Advances and Applications in Bioinformatics and Chemistry Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Toward Understanding the Anticancer Activity of the Phytocompounds from *Eugenia uniflora* Using Molecular Docking, in silico Toxicity and Dynamics Studies

Pallab Kar[1,2], Ayodeji O Oriola[2], Adebola O Oyedeji[1,2]

[1]African Medicinal Flora and Fauna Research Niche, Walter Sisulu University, Mthatha, 5117, South Africa; [2]Department of Chemical and Physical Sciences, Walter Sisulu University, Mthatha, 5117, South Africa

Correspondence: Pallab Kar; Adebola O Oyedeji, Email pallabkar.bio@gmail.com; aoyedeji@wsu.ac.za

Background: The Surinam cherry, *Eugenia uniflora* belongs to the family Myrtaceae, an edible fruit-bearing medicinal plant with various biological properties. Several anticancer studies have been conducted on its essential oils while the non-essential oil compounds including phenolics, flavonoids, and carotenoids have not been fully investigated.

Purpose: Therefore, the study evaluated the in silico anticancer potentials of phenolic, flavonoid, and carotenoid compounds of *E. uniflora* against the MDM2 and Bcl-xL proteins, which are known to promote cancer cell growth and malignancy. The physicochemical parameters, validation, cytotoxicity, and mutagenicity of the polyphenols were determined using the SwissADME, pkCSM, ProTox-II, and vNN-ADMET online servers respectively. Lastly, the promising phytocompounds were validated using molecular dynamics (MD) simulation.

Results: An extensive literature search resulted in the compilation of forty-four (44) polyphenols from *E. uniflora*. Top-rank among the screened polyphenols is galloylastragalin, which exhibited a binding energy score of −8.7 and −8.5 kcal/mol with the hydrophobic interactions (Ala93, Val141) and (Leu54, Val93, Ile99), as well as hydrogen bond interactions (Tyr195) and (Gln72) of the proteins Bcl-xL and MDM2 respectively. A complete in silico toxicity assessment revealed that the compounds, galloylastragalin, followed by myricetin, resveratrol, *p*-Coumaroylquinic acid, and cyanidin-3-O-glucoside, were potentially non-mutagenic, non-carcinogenic, non-cytotoxic, and non-hepatotoxic. During the 120 ns MD simulations, the RMSF analysis of galloylastragalin- MDM2 (complex 1) and galloylastragalin- Bcl-xL (complex 2) showed the fewest fluctuations, indicating the conformational stability of the respective complexes.

Conclusion: This study has shown that polyphenol compounds of *E. uniflora* led by galloylastragalin, are potent inhibitors of the MDM2 and Bcl-xL cancer proteins. Thus, they may be considered as candidate polyphenols for further anticancer studies.

Keywords: *Eugenia uniflora*, anticancer, in silico molecular docking, in silico toxicity, molecular dynamics simulation, polyphenols, galloylastragalin

Introduction

Cancer is one of the world's deadliest diseases, with an estimated 9.9 million deaths in 2020 due to the uncontrolled generation of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS).[1] According to reports, cancer is a multifaceted process that manifests as distinct features at the cellular, tissue, and organismic levels.[2] According to Valko et al,[3] ROS can cause base alterations, deletions, and strand breaks that lead to unstable genomes, chromosomal rearrangements, and hyper and hypo-methylation of DNA. A mutant cell may develop into cancer if damaged DNA is not repaired, giving it the ability to live and divide abnormally.[4] As a result, DNA damage has an oxidative character and is thought to be a major factor in the carcinogenesis promotion stage. In addition, the mutant or transformed cells exhibit changed amounts of cell cycle signaling molecules and apoptosis, which leads to unchecked cell growth and tumor

Received: 15 April 2024
Accepted: 22 August 2024
Published: 20 September 2024

development.[5] Six novel systemic hallmarks of cancer cells are the basic tumor metastasis connection, global inflammation, immune suppression, cachexia-causing metabolic changes, thrombosis propensity and neuroendocrine abnormalities.[2] Cancer cells are unable to negatively regulate the cell cycle, which results in uncontrollably continuous proliferation. They may also overcome the apoptotic response through a variety of methods, which reduces the attrition of cells.[6,7] One important regulatory process by which cells die if DNA damage is not properly repaired is called apoptosis, or programmed cell death.[8] Activating apoptotic pathways and negatively regulating the progression of the cell cycle in cancer cells are therefore thought to be the most significant therapeutic approaches for the treatment of cancer, as these processes are fundamentally involved in neoplastic transformation and metastases.[9] The potential of antioxidants, primarily derived from natural sources, to induce the regression of premalignant lesions or prevent their progression into cancer is a topic of extensive research.[10] Consequently, increasing the consumption of antioxidants which have the ability to scavenge free radicals—may be a safer and more effective way to stop cancer cell initiation, a crucial first step in the development of cancer.[4]

Plants are abundant in polyphenols, which are secondary metabolites with redox and antioxidant properties. Reactive oxygen and nitrogen species (ROS and RNS), which are essential for various physiological activities such as gene expression, growth, and infection defense, are combated by polyphenols. ROS detoxification is essential because excessive ROS can cause oxidative stress and increase the risk of cancer. They also guard against oxidizing agents, free radicals, and fatty acids from oxidative degradation.[11] It is firmly thought that regular ingestion of phytochemicals produced from plants may tip the scales in favor of the body having an appropriate level of antioxidants.[12] *Eugenia uniflora* is a member of the Myrtaceae family and is edible. It bears a conical-shaped shrub or small tree. The fruit is high in flavonoids, carotenoids, phenolic compounds, vitamin C, and a variety of minerals, including calcium, iron, magnesium, and phosphorus.[13] There are a number of pharmacological actions associated with the fruit. The fruit essential oils contain characteristics that are antidiabetic, anticancer, analgesic, antifungal, antihypertensive, and antitumor.[14–19] As previously mentioned, *Eugenia uniflora* is a rich source of bioactive chemicals with a range of pharmacological properties. It was interesting to note that several in vitro anticancer studies have been conducted with *Eugenia uniflora* essential oil.[20–22] However, there is no evidence to support the use of a molecular docking approach to investigate the anticancer properties of *Eugenia uniflora* polyphenols. An extensive literature survey resulted forty-four (44) polyphenols from *E. uniflora* (Table S1) have been examined in this study in order to evaluate their potential as cancer treatments.[23–25] This inspired us to use molecular docking to ascertain the precise binding mechanisms by which polyphenols interact with the MDM2 and Bcl-xL proteins, as well as molecular dynamics-based methods to explore the anticancer characteristics.

