Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

The anticancer activity of *Saussurea costus* roots extract against HRT-18 colon cancer cell

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Article info

Received 17.04.2026
Received in revised form 03.05.2026
Accepted 04.06.2026

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Salman, M. I., Farman, M. S., Rashied, R. M., & Almehemdi, A. F. (2026). The anti-cancer activity of Saussurea costus roots extract against HRT-18 colon cancer cell. Regulatory Mechanisms in Biosystems, 17(4), e26082. doi:10.15421/0226082

Colon cancer is considered one of the main causes of cancer-related deaths worldwide, and the search for new therapeutic agents with selective toxicity remains a priority. The present study aimed to evaluate the cytotoxic activity and molecular mechanisms of the *Saussurea costus* roots extract against the human colon cancer cell line HRT-18 compared with normal fibroblast cells NHF. The half-maximal inhibitory concentration (IC_{50}) was determined using the MTT assay after 48 hours of treatment, and the selectivity index (SI) was calculated. Additionally, the pattern of cell death was assessed using quantitative and qualitative analyses of Live/Dead and Acridine Orange/Ethidium Bromide (AO/EB) assays, along with the evaluation of DNA damage. The *Saussurea costus* roots extract showed dose-dependent toxicity against HRT-18 cells with an IC_{50} value of 168.6 µg/mL, with a high selectivity index toward cancer cells (1.61), compared with normal NHF cells. Quantitative analysis of the Live/Dead assay confirmed a highly significant decrease in the viability of treated cells. Furthermore, AO/EB images revealed distinctive morphological changes of apoptosis, including chromatin condensation and formation of apoptotic bodies. In addition to the evidence of DNA damage, the extract exerted a strong inhibitory effect on HRT-18 cell migration in the wound healing assay, causing an expansion of the scratch area by -58.7% compared with its contraction by +22.5% in the control group, indicating that it has dual cytotoxic and migration-inhibiting activity. The results collectively indicate that *Saussurea costus* roots extract has a promising anticancer activity against HRT-18 cells and acts by inducing programmed cell death accompanied by DNA damage while maintaining high selectivity toward cancer cells. These data support conducting further studies to evaluate its potential development as a therapeutic agent. The extract has promising efficacy as an anti-colon cancer agent by inducing apoptosis and inhibiting the invasive property of cells.

Keywords: colon cancer; *Saussurea costus*; DNA damage; HRT18; selective cytotoxicity; apoptosis; cell migration.

Introduction

The second most fatal type of cancer is colon cancer, with a frequency of 4–5% in the Western population, occurring in the colon or rectum as a result of the uncontrolled growth of glandular epithelial cells (Mármol et al., 2017; López-Cabanillas et al., 2025). The global frequency of colon cancer is estimated to rise by 60% between present time and the year 2030, with about 2.2 million new cases recorded, and mortality rate increasing to 1.1 million deaths (Mattiuzzi et al., 2019). Inflammatory bowel diseases such as ulcerative colitis and Crohn's disease can increase the risk of developing colon cancer by two to three times (Shah & Itzkowitz, 2022). Several other factor can be involved in the development of colon cancer, such as family history, physical inactivity, smoking, high fat diet, high alcohol consumption, diabetes, and overweight (Johns & Houlston, 2001; Meyerhardt et al., 2003; Huxley et al., 2009; Siegel et al., 2023).

Saussurea costus is a medicinal plant belonging to the Costaceae family, which can be found in regions of Himalayas, North America, Europe, and Asia (Vijayalakshmi et al., 2022). It has been used for a long time as a medicine to treat throat infection, headache, cough and cold, ulcer, and rheumatism (Lis & Olas, 2019). *Saussurea costus* extract demonstrated potent anticancer action in human neuroblastoma (Tabata et al., 2015), prostate cancer (Kim et al., 2012), liver cancer (Al-Radadi, 2022), gastric cancer, lung cancer (Gao et al., 2024), endometrium cancer (Selek et al., 2018), breast cancer (Binobead et al., 2024), and pancreatic cancer (Pavan Kumar et al., 2016). Its anticancer, anti-inflammatory, antibacterial, antifungal, and antioxidant activities have garnered the attention of scientists, encouraging them to discover the fundamental mechanisms underlying these activities (Shati et al., 2020). This plant has an extended history of traditional use in diverse indigenous health systems for addressing various conditions, demonstrating carminative, expectorant, antiarthritic, antiseptic, aphrodisiac, anodyne, and vermifuge effects, all of which have been observed without any apparent adverse consequences (Madhuri et al., 2012; Ali & Venkatesalu, 2022; Vijayalakshmi et al., 2022).

Conventional therapeutics for cancer, including chemotherapy, radiation, hormonal, and targeted therapies, have been applied. However, these therapeutic options are accompanied by many side effects. Therefore, study of plants that contain biologically active compounds that can treat cancer has become urgent, so the aim of this study was a comprehensive evaluation of the cytotoxic activity and the underlying molecular mechanisms of the growth-inhibitory effect of the of methanolic root extract of *Saussurea costus* on the human colon cancer cell line HRT-18 by determining its half-maximal inhibitory concentration (IC_{50}), assessing its selectivity toward normal fibroblast cells (NHF) through calculating the selectivity index (SI), and identifying the pattern of programmed cell death using Live/Dead and AO/EB assays, while verifying its ability to induce DNA damage as a potential mechanism of its toxicity.

Materials and methods

Dried *S. costus* roots were obtained from the local market and identified in the plant laboratory of the Center of Desert Studies at the University of Anbar. Prior to extraction, the roots were washed to remove dirt and impurities, and then dried in an oven at a low temperature (<40 °C) to prevent loss of volatile compounds. The sample was then ground into a fine powder using an electric mill, with a particle size of ≤1 mm.

The cold soaking solvent method was used to extract the active compounds. Sample to solvent ratio was 50 grams of root powder added to 500 mL of analytical methanol ≥99% (1:10 w/v ratio). A glass vial was placed on a shaking platform for 30 minutes to ensure thorough mixing. The sample was then left to infuse for 48 hours

at room temperature (~25 °C), with the vial shaken daily. After the infusion period, the extract was filtered using a paper filter to remove solids and collected in a clean glass container.

Methanol was evaporated under reduced pressure at 40–45 °C using a rotary evaporator to obtain a concentrated, viscous extract.

The extract was stored in shaded glass vials at 4 °C to prevent oxidation and loss of biological activity (Fig. 2). The resulting extract was ready for chemical analysis using techniques such as GC-MS, as well as for biological studies (antimicrobial, antioxidant, or anticancer activity).

