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Chemical Composition of the Phenolic Profile and Evaluation of Antimicrobial and Cytotoxic Activities of Moroccan Pomegranate (*Punica granatum*) Peel Extract

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Abstract

This study aims to deepen the understanding of the pharmacological properties of the aqueous extract derived from the peel of *Punica granatum* L., sourced from eastern Morocco. Chemical analysis using ultra-high-performance liquid chromatography (UHPLC) led to the identification of 30 compounds, mainly phenolic acids and flavonoids. The major constituents were quercetin (23.32%), gallic acid (12.85%), caffeic acid (12.44%), and hesperetin (12.11%). The antimicrobial activity of the extract was evaluated using the broth microdilution method, revealing strong efficacy against Gram-positive bacteria, particularly *Bacillus cereus* (MIC = 0.781 mg/mL) and *Staphylococcus aureus* (MIC = 1.56 mg/mL), while *Escherichia coli* exhibited higher resistance (MIC = 3.125 mg/mL). Cytotoxicity assessment showed greater inhibition of splenocytes (IC_{50} = 708.7 ± 49.46 µg/mL) compared to myeloma P3 cell lines (IC_{50} = 950.3 ± 43.8 µg/mL), suggesting potential selectivity. An *in silico* study was conducted to explore the pharmacokinetic and toxicological properties of the main compounds. Hesperetin

and gallic acid were predicted to be non-toxic and exhibited strong binding affinities to antibacterial target proteins and to BCL-2, a key regulator of apoptosis. Hesperetin demonstrated more stable and diverse interactions with BCL-2, indicating superior potential as an inhibitor. These findings suggest that pomegranate peel extract may serve as a promising source of bioactive compounds for the development of new antimicrobial and anticancer agents.

Keywords: *Punica granatum*, UHPLC, antimicrobial activity, cytotoxicity, and natural bioactives.

1. Introduction

Pomegranate (*Punica granatum* L.) is a significant fruit crop known for its adaptability to diverse agro-climatic environments. This deciduous, fruit-producing shrub—or occasionally a small tree—belongs to the Punicaceae family. While its origins trace back to Iran, the pomegranate is now widely cultivated in countries such as Tunisia, Turkey, Spain, Egypt, Morocco, the United States, China, India, Argentina, and South Africa (Kahramanoglu and Usanmaz 2016). The pomegranate tree is widely regarded as a medicinal plant, and its fruit—often referred to as nature's power fruit—is globally recognized for its delightful taste and remarkable health benefits (Karimi, Sadeghi, and Kokini 2017). With growing public awareness of its exceptional therapeutic properties, the production and consumption of pomegranate have risen significantly (Kahramanoglu and Usanmaz 2016). Pomegranate is rich in phenolic compounds, which are known for their health-promoting properties. The fruit, shaped like a grenade, contains numerous deep red, juice-filled arils encased in a glossy, leathery peel known as the pericarp, and topped with a distinctive, persistent calyx. It can be enjoyed fresh—either raw or as juice—or incorporated into various food products such as beverages, jams, and jellies. The health benefits of pomegranate extend beyond its edible arils; in fact, the non-edible parts, particularly the peel, contain even higher concentrations of bioactive compounds than the arils themselves (Akhtar et al., 2015, Abid et al., 2017).

The peel of the pomegranate makes up approximately 30–40% of the total fruit and is typically considered a byproduct following juice extraction (Çam, İçyer, and Erdoğan 2014). Currently, fruit peels are recognized as valuable sources of bioactive compounds within the fruit processing industry, gaining increasing attention due to their significant economic potential. There is growing interest in exploring the beneficial phytochemicals found in fruit peels, with the aim of utilizing them in the food, pharmaceutical, and cosmetic industries. Pomegranate peel is known for its rich array of extraordinary phytochemicals, which have both medicinal and nutritional value. In particular, the variety of phenolic compounds present in the peel,

recognized for their natural antioxidant properties, has garnered significant interest from researchers and medical professionals (Singh et al. 2018). Both the consumable and non-consumable parts of the pomegranate plant exhibit significant phytotherapeutic and pharmacological effects, largely attributed to the presence of various phytochemical compounds such as flavonoids, hydrolysable tannins, alkaloids, and phenolic acids (Abdul Qahir et al., 2021, Brighenti et al., 2017, Borges & Crozier, 2012). The bark of *P. granatum*, as highlighted in scientific literature, has shown promising health benefits, including anti-inflammatory, antihypertensive (Wang et al. 2018), antihyperglycemic (Laaraj et al. 2022), antihyperlipidemic and antioxydant (Leesombun et al., 2022, Elkoraichi et al., 2022, Benchagra et al., 2021) and antithrombotic properties (Lafdil et al. 2025).

