overview. *Cancers*, *6*(3): pp.1769-1792.. Available at: https://doi.org/10.3390/cancers6031769.

Huang, M. M.; Yiin, C. L.; Lock, S. S. M.; Chin, B. L. F.; Othman, I., and Chan, Y. H. (2025). Natural deep eutectic solvents (NADES) for sustainable extraction of bioactive compounds from medicinal plants: Recent advances, challenges, and future directions. *Journal of Molecular Liquids*, p.127202.

Ilonga, S.K. (2012) . Anticancer, antioxidant and antimicrobial sreening of extracts from Ziziphus Mucronata, Heliotropium Ciliatum and Gnidia Polycephala from the Oshikoto region of Namibia (Doctoral dissertation).

Iqbal, J.; Abbasi, B.A.; Mahmood, T.; Kanwal, S.; Ali, B.; Shah, S.A. and Khalil, A.T. (2017). Plant-derived anticancer agents: A green anticancer approach. *Asian Pacific Journal of Tropical Biomedicine*, *7*(12): pp.1129-1150. Available at: https://doi.org/10.1016/j.apjtb.2017.10.016.

Jaganathan, S.K. and Supriyanto, E. (2012). Antiproliferative and Molecular Mechanism of Eugenol-Induced Apoptosis in Cancer Cells. *Molecules*, 17(6): pp. 6290–6304. Available at: https://doi.org/10.3390/molecules17066290.

Jayabalan, R.; Subathradevi, P.; Marimuthu, S.; Sathishkumar, M. and Swaminathan, K. (2008) . Changes in free-radical scavenging ability of kombucha tea during fermentation. *Food Chemistry*, *109*(1): pp.227-234.. Available at: https://doi.org/10.1016/j.foodchem.2007.12.037.

Joshi, R.K. (2020). GC-MS Analysis of Volatile Organic Constituents of Traditionally Used Medicinal Plants from the Western Ghats of India: Blumea lanceolaria (Roxb.) Druce., Heliotropium indicum L. and Triumfetta rhomboidea Jacq. *Journal of the Mexican Chemical Society*, 64(2): pp. 74–82. Available at: https://doi.org/10.29356/jmcs.v64i2.1093.

Kararenk, A. C.; Sönmez, H. R.; Asgarli, T.; Tekman, E.; Taeb Dişli, F.; Yuca, H. and Güvenalp, Z. (2025). Comprehensive Analysis of Elemental and Metabolite Composition in Boraginaceae Species From Türkiye. *Chemistry & Biodiversity*, p*22*(5): e202402331.

Katalinic, V.; Milos, M.; Kulisic, T. and Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food chemistry*, *94*(4): pp.550-557.

Kaya, O. (2020). Bud death and its relationship with lateral shoot, water content and soluble carbohydrates in four grapevine cultivars following winter cold. *Erwerbs-Obstbau*, *62*(Suppl 1):pp.43-50. Available at: https://doi.org/10.1007/s10341-020-00495-w.

Kumar, A.; Kaur, S.; Dhiman, S.; Singh, P.P.; Bhatia, G.; Thakur, S.; Tuli, H.S.; Sharma, U.; Kumar, S.; Almutary, A.G. and Alnuqaydan, A.M. (2022). Targeting Akt/NF-κB/p53 pathway and apoptosis inducing potential of 1, 2-benzenedicarboxylic acid, bis (2-methyl propyl) ester isolated from Onosma bracteata Wall. against human osteosarcoma (MG-63) cells. *Molecules*, *Molecules*, 27(11): p. 3478. Available at: https://doi.org/10.3390/molecules27113478.

Lanchhana, D.S.; Ranjit, M. and Kumar, M.S. (2023) . Anticancer activity study of some selected indian medicinal plants used traditionally. *Journal of Pharmaceutical Negative Results*, pp. 1123–1132. Available at: https://doi.org/10.47750/pnr.2023.14.02.144.

Latrach, L.; Farissi, M.; Mouradi, M.; Makoudi, B.; Bouizgaren, A. and Ghoulam, C. (2014) Growth and nodulation of alfalfa-rhizobia symbiosis under salinity: electrolyte leakage, stomatal conductance, and chlorophyll fluorescence *TURKISH JOURNAL OF AGRICULTURE AND FORESTRY*, 38: pp. 320–326. Available at: https://doi.org/10.3906/tar-1305-52.

Li, T.; Mu, T.; Liu, G.; Yang, X.; Zhu, G. and Shang, C. (2022). A method of soil moisture content estimation at various soil organic matter conditions based on soil reflectance. *Remote Sensing*, *14*(10): p.2411. Available at: https://doi.org/10.3390/rs14102411.

Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D. and Abete, P.(2018). Oxidative stress, aging, and diseases. *Clinical interventions in aging*, pp.757-772.

Manivannan, P.; Muralitharan, G. and Balaji, N.P. (2017) 'Prediction aided in vitro analysis of octa-decanoic acid from Cyanobacterium Lyngbya sp. as a proapoptotic factor in eliciting anti-inflammatory properties', *Bioinformation*, 13(9): pp. 301–306. Available at: https://doi.org/10.6026/97320630013301.

