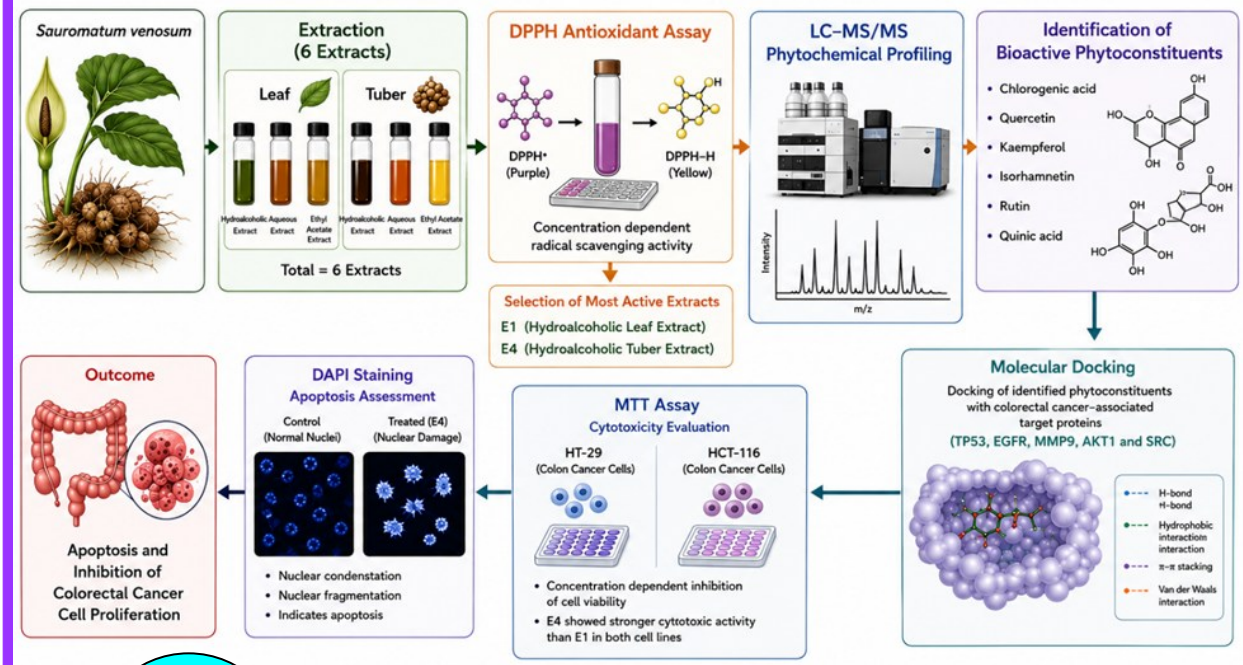

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Integrated LC–MS–guided phytochemical profiling, molecular docking, and *in vitro* validation of *Sauromatum venosum* extracts against colorectal cancer

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Abstract

The antioxidant, phytochemical, and anti-colorectal cancer potential of *Sauromatum venosum* leaf and tuber extracts were investigated using experimental and computational approaches. Hydroalcoholic, aqueous, and ethyl acetate extracts were evaluated using DPPH assay. Hydroalcoholic leaf (L1) and tuber (T1) extracts exhibited superior concentration-dependent antioxidant activity. The LC–MS analysis identified bioactive phytoconstituents, including quinic acid, chlorogenic acid, quercetin, kaempferol, isorhamnetin, and rutin. Molecular docking revealed favorable interactions of these compounds with colorectal cancer-associated targets, particularly quercetin, kaempferol, and isorhamnetin. The T1 extract exhibited the strongest antiproliferative activity against HT-29 and HCT-116 cells, while DAPI staining demonstrated apoptosis-associated nuclear changes. These finding suggest that *S. venosum* is a promising source of bioactive compounds with potential for the development of future anti-cancer drugs.

Introduction

Colorectal cancer is a major cause of cancer-related morbidity and mortality worldwide and remains a significant public health challenge (Zhou et al., 2025). Despite advances in surgery, chemotherapy, radiation, targeted therapy, and immunotherapy, treatment is often limited by adverse effects, multidrug resistance, tumor progression, and poor selectivity (Randive et al., 2023; Kumbhar et al., 2025; Wang et al., 2025). These limitations have intensified the search for safer and more effective therapeutic agents from natural sources.

Natural products and medicinal plants have historically contributed to the discovery of anti-cancer drugs (Nadaf et al., 2020; Nangare et al., 2025). Plant secondary metabolites, including flavonoids, alkaloids, phenolic compounds, terpenoids, and glycosides, possess antioxidant, anti-inflammatory, antiproliferative, and apoptosis-inducing activities (Hilal et al., 2024; Nadaf and Killedar, 2018). Several clinically important anti-cancer drugs, such as paclitaxel, vincristine, vinblastine, and camptothecin derivatives, are derived from medicinal plants (Deep et al., 2023). Oxidative stress contributes to colorectal carcinogenesis through reactive oxygen species-mediated DNA damage, lipid peroxidation, mitochondrial dysfunction, and oncogenic signaling, whereas chronic inflammation promotes tumor progression (Liu et al., 2025; Akter et al.,

2026). Therefore, antioxidant-rich medicinal plants may offer promising therapeutic potential (Jomova et al., 2025).

Sauromatum venosum (Aiton) Kunth (Araceae), commonly known as voodoo lily, is traditionally used to treat inflammatory disorders, snake bites, and other ailments. Phytochemical studies have reported that flavonoids, phenolics, alkaloids, terpenoids, saponins, and lectins are associated with antioxidant, antimicrobial, anti-inflammatory, and anti-cancer activities (Kahar et al., 2024). Tuber lectin has demonstrated potent antiproliferative activity against various cancer cell lines (Singh et al., 2005). Further supporting this potential, GC–MS analysis identified several tuber phytoconstituents, among which 12-O-acetylingol 8-tiglate showed potential anti-cancer activity through molecular docking (Kahar et al., 2024). Similarly, the concentration-dependent cytotoxicity of the acetone extract of *S. venosum* exhibited against the human pancreatic cancer cell line (MiaPaCa-2), further strengthening the anti-cancer potential of the species (Ghurde et al., 2025).

However, the systematic evaluation of different S. venosum extracts against colorectal cancer remains limited. Since solvent polarity influences phytoconstituent recovery, extraction using solvents of different polarities can provide a broader chemical profile for biological evaluation (Bitwell et al., 2023). Accordingly, the present study evaluated the antioxidant activity of hydroalcoholic, aqueous, and ethyl acetate extracts of *S. venosum* leaves and tubers. The most active extracts were subjected to LC–MS profiling, and the identified constituents were investigated by molecular docking against colorectal cancer-associated targets. Their anti-cancer potential was further validated using MTT assay and DAPI staining in human colorectal cancer cell lines. This integrated approach provides insights into the phytochemical composition, antioxidant activity, molecular interactions, and anti-cancer potential of *S. venosum* as a source of potential therapeutic agents.

Materials and Methods

Collection and identification of plant

The whole plant of *S. venosum* was collected from the Kolhapur district of Maharashtra, India (16.6913° N, 74.2448° E) during the rainy season. The collected plant material was authenticated by Mr. D. L. Shirodkar, Botanical Survey of India. A voucher herbarium specimen was prepared and deposited following standard Botanical Survey of India guidelines for future reference.

Preparation of plant material

Fresh leaves and tubers of *S. venosum* were separated, thoroughly washed with distilled water to remove adhering debris and extraneous matter, and cut into small uniform pieces. The plant materials were shade dried under ambient conditions for 10–15 days to minimize degradation of thermolabile phytoconstituents. The dried materials were pulverized separately using a mechanical grinder to obtain coarse powder and stored in airtight containers protected from moisture and light until extraction.

