SINAI International Scientific Journal (SISJ)
Volume 2, Issue 2, October 2025

The Protective Effects of Arbutin Against Colon Cancer: in Silico and in Vitro Studies

*Mohamed H.A. Gadelmawla [1 *], Hossam H. Nasrallah [2]*

[1]Life Sciences Department, Faculty of Biotechnology, Sinai University, Kantara Campus, Ismailia, Egypt
[2]Chemistry Department, Faculty of Biotechnology, Sinai University, Kantara Campus, Ismailia, Egypt
**Corresponding author*

Correspondence:

Mohamed H.A. Gadelmawla

Email: Mohamed.hassany@su.edu.eg

Citation:

Gadelmawla, M. H.A., Nasrallah, H. H. "The Protective Effects of Arbutin Against Colon Cancer: in Silico and in Vitro Studies", SINAI International Scientific Journal (SISJ), vol.2(2), pp.80-94, 2025

Received: 19 August 2024

Accepted: 20 December 2025

ABSTRACT

After lung cancer, colon cancer is the second most prevalent cause of death globally and is one of the most common digestive cancers detected. Phytomedicines are strong substitutes for conventional drugs that have numerous adverse effects. Arbutin is a glycoside phytochemical derived from plants and is a member of the Ericaceae family. It has several pharmacological effects. This study aims to assess the toxic effect towards cancer cells and the anti-inflammatory effects of arbutin against the Caco-2 colon cancer cell line. The materials used are Molecular docking of Arbutin with the Caspase-3 enzyme (PDB code: 2XYG), TNF-α (PDB code: 1TNF) and pharmacokinetic assessments through SwissADME. Arbutin cytotoxicity potential was assessed using an MTT assay on the Caco-2 colon cancer cell line. The effect of ARB on the gene expression of P53, Caspase-3, TNF-α, and NF-κB were evaluated using the real-time PCR method. The results shows that there was a cytotoxic effect of the arbutin against Caco-2 cells with a significant potential impact of arbutin with IC50 of 29.05 µg/ml. The gene expression analysis showed statistically significant differences regarding p53, Caspase-3, TNF-α, and NF-κB between control and experimental groups (Control, 5FU, and Arbutin) ($p<0.05$). In regarding the previous findings, ARB revealed a potential ability to promote apoptosis in neoplastic cells and regulate the cell cycle. Further investigations are required to confirm our findings we ARB could be a promising anticancer agent.

KEYWORDS: Arbutin, colon cancer, anti-cancer, apoptosis

1. INTRODUCTION

As a malignant tumor, colon cancer or colorectal cancer (CRC) is a common cause of death globally due to its high rate of morbidity and mortality [1]. Despite significant advancements in colon cancer surgery and therapies, the 5-year relative survival rate for these individuals has not altered significantly over the previous few decades [2]. The main therapeutic approaches are radiation, chemotherapy, and surgery; however, these treatments frequently have adverse effects that impair liver and kidney function in addition to the immune system [3].

Traditional cancer treatments' negative pharmacological and physicochemical characteristics, hazardous effects on healthy cells and tissues, low water affinity, and the emergence of multi-drug resistance all harm bodily organs' effectiveness [4]. Chemotherapy is an important part of cancer treatment. Many novel chemotherapy drugs have recently been employed to treat various cancer types [5]. Since phytochemicals have safe anti-cancer properties, there is growing interest in their potential for cancer prevention and treatment. The glycosylated hydroquinone arbutin (ARB) is a bioactive polyphenol (Fig. 1) that occurs naturally in various plant species. Arbutin's β-glucose unit, rather than the α-glucose unit, structurally separates it from its isomer α-arbutin [6]. Other biologically significant therapeutic

Online ISSN: 3009-7800
Print ISSN: 3009-6324

qualities of arbutin have been demonstrated to include antioxidant, antibacterial, anticancer, and anti-inflammatory activities [7–11].

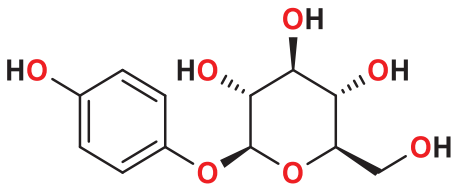


Fig. 1: Chemical structure of arbutin.

Moreover, ARB has therapeutic potential against cyclophosphamide-induced hepatotoxicity by lowering inflammation and oxidative stress [12]. ARB demonstrated proficiency in reducing intestinal pathological symptoms by blocking myeloperoxidase and pro-inflammatory cytokines in ulcerative colitis [13]. Reducing inflammation and prooxidant processes also contribute to its beneficial defense against ovarian damage induced by cisplatin [14] and acute renal damage caused by lipopolysaccharide [15]. ARB improved spermatogenesis and semen quality by re-establishing the balance between JAK2/STAT3 cascades, excessive ROS generation, and the pro-inflammatory NLRP3/caspase-1. It also enhanced the PK2/PKR2 pathway, which is cytoprotective [16]. The current study examined the potential ameliorative effect of the hydroquinone glycoside arbutin against colon cancer. We assessed the therapeutic prospects of arbutin through both in silico and in vitro approaches. We also evaluated the anticancer properties of the Caco-2 cell line.

2. MATERIALS AND METHODS

2.1. In Silico Studies

Drug likeness results from a difficult balancing act between structural and molecular characteristics. These characteristics, which include bioavailability, dispersion, affinity for proteins, reactivity, and many more, influence how a molecule behaves in a living thing. The chemical structure of the arbutin was added to the online Swiss ADME web tool to identify the substructure's features, which in turn dictate its physicochemical attributes [17,18]. The Swiss Target Prediction online tool predicted target–ligand interactions [19,20].

2.2. Molecular Docking

The molecular operation environment (MOE) application (version 2015.10) was used for docking studies, Caspase-3 (PDB code: 2XYG) [3], and TNF-α (PDB code: 1TNF) [21] (Homo sapiens) obtained from the protein data bank (PDB). The quality of the protein data was assessed using temperature factors and using Ramachandran plot. All residues show phi and psi angles in the allowed area in the plot. Residues of the terminal chain Thr174A and His277B were capped by N-CH3. The water molecules and the bound ligand were deleted. Hydrogens were added and their positions were optimized. Protein was minimized using tethers and restraints. Until a root mean square deviation (RMSD) gradient of 0.01 Kcal/mol/A was reached using the Merck molecular force field (MMFF94), all minimizations were carried out with MOE, and partial charges were computed automatically. The positions were established employing the Alpha Triangle placement, which generates positions through a random arrangement of ligand atom triplets superposed on alpha sphere dummies within the receptor

Online ISSN: 3009-7800
Print ISSN: 3009-6324

site. The London ΔG scoring function calculates the free energy required for the ligand to bind from a specific position [23]. The results were improved after refining the results using the MMFF94 force field. The output database's dock file, the last stage score that has not been set to zero, was created using various ligand poses and sorted using the S function [23,24].