The chemical constituents of the *Saussurea costus* root extract were analyzed using gas chromatography–mass spectrometry (GC-MS). The test was performed with a GC-MS system equipped with an autosampler. The GC investigation was performed by means of a Shimadzu GC-2010 gas chromatograph joined with a QP2010 Plus mass spectrometer. The injector temperature was maintained at 280 °C, and the oven temperature was primarily set at 80 °C and held for 2 min, then augmented at a rate of 10 °C/min until reaching 300 °C, where it was held for 6 min. The injection was done in split mode with a split ratio of 15:1. The carrier gas flow was managed under pressure at 100 kPa, with a total flow rate of 26.4 mL/min and a column flow of 1.46 mL/min. The linear velocity of the carrier gas was 44.5 cm/s, while the purge flow was managed at 3.0 mL/min.

The mass spectrometer was operated with an ion source temperature of 200 °C and an interface temperature of 250 °C. The solvent cut time was set to 2.0 min to prevent solvent interference. Mass spectra were recorded in scan mode within a mass range of m/z 40–550. The scan speed was 1111 amu/s, with an event time of 0.5 s.

Samples were injected using an AOC-20i autosampler. The injection was performed under normal injection mode with a syringe washing volume of 8 µL, and the system was equilibrated for 3 minutes before analysis to ensure stable baseline conditions.

The identification of chemical constituents was carried out by comparing the obtained mass spectra with those available in the NIST Mass Spectral Library database. The mass spectra of the detected compounds were matched with reference spectra in the library, and identification was based on the similarity index together with the comparison of retention times.

In addition, the relative percentage of each compound was calculated based on the peak area normalization method from the total ion chromatogram (TIC), assuming that the sum of all detected peak areas represents 100% of the volatile components present in the sample.

To improve the reliability of compound identification, the retention indices (RI) of the detected compounds were calculated and compared with those reported in the literature. The retention index values were determined using a homologous series of n-alkanes analyzed under the same chromatographic conditions.

The calculated RI values were then compared with published RI data and those available in the NIST Mass Spectral Library database. Compound identification was considered reliable when both the mass spectral matching and the retention index values were consistent with reference data.

Derived from the intestinal tissue of a 67-year-old male with adenocarcinoma, HCT-8 (also known as HRT-18) cells were employed in oncological and toxicological studies. In addition, normal human fibroblasts (NHF) sourced from adipose tissue were used.

Exponentially growing cells were cultured in MEM (US Biological, USA) containing 10% (v/v) fetal bovine serum and 100 U/mL penicillin-streptomycin (Capricorn-Scientific, Germany). The culture was maintained at 37 °C in a humidified incubator (Salman et al., 2021).

For the monolayer confluence, cells were cultured in 96-well plates (NEST Biotech, China) at a concentration of 10,000 cells/well. The plates were then maintained at 37 °C for 72 hours. Cell viability and potential cytotoxicity were assessed using the MTT. Exposure of cells to *S. costus* methanol extract was performed at concentrations of 1000, 500, 250, 125, 62, and 31 µg. At 72 h posttreatment, the wells were treated with 28 µL of a 2 mg/ml MTT solution. The mixture was incubated for three hours; then each well was treated with 100 µL of DMSO and subsequently incubated for 15 min. A microplate reader was employed to determine the optical density at 492 nm. The percentage of cytotoxicity was determined using the formula below:

$$\text{Cytotoxicity \%} = (\text{OD Control} - \text{OD sample})/\text{OD Control} \times 100$$

where OD control refers to the average optical density of untreated wells, while OD sample represents the OD of treated wells.

Apoptosis induction in the control and treated cell lines was assessed using Acridine Orange/Propidium Iodide (AO/PI) staining. Cells were seeded at a density of 5×10^4 cells/well and treated with the root extract for 24 hours at 37 °C. To perform conventional dual staining, 50 µL of AO/PI staining solution was added to each test well. The incubation was conducted at room temperature for a duration of 30 seconds. Once the stain was washed away, sample imaging was acquired using a Leica fluorescent microscope (1). Fluorescence intensity was quantified using the Image software.

The effect of the *Saussurea costus* root extract on the migratory ability of HRT-18 colon cancer cells was evaluated using the wound healing assay. Cells were seeded in 6-well plates at a density of 3×10^5 cells/well and incubated at 37 °C and 5% CO_2 until a complete 100% monolayer formed. A uniform linear scratch was made in the center of each well using a sterile 200 µL pipette tip. After washing with PBS to remove floating cells, fresh medium containing the half-maximal inhibitory concentration (IC_{50}) of the extract was added to the treatment group, and an extract-free medium was added to the control group. The scratch area was imaged at zero time (0 h) and after 72 hours of incubation using an inverted microscope equipped with a camera at 10X magnification. After 72 hours, the cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet for 10 minutes to enhance contrast. The scratch width (µm) was measured in three random fields per well using ImageJ software (NIH, USA). The scratch shrinkage percentage was calculated using the equation:

$$\text{Scratch shrinkage (\%)} = W0h - W72h \,/\, W0h \times 100,$$

where W0h represents the scratch width at time zero and W72h represents the width after 72 hours. Positive values indicate scratch contraction and cell migration, while negative values indicate scratch expansion and cell death. The experiment was performed in three independent replicates, and the results were statistically analyzed using ANOVA, with differences considered significant at $P < 0.05$. Images of the scratch were captured using an inverted microscope at 10X magnification at time zero (0 h) and after 72 hours of incubation. The images were analyzed qualitatively and quantitatively.

The obtained data were statically analyzed using Tukey's ANOVA multiple comparisons test with GraphPad Prism 8. The values were presented as the mean ± SD of triplicate measurements.

Ethical approval for the study was obtained from the Institutional Research Ethics Committee, and all procedures were conducted in accordance with the principles of the Declaration of Helsinki.

Results

GC-MS analysis of *Saussurea costus* root extract revealed 31 chemical compounds. The most abundant compounds in the sample were lignocerol (30.51%), cyclotetradecane (9.21%), 5-hydroxymethyl-2-furaldehyde (9.20%), (Z)-18-octadec-9-enolide (8.66%), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one (5.87%). These peaks represent approximately 63–70% of the total volatile compounds, indicating that the biological activity of the extract may be primarily related to them. The compounds were typically identified by comparing their mass spectra with the NIST Mass Spectral Library database. The intermediate concentration compounds (1–5%) include peaks 1, 2, 3, 6, 9, 10, 11, 17, 19, 20, 22, 25, 26, and 27. These indicate the presence of a variety of secondary compounds. The low concentration compounds (<1%) include peaks 8, 12, 13, 14, 15, 18, 21, 28, 29, 30, and 31 (Table 1, Fig. 3). These are trace compounds that may possess biological activity despite their low concentrations. GC-MS results indicate that the extract contains a high number of volatile and semivolatile compounds, which may be responsible for the plant's biological properties, such as antibacterial, antioxidant, and antitumor activities.