Soxhlet extraction of plant material

Different extracts of *S. venosum* were prepared using Soxhlet extraction with solvents of varying polarity. Briefly, 50 g of dried powdered leaf and tuber materials were separately packed into cellulose thimbles and extracted under reflux conditions until complete exhaustion of the plant material, as indicated by a colorless siphon tube solvent.

Hydroalcoholic extract (L1), aqueous extract (L2), and ethyl acetate extract (L3) were prepared using 400 mL of 70% ethanol (ethanol: water, 70:30, v/v), 500 mL of distilled water, and 400 mL of ethyl acetate, respectively, for leaf extraction. Similarly, hydroalcoholic extract (T1), aqueous extract (T2), and ethyl acetate extract (T3) of the tuber were prepared using the respective solvents under identical extraction conditions.

Hydroalcoholic and ethyl acetate extractions were done for 6–8 hours at their respective boiling temperatures, while aqueous extraction was done for 8–10 hours at about 100°C. Following extraction, the obtained extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extracts were further dried on a water bath to obtain semisolid masses. All dried extracts were weighed, percentage yields were calculated, and the extracts were stored in properly labeled amber-colored glass containers under refrigerated conditions (4°C) until further phytochemical and biological investigations (Nikhal et al., 2010; Killedar et al., 2014; Bhavikatti et al., 2021).

Determination of percentage yield

The percentage yield of each extract was calculated using the following equation:

%Yield = (weight of dried extract / weight of plant material taken) x 100

In vitro antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of all six extracts of *S. venosum* was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay in a 96-well microplate format. Briefly, the hydroalcoholic leaf extract (L1), aqueous leaf extract (L2), ethyl acetate leaf extract (L3), T1, T2, and T3 extracts were dissolved separately in methanol to obtain different concentra-

tions (250, 500 and 1000 µg/mL). Ascorbic acid was used as the standard antioxidant reference compound and was prepared similarly in methanol. A freshly prepared 0.1 mM DPPH solution in methanol was used for the assay.

In a 96-well microplate, 100 µL of each test sample or standard solution was added in triplicate, followed by the addition of 100 µL of DPPH solution. The control was prepared by mixing 100 µL of methanol with 100 µL of DPPH solution, and the blank wells contained 100 µL of methanol without DPPH. The microplate was covered with aluminum foil and incubated in the dark at room temperature for 30 min, and then the absorbance was measured at 517 nm using a microplate reader (Halarnekar et al., 2023; Bhavikatti et al., 2024).

The percentage DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Ac-As})}{\text{Ac}} \times 100$$

Whereas absorbance of control (Ac), Absorbance of sample or standard (As)

The antioxidant activity was expressed as percentage inhibition, and IC_{50} values were determined from concentration–response curves. All experiments were performed in triplicate and results were expressed as mean ± standard deviation (SD).

LC–MS analysis

Based on the antioxidant screening results, L1 and T1 extract of *S. venosum* were selected for LC–MS/MS analysis for phytochemical characterization. For the sample preparation, 100 mg of each extract was accurately weighed and dissolved separately in 50 mL of methanol followed by sonication for 30 min to ensure complete dissolution of phytoconstituents. The prepared solutions were filtered through a membrane filter before analysis and 10 µL of each sample was injected into the LC–MS system. Chromatographic separation was performed on an Acquity UPLC system (Waters, USA) using an Acquity BEH C18 column (50 × 2.1 mm, 1.7 µm particle size). The mobile phase was 0.1% formic acid in water as mobile phase A and acetonitrile as mobile phase B. Elution was performed by gradient mode at a flow rate of 0.400 mL/min for a total run time of 20 min. The gradient program was optimized to achieve efficient separation of phytoconstituents by gradually increasing the proportion of acetonitrile during the analysis and subsequently re-equilibrating the column to initial conditions.

Mass spectrometric analysis was carried out using a Xevo G2-XS QT of mass spectrometer (Waters, USA). The optimized operating conditions included capillary voltage 3.0 kV, collision energy 20 V, ramp collision energy from 30–90 V, source temperature 150°C, and desolvation temperature 450°C. Nitrogen was used as cone gas and desolvation gas at a flow rate of 50 L/hour and 800 L/hour respectively. Data acquisition and processing were done using MassLynx software version 4.1 (Waters, USA). Tentative identification of the phytoconstituents was performed by comparing retention times, molecular ion peaks, fragmentation patterns with available spectral databases and literature reports.

Molecular docking studies

Molecular docking studies were conducted to evaluate the binding interactions of the selected phytoconstituents with colorectal cancer-associated target proteins. The three-dimensional (3D) structures of the selected phytoconstituents were retrieved from the PubChem database, while the crystallographic structures of the target proteins TP53, EGFR, MMP9, AKT1, and SRC were obtained from the RCSB Protein Data Bank.

Prior to docking, the protein structures were prepared by removing water molecules, co-crystallized ligands, and other heteroatoms, followed by the addition of hydrogen atoms and energy minimization. The ligand structures were similarly optimized before docking analysis. Molecular docking was then performed to predict the binding affinity and interaction patterns of the phytoconstituents with the selected target proteins. Binding energies and intermolecular interactions were analyzed to assess the potential anti-colorectal cancer activity of the identified phytoconstituents.

MTT assay

Cell culture

HT-29 and HCT-116 human colorectal cancer cell lines were procured from the National Centre for Cell Science, Pune, India. Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and cultured at 37°C in a humidified atmosphere containing 5% CO_2. Cells in the exponential growth phase were used for all experiments.

The cytotoxic activity of the test samples was evaluated using the MTT assay. HT-29 and HCT-116 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hours. The cells were then treated with different concentrations of the test samples (6.25–100 µg/mL), while 5-fluorouracil (5-FU) was used as the reference standard at the same concentrations. Vehicle control wells received 0.2% dimethyl sulfoxide. All treatments were performed in triplicate.

After 24 hours of treatment, the medium was removed, and 20 µL of MTT solution (5 mg/mL in PBS) was added to each well. Following incubation for 4 hours at 37°C, the medium was discarded, and the resulting formazan crystals were dissolved in 200 µL DMSO. Absorbance was measured at 570 nm using a microplate reader (Benesphera E21). Cell viability was

expressed as a percentage relative to the untreated control, and IC_{50} values were calculated from the dose-response curves (Bhagwat et al., 2021).

Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100

DAPI staining

The sample exhibiting better cytotoxic activity in the MTT assay was selected for DAPI staining and tested at its respective IC_{50} concentration. HT-29 and HCT-116 cells were seeded onto sterile coverslips placed in 24-well plates and incubated overnight at 37°C in a humidified atmosphere containing 5% CO_2. The cells were treated for 24 hours, washed twice with PBS, fixed with 4% paraformaldehyde for 30 min, and stained with 20 µL of DAPI solution for 5 min in the dark at room temperature. The stained cells were examined under a fluorescence microscope, and cells were counted from randomly selected microscopic fields for both cell lines (Nadaf and Killedar, 2018).

Results

Percentage yield

The percentage yield of different *S. venosum* leaf and tuber extracts was determined to evaluate the extraction efficiency of solvents with different polarities. The extraction yield varied depending on both the plant part and the solvent employed, indicating differences in the solubility of phytoconstituents.

Among the leaf extracts, L1 extract exhibited the highest percentage yield 13.1%, followed by the L2 extract (9.1%), whereas the extract L3 showed the lowest yield (7.6%). Similarly, in the tuber extracts, the T1 extract produced the maximum yield 16.4%, followed by the T2 extract 12.3%, while the T3 extract yielded the least amount 9.3% (Table SI).