2.3. Assay for Cell Viability and Proliferation

Using the 3-(4,5-Dimethylthiazol-2-yl)2, 5-diphenyltetrazolium bromide (MTT) assay, the antiproliferative effect and median inhibitory concentration (IC50) of arbutin, the conventional chemotherapeutic drug, were evaluated on immortalized human colorectal adenocarcinoma cells (Caco-2) cell line [25]. The study was approved by the ethics committee of Sinai University (SU-REC) and according to the Declaration of Helsinki for human subject researchers (2013) and ICH GCP guidelines.

2.3.1. Cell Culture

The VACSERA Cell Culture Department supplemented the Caco-2 cell line. The RPMI-1640 medium, containing 10% FBS, sodium bicarbonate (25 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), and HEPES (20 mM) was used to cultivate Caco-2.

2.3.2. Cytotoxicity

In brief, cells were seeded in 96-well plates at a concentration of 5x104 cells/well (200 µl) and incubated for 24 h at 37°C. The following day, cells were subjected to four repetitions of each of the following doses of arbutin (Sigma, Cat #4256): 10, 20, 30, 40, 50, and 60 µg/mL. The plates were incubated for 48 hours at 37°C, [9,20]. Minimal quantities of DMSO were utilized as a drug solvent. Moreover, 1% DMSO was applied to the Caco-2 cell line as a control group in this study. As an additional negative control group, untreated cells were employed. Following the incubation period, 10 µL of MTT dye solution (Promega, USA) was added to each well, and Dulbecco's Modified Eagle Medium (DMEM) high glucose and 10%FBS medium were aspirated. After the dye solution was removed (after 4 hours of incubation at 37°C), 100 microliters of DMSO were added to each well, and the absorbance was assessed by employing a 96-well plate reader that operated at 560 and 750 nm wavelengths. The following formula was used to calculate the cell viability values:

$$cell\ viability = \frac{A\ sample}{A\ control} \times 100 \quad (1)$$

The logarithmic trend line of the cytotoxicity graph created by the GraphPad PRISM® 8.0 software was used to calculate the IC50 values [24].

2.4. Gene Expression

Additionally, in both treated and untreated Caco-2 cells with IC50, the expression of proapoptotic genes such as p53, TNF-α, NF-κB, and Caspase-3 was evaluated by qRT-PCR. Doses of both 5FU and arbutin were used after an incubation period of 48h to investigate the anti-inflammatory and apoptotic effects. RNA was extracted utilizing the RNeasy Plus Minikit from both arbutin and 5FU-treated cells. RevertAid™ H Minus Reverse Transcriptase used for complementary DNA (cDNA) synthesis. The study uses p53 sequence forward, 5'-CCTCAGCATCTTATCCGAGTGG-3' and reverse, 5'-TGGATGGTGGTACAGTCAGAGC-3', Caspase-3 sequence forward 5'-GGAAGCGAATCAATGGACTCTGG-3' and reverse 5'-GCATCGACATCTGTACCAGACC-3', TNF-α sequence forward 5'- 5-

Online ISSN: 3009-7800
Print ISSN: 3009-6324

CTTCAGGGATATGTGATGGACTC-3-3' and reverse 5'- GGAGACCTCTGGGGAGATGT -3', NF-κB sequence forward 5'- 5- CGCAAAAGGACCTACGAGAC-3-3' and reverse 5'- TGGGGGAAAACTCATCAAAG -3', and the reference gene, GAPDH the sequence, forward, 5'-GTCTCCTCTGACTTCAACAGCG-3' and reverse 5'-ACCACCCTGTTGCTGTAGCCAA-3'. The sequence qRT-PCR was performed utilizing the BioRad SYBR green PCR Master Mix kit in which the Rotor-Gene 6000 instrument was employed. The typical thermal profile is implemented at 95°C for 4 min, followed by 40 cycles of 94°C for 60 s and 55°C for 60 s. The fold differences in gene expression between the control and treatment groups were calculated using $2^{-\Delta\Delta Ct}$ [23,26].

2.5. Statistical Analysis

The data were reported as mean ± SD. A one-way analysis of variance (ANOVA) was used to compare several variables in the statistical package software (SPSS version 20). Duncan's test was utilized to compare the two groups statistically. Statistical significance was considered as a $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. In Silico Studies

Many factors influence a molecule's behavior in live organisms, such as bioactivity, transport characteristics, protein interactions, and reactivity. The SwissADME online tool has been updated to include the physicochemical parameters of arbutin [17]. The additive XLogP3 method [27] was used to predict several physicochemical parameters, including heavy atom count, H-bond donors, acceptors, rotatable bond count and the fraction of carbon bond saturation (Csp3); alternatively, topological polar surface area (TPSA), molar refractivity, parameter LogS (Silicos-IT), water solubility (S), and lipophilicity parameter LogP; or the number of sp3 hybridization carbons/total carbon count. The pharmacokinetic parameters include brain penetration (BBB permeant), skin permeation (Log Kp), P-glycoprotein substrate (P-gp substrate), and gastrointestinal absorption (GI absorption) (Table 1).

Table 1: Predicted physicochemical variables .for Arbutin

Compound	**Arbutin**
Heavy atoms	19
H-bond acceptors	7
H-bond donors	5
Rotatable bonds	3
Solubility LogS (Silicos-IT) (water)	0.36
Fraction Csp3	0.5
Molar refractivity	62.61
XLogP3	-1.35
TPSA(Å^2)	119.61
Log Kp(skin permeation) (cm/s)	-8.92
GI absorption	High
BBB permeant	No
P-gp substrate	No

Employing the Brain or Intestinal calculated Permeation method (BOILED-Egg model), we computed and evaluated the levels of GI, BBB permeant, and P-glycoprotein substrate It is quite likely that the white zone will be passively absorbed through the G [28]I

Online ISSN: 3009-7800
Print ISSN: 3009-6324

tract, while the yellow zone (yolk) will likely penetrate the brain. One does not have to exclude the white and yolk areas from each other.