The primary objective of this study is to deepen the understanding of the pharmacological properties of the aqueous extract derived from the peel of *Punica granatum* L., sourced from the eastern region of Morocco. This will be achieved through the analysis of its chemical composition using ultra-high-performance liquid chromatography (UHPLC), as well as the evaluation of its anticancer potential by assessing its ability to protect against cytotoxicity. Additionally, the antibacterial activity will be examined to identify the extract's antimicrobial properties, potentially paving the way for medicinal applications. Finally, an *in silico* study will be conducted to virtually screen the compounds present in the extract against selected protein targets, offering insights into potential mechanisms of action. Together, these investigations aim to support the potential use of this extract in medical and pharmaceutical research.

2. Materiels and methods

2.1. Collection of plant material and extraction of the aqueous crude extract of P. granatum peel

P. granatum fruits were collected in Guercif (Morocco) in December 2020. The plant was taxonomically identified at the Biology Department of the Faculty of Sciences, Mohammed First University (Oujda, Morocco) by Professor Mostafa ELACHOURI, and a voucher specimen was registered under the reference number HUMPOM237. The entire fruit was first dried, after which the seeds were separated from the bark. A total of 40 g of bark powder was subjected to decoction at 40 °C in 400 ml of distilled water for 72 hours. The mixture was then filtered through filter paper, and the obtained filtrate was concentrated under reduced pressure using a rotary evaporator (Heidolph Instruments, Germany) at 45 °C. The concentrated extract was subsequently dried in an oven at 40 °C overnight. The extraction process yielded 7.25%.

2.2. LC-MS/MS Analysis of the aqueous crude extract of P. granatum peel

The chemical composition of the aqueous extract of *P. granatum* peel (aqE-PGP) was analyzed using ultra-high-performance liquid chromatography (UHPLC) coupled with high-resolution mass spectrometry (LCMS8060, Shimadzu Italy, Milan). Instrumental parameters were set as follows: nebulizing gas flow at 2.9 L/min, heating gas flow at 10 L/min, interface temperature at 300 °C, Linear Ion Trap (LIT) detector temperature at 250 °C, thermal block temperature at 400 °C, and drying gas flow at 10 L/min. A custom internal database of polyphenol derivatives was constructed to facilitate qualitative analysis. Compound separation was achieved using a C18 column (3 × 100 mm, 2.6 µm particle size; Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile (solvent A) and water containing 0.01% formic acid (solvent B). The aqE-PGP was dissolved in a 1:1 mixture of acetonitrile and water. From this solution, 20 µL was further diluted with 980 µL of acetonitrile prior to injection into the analytical system. A compound was considered present if its peak area exceeded that of the blank control. For compounds with closely related structures, identification was based on retention time, with the instrument set to detect molecular mass in the third quadrupole (Kandsi et al. 2021).

2.3. Molecular Docking

An *in-silico* investigation was carried out to assess the principal bioactive compounds, namely: Caffeic acid, Hesperetin, Quercetin, and Gallic acid, which were isolated from the peel of *Punica granatum*. These compounds demonstrated AUC values of 12.44%, 12.11%, 23.32%, and 12.85%, respectively. The physicochemical characteristics and pharmacokinetic profiles of Absorption, Distribution, Metabolism, Excretion, and Toxicity parameters were systematically evaluated using SwissADME and pkcsm servers and Egan's boiled egg model (Ed-Dahmani et al. 2024; Fadili et al. 2022; Kandsi et al. 2022) Compounds identified as safe were further subjected to molecular docking studies targeting four pathogenic strains: *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Bacillus cereus*, then docked to the crystal structure of BCL-2 (PDB ID of 6O0K) (Lakka et al. 2023), to understand how mutations or drug interactions modulate BCL-2's role in apoptosis, which is critical in cancer progression and treatment resistance. Docking simulations were conducted using AutoDock software (El fadili et al. 2024; El fadili et al. 2024) with proteins prepared according to standardized protocols, including the removal of water molecules, the addition of Gasteiger charges, and the elimination of co-crystallized ligands (El fadili et al. 2022; Ed-Dahmani et al. 2024).