Antioxidant activity

The antioxidant potential of the different extracts of *S. venosum* was evaluated by DPPH free radical scavenging assay (Table SII). All extracts showed concentration dependent antioxidant activity with percentage inhibition increased progressively from 200 to 1000 µg/mL. Among the tested samples, the standard ascorbic acid showed highest radical scavenging activity with 91.9% inhibition at 1000 µg/mL. Among the plant extracts, T1 exhibited the strongest antioxidant activity with 82.6% inhibition at 1000 µg/mL, followed by T2 (78.0%), L1 (77.8%), T3 (72.3%), L2 (69.6%) and L3 (61.8%). Based on the relatively better antioxidant activity, the L1 and T1 extracts were selected for further investigations.

LCMS

For instance, for sample L1 extract, a cluster of diagnostic ions at m/z 303.1, 287.1, and 315.1 (Figure 1A) were repeatedly found in different retention times and spectra, which demonstrated the presence of the major flavonoid aglycones, i.e., quercetin, kaempferol and isorhamnetin. Such compounds are reported to have strong antioxidant, anti-inflammatory and metabolic regulatory activities, indicating that the sample has a high bioactivity potential. The repeated finding of the ions in different peaks also suggests the co-existence of several isomeric or related derivatives.

Besides aglycones, some higher molecular weight ions (e.g., m/z 449, 599, 617, 757, 801 and 929) were also observed with characteristic neutral losses corresponding to sugar moieties such as hexose (−162 Da) and rutinoside (−306 Da). This is a strong indication for the presence of mono-, di- and triglycosylated flavonoids. Glycosylation is known to significantly affect the solubility, stability and bioavailability of flavonoids suggesting that the sample may have improved pharmacokinetic properties over aglycone-rich systems.

Negative ion mode analysis verified the compositional profile, showing phenolic acids like chlorogenic acid (m/z 353.1), quinic acid (m/z 191.1), and gallic acid (m/z 169.0). The fragmentation of chlorogenic acid to quinic acid product ion offered confirmatory evidence of hydroxycinnamic acid derivatives. These phenolic acids are widely known to possess antioxidant and free radical scavenging activities that work synergistically to contribute to the overall biological activity of the sample.

The presence of several molecular weight ions above m/z 800 (e.g., 915, 1083, 1267) also indicates the presence of complex secondary metabolites, such as oligomeric polyphenols, tannins, or saponin-like compounds, which are generally present in plant matrices and are known to contribute to various pharmacological effects, such as anti-diabetic, anti-cancer, and immunomodulatory activities.

These results are supported by the chromatographic distribution of compounds where the early eluting peaks correspond to the polar phenolic acids, mid-retention peaks are dominated by flavonoid aglycones and late eluting peaks represent glycosylated and lipophilic conjugates. This gradient-based separation confirms the presence of metabolites with a broad range of polarities, indicative of a complex natural extract.

Altogether, the LC–MS profile suggests that sample A1 is a rich source of structurally diverse polyphenols, particularly flavonoids and their glycosides, and phenolic acids and their higher-order conjugates. Such a composition is typical of plant-derived extracts with high antioxidant capacity and potential therapeutic applications in metabolic disorders such as diabetes and obesity.

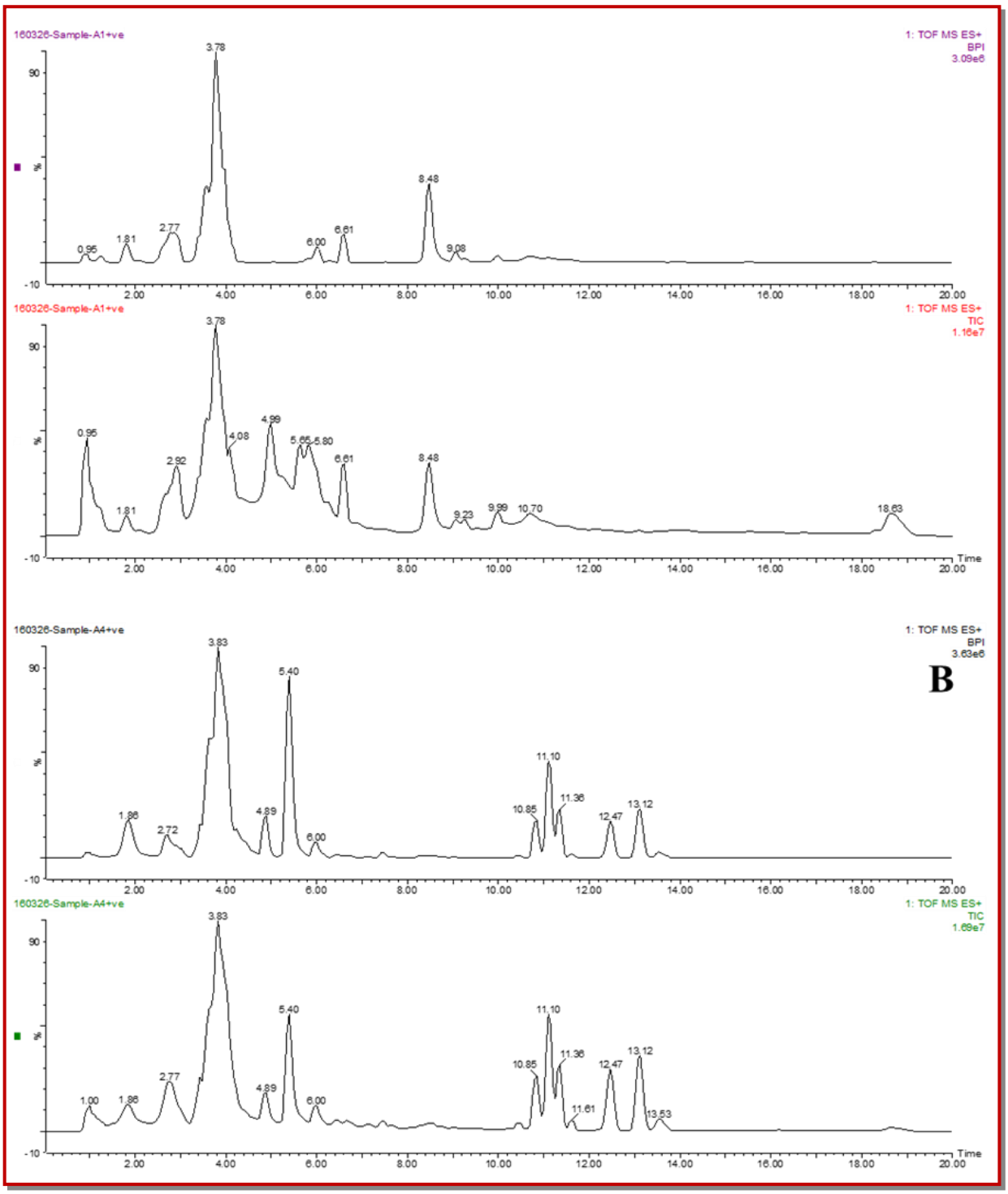

Figure 1: UPLC–QToF–MS/MS base peak intensity (BPI) chromatograms of selected hydroalcoholic extracts of *S. venosum*: leaf extract L1 (A) and tuber extract T1 (B)

In conclusion, the LC–MS analysis provides strong evidence that sample L1 represents a polyphenol-rich (Table SIII) system with significant pharmacological potential.

In case some sample T1, the total ion chromatograms (TIC) and base peak intensity (BPI) chromatograms (Figure 1B) revealed a complex mixture of constituents distributed over a wide retention time (RT) window (1–18 min), indicating the presence of compounds with diverse polarity.