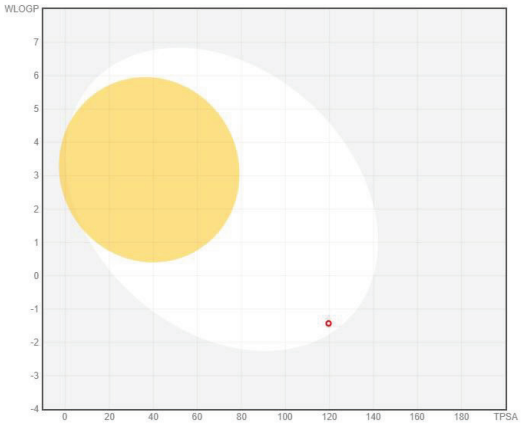
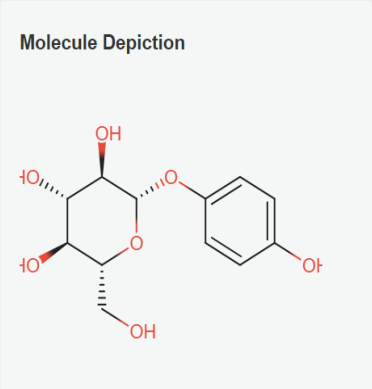


Fig. 2: Boiled-EGG Model for the Arbutin.

This model includes two parameters: (1) the lipophilicity of the substances under study as determined by the Wildman-Crippen method and expressed as a partition coefficient (P) (WLogP) [29]; and (2) the polarity of the compounds, which is represented by TPSA value. This model predicts that points in BOILED-white eggs will passively absorb via the GI tract, while points in BOILED-yolk eggs will passively flow through the BBB. Arbutin is highly absorbed by the GIT and passively permeable across the BBB, according to the BOILED-Egg model (Fig. 2). Veber's rule-based approach was utilized to analyze Arbutin. It was observed that Arbutin with a polar surface area of 140 Å2 or less and ten or fewer rotatable bonds had a greater chance of having good bioavailability. [30,31]. Arbutin that did not show any violations is indicated in Table 1, suggesting that they are suitable therapeutic candidates for studies into their bioactivity. Pharmacokinetics from pkCSM [32], P-glycoprotein was expected to bind to arbutin as a substrate (Fig. 3).



Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-1.173	Numeric (log mol/L)
Absorption	Caco2 permeability	0.125	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	42.175	Numeric (% Absorbed)
Absorption	Skin Permeability	-2.743	Numeric (log Kp)
Absorption	P-glycoprotein substrate	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	No	Categorical (Yes/No)

Fig. 3: Arbutin Expected P-Glycoprotein Substrates using the pkCSM website Tool.

The Swiss Target Prediction online tool allows us to predict the Arbutin's ideal target, with results displayed in Fig. 4, which may include enzyme and lyase receptors, with a prospect of 26.7%, a hydrolysis, and family A G protein-coupled with 13.3%. Other receptors were protease, electrochemical transporter, oxidoreductase, lyase, and ligand-gated ion channel protein, each with 6.7%. The Arbutin Zn-D1 has different targets, indicating higher receptor inhibitory action.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

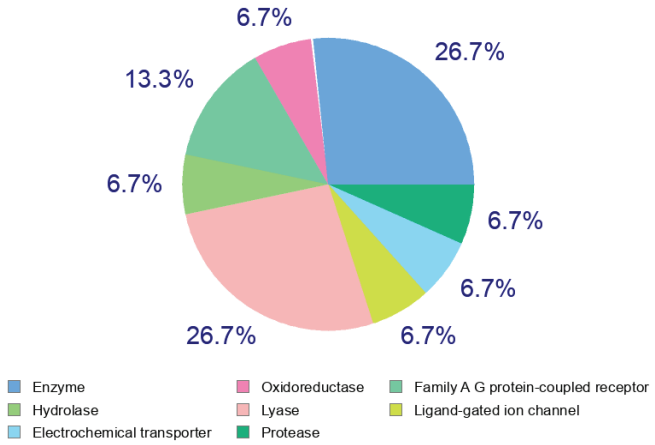


Fig. 4: The Target Prediction of Arbutin.

3.2. The Surface Properties of Arbutin

The best qualities that are crucial for producing drugs are associated with Arbutin's surface features. The interaction between Living and the Arbutin revealed the presence of an active lone pair and a lipophilic characteristic. Three hues represented the active lone pair map: blue for mild polarity, green for hydrophobicity, and violet for H-bonding. Three places were the focus of the H-bonding capacity for Arbutin. The lipophilic map was displayed using three colors: green represents lipophilia, white indicates neutrality, and violet indicates hydrophilia [33,34]. The surface properties of Arbutin are shown in Fig. 5.

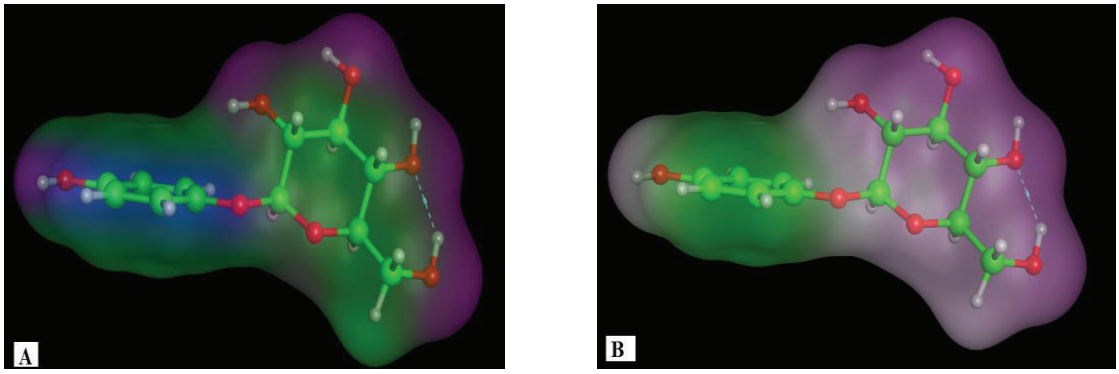


Fig. 5: The Surface Properties of Arbutin (A) Active lone Pair Map, (B) Hydrophilic and Lipophilic Map.