Table 1
GC-MS profile of *Saussurea costus* methanolic root extract

Peak	RT	Profile	Area, %	Class
1	2.041	2,3-dihydroxymaleic acid	1.67	dicarboxylic acids
2	2.412	2,3-butanediol	1.07	alcohol
3	6.458	1,5-dimethylpyrazole-3,4-diamine	2.24	alkaloid
4	7.439	2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	5.87	pyranone
5	8.809	5-Hydroxymethyl-2-furaldehyde	9.20	furaldehyde
6	9.760	Amyl caprylate	1.21	fatty acid ester
7	12.243	glycero-galacto-Heptose	4.83	sugar
8	12.929	D-Gluco-2-heptulose	0.57	sugar
9	13.705	6-Methoxy-1,2,3,4-tetrahydro-1,2-naphthalenediol	2.76	naphthalene
10	14.424	L-Isoleucine b-naphthylamide	1.52	amino acid derivatives
11	14.667	2-Heptyloxirane	3.56	ether
12	15.567	2-(Dimethylamino)phenol	0.58	phenol
13	15.781	D-Gulonic acid γ-lactone	0.79	sugar acid
14	16.140	Epoxychrysanthenone	0.52	terpenoid
15	16.683	8-O-senecioyltovarol	0.50	terpenoid
16	16.998	Cyclotetradecane	9.21	cycloalkane
17	17.417	Methyl 13-methylpentadecanoate	1.00	fatty acid ester
18	17.573	Guaiyl citronellate	0.47	terpenoid
19	17.947	palmitic acid	2.19	fatty acid
20	18.078	Farnesiferol C	1.31	coumarin
21	18.463	3-Isopropyl-6-methyl-2-(2-oxopropyl)cyclohexanone	0.51	terpenoid
22	18.636	Dehydrocostus lactone	2.33	sesquiterpenoid
23	19.108	Lignocerol	30.51	fatty alcohol
24	19.741	(Z)-18-Octadec-9-enolide	8.66	lactone
25	20.878	Pseudoecgonine (6CI,7CI)	1.19	alkaloid
26	21.692	1,4-Diazabicyclo[3.2.2] nonane	1.35	alkaloid
27	23.276	Piperonal, (2,4-dinitrophenyl)hydrazone	1.72	alkaloid
28	24.118	Does not match	0.75	–
29	25.254	Furfuryl glycidyl ether	0.79	lactone ether
30	26.546	2-tert-Butylcyclohexyl acetate	0.67	cycloester
31	28.107	methyl (Z)-octadec-15-enoate	0.46	fatty methyl ester

To evaluate the cytotoxic activity of *Saussurea costus* methanolic root extract toward human rectal tumor, 18 (HRT-18) and normal human fibroblast (NHF) cells were incubated with different doses (1000, 500, 250, 125, 62, 31 μg/mL) of the extract. After 72 hours of incubation, the cell viability was assessed by the MTT assay. The results of the MTT assay showed that the cytotoxicity of the extract to HRT-18 cells was dose-dependent, as the cell growth inhibition rate increased from 8.53% at a concentration of 31.2 μg/mL to 65.15% at a concentration of 1000 μg/mL. By contrast, the extract showed low toxicity for normal NHF cells, with the inhibition percentage ranging from 1.34% at a concentration of 31.2 μg/mL to 18.51% at the highest concentration of 1000 μg/mL (Fig. 4). When comparing the effect on both cell lines at a concentration of 1000 μg/mL, it was observed that the toxicity to cancerous HRT-18 cells, 65.15%, was approximately 3.5 times higher than the toxicity for normal NHF cells, 18.51%, confirming a selective effect of the extract.

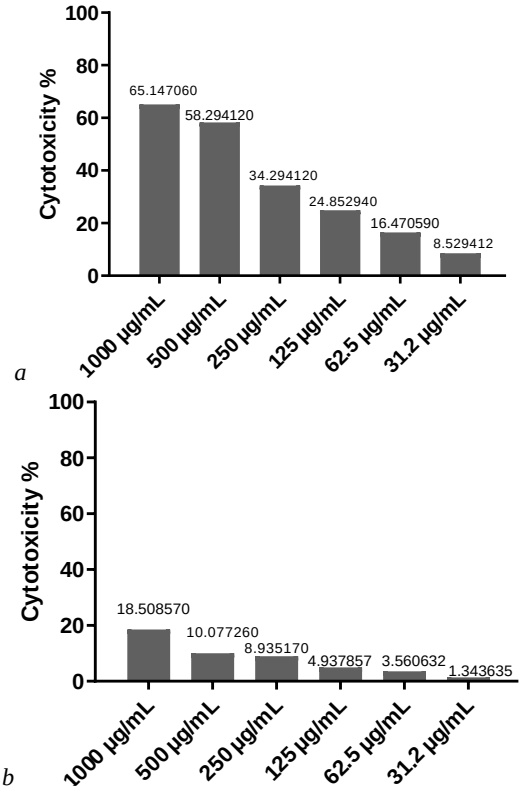

Fig. 1. Dose-response curve showing the reduction in cell viability induced by the methanolic extract of *Saussurea costus* roots in HRT-18 (*a*) and NHF cell line (*b*): Concentration (μg/mL) is plotted on the x-axis, while the corresponding cytotoxicity (%) is shown on the y-axis

The microscopic examination revealed that treatment of HRT-18 cells with the root extract resulted in a marked destruction of the monolayer, with a significant decrease in the cell density and a change to a shrunken, rounded shape, indicating a cytotoxic effect. Meanwhile, the normal NHF cells treated with the same substance showed only minor changes, with most cells retaining their normal spindle shape and high density, suggesting resistance to the cytotoxic effect (Fig. 5a, 5b, 6a, 6b).

The root extract exerted a selective cytotoxicity, effectively killing the HRT-18 cancer cells, but hardly affecting the normal NHF cells. In other words, the *S. costus* root extract targeted the tumor and did not significantly harm the healthy cells.