Table I

Molecular docking binding affinity scores (kcal/mol) of selected phytoconstituents identified from *S. venosum* against selected colorectal cancer-associated target proteins

Phytoconstituent	TP53	EGFR	MMP9	AKT1	SRC
Chlorogenic acid	-6.58	-18.50	-10.46	-19.35	-9.00
Isorhamnetin	-14.52	-22.77	-16.64	-19.54	-31.80
Kaempferol	-7.18	-25.50	-24.17	-28.62	-33.58
Quercetin	-10.96	-26.04	-30.19	-16.48	-31.07
Quinic acid	-11.39	-17.31	-12.91	-23.21	-25.74
Rutin	-4.97	-9.57	-8.20	-5.16	-6.80

Several major ions were observed in the positive ionization mode at different retention times. The characteristic ion at m/z 303.1 was observed to correspond to the protonated molecular ion M+H+ of quercetin and was supported by its typical fragmentation pattern with product ions at m/z 179 and 151. An abundant ion at m/z 287.1 was assigned to kaempferol (M+H+). The prominent ion at m/z 315.0 was assigned to isorhamnetin, a methylated derivative of quercetin with a mass increment of 14 Da relative to quercetin.

In addition to aglycones, several glycosylated flavonoids were also detected. The major ion at m/z 609.1 was assigned to quercetin-3-O-rutinoside (rutin), confirmed by its fragmentation to m/z 303 with the loss of a rutinose moiety (−306 Da). Similarly, the ion at m/z 593.2 was tentatively identified as a kaempferol glycoside, which fragmented to m/z 287 after the loss of the sugar unit. In addition, the ions at m/z 621–637 suggest the presence of flavonoid diglycosides or conjugates with similar structures.

Negative ionization mode also confirmed these findings. The presence of quercetin was confirmed by the deprotonated molecular ion M-H- at m/z 301.0, an ion at m/z 353.1 was assigned to chlorogenic acid (caffeoylquinic acid). The characteristic fragment at m/z 191.1 corresponding to quinic acid supported the identification of chlorogenic acid and related hydroxycinnamic acid derivatives.

We also observed several medium- to high-molecular-weight ions (e.g., m/z 757, 915, and 1083) that could be oligomeric polyphenols, saponins, or lipid-conjugated substances, but since no definitive fragmentation data were available, these compounds were tentatively classified only according to their mass range and chromatographic behavior (Table SIV).

The LC–MS analysis of the sample showed that it is mainly constituted by polyphenolic components, especially flavonoids and phenolic acids, and their glycosylated derivatives. The detection of quercetin, kaempferol, isorhamnetin, rutin and chlorogenic acid indicates a potent antioxidant phytochemical composition, typical of plant-based extracts.

Molecular docking interactions

The molecular docking analysis demonstrated favorable binding interactions of the selected phytoconstituents with colorectal cancer-associated target proteins. The binding affinity scores (Table I) and molecular interactions (Figures 2-6) of the identified phytoconstituents against TP53, EGFR, MMP9, AKT1, and SRC are shown.

Kaempferol exhibited strong binding affinity towards several colorectal cancer-associated target proteins, with the highest binding affinity observed for SRC (-33.6 kcal/mol; Figure 6C) followed by AKT1 (-28.6 kcal/mol; Figure 5C).

Quercetin also demonstrated favorable interactions, showing the strongest binding with MMP9 (-30.2 kcal/mol; Figure 4D) and SRC (-31.1 kcal/mol; Figure 6D).

Isorhamnetin displayed the highest binding affinity towards TP53 (-14.5 kcal/mol; Figure 2B) among the evaluated phytoconstituents for this target.

Quinic acid and chlorogenic acid exhibited moderate binding affinities across the selected protein targets, indicating their potential contribution to the overall biological activity of the extract.

Rutin showed comparatively lower binding affinities against all selected proteins, which may be attributed to steric hindrance caused by its bulky rutinoside moiety, limiting its accommodation within the active binding pockets.

The molecular docking analysis provided a plausible mechanistic basis for the potential anti-colorectal cancer activity of the identified phytoconstituents. Colorectal cancer is a highly heterogeneous malignancy characterized by mutations in tumor suppressor genes and dysregulation of multiple signaling pathways involved in cell proliferation, apoptosis, invasion, and metastasis. The selected colorectal cancer-associated proteins, TP53, EGFR, MMP9, AKT1, and SRC, play critical roles in these pathological processes. The favorable binding interactions of phytoconstituents such as quercetin, kaempferol, and isorhamnetin with these proteins suggest that these compounds may modulate multiple molecular targets involved in colorectal cancer progre-

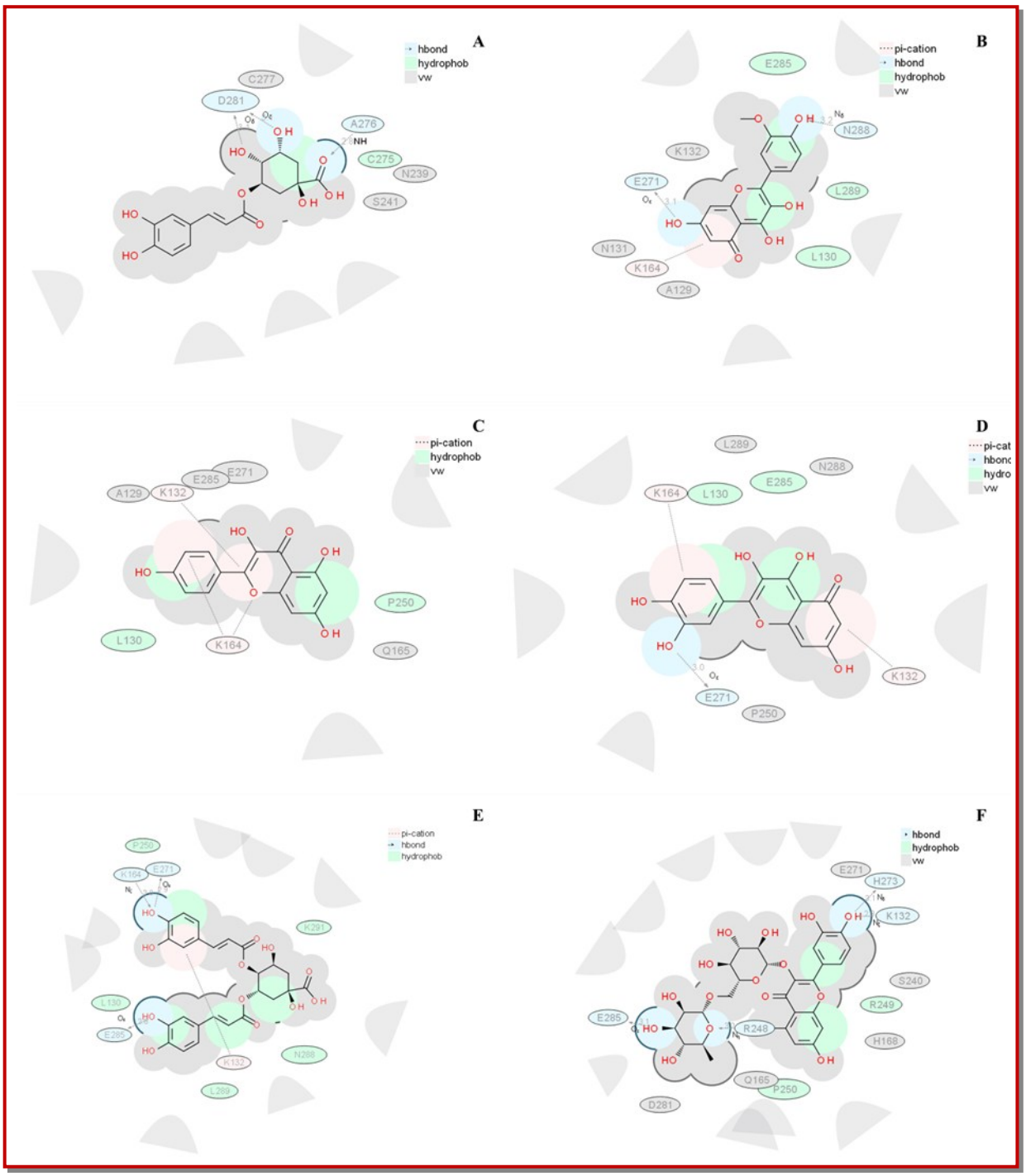

Figure 2: Two-dimensional molecular interactions of TP53 with selected phytoconstituents: (A) Chlorogenic acid, (B) isorhamnetin, (C) kaempferol, (D) quercetin, (E) quinic acid, and (F) rutin

ssion, supporting their potential as promising anti-cancer agents.