Materials and Methods

In silico Molecular Docking

Preparation and Refinement of the Protein and Ligand Structures

The phytochemicals were obtained by screening the extensive literature survey.[23–25] Robust molecular docking research was performed on the chosen phytocompounds (Table S1) against cancer proteins that are currently considered attractive targets for future therapeutic development. The PDB structures of Bcl-xL (PDB ID 2YXJ; 2.20 Å resolution)[26] and MDM2 (PDB ID 3W69; 1.90 Å resolution)[27] were retrieved from the Protein Data Bank (http://www.rcsb.org). The associated inhibitor and water molecules were removed from the appropriate protein structures prior to analysis. To get ready for docking in AutoDockTools, polar hydrogen atoms, and Kollman charges were added to the protein structures. The concerned phytocompounds were downloaded from the NCBI PubChem (https://pubchem.ncbi.nlm.nih.gov/). The Open Babel Server was then used to transform the downloaded sdf structures into pdb structures.[28] The ligand structures underwent energy minimization using the Gromos 96 force field after the PRODRG server was used to optimize their energy.[29]

Molecular Docking

The ligands of interest were molecularly docked at the active binding sites of the relevant proteins utilizing a stiff protein receptor and a flexible ligand docking methodology through the use of a grid-based molecular docking technology.[30–32] A grid box involving the active site residues of the MDM2 protein was created, with center_x = −34.4, center_y = 29.1,

center_z = −11.1, size_x = 15.7, size_y = 18.5, and size_z = 28.9. Similarly, a grid box covering the active binding pocket of Bcl-xL was employed, with center_x= −9.8, center_y= −14.2, center_z= 10.7, size_x= 23.4, size_y= 29.3, and size_z= 16.3. After Autodock Vina finished the molecular docking procedure, the docked complexes were visualized using the Discovery Studio visualization tool.[30–32]

Assessment of Physicochemical Features and Potential Toxicity of the Phytocompounds

For additional physicochemical properties and drug-like feature evaluation, the phytocompounds having binding energy scores < −7.5 kcal/mol with the corresponding cancer proteins in AutoDock Vina were selected.[33] The SwissADME server was utilized to estimate the physicochemical parameters, and the pkCSM server was utilized for validation.[34,35] The compounds' cytotoxicity and mutagenicity (AMES mutagenesis) were evaluated using the ProTox-II and vNN-ADMET online servers.[36,37]

Molecular Dynamics Simulations

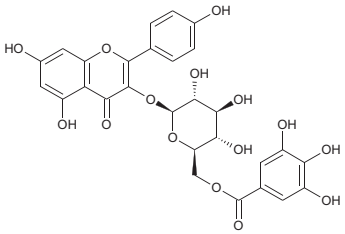
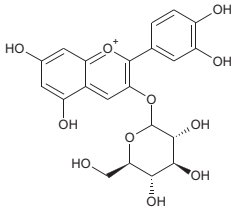
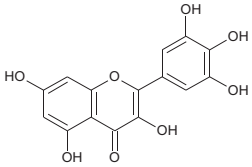
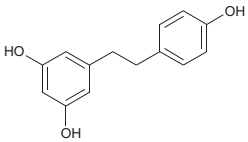
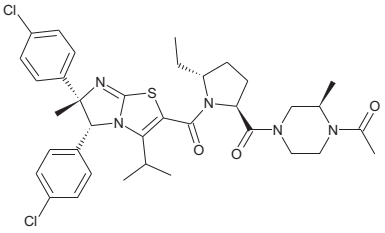
Molecular dynamics simulation (MDS) is an essential step in validating molecular docking data, as it provides an accurate assessment of the potential stability of protein-ligand complexes.[30] The compounds were subjected to MDS for 120 ns using the GROMACS (version 2019) force field parameters (GROMOS96 43a1).[30–32,38] Constant pressure and temperature (NPT) ensemble was used to set the equilibration stages.[39–41] According to Umesh et al,[41] the MD simulations were run at a standard temperature of 300 K and pressure of 1.013 bar. An estimate of the conformational and structural stability of complexes was obtained through the analysis of root-mean-square-deviation/fluctuation (RMSD/F).