3.3. Molecular Docking

The Arbutin had strong interactions with the target protein, Oxygen (O 22) and Nitrogen (O 26), which formed two hydrogen bonds with the Caspase-3 protein through the Glycine (GLY 122) and Histidine (HIS 121) amino acids. The binding energy of Arbutin was found to be - 5.09 kcal/mol (Fig. 6 A, B, and Table 2). The docking of Arbutin with TNF-α protein showed three hydrogen bonds: O18 with GLU 135, O22 with ILE 136, O34 with LEU 26, and one hydrophobic bond: 6-ring with GLU 135 amino acid of the target protein (Fig. 6 C, D, and Table 2). The docking of Arbutin with TNF-α protein showed three hydrogen bonds: O18 with GLU 135, O22 with ILE 136, O34 with LEU 26, and one hydrophobic bond: 6-ring with GLU 135 amino acid of the target protein (Fig. 6 C, D, and Table 2). The Validation shows

Online ISSN: 3009-7800
Print ISSN: 3009-6324

Arbutin (-5.02 kcal/mol, 1.35 Å RMSD) and TQ8 (-5.39 kcal/mol, 0.95 Å RMSD) bind moderately to Caspase-3 (Table 3). TQ8 demonstratesslightly better alignment, while both ligands Arbutin exhibited strong interactions with the target .indicate potential as inhibitors protein, forming two hydrogen bonds, but TQ8 displayed one hydrogen bond, indicating moderate stability with slight alignment superiority for TQ8. The binding energy of Arbutin with TNF-α protein was -5.59 kcal/mol. Arbutin's interaction sites and total binding energy with different proteins showed a better inhibitory effect on Caspase-3 and TNF-α proteins.

Table 2: The interaction parameters of Arbutin with Caspase-3 and B- TNF-α.

Protein	Ligand sites	Receptor sites	Type of the interaction	Distance of bond (Å)	Binding energy (kcal/mol)	Total free binding energy (kcal/mol)
Caspase-3 (PDB code: 2XYG)	O (22)	GLY 122	Side chain acceptor (H-Acceptor)	2.91	-1.2	-5.09
	O (26)	HIS 121	Backbone donor (H-donor)	2.91	-1.5	
TNF-α (PDB code: 1TNF).	O (18)	GLU 135	Side chain donor (H-donor)	3.06	-1.7	-5.59
	O (22)	ILE 136	Backbone acceptor (H-Acceptor)	3.15	-2.0	
	O (34)	LEU 26	Backbone acceptor (H-Acceptor)	3.09	-1.7	
	6-ring	GLU 135	Arene-H (H-pi)	4.23	-0.8	

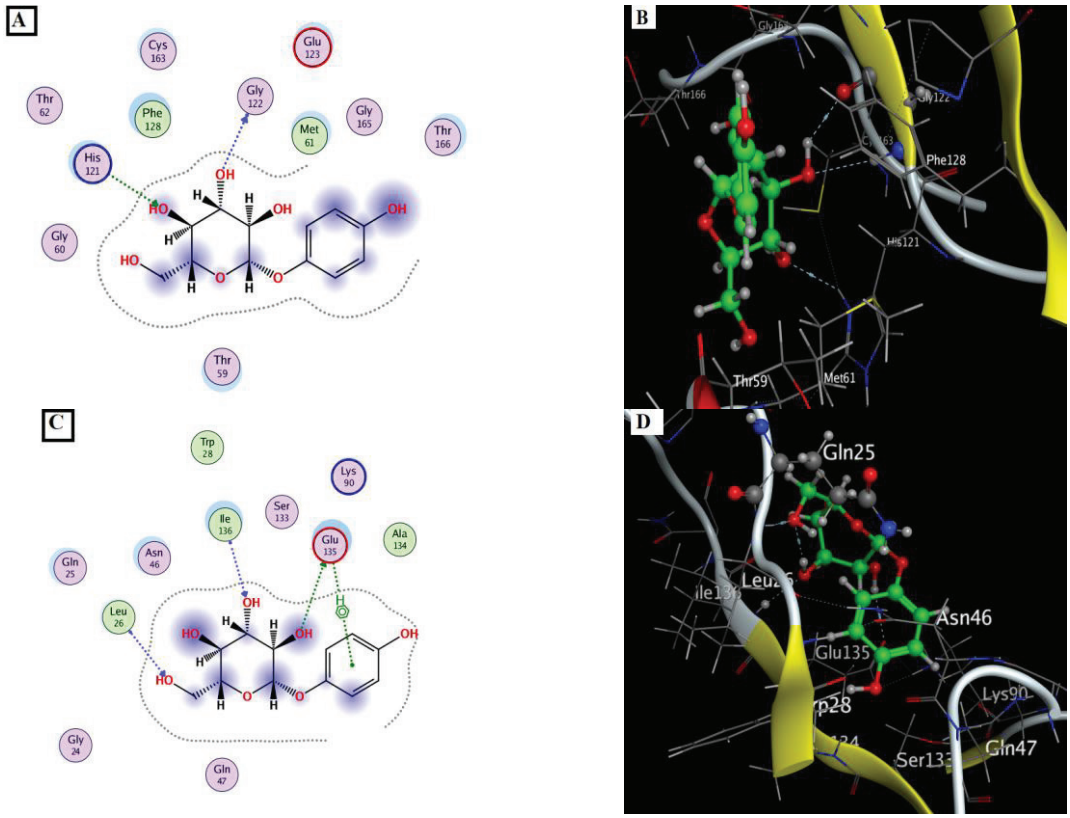


Fig. 6: 2D and 3D Diagram of Docking Arbutin with (A, B) Caspase-3, and (C,D) TNF-α.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

Table 3: The Validation Parameters of Arbutin and Co-Crystallized Ligand (TQ8) with Caspase-3.

Ligand	Ligand sites	Receptor sites	Type of the interaction	Distance of bond (Å)	Binding energy (kcal/mol)	rmsd	Total free binding energy (kcal/mol)
Arbutin	O (22)	GLY 122	Side chain acceptor (H-Acceptor)	2.91	-1.2	1.35	-5.09
	O (26)	HIS 121	Backbone donor (H-donor)	2.91	-1.5		
TQ8	O (14)	Arg 64	Side chain acceptor (H-Acceptor)	2.93	-2.8	0.95	-5.39

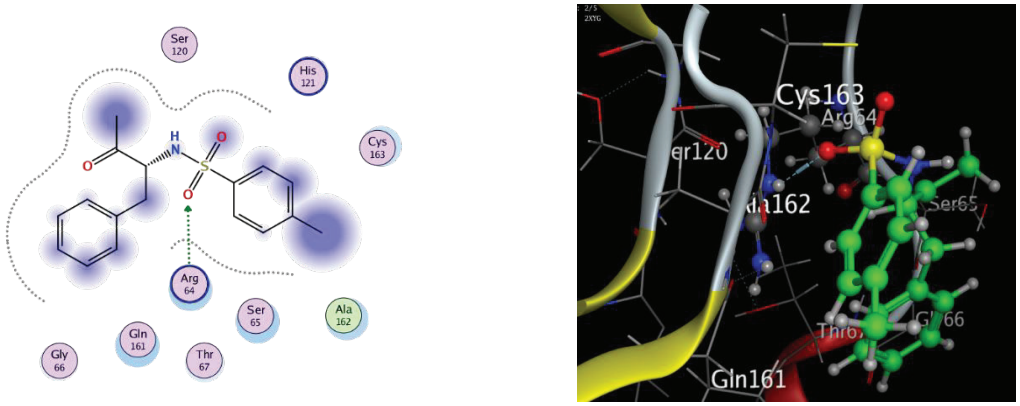


Fig. 7: 2D and 3D diagram of docking TQ8 with Caspase-3.