Targeting the proliferation axis (EGFR and AKT1)

CRC is characterized by overexpression of Epidermal Growth Factor Receptor (EGFR) leading to uncontrolled cell division. Downstream of EGFR, the PI3K/AKT signaling pathway (mediated by AKT1) acts as a major survival and anti-apoptotic cascade. Kaempferol demonstrated excellent dual-inhibition efficacy against this axis with binding scores of -25.5 against EGFR (Figure 3C) and a network-leading -28.6 for AKT1 (Figure 4C). Quinic acid also showed a strong preference for AKT1 (-23.2) (Figure 5E). These compounds may theoretically mimic the effects of synthetic tyrosine kinase inhibitors by simultaneously locking the ATP-binding clefts of

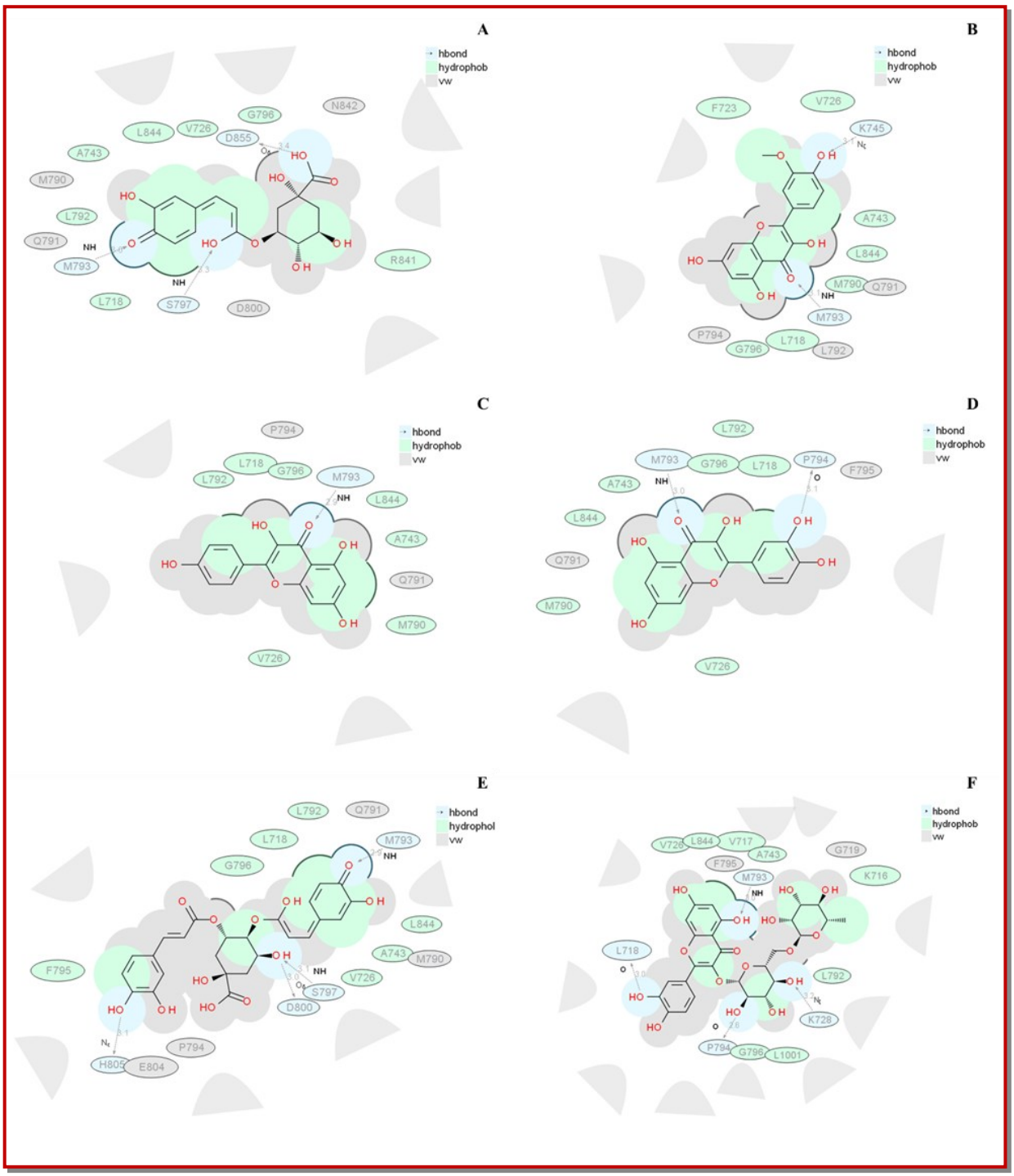

Figure 3: Two-dimensional molecular interactions of EGFR with selected phytoconstituents: (A) Chlorogenic acid, (B) isorhamnetin, (C) kaempferol, (D) quercetin, (E) quinic acid, and (F) rutin

these kinases and by inducing synergistic apoptosis in CRC cells.

Suppressing metastasis and invasion (SRC and MMP9)

Metastatic dissemination is the leading cause of death in CRC. SRC is a proto-oncogenic non-receptor tyrosine kinase that controls cell migration stringently and MMP9 (matrix metalloproteinase 9) degrades the extracellular matrix, enabling tumor invasion. Quercetin exhibited a prominent affinity for MMP9 (-30.2) (Figure 4D), suggesting it can directly inhibit extracellular matrix remodeling. Furthermore, kaempferol (-33.6; (Figure 7C), isorhamnetin (-31.8; Figure 6B), and quercetin (-31.1; Figure 6D) demonstrated strong binding