Results and Discussion

Interaction of the Phytocompounds with the Cancer Proteins

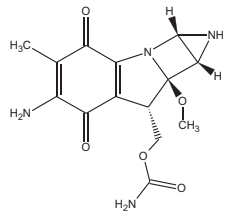
The hyperoxic and hypoxic conditions result in the production of free radicals, including superoxide anions, hydroxyl radicals, and hydrogen peroxide.[42] In addition, various free radicals like H_2O_2 are produced in cells via various pathways due to oxidative biochemical reactions. The reactive oxygen species (ROS) are extremely reactive molecules that contain an unstable oxygen species with unpaired electrons and reactive chemical properties.[42] These free radicals can alter Biomolecules such as proteins, carbohydrates, membrane lipids, DNA, and lipids, leading to eventual cellular dysfunction or death.[43] Apart from the endogenous source, external factors also contribute to ROS production, including smoking cigarettes, ionizing radiation, and hazardous gases. Consequently, the development and progression of numerous diseases are caused by chronic inflammation, which is caused by ROS and different kinds of free radicals. Inflammation is mainly caused by a variety of immunological and inflammatory response factors.[43] Pro-inflammatory cells are often a major source of multiple inflammatory mediators, including ROS and cytokines (TNF-α, IL-1, and IL-6), which contribute to the development of cancer.[43] Nowadays, medicinal plants are becoming more and more significant because of their many therapeutic uses. Several contemporary synthetic medications are also widely accessible and utilized regularly. While its benefits are undeniable, the adverse effects make it too unsafe. Conversely, plant metabolites have different structural characteristics and different biological actions with negligible or no side effects. *Eugenia uniflora* possesses a wide range of pharmacological properties that are mainly attributed to the presence of a broad range of secondary metabolites such as flavonoids, carotenoids, and phenolic compounds.[13] Forty-four (44) phytocompounds in all were tested in this investigation against the cancer proteins (MDM2 and Bcl-xL). Among the 44 phytocompounds, resveratrol, p-Coumaroylquinic acid, and cyanidin-3-O-glucoside are phenolic compounds, whereas galloylastragalin and myricetin are flavonoids, which showed potential binding affinity against MDM2 and Bcl-xL proteins (Tables 1 and 2). According to reports, resveratrol inhibits the expression of β-catenins in multiple myeloma cells, as well as the target genes c-Myc, MMP-7, and survivin. This reduces the proliferation, migration, and invasion of cancer cells.[44] Resveratrol suppressed the Wnt/β-catenin signaling system, which decreased the number of malignant cells and stopped their growth, hence decreasing breast cancer stem-like cells both in vitro and in vivo.[45] In addition to inducing cell death in glioblastoma and colon cancer, cyanidin-3-O-glucoside has been shown to lower ROS levels by controlling the Nrf2 signaling pathway.[46] Human retinal endothelial cells' ability to migrate can be inhibited by cyanidin-3-O-glucoside, and HeLa cell cycle arrest in the G1 phase can be avoided.[47] Myricetin exhibited a dose-dependent 70% reduction in human colon cancer cells (HCT-15), whereas galloylastragalin showed a strong

Table 1 Binding Energy Scores and Interaction Profile of the Phytocompounds with mdm2 Protein

Phytocompounds	Class	Chemical Structure	Binding Energy Scores (kcal/mol)	Interacting Residues
			mdm2 (3W69)	
Galloylastragalin	Flavonoid-3-o-glycoside		−8.5	*Leu54, Val93, Ile99*, **Gln72**
Cyanidin-3-O-glucoside	Anthocyanin		−7.5	*Ile61, Val93, Ile99, Gln72*, Met62*
Myricetin	Flavonol		−7.7	**Leu54, Gly58, Gln72, Val93, His96**, *Met62*
Resveratrol	Polyphenol		−7.6	*Leu54, Gly58, Ile61, Tyr67, Gln72, Val75, Phe91*, Val93#, Ile99#, His96*
(5R,6S)-2-[((2S,5R)-2-{[(3R)-4-acetyl-3-methylpiperazin-1-yl]carbonyl}-5-ethylpyrrolidin-1-yl)carbonyl]-5,6-bis(4-chlorophenyl)-3-isopropyl-6-methyl-5,6-dihydroimidazo[2,1-b][1,3]thiazole **(Inhibitor)**			−10.7	*Leu54, Ile61, Met62, Tyr67, Gln72, Val75, Val93, Ile99*, His96*

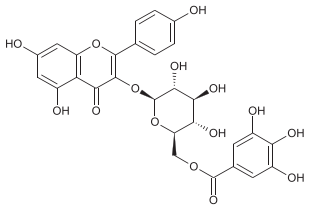
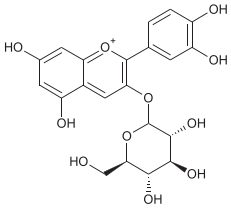
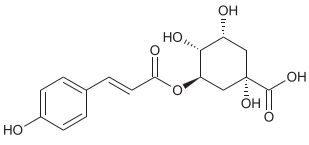
(*Continued*)

Table 1 (Continued).

Phytocompounds	Class	Chemical Structure	Binding Energy Scores (kcal/mol)	Interacting Residues
			mdm2 (3W69)	
Mitomycin C			−5.8	**Leu 54**, *Phe55*

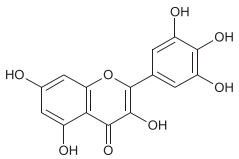
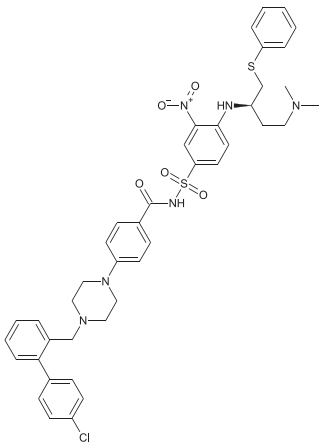
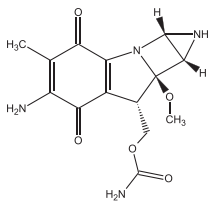
Notes: Hydrophobic interactions are marked in italics, hydrogen bonds are highlighted in bold, π-stakings are displayed with * and Pi-sigma bonds are displayed with #.

Table 2 Binding Energy Scores and Interaction Profile of the Phytocompounds with Bcl-xL Protein

Phytocompounds	Class	Chemical Structure	Binding Energy Scores (kcal/mol)	Interacting Residues
			Bcl-xL (2YXJ)	
Galloylastragalin	Flavonoid-3-o-glycoside		−8.7	*Ala93, Val141*, **Tyr195**, Tyr101*
Cyanidin-3-O-glucoside	Anthocyanin		−7.6	*Ala142*, **Glu129, Leu130, Asp133, Arg139**
p-Coumaroylquinic acid	Cinnamate ester		−7.5	*Ala104, Leu108, Arg139, Ala142*, **Phe105**, Phe97*, Tyr101

(*Continued*)

Table 2 (Continued).