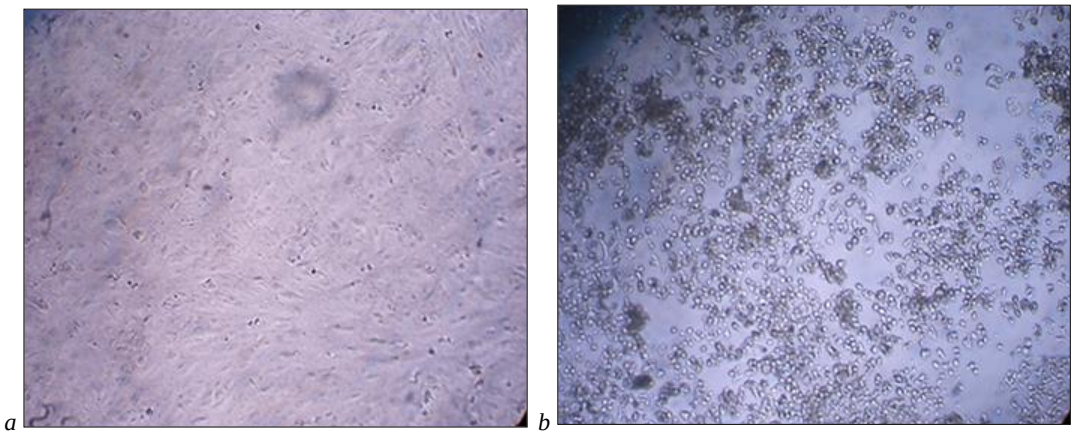

Fig. 2. HRT-18 cells: *a* – untreated, *b* – treated with *Saussurea costus* methanolic root extract

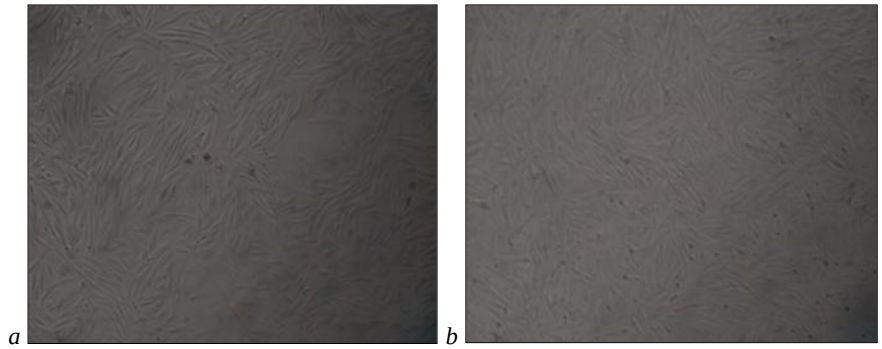

Fig. 3. NHF cells: *a* – untreated (control), *b* – treated with *Saussurea costus* methanolic root extract

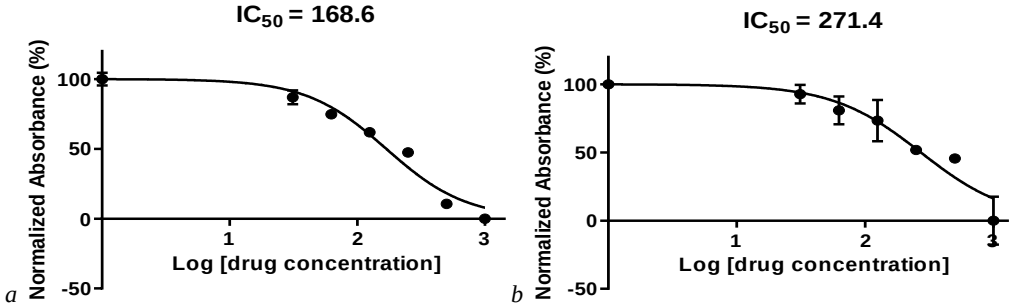

Fig. 4. IC_{50} curve of the *Saussurea costus* methanolic root extract for (*a*) HRT-18 and (*b*) NHF cells: The root extract's potency was determined by graphing the log concentration (x-axis) versus cell inhibition percentage (y-axis), where the signifies 50% reduction in viability

The inhibitory concentration value for 50% of the cells (IC_{50}) was calculated from the dose-response curves. The IC_{50} value of the substance for HRT-18 cancer cells was 168.6, while it was 271.4 for normal NHF cells.

To calculate the selectivity of the substance toward cancer cells, the following equation was used: SI = IC_{50} NHF/ IC_{50} HRT-18 = 271.4 / 168.6 = 1.61. The selectivity index value of 1.61 indicates that the substance has moderate selectivity for HRT-18 colon cancer cells, as it requires a concentration 1.6 times higher to inhibit 50% of normal cells compared with cancer cells.

The results of photometric absorbance showed a significant decrease ($P < 0.0001$) in the viability of HRT-18 cells starting from a concentration of 125 μg/mL and above compared with the untreated control cells, while the effect was not significant at a concentration of 31.25 μg/mL (Fig. 4). This indicates that the minimum effective concentration of the extract is 62.5 μg/mL ($P < 0.01$).

The optical density values of NHF cells showed an insignificant decrease in the cell viability at low concentrations of 31.25 and 62.5 μg/mL ($P > 0.05$, Fig. 8). A slight significant effect was observed ($P < 0.05$) at 125 and 250 μg/mL, while a highly significant effect ($P < 0.0001$) appeared only at high concentrations of 500 and 1000 μg/mL. Nevertheless, the optical density values of NHF cells remained much higher than those of HRT-18 at all tested concentrations, confirming the relative selectivity of the extract.

To confirm the effect of the extract on the viability of HRT-18 cells and to determine the pattern of cell death, the calcein-AM/propidium Iodide assay was used after treatment with the IC_{50} concentration (168.6 μg/mL) for 48 hours. The results showed a highly significant decrease in the fluorescence intensity of live cells ($P < 0.01$) in the treated group compared with the control, with an observed increase in the fluorescence intensity of dead cells, although it did not reach statistical significance (Fig. 5). This result confirms that the substance induces death in HRT-18 cancer cells and supports the results of the MTT assay.

HRT-18 cells were treated with *Saussurea costus* methanolic root extract at IC_{50} concentration (168.6 μg/mL) for 48 h and stained with Acridine Orange/Ethidium Bromide. (A) Untreated control cells exhibited uniform green fluorescence, indicating viable cells with intact DNA and membrane. (B) Treated cells exhibited orange/red fluorescence, characteristic of early and late apoptotic cells with DNA fragmentation and membrane blebbing.

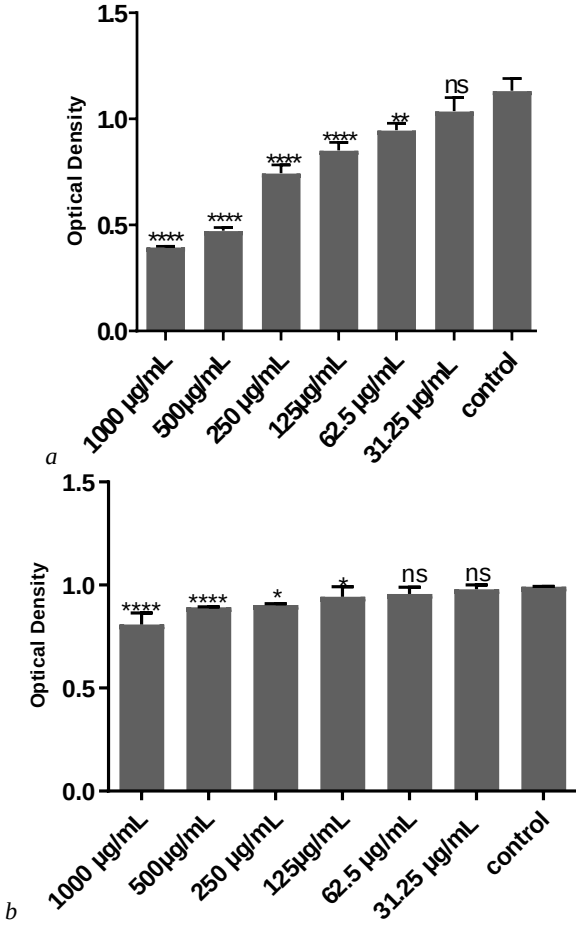

Fig. 5. Time-dependent optical density (OD) of cells exposed to *Saussurea costus* methanolic root extract: *a* – HRT-18 cell line; *b* – NHF cell line; absorbance (492 nm) is plotted against concentration in μg/mL; data points are shown as the mean ± SD, based on three independent measurements