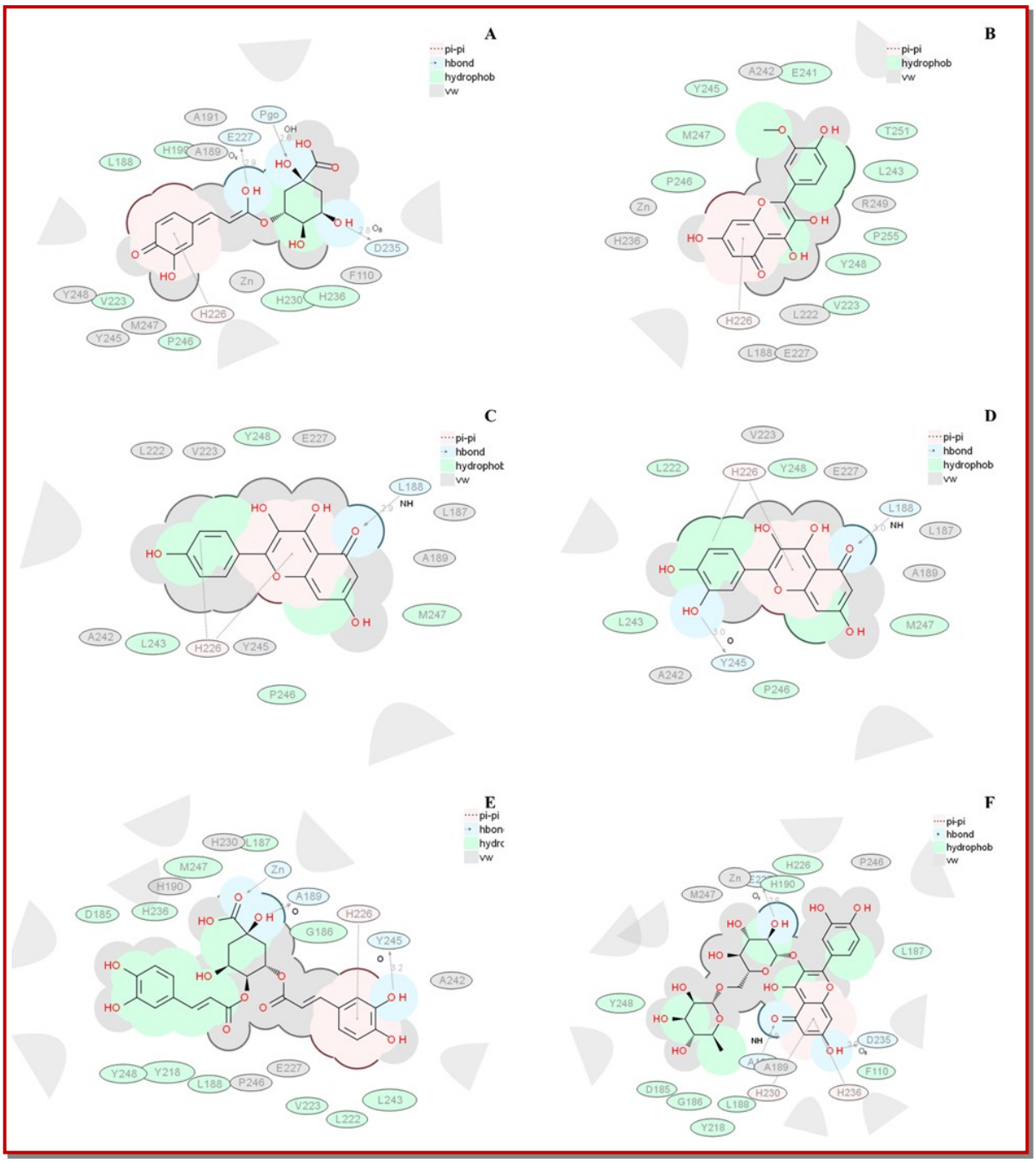

Figure 4: Two-dimensional molecular interactions of MMP9 with selected phytoconstituents: (A) Chlorogenic acid, (B) isorhamnetin, (C) kaempferol, (D) quercetin, (E) quinic acid, and (F) rutin

affinity towards SRC. This underscores a clear role for this specific aglycone flavonoids in arresting CRC metastasis.

Modulation of tumor suppressors (TP53)

TP53 is the most frequently mutated gene in CRC, resulting in the loss of cell cycle checkpoints. While targeting p53 with small molecules is notoriously difficult, isorhamnetin (-14.52; Figure 2B) and quinic acid (-11.39; Figure 2E) exhibited significant interactions with the p53 structure. Depending on the docking site (which needs to be further profiled), these compounds may act as stabilizers of the wild-type p53 or help to restore mutant p53 conformation and to restore apoptotic signaling.

MTT assay

The cytotoxic effects of extracts L1 and T1 against HT-

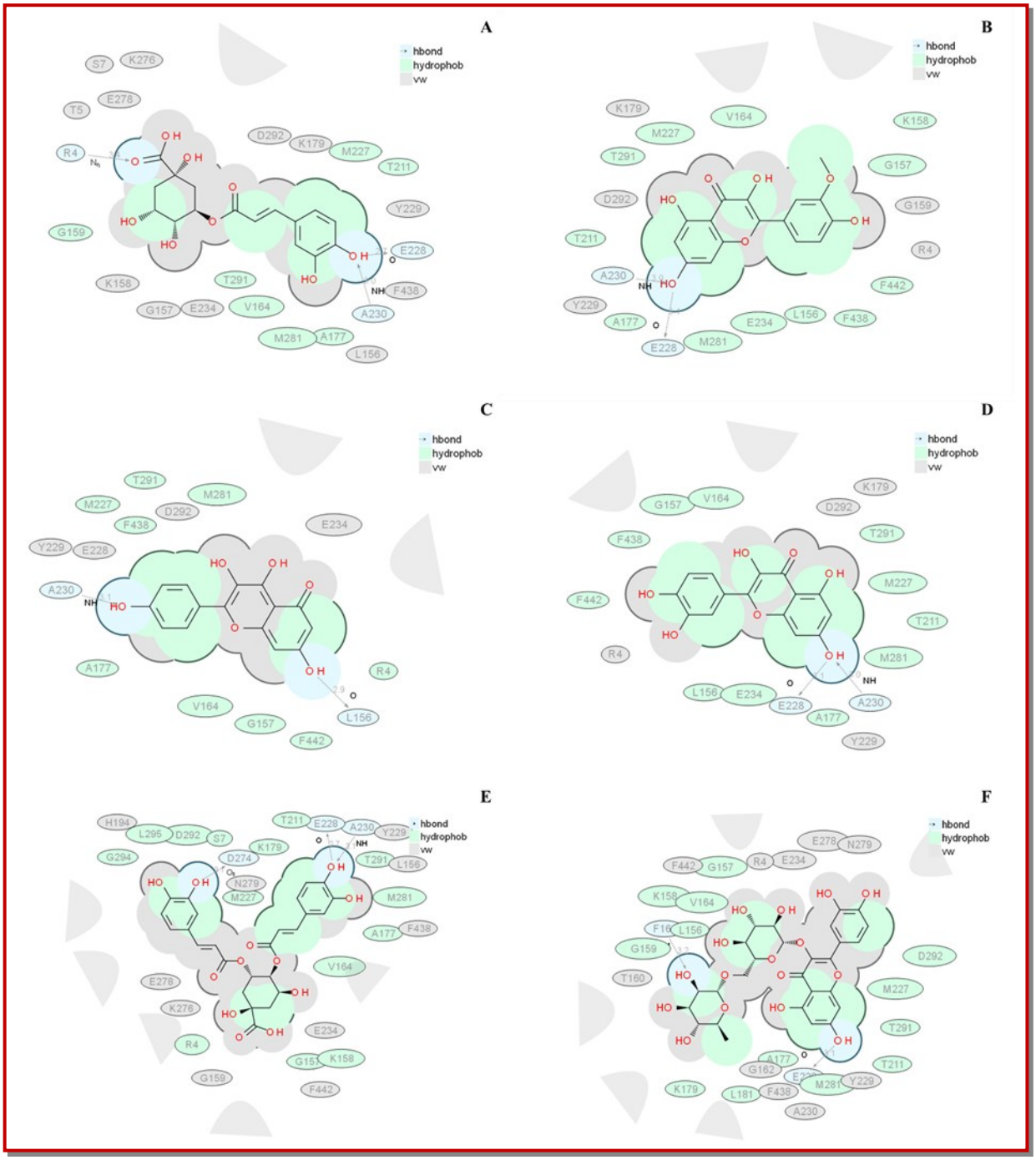

Figure 5: Two-dimensional molecular interactions of AKT1 with selected phytoconstituents: (A) Chlorogenic acid, (B) isorhamnetin, (C) kaempferol, (D) quercetin, (E) quinic acid, and (F) rutin

29 and HCT-116 human colorectal cancer cell lines were evaluated using the MTT assay and compared with the reference drug 5-FU. Both extracts exhibited concentration-dependent inhibition of cell viability in both cell lines (Table SV). Among the test samples, T1 extract demonstrated greater cytotoxic activity than L1 extract, with lower IC_{50} values against HT-29 (20.1 µg/mL vs. 32.0 µg/mL) and HCT-116 (36.5 µg/mL vs. 57.0 µg/mL). At the highest tested concentration (100 µg/mL), T1 extract reduced cell viability to 31.3% and 36.8% in HT-29 and HCT-116 cells, respectively, whereas L1 extract reduced cell viability to 35.3% and 41.1%. The standard drug 5-FU exhibited the highest cytotoxic potency, with IC_{50} values of <6.3 µg/mL against HT-29 and 29.9 µg/mL against HCT-116, reducing cell viability to 17.1% and 22.5%, respectively, at 100 µg/mL.