Table 4: The cytotoxic effect of the arbutin against Caco-2 cells

	OD1	OD2	OD3	Mean O.D	±SE	Viability %	Toxicity %	IC_{50} (ug/ml)
0	0.752	0.755	0.761	0.756	0.005	100.000	0.000	29.05± 0.57
10	0.025	0.024	0.026	0.025	0.001	3.307	96.693	
20	0.029	0.033	0.025	0.029	0.004	3.836	96.164	
30	0.036	0.031	0.029	0.032	0.004	4.233	95.767	
40	0.11	0.128	0.107	0.115	0.011	15.212	84.788	
50	0.31	0.3	0.32	0.310	0.010	41.005	58.995	
60	0.55	0.56	0.51	0.54	0.026	71.429	28.571	

3.4. The Cell Viability and Proliferation Assay

IC50 values were determined using an MTT colorimetric screening test against the most susceptible colon cancer cell line, Caco-2, about the reference medication, 5-Fluorouracil (5-FU), to investigate arbutin's possible toxicity and selectivity. In both the adjuvant and palliative stages of CRC, 5-FU is a significant component of the systemic chemotherapy regimen. 5FU was the best choice here, as it is an approved chemotherapeutic drug against colon cancer and other various types of solid tumors [35]. The MTT experiment revealed that the IC50 values of 5-FU on Caco-2 cells were 34.65 µg/ml [36]. The cytotoxic activity of arbutin was screened against the colon cancer (Caco-2) cell line at varying concentrations)10 ,20 ,30 ,40 ,50 ,and µg/mL) for 48 h usin 60g an MTT assay. The findings, as indicated in Table 4 and Fig. 8, showed the cytotoxic effect of arbutin against Caco-2 cells, with a significant potential impact of arbutin with an IC50 of 29.05 µg/ml.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

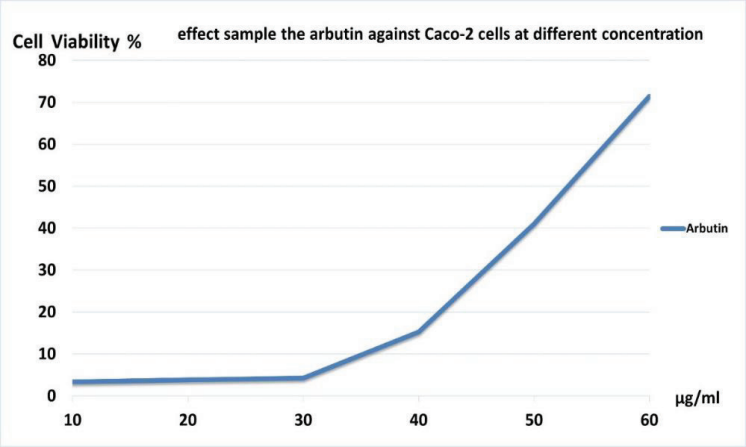


Fig. 8: The cytotoxic Effect of Arbutin Against the Caco-2 cell Line.

According to Kamei *et al*., arbutin exhibited cytotoxicity towards the quasi-diploid human HCT-15 cell line obtained from the large intestine of a male patient with CRC. Before counting the number of cells, culture cells were subjected to a four-day incubation period in an incubator with 5% CO2 and varied doses of this hydroquinone glycoside of less than 12.5 micrograms/ml in the 50% suppression dose [37]. Even though colon cancer is prevalent in adults over 60, no additional information regarding the cytotoxic effect of arbutin against different CRC cell lines has been released in previous investigations since this initial cytotoxicity result [6]. This study is the first one to assess arbutin's cytotoxic effects on the Caco-2 cell line.

3.5. Gene Expression Analysis

There were statistically significant differences regarding p53, Caspase-3, TNF-α, and NF-κB between control and experimental groups (Control, 5FU, and Arbutin) ($p<0.05$) (Fig. 9). The most altered gene in human tumors is p53. Additionally, p53 is deactivated or suppressed in a significant percentage of tumors carrying wild-type p53 via a variety of methods, such as aberrant degradation or dysregulation of activators, effectors, or repressors, which results in the majority of malignancies, if not all of them, evading the p53 signaling pathway [23].

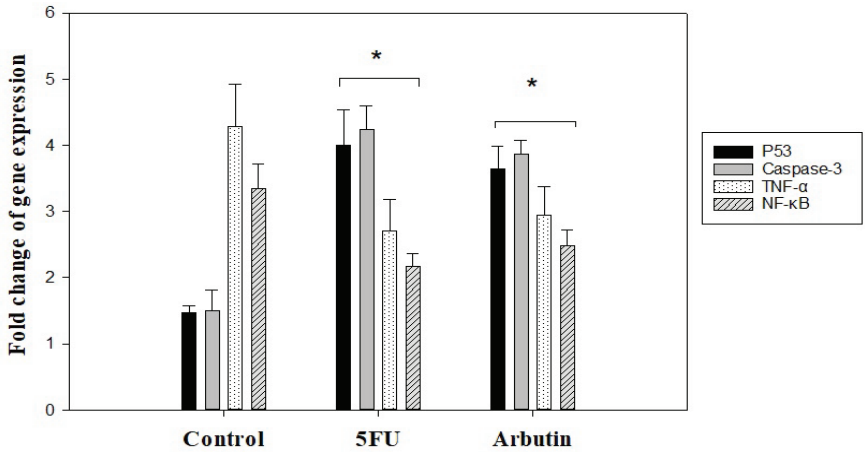


Fig. 9: The Gene Expression in Caco-2. Expression of P53, Caspase-3, TNF-α, and NF-κB were Evaluated using qRT-PCR, Values are Mean ± SD.*, Significance Against Control.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

P53 is triggered in normal cells by various stress-induced stimuli, specifically DNA damage. It regulates a vast network of target genes, mostly through which it suppresses tumors [38,39]. Many biological processes are influenced by p53, including hormone-induced activities, transcription, autophagy, inflammatory response, differentiation, cell cycle control, immunological response, metabolism, senescence and epigenome [40]. From the previous data, our findings suggested that there is an antiproliferative effect of arbutin against colon cancer. There were non-significant differences between 5FU and arbutin regarding p53, Caspase-3, TNF-α, and NF-κB ($p=0.0001$, 0.0001, and 0.028, respectively). These findings assumed that arbutin has an approximately similar effect to 5FU, a significant anticancer drug.