2.4. Antimicrobial activity assay of P. granatum peel

The antimicrobial activity was evaluated against a panel of bacterial and fungal strains using the broth microdilution method. The bacterial strains employed in this study were previously

isolated from food sources and referenced as American Type Culture Collection (ATCC). These included *Escherichia coli* ATCC 25922 (Gram-negative), *Staphylococcus aureus* ATCC 29213, and *Bacillus subtilis* ATCC 6633 (both Gram-positive). In addition, *Candida albicans* obtained as a clinical isolate, was included in the screening.

2.4.1. Determination of the Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MICs) was determined in sterile 96-well microplates using microdilution assay, as reported by (Chraibi et al. 2021). Bacteriological agar at 0.15% (w/v) was used as an emulsifier for the tested extracts, previously dissolved in DMSO. First, 50 µL of Muller Hinton broth was added to all test wells, except the first well, which contained only 100 µL of the extracts (25 mg/mL). The extracts were serially diluted by transferring 50 µL from the first well to the to the 10th well (Concentrations ranging from 0.097 to 50 mg/mL). The 11th well served as growth control, and the 12th was used as sterility control. Then, 50 µl of bacterial inoculum, adjusted to 0.5 McFarland standard (10^6 CFU/ml), was added to each well (from the first to 11th). After incubation at 37°C for 20-24 hours, 15 µl of the bacterial growth indicator (resazurin) was added to each well. For yeast, the MIC was determined according to the protocol described by (Zhang et al. 2008) with slight modifications. First, the extracts were serially diluted in broth (YPG), supplemented with agar at 0.15% (w/v), to achieve final concentrations ranging from 0.097 to 50 mg/mL. Then, 50 µl of inoculum was added to each well to achieve a final concentration of 10^3 CFU/ml. Finally, the microplate was incubated at 30 ◦C for 48 h.

2.4.2. Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The Minimal Bactericide and Fungicide Concentration (MBC/MFC) were determined by inoculating 6 µl from each negative well onto LB plates for bacteria, which were incubated at 37°C for 20-24 hours, and onto YPG plates for yeast, that were incubated at 30°C for 48 hours. All experiments were performed in triplicate.

2.5. Evaluation of cytotoxicity Assay

2.5.1. Ethical statement

All animal procedures were conducted in accordance with internationally recognized ethical standards, including the guidelines of the International Association for the Study of Pain (IASP) and national regulations governing the care and use of laboratory animals. Prior to the start of the study, a detailed ethics application was submitted to the Comité d'Éthique de la Recherche Biomédicale (CERB) of the Faculty of Medicine and Pharmacy, Hassan II University

of Casablanca. The protocol received formal approval under the registered IRB number IRB00002504. This study is reported in compliance with the ARRIVE guidelines (https://arriveguidelines.org) to ensure rigorous and transparent reporting of animal research.

2.5.2. Cell line and culture conditions

Murine myeloma cells, P3X63Ag8.653, were acquired from the Laboratory of Genetic and Cellular Engineering, Faculty of Medicine and Pharmacy, Casablanca, Morocco. Standard culture conditions were established using RPMI 1640 medium, supplemented with 10% FBS to provide essential growth factors. To ensure optimal cellular metabolism and prevent bacterial contamination, the medium was further supplemented with D-(+) glucose, L-glutamine, sodium pyruvate, HEPES, Na_2EDTA, $NaHCO_3$, and antibiotics (penicillin and streptomycin sulfate). Trypan blue dye exclusion was employed to determine cell viability, differentiating between live and dead cells. Cells were incubated at 37°C within a humidified, 5% CO_2 environment (Souhaili et al. 2008).

2.5.3. Splenic mononuclear cells and culture conditions

To assess the cytotoxic potential of the extracts, splenic mononuclear cells, were isolated from the spleens of two adult BALB/c mice (6 weeks old, 27 g). Mice were euthanized using an intraperitoneal injection of ketamine (100 mg/kg) to ensure rapid and humane sacrifice, and their spleens were aseptically excised and placed in separate Petri dishes. The spleens were then rinsed and cells were released from the spleen tissue by gentle perfusion with 15 mL of RPMI medium. To remove erythrocytes, the cell suspension was treated with a red blood cell lysis solution. The resulting cell suspension was then diluted to 10 mL with RPMI medium and centrifuged at 1500 rpm for 10 minutes. This centrifugation and supernatant aspiration process was repeated twice to ensure a pure cell population (Zandonai et al. 2010).