Phytocompounds	Class	Chemical Structure	Binding Energy Scores (kcal/mol)	Interacting Residues
			Bcl-xL (2YXJ)	
Myricetin	Flavonol		−7.9	*Ala142*, **Leu130, Gly138, Arg139**
4-{4-[(4'-Chlorobiphenyl-2-yl)methyl] piperazin-1-yl}-n-{[4-({(1r)-3-(dimethylamino)-1-[(phenylthio) methyl]propyl}amino)-3-nitrophenyl] sulfonyl}benzamide			−12.2	*Tyr101, Ala104, Leu108, Arg139, Val141, Ala142*, **Phe97, Tyr195*, Asn197**, Leu130#
Mitomycin C			−5.8	*Ala93*, **Gly138**, Tyr195*

Notes: Hydrophobic interactions are marked in italics, hydrogen bonds are highlighted in bold, π-stakings are displayed with * and Pi-sigma bonds are displayed with #.

anticancer effect.[48–50] The MDM2 gene encodes human MDM2, also known as E3 ubiquitin-protein ligase MDM2, which degrades the p53 tumor suppressor by proteasomal means. Here, MDM2 raises the risk of cancer by acting as a negative regulator on the p53 tumor suppressor gene. Through its binding to tumor suppressors, degradation of cell-cycle inhibitors, and induction of genomic abnormalities, MDM2 stimulates both genomic instability and cell proliferation. Moreover, it binds and breaks down Rb directly, preventing Rb-E2F1 interaction.[51] Table 1 displays the binding energy scores of the phytocompounds against the mdm 2 protein (binding energy scores ≤ −7.5 kcal/mol), and Table S2, provides a summary of the binding energy values of all 44 phytocompounds. A binding energy score of −8.5 kcal/mol was seen in the interaction of galloylastragalin with the hydrophobic interactions (Leu54, Val93, Ile99) and hydrogen bonds (Gln72) of the MDM2 protein. The binding energy score of typical Mitomycin C with hydrophobic (Phe55) and hydrogen bond (Leu54) interactions was −5.8 kcal/mol. Using the proposed inhibitor (5R,6S)-2-[((2S,5R)-2-{[(3R)-4-acetyl-3-methylpiperazin-1-yl]carbonyl}-5-ethylpyrrolidin-1-yl)carbonyl]-5-(6-chlorophenyl)-bis6-methyl-5,6-dihydroimidazo-3-isopropyl-6-methyl [2,1-b][1,3] thiazole, we investigated how bonded to the

MDM2 protein and compared its binding pattern to the relevant phytochemicals.[27] The inhibitor exhibited a significant binding potential with a binding energy value of −10.7 kcal/mol. It was shown to be associated with residues Leu54, Ile61, Met62, Tyr67, Gln72, Val75, Val93, Ile99 (hydrophobic contacts), and His96 (π-stakings) at the active binding pocket of the MDM2 protein (Table 1). Interestingly, Table 1 and Figure 1A show that these active site residues are associated with galloylastragalin, a substance that has been shown to have a significant role in the interaction between MDM2 and small molecule inhibitors.[27] A defective apoptotic mechanism, which intentionally reduces the cell's advantages for survival, is the primary cause of most of these cancer cases.[52] One important protein family associated with apoptosis is the Bcl-2 family. In cancer cells, Bcl-xL inhibits the multi-domain pro-apoptotic proteins Bak and Bax that are associated with the mitochondria, which eventually promotes cell growth and malignancy.[53] Thus, it is believed that the most important therapeutic strategy for the treatment of cancer involves inhibiting the Bcl-xL protein and triggering apoptotic pathways. The detailed binding energy scores of all the concerned phytocompounds have been provided in Table 2 (compounds displaying binding energy scores ≤ −7.5 kcal/mol) and Table S3. In this investigation, the compound galloylastragalin interacts with the hydrophobic interaction (Ala93, Val141) and hydrogen bond interactions (Tyr195) of the protein Bcl-xL returning a binding energy score of −8.7 kcal/mol (Table 2 and Figure 1B). The suggested inhibitor interacts with Bcl-xL protein with Tyr101, Ala104, Leu108, Arg139, Val141, Ala142 (hydrophobic interaction) and Phe97, Tyr195, Asn197 (hydrogen bond) returning a binding energy score of −12.2 kcal/mol, whereas the standard Mitomycin C showed binding energy score of −5.8 kcal/mol as well. The active site residues Tyr101, Val141, and Tyr195 of Bcl-xL were involved in the interactions between galloylastragalin and the enzyme, which is essential for binding the putative inhibitor. The presence of a carbonyl functional group and extra trihydroxybenzoic ring in galloylastragalin unlike cyanidin-3-O-glucoside may be attributed to its highest binding affinity, as these functional groups have been reported to improve anticancer activity.[54,55]