Additionally, it was demonstrated that arbutin might inactivate extracellular signal-regulated kinase (ERK), a crucial mediator of p53-dependent cell cycle arrest and a core regulator of cellular division, and time-dependently interrupt the cell cycle. By down-regulating ERK and upregulating p21, arbutin may prevent bladder cancer cells from proliferating in vitro [6]. Nevertheless, rats with induced liver cancer treated with arbutin showed improved liver enzymes, declined liver injury markers, and increased caspase-8 and p53 contents when arbutin was administered [11]. Inflammation and programmed cell death are related to the caspase family of 15 cysteine proteases. The most famous among them is Caspase-3, an apoptotic executor that cleaves many other essential proteins in the cell to cause apoptosis when it is activated by the initiator Caspase-8 or Caspase-9 [41]. Activating Caspase-3 as one-way certain anticancer treatments, including immunotherapy, radiation, and cytotoxic medications, might kill tumor cells. For this reason, several researchers employ Caspase-3 activation as a proxy sign for the effectiveness of cancer treatments [16,39]. Otherwise, Caspase-3 showed a statistically significant difference between arbutin and untreated colon cancer cell lines ($p<0.0001$). Arbutin showed a potential role in inducing apoptosis, which was proved by the upregulation of Caspase-3. Nawarak *et al*. [42] showed that arbutin is a hydroquinone-D-glucopyranoside that causes human melanoma cells to produce apoptosis genes. Hazman *et al*. [43] found that giving arbutin to HepG2 cells will increase oxidative stress, genotoxicity, inflammation, and apoptosis and reduces proliferation; this would have anticarcinogenic benefits.

In an experimental study, Zeng *et al*. [11] assessed the anticarcinogenic activity of arbutin against induced hepatocellular carcinoma in rats. Arbutin enhanced the amounts of c-JNK, TRAIL, caspase-8, and p53 while reducing the activity of liver damage marker enzymes. Tumor necrosis factor-α (TNF-α) is a cytokine that promotes inflammation and is mostly produced by tumor cells and macrophages. In colorectal cancer (CRC) tissues, increased TNF-α expression could encourage tumor development, invasion, and metastasis [44,45]. As a regulator of inflammation and the immune system, the nuclear factor-kappa B (NF-κB) signaling pathway has been correlated with the carcinogenic process [46-48]. Regarding the current findings, arbutin showed a substantial decline in inflammatory markers, which agreed with many studies [9,16]. Additionally, Yang *et al*. [10] showed that arbutin prevented the cell adhesion characteristic of C6 glioma cells and produced excessive ROS, damaging the mitochondrial membrane and causing cell death. According to a qPCR study, Arbutin boosts the genes involved in apoptosis and decreases the molecules involved in inflammation and PI3K/mTOR signaling. In their study, Zeng *et al*. assess the anticancer effect using ARB on rats with diethylnitrosamine-induced liver cancer. This revealed that arbutin also enhanced the levels of c-JNK, TRAIL, Caspase-8, and p53 and decreased the activity of liver injury marker enzymes [11], which came in line with the present findings in proposing the upregulation of the Caspase-3 and p53 and decreasing inflammatory markers. Additionally, in the current

Online ISSN: 3009-7800
Print ISSN: 3009-6324

study, arbutin attenuated the CRC-induced gene expression and the release of pro-inflammatory cytokines such as TNF-α. Furthermore, this suppression was associated with a decrease in NF-κB transcriptional activity, which induces p53 expression and in turn activates Caspase-3 and leads to apoptosis. Taken together, our data suggest an anti-inflammatory effect of arbutin on CRC cells.

The current study has limitations, such as missing cell viability data over different periods and using different types of colon cancer cell lines to screen the effect of arbutin over time on cell lines. Nevertheless, further in vivo investigations are required to explore the mechanisms underlying the impact of ARB.

4. CONCLUSION

The effects of active ingredients, such as arbutin, on various disease models, especially in cell culture media, must be ascertained. Subsequently, we assume the active ingredient under investigation may have advantageous impacts. Considering this study, it was determined that arbutin, when used as an active ingredient, may have any effect on colon cancer (Caco-2) cells. Concerning the previous findings, ARB revealed a potential ability to promote apoptosis in neoplastic cells and inhibit inflammatory cytokines. Additional studies are required to clarify the association between ARB and other cancer types and their possible mechanism/s of action. more research using models developed with experimental animals is needed, followed by clinical phase studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Tie J, Cohen JD, Lahouel K, Lo SN, Wang Y, Kosmider S, et al. Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer. N Engl J Med. 2022;386:2261–72. DOI: 10.1056/NEJMoa2200075

[2] Shang L, Wang Y, Li J, Zhou F, Xiao K, Liu Y, et al. Mechanism of Sijunzi Decoction in the treatment of colorectal cancer based on network pharmacology and experimental validation. J. Ethnopharmacol. 2023;302:115876. DOI: 10.1016/j.jep.2022.115876

[3] Wu D, Fu Z, Liu W, Zhao Y, Li W, Liu Q, et al. Bioinformatics analysis and identification of upregulated tumor suppressor genes associated with suppressing colon cancer progression by curcumin treatment. Front Pharmacol. 2023;14:1218046. DOI: 10.3389/fphar.2023.1218046

[4] Gadelmawla MHA, Nasrallah HH. Recent Biological Activity of Ferulic Acid. SISJ; 2025;2:102–14. DOI: 10.21608/sisj.2025.442506

[5] Emran TB, Shahriar A, Mahmud AR, Rahman T, Abir MH, Siddiquee MohdF-R, et al. Multidrug Resistance in Cancer: Understanding Molecular Mechanisms, Immunoprevention and Therapeutic Approaches. Front. Oncol. 2022;12. DOI: 10.3389/fonc.2022.891652