2.5.4. Treatment with the aqueous crude extract of P. granatum peel

For dose-dependent viability assessment, 100 µL P3 cells (4 x 10^4 cells/well in 96-well plates) were seeded and incubated for 24 hours to achieve a stable growth phase, as described by (Elouaddari et al. 2019). Subsequently, aqE-PGP, prepared in 100 µL of RPMI medium at concentrations ranging from 125 to 2000 µg/mL, were applied for 48 hours. In contrast, PBMC cells were seeded at a density of 8.10^5 cells/well and treated immediately without a pre-incubation period. Melphalan, a standard chemotherapeutic, was included as a positive control to verify the assay's capacity to accurately detect cell death. To control for potential medium-induced effects, cells were also treated with culture medium alone (negatif control). Cell mortality was expressed as a percentage, determined by the following formula:

$$\%\ Cell\ mortality = 100 - \frac{Absorbance\ of\ treated\ cells - Blank\ absorbance}{Absorbance\ of\ untreated\ cells - Blank\ absorbance}\ X\ 100$$

2.5.5. MTT assay

Cellular viability was assessed by quantifying mitochondrial activity through the colorimetric reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) after 48h of exposure. Following treatment, cells were incubated 4h with MTT, leading to the formation of formazan crystals in metabolically active cells (Mosmann 1983). The resulting crystals were then solubilized in dimethyl sulfoxide (DMSO), and absorbance, directly correlating with viable cell number, was measured spectrophotometrically at 492 nm.

3. Results

3.1. Phytochemical Analysis

The phytochemical analysis of the aqueous extract of pomegranate peel (aqE-PGP) led to the identification of a total of 30 compounds, detailed in **Table 1**. The corresponding chromatograms are provided as Supplementary File S1. The detected compounds primarily belong to the phenolic acids and flavonoid families, both well-known for their bioactive properties. Among these, four compounds were found in relatively high abundance, as estimated by the proportion of the area under the chromatographic curve. These major constituents include quercetin (23.32%), gallic acid (12.85%), caffeic acid (12.44%), and hesperetin (12.11%).

Table 1: Phytochemical composition of the aqueous extract of *P. g* revealed by LC-MS/MS

Compounds	**Formula**	**Classes of compounds**	**$(M-H)^-$**	**AUC**	**% AUC**
Syringic acid	$C_9H_{10}O_5$	Phenolic acid	198.9000	803904	0.55
p-coumaric acid	$C_9H_8O_3$	Phenolic acid	162.9000	3965031	2.75
Trans-ferulic acid	$C_{10}H_{10}O_4$	Phenolic acid	193.0000	1427040	0.98
Arbutin	$C_{12}H_{16}O_7$	glycoside	271.2000	1052242	0.72
Rosmarinic acid	$C_{18}H_{16}O_8$	Phenolic acid	359.0000	810608	0.56
Apigenin	$C_{15}H_{10}O_5$	flavonoid	269.0000	1168327	0.81
Amentoflavone	$C_{30}H_{18}O_{10}$	flavonoid	537.1000	1318641	0.91
Luteolin	$C_{15}H_{10}O_6$	Flavonoid	284.9000	553957	0.38
Quercetin-3'-o-glucuronide	$C_{21}H_{18}O_{13}$	flavonoid	477.0000	1140592	0.79