Images were captured at 200x magnification. Representative images from three independent experiments are presented. These visual results correspond with the significant decrease in viable cells observed in the Live/Dead assay (Fig. 7) and indicate that the main mechanism of the substance's toxicity is the induction of apoptosis.

The antimigratory effect of *Saussurea costus* root extract was evaluated using a wound healing assay. Microscopic images showed that cells in the untreated control group had active migratory ability, as a clear contraction in the scratch area was observed after 72 hours of incubation due to the migration of cells from the wound edges toward the center. By contrast, treatment of the cells with the IC_{50} inhibitory concentration of the extract resulted in a complete inhibition of migration, and instead a noticeable widening of the scratch area was observed, along with a decrease in cell density at the edges and the appearance of irregular morphology, indicating the occurrence of a cytotoxic effect. When analyzing the results quantitatively, cell migration was expressed as a percentage of scratch closure. The control group showed a positive scratch closure of +22.5 ± 18.3%, reflecting the natural migration of cancer cells. By contrast, the treated group showed a negative scratch closure of – 58.7 ± 32.1%, indicating that the extract not only inhibited migration but caused the scratch to widen due to cell death at its edges. The difference in scratch closure percentage between the treated group and the control group was highly significant ($P < 0.0001$). Together, these results indicate that the extract has a dual effect, both inhibiting migration and being cytotoxic to the HRT-18 cell line.

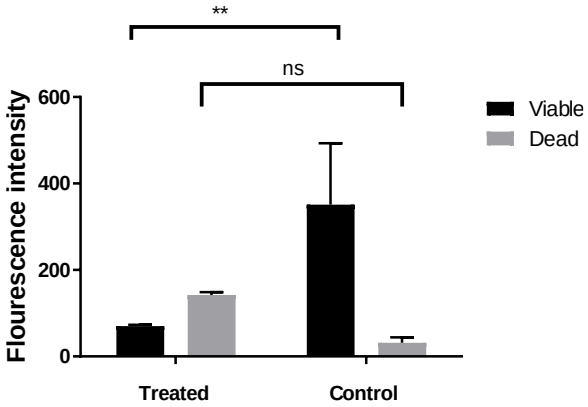

Fig. 6. Live/dead fluorescence assay of HRT-18 cells treated with *Saussurea costus* methanolic root extract

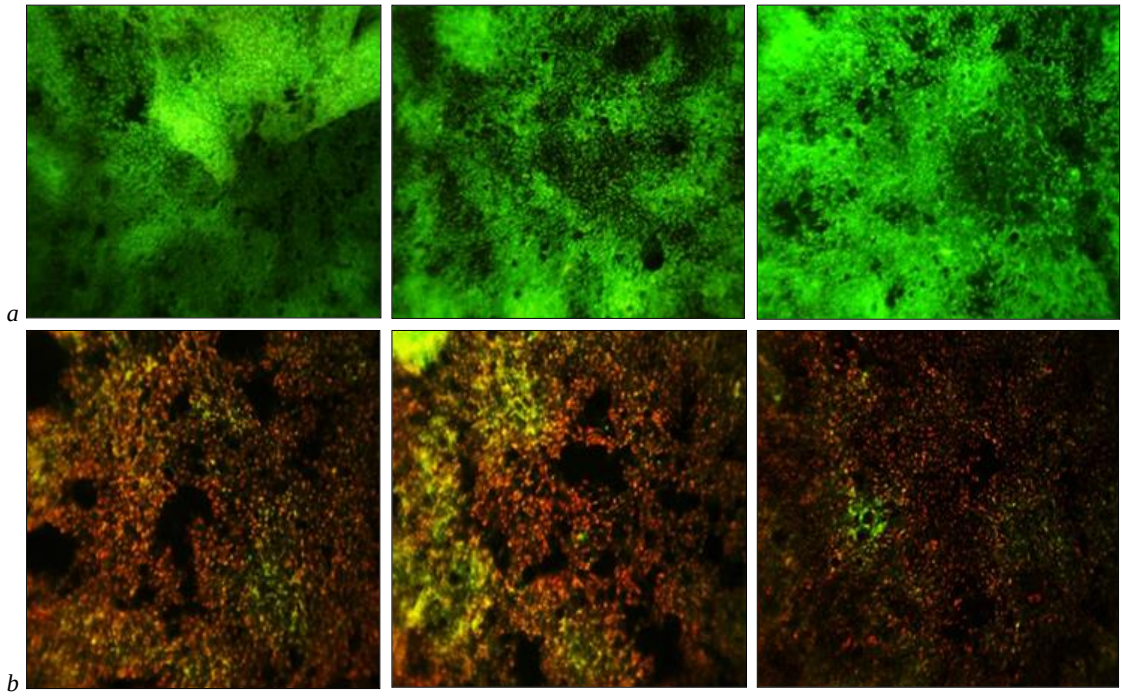

Fig. 7. Morphological assessment of apoptosis in HRT-18 cells using AO/EB dual staining: *a* – HRT-18 cells control (untreated); *b* – HRT-18 cells treated

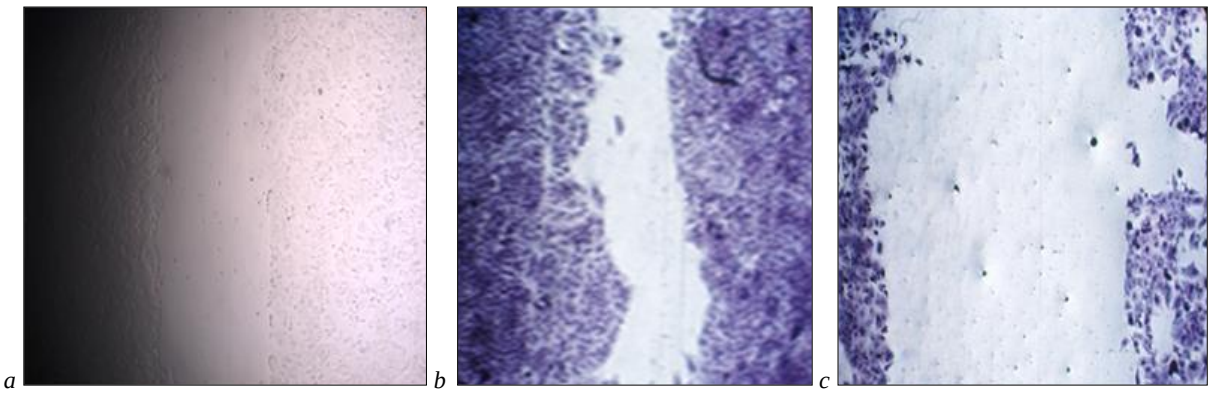

Fig. 8. Representative microscopic images (10^X magnification, crystal violet stain) showing the scratch area at time zero (0 h), and after 72 hours in untreated cells and cells treated with the IC_{50} concentration; *a* – 0 time; *b* – untreated; *c* – treated