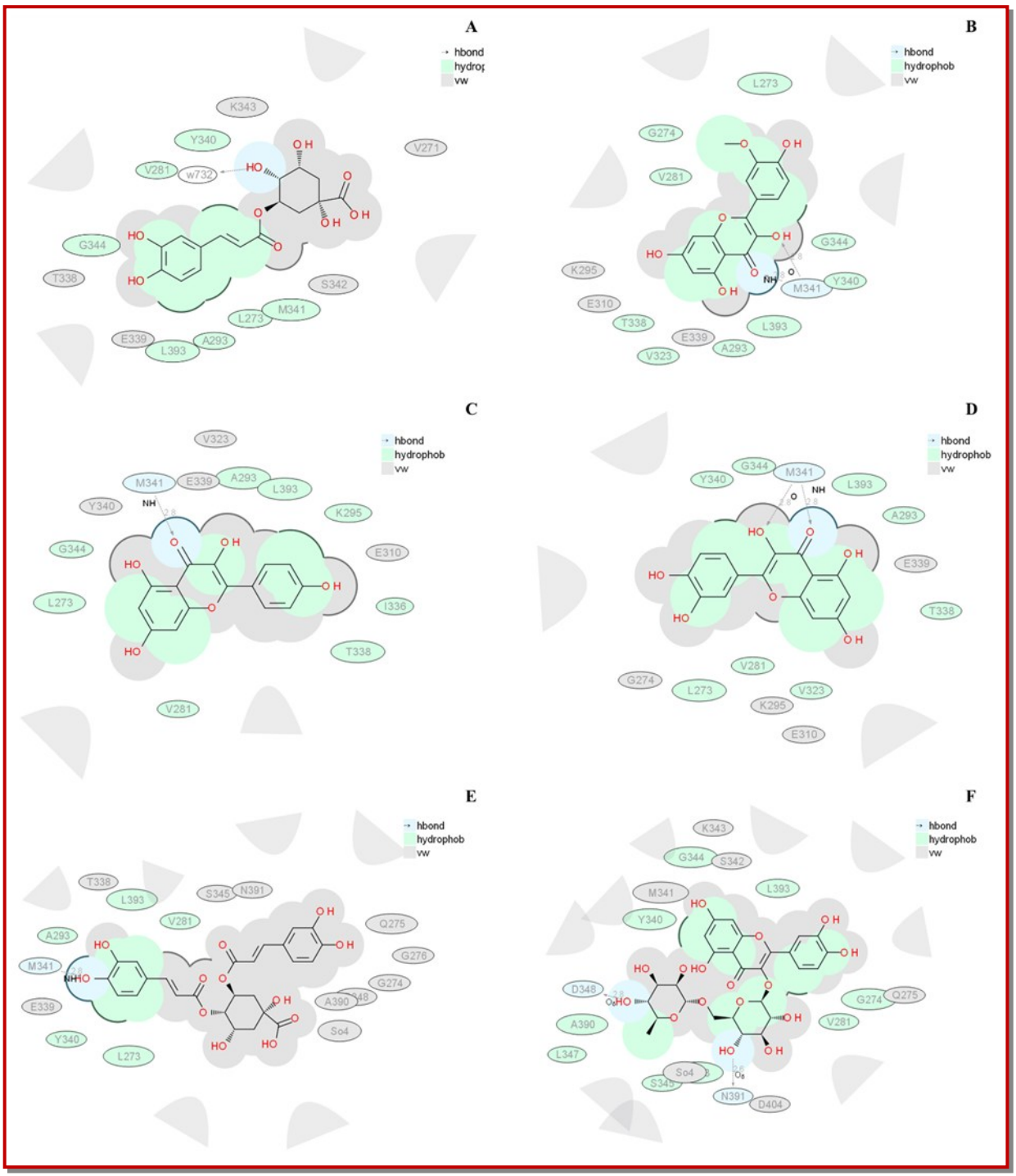

Figure 6: Two-dimensional molecular interactions of SRC with selected phytoconstituents: (A) Chlorogenic acid, (B) isorhamnetin, (C) kaempferol, (D) quercetin, (E) quinic acid, and (F) rutin

Overall, both test samples showed greater cytotoxic activity against HT-29 cells than HCT-116 cells, with T1 extract consistently exhibiting superior antiproliferative activity compared to L1 extract, although both remained less potent than the reference drug 5-FU.

DAPI staining

DAPI staining revealed distinct differences in nuclear morphology between the control and T1 extract-treated cells. The control cells (Figure 7A) exhibited intact, uniformly stained nuclei with normal morphology, indicating healthy and viable cells. In contrast, cells treated with T1 extract (Figure 7B) showed a marked reduction in cell number along with condensed and fragmented nuclei, accompanied by increased nuclear shrinkage and loss of normal cellular architecture.

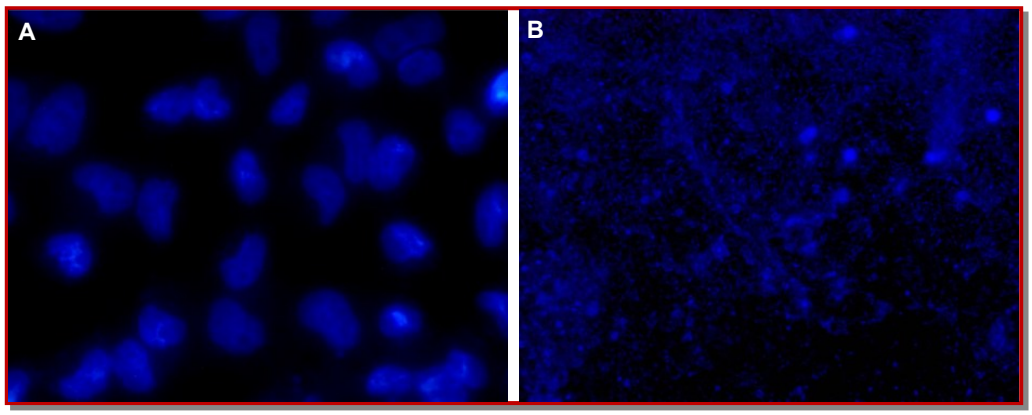

Figure 7: Microphotographs of DAPI stained images control cells (A) and cells treated with T1 extract (B)

Discussion

The present study demonstrated that *S. venosum* leaves and tubers possess significant antioxidant and potential anti-colorectal cancer activities. Among the six extracts evaluated, the T1 extract showed the highest extraction yield (16.4%) and exhibited the strongest DPPH radical-scavenging activity, with 82.6% inhibition at 1000 µg/mL, followed by T2 and L1 extracts. The comparatively higher antioxidant activity of L1 and T1 extracts provided a rationale for their selection for further phytochemical characterization. The higher activity observed with T1 extract may be associated with the hydro-alcoholic solvent system, which facilitates the extraction of a broad range of polar and moderately polar phytoconstituents, particularly phenolic acids and flavonoids. These findings indicate that *S. venosum*, particularly its tubers, may represent a promising source of naturally occurring antioxidant constituents.