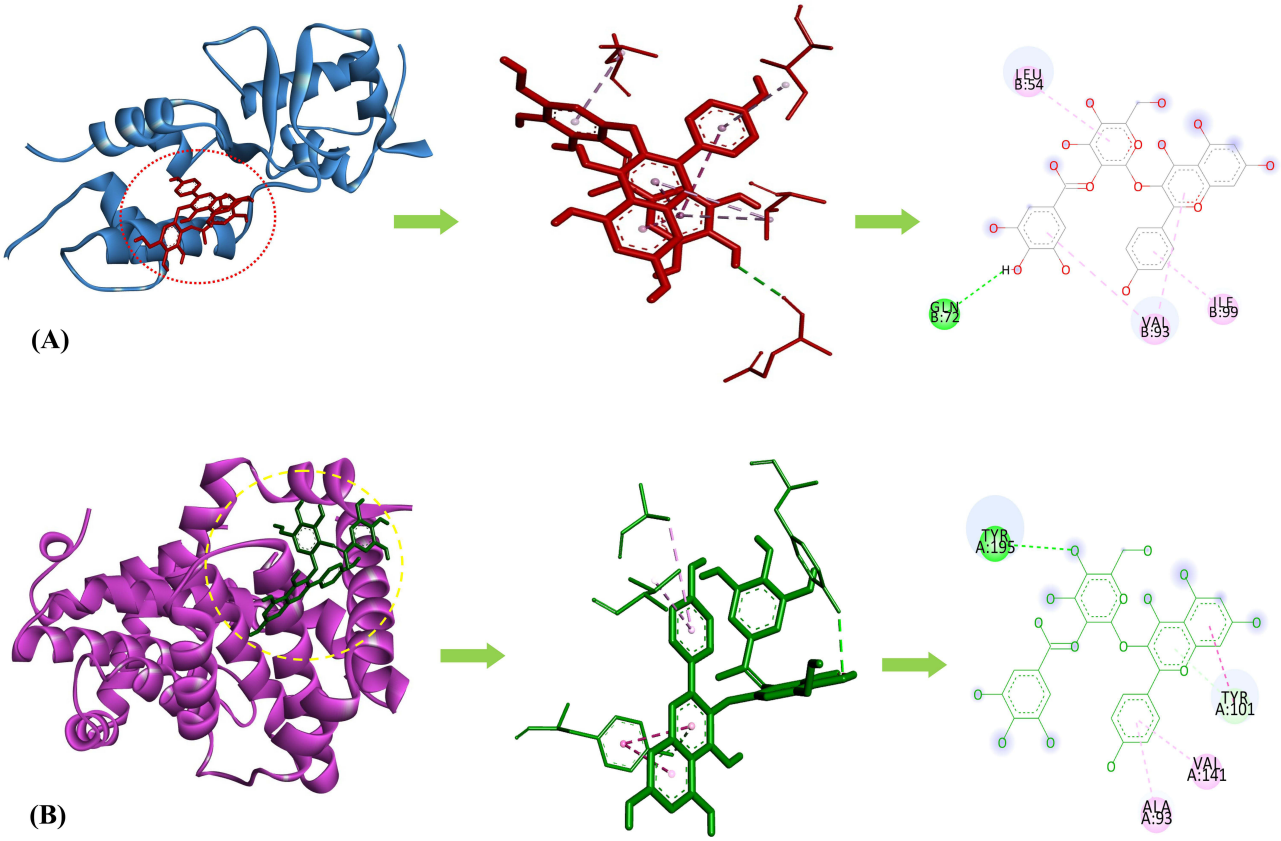


Figure 1 The mode of interaction of galloylastragalin with **(A)** mdm2 and **(B)** Bcl-xL protein. The blue and pink ribbon represents the mdm2 and Bcl-xL protein. Galloylastragalin has been illustrated as a red and green stick.

Table 3 Physicochemical Features and Toxicity Estimation of the Selected Phytocompounds

Phytocompound	Molecular weight (Dalton)	Number of Hydrogen Bond Acceptor (HBA)	Number of Hydrogen Bond Donor (HBD)	Log P	Solubility	GI Absorption	Toxicity			
							Mutagenicity (AMES Mutagenesis)	Cytotoxicity	Carcinogenicity	Hepatotoxicity
Galloylastragalin	600.48	15	9	0.737	Soluble	Low	No	No	No	No
Myricetin	318.24	8	6	1.69	Soluble	Low	No	No	No	No
Resveratrol	228.24	3	3	2.97	Soluble	High	No	No	No	No
p Coumaroylquinic acid	338.31	8	5	−0.3515	Soluble	Low	No	No	No	No
Cyanidin-3-O-glucoside	484.84	10	8	−2.614	Soluble	Low	No	No	No	No

Notes: log P- Logarithm of partial coefficient.

In silico Analysis of Physicochemical Features and Toxicity Indices

Most often, the physicochemical qualities of a substance are evaluated to estimate the pharmacokinetics of pharmacological leads.[30,33] We evaluated the physicochemical properties of the compounds in accordance with Lipinski's rule of five and Veber's rule.[56] The phytocompounds with binding energy scores of ≤ −7.5 kcal/mol with the relevant proteins were chosen for further drug-likeness evaluation (Tables 1 and 2). All compounds had slight deviations from Lipinski's rule of five like molecular weights > 500 Dalton, number of H-bond acceptors > 10, number of H-bond donors > 5, or log P values > 5 (Table 3). Under this pretext, it is pertinent to note that medications often used in the fight against cancer have apparently demonstrated slight deviations from Lipinski's rule of five (RO5).[30,57] One of the most important phases in the development of new drugs nowadays is preclinical toxicity.[34] The detrimental effects of chemical exposure that result in genetic alterations are referred to as mutagenicity.[30,33] A complete in silico toxicity assessment revealed that the compounds galloylastragalin, myricetin, resveratrol, *p*-Coumaroylquinic acid and cyanidin-3-O-glucoside were potentially non-mutagenic, non-carcinogenic, non-cytotoxic, and non-hepatotoxic (Table 3) and our results were consistent with previous reports.[58,59]

MD Simulations

Based on binding energy ratings, the substances galloylastragalin interacted with the MDM2 and Bcl-xL protein the best (Tables 1 and 2). This led to the execution of 120 ns molecular dynamics (MD) simulations of the corresponding protein-ligand complexes. The complexes including galloylastragalin - MDM2 protein complexes (complex 1), galloylastragalin - Bcl-xL protein (complex 2), had mean RMSD values of 0.79 and 0.73 Å respectively. In contrast, for the equivalent complexes, the mean RMSF values were 1.33 and 1.12 Å. In complexes 1 and 2 the RMSD values of the C atoms fluctuated at first, but after 105 and 110 ns they stabilized and stayed in equilibrium (Figure 2A and C). However, during the 120 ns MD simulations, the RMSF analysis of complexes 1 and 2 showed the fewest fluctuations and was generally steady, indicating the conformation stability of the respective complexes (Figure 2B and D).