quercetin-3-O-deoxyhexosyl(1-2)pentoside	$C_{26}H_{28}O_{15}$	flavonoid	609.1000	3150699	2.18
Isorhamnetin-3-O-rutinoside	$C_{28}H_{32}O_{16}$	flavonoid	623.1000	883420	0.6
Vanillic acid	$C_8H_8O_4$	Phenolic acid	167.0000	383684	0.26
Salicylic Acid	$C_7H_6O_3$	Phenolic acid	137.0000	805604	0.55
Sinapic acid	$C_{11}H_{12}O_5$	Phenolic acid	223.0000	987753	0.68
Ferulic acid	$C_{10}H_{10}O_4$	Phenolic acid	193.0000	1035911	0.71
catechin gallate	$C_{22}H_{18}O_{10}$	flavonoid	441.0000	842047	0.58
Procyanidin	$C_{30}H_{26}O_{13}$	flavonoid	577.0000	860153	0.59
Myricetin	$C_{15}H_{10}O_8$	Flavonoid	317.0000	619298	0.42
kaempferol	$C_{15}H_{10}O_6$	Flavonoid	285.0000	605209	0.41
Naringin	$C_{27}H_{32}O_{14}$	Flavonoid	579.0000	4611913	3.19
rutin	$C_{27}H_{30}O_{16}$	Flavonoid	609.0000	3097288	2.14
Gallocatechin	$C_{15}H_{14}O_7$	Flavonoid	305.0000	4964368	3.44
Caffeic acid	$C_9H_8O_4$	Phenolic acid	179.0000	17947221	12.44
Luteolin 7-O-glucoside	$C_{21}H_{20}O_{11}$	Flavonoid	447.1000	6017591	4.17
isorhamnetin 7-O-glucoside	$C_{22}H_{22}O_{12}$	Flavonoid	447.1000	6045113	4.19
Kaempferol-3-O-glucoside	$C_{21}H_{20}O_{11}$	Phenolic acid	609.1000	3133379	2.17
Quercetin-3-O-glucoside	$C_{21}H_{20}O_{12}$	Flavonoid	463.1000	6319954	4.38
hesperetin	$C_{16}H_{14}O_6$	Flavonoid	301.3000	17467657	12.11
quercetin	$C_{15}H_{10}O_6$	Flavonoid	301.0000	33626392	23.32
Gallic acid	$C_7H_6O_5$	Phenolic acid	168.9000	18529726	12.85

These findings are generally consistent with previously published data, although certain differences were noted regarding the relative abundance of compounds and the presence of specific molecules. Such variations are commonly observed and may be attributed to factors such as environmental conditions at the time of harvest, the botanical variety of the fruit, and the extraction methods employed. (Cruz-Valenzuela et al. 2022) conducted a maceration-based extraction of pomegranate peels collected in Mexico. Analysis of aqueous and ethanolic extracts using UPLC-DAD revealed seven polyphenolic compounds, with chlorogenic acid and gallic acid being the most abundant. Similarly, investigated 14 cultivated pomegranate varieties from the United States. The peels underwent hydroalcoholic extraction, followed by HPLC analysis. Out of 14 tested standards, four phenolic compounds were identified and quantified:

ellagic acid, gallic acid, punicalagin A, and punicalagin B (Shahkoomahally et al. 2023). In Tunisia, (Khemakhem et al. 2021) analyzed pomegranate peels from three distinct regions. HPLC analysis revealed the presence of 16 phenolic compounds, among which rutin, isoquercitrin, ferulic acid, and chlorogenic acid were particularly abundant. Furthermore, (Özcan et al. 2021) conducted a study on pomegranate seeds harvested in Turkey. HPLC analysis identified 16 polyphenolic compounds, with the most abundant being gallic acid, 3,4-dihydroxybenzoic acid, (+)-catechin, 1,2-dihydroxybenzene, and quercetin. Although the plant matrices differ (seeds vs. peels), the recurrence of certain compounds such as quercetin and gallic acid highlights their ubiquity across various parts of the fruit. Taken together, these findings are in line with our results and support the hypothesis that polyphenols—particularly those found in high concentrations in aqE-PGP—may significantly contribute to the associated biological activities, notably antioxidant, antimicrobial, and anti-inflammatory effects, as widely reported in the literature(Bahmanzadegan et al. 2023; Aleksandrova et al. 2023; Kiran et al. 2024).

3.2. In silico predictions of ADMET features

The prediction of the physicochemical properties of the main compounds extracted from the peel of *Punica granatum* indicates that Caffeic acid, Hesperetin, Quercetin, and Gallic acid, labeled as C1, C2, C3, and C4 respectively, possess the desired physicochemical characteristics, fully complying with Lipinski's rules (Aloui et al. 2024; El fadili et al. 2023) as shown in **Table 2**. The compounds were also evaluated for their ADMET pharmacokinetic profiles (El fadili, Er-rajy, Ali Eltayb, et al. 2023) revealing good human intestinal absorption (HIA), particularly for Hesperetin and Quercetin. Metabolism studies showed that none of the major compounds inhibit cytochrome enzymes 1A2, 2C9, 2C19, 2D6, and 3A4, except for Quercetin (C3), which demonstrated a positive inhibitory effect on cytochromes 1A2 and 3A4. Toxicity assessments confirmed that Hesperetin (C2) and Gallic acid (C4) are free from risks of skin sensitization and hepatotoxicity. Although Caffeic acid (C1) and Quercetin (C3) were also predicted to be non-hepatotoxic and non-sensitizing, they tested positive in the AMES prediction, suggesting a potential mutagenic risk by inducing DNA mutations in bacteria, which could imply carcinogenic potential in humans, as detailed in **Table 3**. Additionally, the predictive model of the boiled egg of Egan as a fast and visual way to predict if a molecule can be absorbed by the intestine or cross into the brain based on just its size and polarity, confirms that all four examined compounds are failing into the white area of the Egan egg, which demonstrates that are supposed to have good intestinal absorptions, as shown in **Figure 1**.