LC–MS/MS profiling of the selected extracts revealed several phenolic acids and flavonoids, including quinic acid, chlorogenic acid, quercetin, kaempferol, isorhamnetin and rutin, together with their glycosylated derivatives. The presence of these compounds is relevant because phenolic acids and flavonoids are widely associated with free-radical scavenging, modulation of oxidative stress and regulation of cellular signaling pathways. The occurrence of multiple structurally diverse phenolic constituents may therefore explain, at least in part, the strong antioxidant activity observed in T1 extract. The biological activity of the extract may also result from the combined effects of several constituents rather than from the activity of a single compound acting independently. However, such additive or synergistic interactions require experimental confirmation.

The findings of the present study are generally consistent with previous investigations demonstrating the biological potential of *S. venosum*, although differences were observed in the plant material, extraction procedures, analytical techniques, cancer models and experimental approaches. Lectin isolated from *S. venosum* tubers has exhibited antiproliferative activity against several human and murine cancer cell lines, supporting the presence of biologically active anti-cancer constituents in the tubers (Singh et al., 2005). Similarly, in another work, tuber extract has shown cytotoxic potential associated with 12-O-acetylingol 8-tiglate (Kahar et al., 2024). In contrast, the present study predominantly identified phenolic acids, flavonoids and flavonoid glycosides through LC–MS/MS. The differences in the compounds detected may be attributable to variations in extraction solvents, plant material, geographical conditions and analytical platforms, as well as differences in the chemical characteristics of compounds detectable by GC–HRMS and LC–MS/MS.

Acetone extract of *S. venosum* corm has shown cytotoxic effect against MiaPaCa-2 pancreatic cancer cells, with an IC_{50} value of approximately 93.68 µg/mL (Ghurde et al., (2025). In comparison, the present study demonstrated greater cytotoxic activity of the hydro-alcoholic tuber extract T1 against the colorectal cancer cell lines HT-29 and HCT-116, with IC_{50} values of 20.1 and 36.5 µg/mL, respectively. Although direct comparison between these findings should be made cautiously because of differences in plant parts, extraction solvents, cancer cell lines and assay conditions, the findings collectively support the potential of *S. venosum* as a source of cytotoxic phytoconstituents. The stronger antioxidant activity observed in the present study is also consistent with the reported radical-scavenging potential of phenolic acids and flavonoids. Thus, the present investigation extends the existing evidence on *S. venosum* by integrating phytochemical profiling, molecular docking and *in vitro* validation against colorectal cancer cell lines.

The molecular docking results provided additional insight into the possible mechanisms underlying the observed anti-cancer activity. Several identified phyto-constituents showed favorable interactions with colorectal cancer-associated targets involved in cellular proliferation, survival, invasion and metastasis. In

particular, kaempferol demonstrated favorable interactions with SRC and AKT1, whereas quercetin showed favorable interactions with MMP9 and SRC. Isorhamnetin and other identified flavonoids also showed favorable interactions with important molecular targets, including EGFR, AKT1, MMP9, SRC and TP53. These findings suggest that the identified phytoconstituents may potentially influence multiple signaling pathways associated with colorectal cancer progression. The multi-target interaction profile is noteworthy because colorectal cancer involves complex and interconnected molecular pathways rather than a single therapeutic target. Nevertheless, the docking results represent predicted interactions and should therefore be considered hypothesis-generating rather than direct evidence of target engagement.

The *in vitro* findings further supported the potential biological activity of the T1 extract. It exhibited greater cytotoxicity than L1 extract against both HT-29 and HCT-116 cells, with IC_{50} values of 20.1 and 36.5 µg/mL, respectively. The greater sensitivity of HT-29 cells may reflect differences in their genetic characteristics, receptor expression, intracellular signaling pathways and susceptibility to phytochemical-induced cellular stress. The greater cytotoxic activity of T1 extract may also be associated with its higher extraction yield and the resulting availability of phenolic and flavonoid constituents. However, because T1 extract represents a complex mixture of phytochemicals, the observed cytotoxicity cannot be attributed to any individual constituent without further fractionation and biological evaluation.

The morphological changes observed following DAPI staining provide further evidence of apoptosis-associated cellular alterations. Treatment with T1 extract resulted in nuclear condensation, cellular shrinkage and nuclear fragmentation, which are characteristic morphological features associated with apoptotic cell death. These morphological alterations are consistent with the reduction in cell viability observed in the MTT assay and suggest that apoptosis-associated mechanisms may contribute to the cytotoxic effect of T1 extract. Jan et al. (2022) reported anti-cancer and apoptosis-related effects associated with quercetin and kaempferol, supporting the possible contribution of these phytoconstituents to the biological activity observed in the present study. Since quercetin and kaempferol were detected in T1 extract by LC–MS/MS, their presence may contribute to the observed cytotoxic and apoptosis-associated effects. However, this association should be interpreted cautiously because the present study did not establish the individual contribution of these compounds. Furthermore, DAPI staining alone cannot conclusively confirm apoptosis. Additional investigations using annexin V/PI staining, caspase activity assays, mitochondrial membrane potential analysis and evaluation of apoptosis-related proteins would be required to confirm the underlying mechanism.

The observed biological activities may therefore be associated with the presence and combined effects of phenolic acids and flavonoids detected in the hydroalcoholic extracts. Compounds such as quercetin, kaempferol, isorhamnetin, chlorogenic acid and rutin have been associated with antioxidant and diverse pharmacological activities and may collectively contribute to the effects observed in the present study. The stronger activity of T1 extract may be related to the greater extraction efficiency of the hydroalcoholic solvent and the resulting enrichment of polar and moderately polar bioactive constituents. In addition, interactions among multiple phytoconstituents may contribute to the overall biological response of the extract. However, the presence of a compound in an extract and its reported biological activity in previous studies do not, by themselves, establish its specific contribution to the activity of the present extract.

Overall, the present study provides evidence supporting the antioxidant and potential anti-colorectal cancer activities of *S. venosum*, particularly its hydroalcoholic tuber extract (T1). The integration of antioxidant evaluation, LC–MS/MS profiling, molecular docking, cytotoxicity assessment and DAPI staining suggests that the observed activity may be associated with multiple phenolic and flavonoid constituents. However, the LC–MS/MS assignments and docking findings require further experimental validation. Isolation and characterization of individual compounds, mechanistic studies, apoptosis-specific assays and *in vivo* investigations are warranted to confirm the anti-cancer potential of *S. venosum*.

The limitations of this study are that the compound identifications presented here are putative, relying on accurate mass measurements, fragmentation patterns, and matching with spectral databases like METLIN, HMDB, and MassBank. Further validation through authentic reference standards, tandem mass spectrometry (MS/MS) spectral matching, and complementary spectroscopic methods (e.g., NMR) is advised for definitive structural characterization.

Conclusion

S. venosum extracts possess significant antioxidant and potential anti-colorectal cancer activities, with T1 extract showing superior antioxidant and cytotoxic effects. It may serve as a promising source of bioactive phytoconstituents for colorectal cancer therapy.

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Ethical Issue

The development, acquisition, authentication, cryopreservation, and transfer of cell lines between laboratories were followed according to the guidelines published in British journal of Cancer, 2014

Conflict of Interest

Authors declare no conflict of interest

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