Mouffouk, S.; Mouffouk, C.; Bensouici, C. and Haba, H. (2020). *In vitro* Cytotoxic Effect, Hemolytic, and Antioxidant Activities of the Algerian Species Nonea vesicaria Rchb. *Current Bioactive Compounds*,, *Current Bioactive Compounds*, 16(8): pp.1197–1204. Available at: https://doi.org/10.2174/1573407216666200109120431.

Mwamatope, B.; Tembo, D.; Kampira, E.; Maliwichi-Nyirenda, C. and Ndolo, V. (2021). Seasonal variation of phytochemicals in four selected medicinal plants. *Pharmacognosy Research*, *13*(4). *Pharmacognosy Research*, 13(4): pp. 218–226. Available at: https://doi.org/10.5530/pres.13.4.14.

Nyamai, D.W.; Arika, W.; Ogola, P.E.; Njagi, E.N. and Ngugi, M.P. (2016). Medicinally important phytochemicals: an untapped research avenue. *Journal of pharmacognosy and phytochemistry*, *4*(4): pp.2321-6182.

Ogwu, M. C.; Izah, S. C. and Joshua, M. T. (2025). Ecological and environmental determinants of phytochemical variability in forest trees. Phytochemistry Reviews, pp. 1-29.

Paul, S.; Chakraborty, S.; Mukherjee, A. and Kundu, R. (2015).Evaluation of cytotoxicity and DNA damaging activity of three plant extracts on cervical cancer cell lines. *Int J Pharm Sci Rev Res*, *31*(1): pp.183-189.

Paul, S. and Kundu, R. (2018) . ROS mediated DNA damage and induction of apoptosis in cervical cancer cells by Heliotropium indicum L. *Journal of Applied Pharmaceutical Science*, *8*(8): pp.092-106.

Plummer, D.T., (1990). Introduction to practical biochemistry. 3rd Ed. India: Tata McGraw Hill Publishing Company Limited.

Pothiraj, C.; Balaji, P.; Shanthi, R.; Gobinath, M.; Babu, R.S.; Munirah, A.A.D.; Ashraf, A.H.; Kumar, K.R.; Veeramanikandan, V. and Arumugam, R. (2021). Evaluating antimicrobial activities of Acanthus ilicifolius L. and Heliotropium curassavicum L against bacterial pathogens: an *in-vitro* study. *Journal of infection and public health*, *14*(12): pp.1927-1934.

Ranneh, Y.; Bakar, M.F.A.; Akim, A.M.; Baharum, Z.B.; Ellulu, M.S. and Fadel, A. (2023). Induction of apoptosis and modulation of caspase activity on MCF-7 human breast cancer cells by bioactive fractionated cocoa leaf extract. *Asian Pacific Journal of Cancer Prevention: APJCP*, *24*(7): p.2473.

Radwan, D.E.D. and El-Shabasy. (2020). Comparative analysis of five Heliotropium species in phenotypic correlations, biochemical constituents and antioxidant properties. *Catrina: The International Journal of Environmental Sciences*, *21*(1): pp.1-8.

Roy, A. (2015).Pharmacological activities of Indian Heliotrope (Heliotropium indicum L.): a review. *Journal of Pharmacognosy and Phytochemistry*, *4*(3).

Santini, T.C.; Fey, M.V. and Smirk, M.N. (2013). Evaluation of soil analytical methods for the characterization of alkaline Technosols: I. Moisture content, pH, and electrical conductivity.*Journal of Soils and Sediments*, 13(7): pp. 1141–1149. Available at: https://doi.org/10.1007/s11368-013-0708-0.

Sarkar, C.; Mondal, M.; Khanom, B.; Hossain, M.M.; Hossain, M.S.; Sureda, A.; Islam, M.T.; Martorell, M.; Kumar, M.; Sharifi-Rad, J. and Al-Harrasi, A. (2021) . Heliotropium indicum L.: from farm to a source of bioactive compounds with therapeutic activity. *Evidence-Based Complementary and Alternative Medicine*, *2021*(1): p.9965481.Available at: https://doi.org/10.1155/2021/9965481.

Senthilraja, P. and Kathiresan, K.J.J.A.P.S. (2015). *In vitro* cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. *Journal of applied pharmaceutical science*, *5*(3): pp.080-084. Available at: https://doi.org/10.7324/JAPS.2015.50313.

Sharma, R.A.; Singh, B.; Singh, D. and Chandrawat, P.(2009). Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. *J Med Plant Res*, *3*(13): pp.1153-75.

Shin, S.H.; Park, J.H.; Kim, G.C.; Park, B.S.; Gil, Y.G. and Kim, C.H. (2007). The mechanism of apoptosis induced by eugenol in human osteosarcoma cells. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*, *33*(1): pp.20-27.

Silva, M.C.; Madeira, V.M.; Almeida, L.M. and Custódio, J.B. (2000). Hemolysis of human erythrocytes induced by tamoxifen is related to disruption of membrane structure. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1464*(1): pp.49-61.