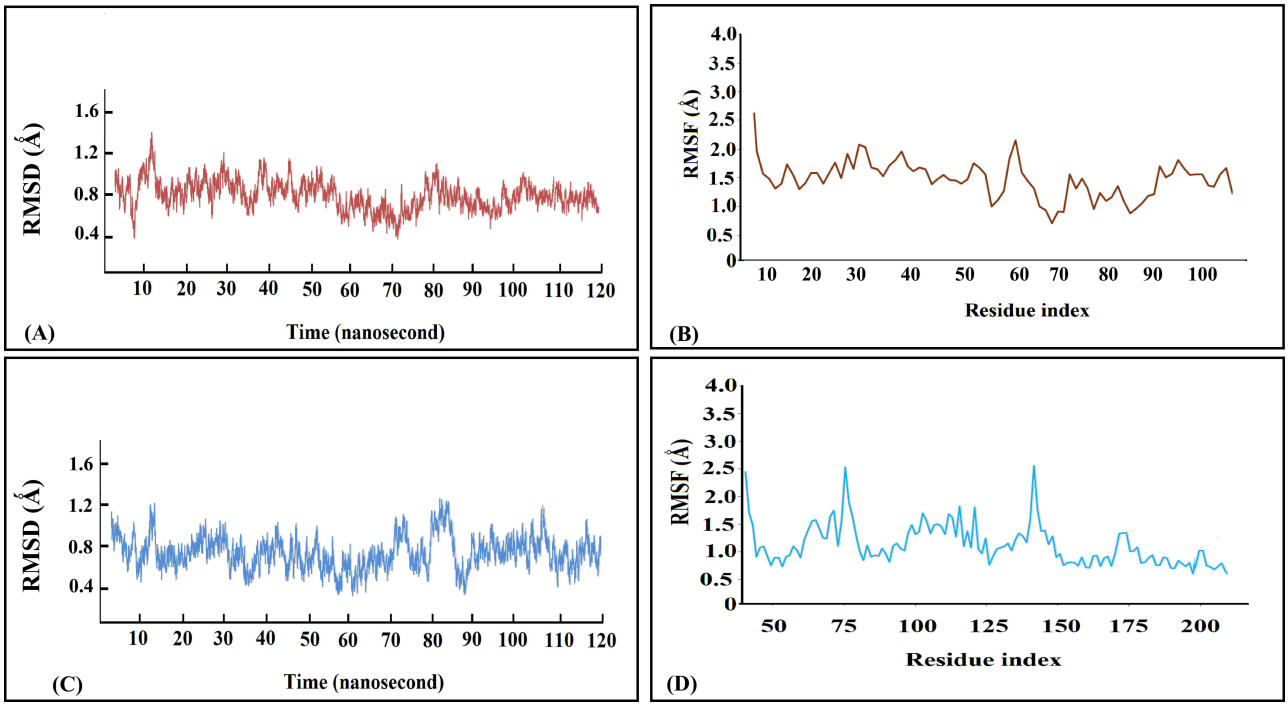


Figure 2 (A) RMSD analysis of the complex galloylastragalin - mdm2 protein. **(B)** RMSF analysis of the complex galloylastragalin - mdm2 protein. **(C)** RMSD analysis of the complex galloylastragalin - Bcl-xL protein. **(D)** RMSF analysis of the complex galloylastragalin - Bcl-xL protein.

Conclusion

A total of 44 phytocompounds of *Eugenia uniflora* were molecularly docked against MDM2 and Bcl-xL proteins, to evaluate the anticancer potential of the plant. Among the phytocompounds, galloylastragalin, a previously reported bioactive molecule, was the best-scoring lead compound with exceptional inhibitory capabilities against the cancer proteins based on thorough molecular docking, in silico toxicity, and MD simulations studies. An in vivo anticancer study followed by the molecular mechanism of action will further assist in potentiating galloylastragalin for possible anticancer drug candidates.

Ethics Statement

The study does not include human or animal subjects.

Acknowledgments

The authors would like to express their gratitude to the African Medicinal Flora and Fauna, and the Department of Chemical and Physical Sciences, Faculty of Natural Science, Walter Sisulu University for their support in conducting this research.

Funding

The APC was funded by the Directorate of Research Development and Innovation, Walter Sisulu University, South Africa.

Disclosure

The authors declare no conflict of interest.