Table 2. Lipinski's rules for the major compounds extracted from the Punica granatum peel plant.

	TPSA	n- Rotatable Bonds	MW	Log P	n-HBA	n-HBD	Violations Lipinski
Rule	**<140 A²**	**<10**	**<500 D.a**	**≤5**	**<10**	**<5**	**≤2**
C1	77.76	2	180.16	0.97	4	3	**Yes**
C2	77.83	1	302.28	2.51	6	3	**Yes**
C3	131.36	1	302.24	1.63	7	5	**Yes**
C4	97.99	1	170.12	0.21	5	4	**Yes**

Table 3. ADME and Toxicity pharmacokinetic features of four major compounds extracted from the Punica granatum peel plant.

models	ADME and Toxicity Properties													
	Absorption	Distribution		Metabolism							Excretion	Toxicity		
				CYP450										
				Substrate		Inhibitor								
	Intestinal absorption (human)	BBB permeability	CNS permeability	2D6	3A4	1A2	2C19	2C9	2D6	3A4	Total clearance	AMES toxicity	Hepatot oxicity	Skin sensitization
Unity	Numeric (%absorbed)	Numeric (Log BB)	Numeric (Log PS)	Categorical (YES/NO)							Numeric (log mL $min^{-1}kg^{-1}$)	Categorical (yes/no)		
	Predicted values													
C1	59.008	-0.816	-3.361	No	No	No	No	No	No	No	0.558	**Yes**	No	No
C2	78.513	-0.952	-3.356	No	No	No	No	No	No	No	0.473	No	No	No
C3	74.994	-1.355	-3.432	No	No	**Yes**	No	No	No	**Yes**	0.578	**Yes**	No	No
C4	40.154	-1.111	-4.157	No	No	No	No	No	No	No	0.625	No	No	No