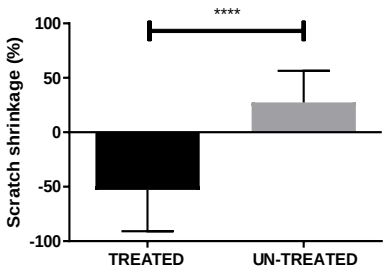

Fig. 9. Quantitative analysis of cell migration expressed as a percentage of scratch contraction: Negative values indicate scratch expansion due to cell death at the edges; data are presented as mean ± standard deviation (n = 3); * – P < 0.0001 by unpaired t-test

Discussion

Many studies have confirmed the anticancer activity of *Saussurea costus* root extract toward several kinds of cancer cells, including HepG2 (Alotaibi et al., 2021), breast cancer cells (MCF7), and human colon cancer (Patel et al., 2020). Therefore, *Saussurea costus* has emerged as an important candidate for the creation of active and secure anticancer medicines. Phytochemical tests have documented *S. costus* root as a unique source of bioactive complexes, such as sesquiterpenes, flavonoids, lignans, phytosterols alkaloids, terpenes anthraquinones, and flavonoids (Hassan & Masoodi, 2020). The results showed the presence of trepenoids compounds, such as epoxychrysanthenone, 8-O-senecioyltovarol, guaiy-l citronellate, and 3-isopropyl-6-methyl-2-(2-oxopropyl) cyclohexanone. Terpenoids display anticancer features by inducing cancer cell apoptosis through the activation of caspases, augmenting ROS, and promoting mitochondrial membrane potential impairment (Amit et al., 2025). Terpenoids hinder critical cell signaling paths in the cancer cells (including MAPK, PI3K, and AKT) that initiate cancer cell development and maintain their durability (Guo et al., 2024). Also, terpenoids restrict the ability of cancer cells to create new blood vessels (angiogenesis) and reduce epithelial-mesenchymal transition, the two mechanism required for cancer metastasis (Fakhri et al., 2023). Many terpenoids exert antagonistic effect for p53-MDM2, helping to effectivey restore p53 (particularly in colon cancer) by targeting protein kinase B (Muhseen & Li, 2020).

Alkaloids, a prominent component of *S. costus*, display anticancer effects due to their antimitotic activity, and are able to disrupt the cancer cell division by binding to tubulin proteins, inhibiting the creation of microtubules, which are crucial components for chromosome segregation during mitosis. Thus, the cancer cell cycle is blocked in the M or G2/M phase (Alam et al., 2017; Sun et al., 2019). Alkaloids prompt apoptosis in cancer cells through several internal and external signaling paths that are critical for cancer cell endurance and proliferation. In addition, they can moderate the p53 path, improving its tumor-inhibiting effects by stimulating apoptosis as a result of cellular stress and DNA damage in cancer cells. Furthermore, several alkaloids inhibit the NF-κB path, thereby suppressing tumor endurance and proliferation (Olofinsan et al., 2023). Alkaloids also play a major role in inhibiting angiogenesis, the creation of new blood vessels that facilitate tumor metastasis. Some alkaloids, such as allicin, have the ability to reduce the action of matrix metalloproteinases (MMPs), which are implicated in the breakdown of extracellular matrix constituents essential for tumor invasion and migration (Rizeq et al., 2020).

Numerous studies have explored the mechanisms of the *in vivo* anticancer activity of *S. costus*. It was found to be associated mostly with the initiation of apoptosis through triggering pro-apoptotic proteins such as p53 and Bax, while constraining anti-apoptotic proteins such as Bcl-2. These actions result in cancer cell apoptosis and cell cycle arrest. In addition, it modifies definite signaling paths such as EGFR, inhibiting the cancer cell proliferation and invasion. It also reduces PI3K/Akt and MEK/P38 paths, thereby lessening the inflammatory response by reducing the concentrations of TNF-α and NF-κB. Furthermore, *S. costus* was observed to stimulate the production of reactive oxygen species (Alghamdi et al., 2025).

6-Methoxy-1,2,3,4-tetrahydro-1,2-naphthalenediol damages cancer cells principally by prompting apoptosis through mitochondrial dysfunction and cell cycle arrest. It affects cancer cells chiefly by generating reactive oxygen species (ROS) and increasing oxidative stress levels, thus leading to broad DNA injury and hindering mitochondrial ATP creation (Saleem et al., 2025). Moreover, naphthalene derivatives, such as naphthalene diimide, have been revealed to inhibit the catenin/c-myc paths and decrease cancer cell proliferation, especially in gastric cancer models (Kumar et al., 2022).

Dehydrocostus lactone is a natural sesquiterpene lactone that affects cancer mainly by inhibiting the NF-κB/COX-2 way by directing IKKβ and suppressing the STAT3 signaling path in glioblastoma, which is critical for cancer cell growth (Wang et al., 2017). In another study, dehydrocostus lactone inhibited the proliferative and metastatic abilities of HepG2 and SK-HEP-1 cells. These effects were achieved by initiating DNA damage, blockage of cancer cell cycle, and induction of cell apoptosis by activating the p53/p21 path and inhibiting epithelial-mesenchymal transition (EMT) (Tian et al., 2023). It was recognized that dehydrocostus lactone suppresses MMP-9 excretion and initiates TIMP-2 excretion to inhibit migration of DU145 and TRAMP-C2 cells (Kim et al., 2008).

The GC-MS investigation identified (Z)-18-octadec-9-enolide as a notable compound in the *S. costus* roots. In another study, the same compound was derived from *Lactiplantibacillus plantarum* K25 and displayed a promising role in treating colorectal cancer by modifying aquaporin-8 (AQP8) as well as modulating gut microbial-membrane ecosystem. This metabolite is able to suppress tumor development by decreasing tumor cell proliferation, promoting apoptosis, and changing the tumor microenvironment, with little toxicity and great specificity (Aziz et al., 2025).