References

1. Garcia-Oliveira P, Otero P, Pereira AG, et al. Status and Challenges of Plant-Anticancer Compounds in Cancer Treatment. *Pharmaceuticals*. 2021;14(2):157. doi:10.3390/ph14020157
2. Piha-Paul SA, Oh DY, Ueno M, et al. Efficacy and safety of pembrolizumab for the treatment of advanced biliary cancer: results from the KEYNOTE-158 and KEYNOTE-028 studies. *Int J Cancer*. 2020;147(8):2190–2198. doi:10.1002/ijc.33013
3. Valko M, Izakovic M, Mazur M, et al. Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem*. 2004;266(1–2):37–56. doi:10.1023/B:MCBI.0000049134.69131.89
4. Wolfe KL, Kang X, He X, et al. Cellular antioxidant activity of common fruits. *J Agric Food Chem*. 2008;56(18):8418–8426. doi:10.1021/jf801381y
5. Koskenkorva-Frank TS, Weiss G, Koppenol WH, Burckhardt S. The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. *Free Radic Biol Med*. 2013;65:1174–1194. doi:10.1016/j.freeradbiomed.2013.09.001
6. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57–70. doi:10.1016/S0092-8674(00)81683-9
7. Fouad YA, Aanei C. Revisiting the hallmarks of cancer. *Am J Cancer Res*. 2017;7(5):1016–1036.
8. Lowe SW, Lin AW. Apoptosis in cancer. *Carcinogenesis*. 2000;21(3):485–495. doi:10.1093/carcin/21.3.485
9. Chaudhary T, Chahar A, Sharma JK, et al. Phytomedicine in the treatment of cancer: a health technology assessment. *J Clin Diagn Res*. 2015;9(12):C04.
10. Langseth H, Andersen A. Cancer incidence among women in the Norwegian pulp and paper industry. *Am J Ind Med*. 1999;36(1):108–113. doi:10.1002/(SICI)1097-0274(199907)36:1<108::AID-AJIM15>3.0.CO;2-N
11. Ignat I, Volf I, Popa VI. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chem*. 2011;126(4):821–1835. doi:10.1016/j.foodchem.2010.12.026
12. Mahomoodally FM, Subratty AH, Gurib-Fakim A, Choudhary MI. Antioxidant, antiglycation and cytotoxicity evaluation of select-ed medicinal plants of the Mascarene Islands. *BMC Complement Altern Med*. 2012;12(1):1–12. doi:10.1186/1472-6882-12-165
13. Franzon RC, Carpenedo S, Viñoly MD, Do CB Raseira M. Pitanga—*Eugenia uniflora* L. In: *Exotic Fruits*. Academic Press; 2018:333–338.
14. Matsumura T, Kasai M, Hayashi T, et al. a-glucosidase Inhibitors From Paraguayan Natural Medicine, Nangapiry, The Leaves Of *Eugenia Uniflora*. *Pharm Biol*. 2000;38(4):302–307. doi:10.1076/1388-0209(200009)3841-AFT302
15. Ogunwande IA, Olawore NO, Ekundayo O, et al. Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. *Int J Aromather*. 2005;15(3):147–152. doi:10.1016/j.ijat.2005.07.004
16. Amorim AC, Lima CK, Hovell AM, et al. Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian Pitanga). *Phytomedicine*. 2009;16(10):923–928. doi:10.1016/j.phymed.2009.03.009
17. Costa DP, Filho EG, Silva LM, et al. Influence of fruit biotypes on the chemical composition and antifungal activity of the essential oils of *Eugenia uniflora* leaves. *J Braz Chem Soc*. 2010;21(5):851–858. doi:10.1590/S0103-50532010000500012
18. Lim TK. *Eugenia uniflora*". In: *Fruits. Edible Medicinal and Non Medicinal Plants*. Springer. 2013;3:620–629. ISBN 978-9400725348