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[6] Nahar L, Al-Groshi A, Kumar A, Sarker SD. Arbutin: Occurrence in Plants, and Its Potential as an Anticancer Agent. Molecules. 2022;27:8786. DOI: 10.3390/molecules27248786
[7] Xu K-X, Xue M-G, Li Z, Ye B-C, Zhang B. Recent Progress on Feasible Strategies for Arbutin Production. Front Bioeng Biotechnol. 2022;10:914280. DOI: 10.3389/fbioe.2022.914280
[8] Shen X, Wang J, Wang J, Chen Z, Yuan Q, Yan Y. High-level De novo biosynthesis of arbutin in engineered Escherichia coli. Metabolic Engineering. 2017;42:52–8. DOI: 10.1016/j.ymben.2017.06.001
[9] Hazman Ö, Sarıova A, Bozkurt MF, Ciğerci İH. The anticarcinogen activity of β-arbutin on MCF-7 cells: Stimulation of apoptosis through estrogen receptor-α signal pathway, inflammation and genotoxicity. Mol Cell Biochem. 2021;476:349–60. DOI: 10.1007/s11010-020-03911-7
[10] Yang Z, Shi H, Chinnathambi A, Salmen SH, Alharbi SA, Veeraraghavan VP, et al. Arbutin exerts anticancer activity against rat C6 glioma cells by inducing apoptosis and inhibiting the inflammatory markers and P13/Akt/mTOR cascade. J Biochem Mol Toxicol. 2021;35:e22857. DOI: 10.1002/jbt.22857.
[11] Zeng X, Liu H, Huang Z, Dong P, Chen X. Anticancer Effect of Arbutin on Diethylnitrosamine-Induced Liver Carcinoma in Rats via the GRP and GADD Pathway. J Environ Pathol Toxicol Oncol. 2022;41:15–26. DOI: 10.1615/JEnvironPatholToxicolOncol.2021039772.
[12] Alruhaimi RS. Protective effect of arbutin against cyclophosphamide-induced oxidative stress, inflammation, and hepatotoxicity via Nrf2/HO-1 pathway in rats. Environ Sci Pollut Res Int. 2023;30:68101–10. DOI: 10.1007/s11356-023-27354-x.
[13] Zhang C, Zhu H, Jie H, Ding H, Sun H. Arbutin ameliorated ulcerative colitis of mice induced by dextran sodium sulfate (DSS). Bioengineered. 12:11707–15. DOI: 10.1080/21655979.2021.2005746.
[14] Demir EA, Mentese A, Yilmaz ZS, Alemdar NT, Demir S, Aliyazicioglu Y. Evaluation of the therapeutic effects of arbutin on cisplatin-induced ovarian toxicity in rats through endoplasmic reticulum stress and Nrf2 pathway. Reprod Biol. 2023;23:100824. DOI: 10.1016/j.repbio.2023.100824.
[15] Zhang B, Zeng M, Li B, Kan Y, Wang S, Cao B, et al. Arbutin attenuates LPS-induced acute kidney injury by inhibiting inflammation and apoptosis via the PI3K/Akt/Nrf2 pathway. Phytomedicine. 2021;82:153466. DOI: 10.1016/j.phymed.2021.153466.
[16] Arab HH, Alsufyani SE, Ashour AM, Gad AM, Elhemiely AA, Gadelmawla MHA, et al. Targeting JAK2/STAT3, NLRP3/Caspase-1, and PK2/PKR2 Pathways with Arbutin Ameliorates Lead Acetate-Induced Testicular Injury in Rats. Pharmaceuticals. 2024;17:909. DOI: 10.3390/ph17070909.
[17] Abbas AM, Nasrallah HH, Aboelmagd A, Boyd WC, Kalil H, Orabi AS. Novel Trimethoprim-Based Metal Complexes and Nanoparticle Functionalization: Synthesis, Structural Analysis, and Anticancer Properties. Inorganics; 2025;13:144. DOI: 10.3390/inorganics13050144.
[18] Abbas AM, Nasrallah HH, Aboelmagd A, Kishk SM, Boyd WC, Kalil H, et al. Design, Synthesis, Anti-Inflammatory Activity, DFT Modeling and Docking Study of New Ibuprofen Derivatives. IJMS. 2024; 25:3558. DOI: 10.3390/ijms25063558.
[19] Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucl Acids Res. 2019;47:W357–64. DOI: 10.1093/nar/gkz382.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[20] Saad SS, El-Sakka SSA, Soliman MHA, Gadelmawla MHA, Mahmoud NM. Thiazolidin-4-one derivatives as antitumor agents against (Caco-2) cell line: synthesis, characterization, in silico and in vitro studies. Phosphorus, Sulfur, Silicon Relat Elem. 2024;199:1–14. DOI: 10.1080/10426507.2024.2419634
[21] Bank RPD. RCSB PDB - 2XYG: Caspase-3:CAS329306 [Internet]. [cited 2024 Jul 29]. Available from: https://www.rcsb.org/structure/2xyg
[22] Bank RPD. RCSB PDB - 1TNF: The Structure of Tumor Necrosis Factor-Alpha At 2.6 Angstroms Resolution. Implications for Receptor Binding. 2024. Available from: https://www.rcsb.org/structure/1TNF
[23] Gadelmawla MHA, Alazzouni AS, Farag AH, Gabri MS, Hassan BN. Enhanced effects of ferulic acid against the harmful side effects of chemotherapy in colon cancer: docking and in vivo study. JOBAZ. 2022;83:28. DOI: 10.1186/s41936-022-00293-8
[24] Abbas AM, Aboelmagd A, Kishk SM, Nasrallah HH, Boyd WC, Kalil H, et al. A Novel Ibuprofen Derivative and Its Complexes: Physicochemical Characterization, DFT Modeling, Docking, In Vitro Anti-Inflammatory Studies, and DNA Interaction. Molecules. 2022;27:7540. DOI: 10.3390/molecules27217540
[25] Abbass HS, Ragab E, Mohammed A, El-Hela A, Elsaady MT, Elaraby A. In silico target fishing, molecular docking and in vitro cytotoxicity evaluation of a new isolated compound Sprague anone from Ficus sprageuana. SISJ. 2025;2:5–20. DOI: 10.21608/sisj.2024.319771.1024
[26] Gaafar SS, El Mekkawi ARO, Farag RA, Gadelmawla MHA, Hussein AMHM, Sayed M, et al. Comparative analysis of the inflammatory response of human gingival fibroblasts to NeoSEALER Flo and CeraSeal bioceramic sealers: an in vitro study. BMC Oral Health. 2025;25:395. DOI: 10.1186/s12903-025-05692-1
[27] Cheng T, Zhao Y, Li X, Lin F, Xu Y, Zhang X, et al. Computation of Octanol−Water Partition Coefficients by Guiding an Additive Model with Knowledge. J Chem Inf Model. 2007; 47:2140–8. DOI: 10.1021/ci700257y
[28] Daina A, Zoete V. A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. ChemMedChem. 2016; 11:1117–21. DOI: 10.1002/cmdc.201600182
[29] Wildman SA, Crippen GM. Prediction of Physicochemical Parameters by Atomic Contributions. J Chem Inf Comput Sci. 1999; 39:868–73. DOI: 10.1021/ci990307l
[30] Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. J Med Chem. 2002; 45:2615–23. DOI: 10.1021/jm020017n
[31] Brito MA de. Pharmacokinetic study with computational tools in the medicinal chemistry course. Braz J Pharm Sci. 2011; 47:797–805. DOI: 10.1590/S1984-82502011000400017
[32] Pires DEV, Blundell TL, Ascher DB. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. J Med Chem. 2015; 58:4066–72. DOI: 10.1021/acs.jmedchem.5b00104
[33] Hisaindee S, Al-Kaabi L, Ajeb S, Torky Y, Iratni R, Saleh N, et al. Antipathogenic effects of structurally-related Schiff base derivatives: Structure–activity relationship. Arab. J. Chem. 2015; 8:828–36. DOI: 10.1016/j.arabjc.2013.03.013.
[34] Tyagi P, Tyagi M, Agrawal S, Chandra S, Ojha H, Pathak M. Synthesis, characterization of 1,2,4-triazole Schiff base derived 3d- metal complexes: Induces cytotoxicity in HepG2, MCF-7 cell line, BSA binding fluorescence and DFT study. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.