By using GC-mass analysis, the compound 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was found in *S. costus* root extract. It was observed to induce cancer decay mainly by prompting apoptosis and preventing the proliferation of cancer cells, chiefly in colon cancer. Furthermore, it inhibits the transcription factor NF-κB, defeating anti-apoptotic genes such as Bcl-2, and triggering pro-apoptotic proteins such as Bax plus caspase-3 (Ban et al., 2007).

D-Glycero-D-galacto-heptose is a bioactive complex able of hindering cancer cell growth, mainly in triple-negative breast cancer. It acts mainly by hindering the cyclooxygenase-2 enzyme, which is often overregulated in tumor cells and participates in inflammation as well as tumor progression (Clarion et al., 2014).

The GC-MS results indicate the presence of 5-hydroxymethyl-2-furaldehyde compound in *S. costus* root extract. It is a natural furan compound with the ability to reduce cancer cell metastasis, migration, invasion, and angiogenesis by blocking the AQP1 channels, critical for cancer cell motility. In addition, 5-hydroxymethyl-2-furaldehyde exhibits antioxidant and anti-inflammatory properties and is able to induce apoptosis and affect the cancer cell cycle by arresting the G0/G1 phase (Zhao et al., 2013; Chow et al., 2020).

Coumarin is another compound that GC-MS analyses have shown to be present in the root extract of the *S. costus*. It inhibits cancer cell growth by inducing apoptosis through the disruption of the mitochondrial membrane, causing the release of cytochrome *c* and the triggering of caspase enzymes, thus dismantling cancer cells. Furthermore, coumarin exerted anti-angiogenesis effect by defeating the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), which are crucial for creating new blood vessels and invading neighboring tissue (Shahbaz et al., 2024; Kubrak et al., 2025). Palmitic acid can prevent the growth of many cancer cells, primarily by prompting apoptosis and activating cellular stress. In addition, it can disrupt many signaling paths crucial for tumor cell endurance, including mitochondrial pathway. Also, it promotes ferroptosis, an iron-dependent system of cell death resulting from accumulation of reactive oxygen species (Wang et al., 2023).

Conclusion

The present study showed that the *S. costus* methanolic root extract possesses selective antiproliferative activity against human colon

cancer HRT-18 cells with a high selectivity index. This effect was exerted through multiple mechanisms, including induction of programmed cell death through DNA damage, in addition to strong inhibition of the cancer cells' migratory ability. Our observation of negative values for the scratch closure rate in the wound healing assay confirms that the compound not only inhibits migration but also exerts an additional cytotoxic effect on cells at the wound edges. This dual cytotoxic and antimigratory activity makes *Saussurea costus* root extract a promising candidate for the development of anticancer drugs for colon cancer and a potential metastasis inhibitor. We recommend conducting future investigations to determine the precise molecular pathways responsible for migration inhibition and to evaluate its effectiveness in animal models.

References

Alam, M. M., Naeem, M., Khan, M. M. A., & Uddin, M. (2017). Vincristine and vinblastine anticancer *Catharanthus* alkaloids: Pharmacological applications and strategies for yield improvement. In: Naeem, M., Aftab, T., & Khan, M. M. A. (Eds.). *Catharanthus roseus*. Springer, Cham. Pp. 277–307.

Alghamdi, S. S., Alshkrh, R., Mohammed, A. E., & Alowais, S. A. (2025). From herb to hope: A systematic exploration of medicinal plants' role in cancer therapy. Journal of Cancer, 16(14), 4081–4098.

Al-Radadi, N. S. (2022). *Saussurea costus* for sustainable and eco-friendly synthesis of palladium nanoparticles and their biological activities. Arabian Journal of Chemistry, 15(11), 104294.

Aziz, T., Shabbir, M. A., Sarwar, A., Yang, Z., Lin, L., Al-Megrin, W. A. I., Shami, A., Alwethaynani, M. S., Alhhazmi, A. A., Al-Asmari, F., Al-Joufi, F. A., & Fallatah, D. (2025). Revealing *Lactiplantibacillus plantarum* K25 derived (Z)-18-octadec-9-enolide in modulating aquaporin-8 in colorectal cancer by bridging the gut microbiome and membrane biology. Cell Biochemistry and Biophysics, 83(4), 5339–5354.

Ban, J. O., Hwang, I. G., Kim, T. M., Hwang, B. Y., Lee, U. S., Jeong, H.-S., Yoon, Y. W., Kim, D. J., & Hong, J. T. (2007). Anti-proliferate and pro-apoptotic effects of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyranone through inactivation of NF-κB in human colon cancer cells. Archives of Pharmacal Research, 30(11), 1455–1463.

Binobead, M. A., Aziz, I. M., Ibrahim, S. M., & Aljowaie, R. M. (2024). Chemical composition and bioactivities of the methanol root extracts of *Saussurea costus*. Open Chemistry, 22(1), 20240002.

Chow, P. H., Kourghi, M., Pei, J. V., Nourmohammadi, S., & Yool, A. J. (2020). 5-Hydroxymethyl-furfural and structurally related compounds block the ion conductance in human aquaporin-1 channels and slow cancer cell migration and invasion. Molecular Pharmacology, 98(1), 38–48.

Clarion, L., Jacquard, C., Sainte-Catherine, O., Decoux, M., Loiseau, S., Rolland, M., Lecouvey, M., Hugnot, J.-P., Volle, J.-N., Virieux, D., Pirat, J.-L., & Bakalara, N. (2014). C-glycoside mimetics inhibit glioma stem cell proliferation, migration, and invasion. Journal of Medicinal Chemistry, 57(20), 8293–8306.

Fakhri, S., Moradi, S. Z., Faraji, F., Farhadi, T., Hesami, O., Iranpanah, A., Webber, K., & Bishayee, A. (2023). Current advances in nanoformulations of therapeutic agents targeting tumor microenvironment to overcome drug resistance. Cancer and Metastasis Reviews, 42(3), 959–1020.

Gao, K., Chen, Z., Zhang, N., & Jiang, P. (2024). High throughput virtual screening and validation of Plant-Based EGFR L858R kinase inhibitors against non-small cell lung cancer: An integrated approach utilizing GC–MS, network pharmacology, docking, and molecular dynamics. Saudi Pharmaceutical Journal, 32(9), 102139.

Guo, J., Huang, M., Hou, S., Yuan, J., Chang, X., Gao, S., Zhang, Z., Wu, Z., & Li, J. (2024). Therapeutic potential of terpenoids in cancer treatment: Targeting mitochondrial pathways. Cancer Reports, 7(9), e70006.

Gurung, S. K., Kumari, S., Dana, S., Mandal, K., Sen, S., Mukhopadhyay, P., & Mondal, N. (2022). DNA damage, cell cycle perturbation and cell death by naphthalene diimide derivative in gastric cancer cells. Chemico-Biological Interactions, 358, 109881.