19. Consolini AE, Baldini OA, Amat AG. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. *J Ethnopharmacol*. 1999;66(1):33–39. doi:10.1016/S0378-8741(98)00194-9
20. Sobeh M, Braun MS, Krstin S, et al. Chemical Profiling of the Essential Oils of Syzygium aqueum, Syzygium samarangense and *Eugenia uniflora* and Their Discrimination Using Chemometric Analysis. *Chem Biodivers*. 2016;13(11):1537–1550. doi:10.1002/cbdv.201600089
21. Rodrigues KADF, Amorim LV, Oliveira JMGD, et al. *Eugenia uniflora* L. essential oil as a potential anti-Leishmania agent: effects on Leishmania amazonensis and possible mechanisms of action. *Evid Based Complement Altern Med*. 2013;2013(1):279726. doi:10.1155/2013/279726
22. Fidelis EM, Savall ASP, de Oliveira Pereira F, et al. Pitanga (*Eugenia uniflora* L.) as a source of bioactive compounds for health benefits: a review. *Arab J Chem*. 2022;15(4):103691. doi:10.1016/j.arabjc.2022.103691
23. Bagatini L, Zandoná GP, Hoffmann JF, et al. Evaluation of *Eugenia uniflora* L. leaf extracts obtained by pressurized liquid extraction: identification of chemical composition, antioxidant, antibacterial and allelopathic activity. *Sustain Chem Pharm*. 2023;35:101214. doi:10.1016/j.scp.2023.101214
24. Pereira EDS, Raphaelli CDO, Radünz M, Camargo TM, Vizzotto M. Biological activity and chemical composition of native fruits: a review. *Agrocienc Urug*. 2021;25:NSPE2. doi:10.31285/agro.25.815
25. Sobeh M, Hamza MS, Ashour ML, et al. A polyphenol-rich fraction from *Eugenia uniflora* exhibits antioxidant and hepatoprotective activities in vivo. *Pharmaceuticals*. 2020;13(5):84. doi:10.3390/ph13050084
26. Lee EF, Czabotar PE, Smith BJ, et al. Crystal structure of ABT-737 complexed with Bcl-xL: implications for selectivity of antagonists of the Bcl-2 family. *Cell Death Differ*. 2007;14(9):1711–1713. doi:10.1038/sj.cdd.4402178
27. Miyazaki M, Naito H, Sugimoto Y, et al. Synthesis and evaluation of novel orally active p53–MDM2 interaction inhibitors. *Bioorg Med Chem*. 2013;21(14):4319–4331. doi:10.1016/j.bmc.2013.04.056
28. O'Boyle NM, Banck M, James CA, et al. Open Babel: an open chemical toolbox. *J Cheminf*. 2011;3:33. doi:10.1186/1758-2946-3-33
29. Sch€uttelkopf AW, Van Aalten DM. PRODRG: a tool for high throughput crystallography of protein-ligand complexes. *Acta Crystallogr D Biol Crystallogr*. 2004;60(8):1355–1363. doi:10.1107/S0907444904011679
30. Kar P, Sharma NR, Singh B, Sen A, Roy A. Natural compounds from Clerodendrum spp. as possible therapeutic candidates against SARS-CoV-2: an in silico investigation. *J Biomol Struct Dyn*. 2021;39(13):4774–4785. doi:10.1080/07391102.2020.1780947
31. Kar P, Kumar V, Vellingiri B, et al. Anisotine and amarogentin as promising inhibitory candidates against SARS-CoV-2 proteins: a computational investigation. *J Biomol Struct Dyn*. 2022a;40(10):4532–4542. doi:10.1080/07391102.2020.1860133
32. Kar P, Saleh-E-In MM, Jaishee N, et al. Computational profiling of natural compounds as promising inhibitors against the spike proteins of SARS-CoV-2 wild-type and the variants of concern, viral cell-entry process, and cytokine storm in COVID-19. *J Cell Biochem*. 2022b;123 (5):964–986. doi:10.1002/jcb.30243
33. Naidoo D, Roy A, Kar P, Mutanda T, Anandraj A. Cyanobacterial metabolites as promising drug leads against the Mpro and PLpro of SARS-CoV-2: an in silico analysis. *J Biomol Struct Dyn*. 2020;39(16):6218–6230. doi:10.1080/07391102.2020.1794972
34. Daina A, Michielin O, Zoete V. Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7(1):42717. doi:10.1038/srep42717
35. Pires DE, Blundell TL, Ascher DB. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem*. 2015;58(9):4066–4072. doi:10.1021/acs.jmedchem.5b00104
36. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2018;46:257–263. doi:10.1093/nar/gky318
37. Schyman P, Liu R, Desai V, Wallqvist A. vNN web server for ADMET predictions. *Front Pharmacol*. 2017;8:889. doi:10.3389/fphar.2017.00889
38. Abraham MJ, Murtola T, Schulz R, et al. GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 2015;1–2:19–25. doi:10.1016/j.softx.2015.06.001
39. Elfiky AA, Azzam EB. Novel guanosine derivatives against MERS CoV polymerase: an in silico perspective. *J Biomol Struct Dyn*. 2020;39 (8):2923–2931. doi:10.1080/07391102.2020.1758789
40. Enayatkhani M, Hasaniazad M, Faezi S, et al. Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19: an in silico study. *J Biomol Struct Dyn*. 2020;39(8):2857–2872. doi:10.1080/07391102.2020.1756411
41. Umesh Kundu D, Selvaraj C, Singh SK, Dubey VK. Identification of new anti-nCoV drug chemical compounds from Indian spices exploiting SARS-CoV-2 main protease as target. *J Biomol Struct Dyn*. 2020;39(9):3428–3434. doi:10.1080/07391102.2020.1763202
42. Kris-Etherton PM, Lichtenstein AH, Howard BV, et al. Antioxidant vitamin supplements and cardiovascular disease. *Circulation*. 2004;110 (5):637–641. doi:10.1161/01.CIR.0000137822.39831.F1
43. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem*. 2015;30(1):11–26. doi:10.1007/s12291-014-0446-0
44. Fu Y, Chang H, Peng X, et al. Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/β-catenin signaling pathway. *PLoS One*. 2014;9(7):e102535. doi:10.1371/journal.pone.0102535
45. Geng W, Guo X, Zhang L, et al. Resveratrol inhibits proliferation, migration and invasion of multiple myeloma cells via NEAT1-mediated Wnt/β-catenin signaling pathway. *Biomed Pharmacother*. 2018;107:484–494. doi:10.1016/j.biopha.2018.08.003
46. Hosseini MM, Karimi A, Behroozaghdam M, et al. Cytotoxic and Apoptogenic Effects of Cyanidin-3-Glucoside on the Glioblastoma Cell Line. *World Neurosurg*. 2017;108:94–100. doi:10.1016/j.wneu.2017.08.133
47. Sun W, Zhang ND, Zhang T, et al. Cyanidin-3-O-Glucoside Induces the Apoptosis of Human Gastric Cancer MKN-45 Cells through ROS-Mediated Signaling Pathways. *Molecules*. 2023;28(2):652. doi:10.3390/molecules28020652
48. Jiang M, Zhu M, Wang L, Yu S. Anti-tumor effects and associated molecular mechanisms of myricetin. *Biomed Pharmacother*. 2019;120:109506. doi:10.1016/j.biopha.2019.109506
49. Rahmani AH, Almatroudi A, Allemailem KS, et al. Myricetin: a significant emphasis on its anticancer potential via the modulation of inflammation and signal transduction pathways. *Int J Mol Sci*. 2023;24(11):9665. doi:10.3390/ijms24119665
50. Patil S, Deshmukh S. A review on the ethnobotany, phytochemistry and pharmacological activities of *Caesalpinia decapetala* (Roth) Alston. *World J Pharm Res*. 2023;12(9):528–560.
51. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol*. 2014;24(10):453–462. doi:10.1016/j.cub.2014.03.034
52. Cory S, Huang D, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene*. 2003;22(53):8590–8607. doi:10.1038/sj.onc.1207102

53. Chan SL, Yu VC. Brief review proteins of the bcl-2 family in apoptosis signalling: from mechanistic insights to therapeutic opportunities. *Clin Exp Pharmacol Physiol*. 2004;31(3):119–128. doi:10.1111/j.1440-1681.2004.03975.x
54. Liew SK, Malagobadan S, Arshad NM, Nagoor NH. A Review of the Structure-Activity Relationship of Natural and Synthetic Antimetastatic Compounds. *Biomolecules*. 2020;10(1):138. doi:10.3390/biom10010138
55. Afnan, Saleem A, Akhtar MF, et al. Cardio-Protective and Anti-Inflammatory Potential of Natural-Sources-Derived Phenolic Acids. *Molecules*. 2022;27(21):7286. doi:10.3390/molecules27217286
56. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001;46(1–3):3–26. doi:10.1016/S0169-409X(00)00129-0
57. Saleh-e-In MM, Roy A, Al-Mansur MA, et al. Isolation and in silico prediction of potential drug-like compounds from Anethum sowa L. root extracts targeted towards cancer therapy. *Comput Biol Chem*. 2019;78:242–259. doi:10.1016/j.compbiolchem.2018.11.025
58. Sreeshma LS, Nair BR. A simple protocol for the isolation of amentoflavone from two species of Biophytum DC.(Oxalidaceae) and evaluation of its antiproliferative potential. *Ind Crop Prod*. 2021;160:113099. doi:10.1016/j.indcrop.2020.113099
59. Do Nascimento RP, De Jesus LB, Oliveira-Junior MS, et al. Agathisflavone as a single therapy or in association with mesenchymal stem cells improves tissue repair in a spinal cord injury model in rats. *Front Pharmacol*. 2022;13:858190. doi:10.3389/fphar.2022.858190