Online ISSN: 3009-7800
Print ISSN: 3009-6324

2017;171:246–57. DOI: 10.1016/j.saa.2016.08.008
[35] Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P, Vymetalkova V. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. Pharmacol Ther. 2020;206:107447. DOI: 10.1016/j.pharmthera.2019.107447
[36] Othman MS, Al-Bagawi AH, Obeidat ST, Fareid MA, Habotta OA, Moneim AEA. Antitumor Activity of Zinc Nanoparticles Synthesized with Berberine on Human EpithelialColorectal Adenocarcinoma (Caco-2) Cells through Acting on Cox-2/NF-kBand p53 Pathways. ACAMC. 2022; 22:2002–10. DOI: 10.2174/1871520621666211004115839
[37] Kamei H, Koide T, Kojima T, Hashimoto Y, Hasegawa M. Inhibition of cell growth in culture by quinones. Cancer Biother Radiopharm. 1998;13:185–8. DOI: 10.1089/cbr.1998.13.185
[38] Alazzouni AS, Dkhil MA, Gadelmawla MHA, Gabri MS, Farag AH, Hassan BN. Ferulic acid as anticarcinogenic agent against 1,2-dimethylhydrazine induced colon cancer in rats. J. King Saud Univ. Sci. 2021;101354. DOI: 10.1016/j.jksus.2021.101354
[39] Abdel-Wahhab KG, Ashry M, Hassan LK, Gadelmawla MHA, Elqattan GM, El-Fakharany EM, et al. Nano-chitosan/bovine lactoperoxidase and lactoferrin formulation modulates the hepatic deterioration induced by 7,12-dimethylbenz[a]anthracene. Comp Clin Pathol. 2023;32,:981–991 DOI: 10.1007/s00580-023-03510-0
[40] Hassan BN, Azzuni ASE, Abdelfattah MS, Elgabri MS, Ahmed AS, Abdo SM. Anticancer Effect of Actinomycetes Secondary Metabolite Against Breast Cancer Cell Line (MCF-7); Cytological and Molecular Studies. Pharmacophore. 2023;14:23–34. DOI: 10.51847/jMXpFOxF3o
[41] Elhemiely AA, El-Fayoumi SH, Gadelmawla MHA, Mahran NA, Gad AM. Hesperidin Reduces Hepatic Injury Induced by Doxorubicin in Rat Model Through Its Antioxidative and Anti-Inflammatory Effects, Focusing on SIRT-1/NRF-2 Pathways. J Biochem Mol Toxicol. 2025;39:e70465. DOI: 10.1002/jbt.70465
[42] Nawarak J, Huang-Liu R, Kao S-H, Liao H-H, Sinchaikul S, Chen S-T, et al. Proteomics analysis of A375 human malignant melanoma cells in response to arbutin treatment. Biochim Biophys Acta. 2009;1794:159–67. DOI: 10.1016/j.bbapap.2008.09.023
[43] Hazman Ö, Evin H, Bozkurt MF, Ciğerci İH. Two faces of arbutin in hepatocellular carcinoma (HepG2) cells: Anticarcinogenic effect in high concentration and protective effect against cisplatin toxicity through its antioxidant and anti-inflammatory activity in low concentration. Biologia. 2022;77:225–39. DOI: 10.1007/s11756-021-00921-8
[44] Nasrallah HH, Orabi AS, Abbas AM, Aboelmagd A. Trimethoprim drug derivatives: A review of synthesis, metal complexes, nanoparticles, and biological activity. Frontiers in Scientific Research and Technology [Internet]. 2025 [cited 2025 Oct 13]; DOI: 10.21608/fsrt.2025.353037.1147
[45] Abdel Salam HM, Soliman SME, Gadelmawla MHA, Mahmoud Ashry MA. The Antioxidant and Anti-inflammatory Effects of Lactoferrin Nanoparticles on the Aflatoxin B1-induced Hepatotoxicity in Male Rats. Iran J Toxicol. 2023;17:42–9. DOI: 10.61186/IJT.17.4.42
[46] Ghobar S, Gadelmawla MHA, Shaheen SD. Comparative In-Vitro Study Of Sound And Carious Primary And Permanent Enamel Content Of Selected Trace Elements. AFJBS. 2024;6:1346–52. DOI: 10.33472/AFJBS.6.2.2024.1346-1352

Online ISSN: 3009-7800
Print ISSN: 3009-6324

[47] Abdel-Wahhab KG, Ashry M, Hassan LK, El-Azma MH, Elqattan GM, Gadelmawla MHA, et al. Hepatic and immune modulatory effectiveness of lactoferrin loaded Selenium nanoparticles on bleomycin-induced hepatic injury. Sci Rep. 2024;14:21066. DOI: 10.1038/s41598-024-70894-6.

[48] Slattery ML, Mullany LE, Sakoda L, Samowitz WS, Wolff RK, Stevens JR, et al. The NF-κB signalling pathway in colorectal cancer: associations between dysregulated gene and miRNA expression. J Cancer Res Clin Oncol. 2018;144:269–83. DOI: 10.1007/s00432-017-2548-6.

Online ISSN: 3009-7800
Print ISSN: 3009-6324