Singh, B.; Sahu, P.M.; Jain, S.C. and Singh, S. (2002) . Antineoplastic and antiviral screening of pyrrolizidine alkaloids from Heliotropium subulatum. *Pharmaceutical biology*, *40*(8): pp.581-586. Available at: https://doi.org/10.1076/phbi.40.8.581.14659.

Shoily, M.S.; Islam, M.E.; Rasel, N.M.; Parvin, S.; Barmon, J.; Hasan Aqib, A.; Nath Roy, D. and Parvin, M.S. (2025) .Unveiling the biological activities of Heliotropium indicum L. plant extracts: anti-inflammatory activities, GC–MS analysis, and in-silico molecular docking. *Scientific Reports*, 15(1): p. 3285. Available at: https://doi.org/10.1038/s41598-024-79559-w.

Soomro, S. and Sibgatullah Sangi, A.A.M. (2019). *In Vitro* Biological Activity of Ethanolic Extract of Maramiyah (*Salvia libanotica*) and Its Combination with Essential Oil.International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR), 9(2): pp.32-37.

Sultana, S.; Asif, H.M.; Nazar, H.M.I.; Akhtar, N.; Rehman, J.U. and Rehman, R.U. (2014) . Medicinal plants combating against cancer-a green anticancer approach. *Asian Pacific Journal of Cancer Prevention*, *15*(11): pp.4385-4394. Available at: https://doi.org/10.7314/APJCP.2014.15.11.4385.

Suthar, R. and Solanki, H. (2021). Phytochemical Screening of Halophytic Plant Heliotropium curassavicum L. *International Journal of Scientific Research in Science and Technology.[Internet]*, *8*(2): pp.141-145.

Tawfik, M.M., Eissa, N., Althobaiti, F., Fayad, E. and Abu Almaaty, A.H.(2021). Nomad jellyfish Rhopilema nomadica venom induces apoptotic cell death and cell cycle arrest in human hepatocellular carcinoma HepG2 cells. *Molecules*, *26*(17): p.5185.

Tawfik, M.M., Galal, B., Nafie, M.S., El Bous, M.M. and El-Bana, M.I. (2023). Cytotoxic, apoptotic activities and chemical profiling of dimorphic forms of Egyptian halophyte Cakile maritima scop. *Journal of Biomolecular Structure and Dynamics*, *41*(1): pp.147-160.

Wang, H.; Liu, Z.; Li, X.; Zhao, R.; Pu, Y.; Wu, H. and Guan, W. (2018**)**. Shikonin causes apoptosis by disrupting intracellular calcium homeostasis and mitochondrial function in human hepatoma cells. *Experimental and therapeutic medicine*, *15*(2): pp.1484-1492. Available at: https://doi.org/10.3892/etm.2017.5591.

Wu, Y.Y.; Wan, L.H.; Zheng, X.W.; Shao, Z.J.; Chen, J.; Chen, X.J.; Liu, L.T.; Kuang, W.J.; Tan, X.S. and Zhou, L.M. (2012). Inhibitory effects of β, β-dimethylacrylshikonin on hepatocellular carcinoma *in vitro* and *in vivo*. *Phytotherapy Research*, *26*(5): pp.764-771.. Available at: https://doi.org/10.1002/ptr.3623.

Ye, X.; Franco, A.A.; Santos, H.; Nelson, D.M.; Kaufman, P.D. and Adams, P.D. (2003) . Defective S phase chromatin assembly causes DNA damage, activation of the S phase checkpoint, and S phase arrest. *Molecular cell*, *11*(2): pp.341-351.Available at: https://doi.org/10.1016/S1097-2765(03)00037-6.

You, L.; Dong, X.; Yin, X.; Yang, C.; Leng, X.; Wang, W. and Ni, J. (2018). Rhein induces cell death in HepaRG cells through cell cycle arrest and apoptotic pathway. *International journal of molecular sciences*, *19*(4): p.1060. Available at: https://doi.org/10.3390/ijms19041060.

Zhao, Y.; Xiang, S.; Dai, X. and Yang, K. (2013). A simplified diphenylamine colorimetric method for growth quantification. *Applied microbiology and biotechnology*, *97*:pp.5069-5077 Available at: https://doi.org/10.1007/s00253-013-4893-y.

Zhao, Y.; Wang, J.; Jiang, H.; Yu, Z.; Li, X. and Shi, J. (2015). annexin 1 nuclear translocation and subsequent induction of apoptosis in neurons are assisted by myosin IIA in a TRPM7 kinase-dependent manner. *Molecular Neurobiology*, *51*: pp.729-742..Available at: https://doi.org/10.1007/s12035-014-8781-y.

Zohra, M. and Fawzia, A. (2014). Hemolytic activity of different herbal extracts used in Algeria. *Int J Pharm Sci Res*, *5*(8), pp.495-500.*t J Pharm Sci Res*, 5(8): pp. 495–500 .