Huang, H., Park, S., Zhang, H., Park, S., Kwon, W., Kim, E., Zhang, X., Jang, S., Yoon, D., Choi, S.-K., Yi, J.-K., Kim, S.-H., Dong, Z., Lee, M.-H., Ryoo, Z., & Kim, M. O. (2021). Targeting AKT with costunolide suppresses the growth of colorectal cancer cells and induces apoptosis *in vitro* and *in vivo*. Journal of Experimental and Clinical Cancer Research, 40(1), 114.

Keshari, A. K., Singh, V., Patni, T., Mishra, T., Mishra, A., & Gupta, R. K. (2025). A new frontier: Terpenes in the fight against colon cancer. ASPET Discovery, 1, 100016.

Kim, E. J., Hong, J. E., Lim, S. S., Kwon, G. T., Kim, J., Kim, J.-S., Lee, K. W., & Park, J. H. Y. (2012). The hexane extract of *Saussurea lappa* and its active principle, dehydrocostus lactone, inhibit prostate cancer cell migration. Journal of Medicinal Food, 15(1), 24–32.

Kim, E. J., Lim, S. S., Park, S. Y., Shin, H.-K., Kim, J.-S., & Park, J. H. Y. (2008). Apoptosis of DU145 human prostate cancer cells induced by dehydrocostus lactone isolated from the root of *Saussurea lappa*. Food and Chemical Toxicology, 46(12), 3651–3658.

Kubrak, T. P., Makuch-Kocka, A., & Aebisher, D. (2025). Coumarins in anticancer therapy: Mechanisms of action, potential applications and research perspectives. Pharmaceutics, 17(5), 595.

Muhseen, Z. T., & Li, G. (2019). Promising terpenes as natural antagonists of cancer: An *in-silico* approach. Molecules, 25(1), 155.

Olofinsan, K., Abrahamse, H., & George, B. P. (2023). Therapeutic role of alkaloids and alkaloid derivatives in cancer management. Molecules, 28(14), 5578.

Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2007). *Saussurea costus*: Botanical, chemical and pharmacological review of an ayurvedic medicinal plant. Journal of Ethnopharmacology, 110(3), 379–390.

Pavan Kumar, C., Devi, A., Ashok Yadav, P., Rao Vadaparthi, R., Shankaraiah, G., Sowjanya, P., Jain, N., & Suresh Babu, K. (2016). "Click" reaction mediated synthesis of costunolide and dehydrocostuslactone derivatives and evaluation of their cytotoxic activity. Journal of Asian Natural Products Research, 18(11), 1063–1078.

Rizeq, B., Gupta, I., Ilesanmi, J., AlSafran, M., Rahman, M. M., & Ouhtit, A. (2020). The power of phytochemicals combination in cancer chemoprevention. Journal of Cancer, 11(15), 4521–4533.

Saleem, R. M., Alqahtani, A. S., Alsulami, R. N., & Zaki, I. (2025). New naphthalene-containing enamides: Synthesis, structural insights and biological screening as potential anticancer agents against Huh-7 cancer cell line. RSC Advances, 15(58), 50166–50178.

Salman, M. I., Emran, M. A., & Al-Shammari, A. M. (2021). Spheroid-formation 3D engineering model assay for *in vitro* assessment and expansion of cancer cells. Proceedings of the III International conference on advanced technologies in materials science, mechanical and automation engineering: MIP: Engineering-III – 2021. AIP Publishing. Vol. 2402, 30011.

Selek, S., Koyuncu, I., Caglar, H. G., Bektas, I., Yilmaz, M. A., Gonel, A., & Akyuz, E. (2018). The evaluation of antioxidant and anticancer effects of *Lepidium sativum* subsp. *spinescens* L. methanol extract on cancer cells. Cellular and Molecular Biology, 64(3), 72–80.

Shahbaz, M., Perween, A., Momal, U., Imran, M., Ul Hassan, M. H., Naeem, H., Mujtaba, A., Hussain, M., Alsagaby, S. A., Al Abdulmonem, W., Abdelgawad, M. A., El-Ghorab, A. H., Selim, S., Mostafa, E. M., & Al Jbawi, E. (2024). Recent perspectives on anticancer potential of coumarin against different human malignancies: An updated review. Food Science and Nutrition, 13(1), e4696.

Shati, A. A., Alkahtani, M. A., Alfaifi, M. Y., Elbehairi, S. E. I., Elsaid, F. G., Prasanna, R., & Mir, M. A. (2020). Secondary metabolites of *Saussurea costus* leaf extract induce apoptosis in breast, liver, and colon cancer cells by caspase-3-dependent intrinsic pathway. BioMed Research International, 1, 1608942.

Sun, G.-C., Chen, H.-H., Liang, W.-Z., & Jan, C.-R. (2019). Exploration of the effect of the alkaloid colchicine on Ca^{2+} handling and its related physiology in human oral cancer cells. Archives of Oral Biology, 102, 179–185.

Tabata, K., Nishimura, Y., Takeda, T., Kurita, M., Uchiyama, T., & Suzuki, T. (2015). Sesquiterpene lactones derived from *Saussurea lappa* induce apoptosis and inhibit invasion and migration in neuroblastoma cells. Journal of Pharmacological Sciences, 127(4), 397–403.

Tian, Y., Ma, B., Zhao, X., Tian, S., Li, Y., Pei, H., Yu, S., Liu, C., Lin, Z., Zuo, Z., & Wang, Z. (2023). Dehydrocostus lactone inhibits the proliferation and metastasis of hepatocellular carcinoma cells via modulating p53-p21-CDK2 signaling pathway. Arabian Journal of Chemistry, 16(8), 104994.

Vijayalakshmi, M., Umamaheswari, A., Agalya, B., Jegasubramaniam, S. N., & Prabu, S. L. (2022). Foresight on phytoconstituents and associated pharmacological activities of traditional medicinal plant: *Saussurea costus* (Falc.) Lipschitz. Current Pharmacology Reports, 8(4), 281–289.

Wang, X., Yu, Z., Wang, C., Cheng, W., Tian, X., Huo, X., Wang, Y., Sun, C., Feng, L., Xing, J., Lan, Y., Sun, D., Hou, Q., Zhang, B., Ma, X., & Zhang, B. (2017). Alantolactone, a natural sesquiterpene lactone, has potent antitumor activity against glioblastoma by targeting IKKβ kinase activity and interrupting NF-κB/COX-2-mediated signaling cascades. Journal of Experimental and Clinical Cancer Research, 36(1), 1009–1011.

Wang, X., Zhang, C., & Bao, N. (2023). Molecular mechanism of palmitic acid and its derivatives in tumor progression. Frontiers in Oncology, 13, 1224125.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., & Chen, T. (2013). *In vitro* antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. Journal of Agricultural and Food Chemistry, 61(44), 10604–10611.