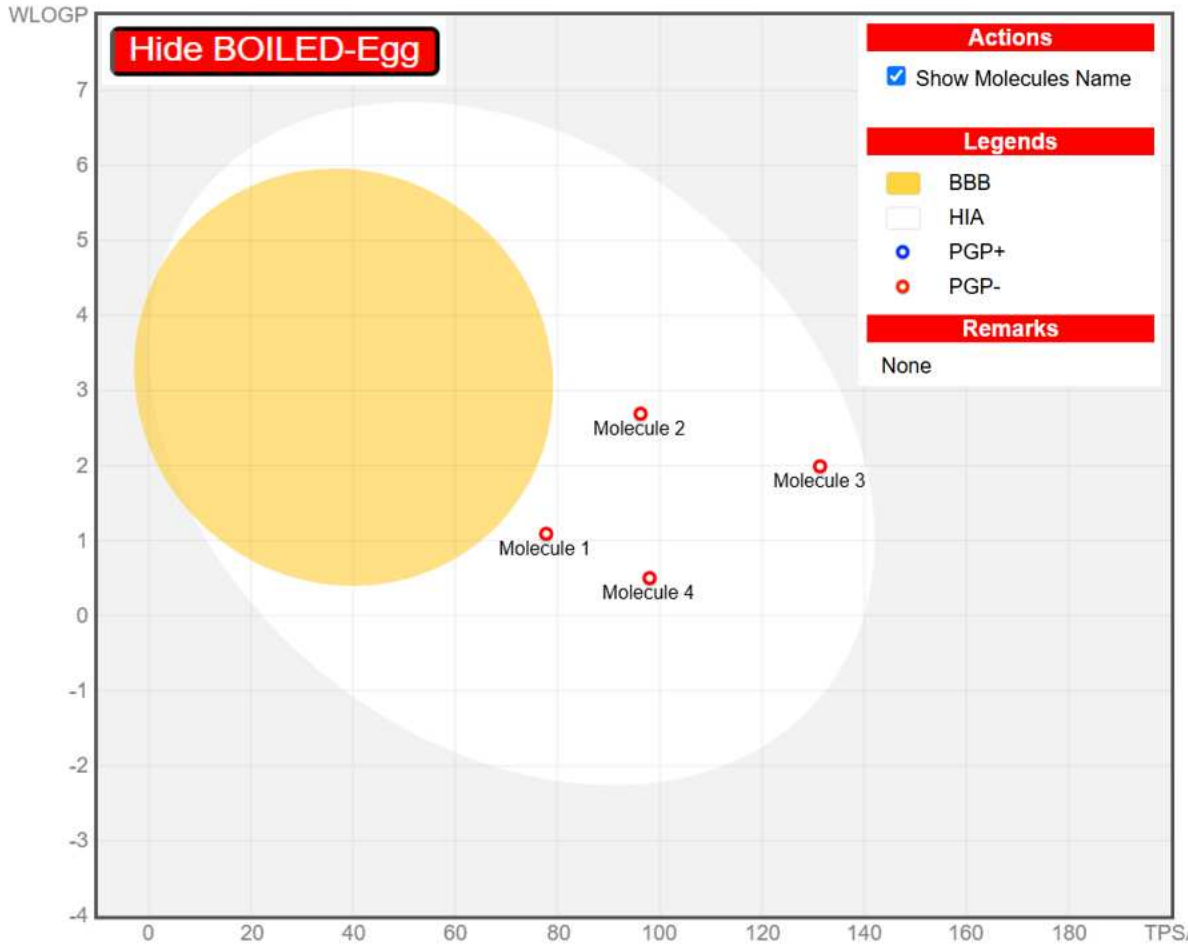


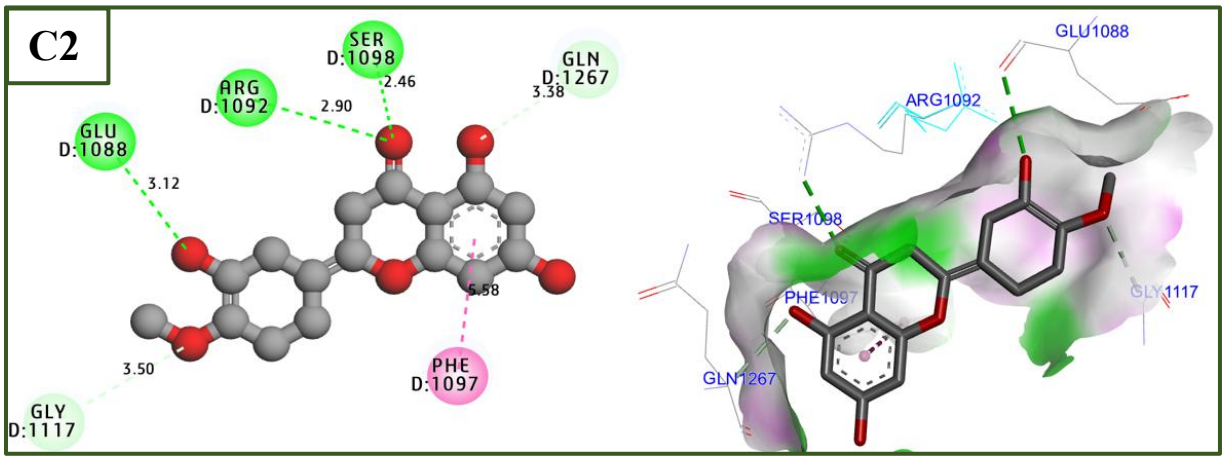
Figure 1. The BOILED-Egg predictive model for Caffeic acid (molecule 1), Hesperetin (molecule 2), Quercetin (molecule 3), and Gallic acid (molecule 4).

3.3. Molecular docking simulations

Docking simulations were carried out to explore the potential binding modes and affinities of the safe extracted compounds toward selected biological targets. For this purpose, Hesperetin (C2) and Gallic acid (C4), predicted as safe agents with good physicochemical and pharmacokinetic profiles, were docked to the crystal structure of Staphylococcus aureus gyrase (**5BS3**.pdb)(Lebrazi et al. 2025) the crystal structure of the FimH lectin domain from Escherichia coli (**4XO8**.pdb) (Jeddi et al. 2023; Nouioura et al. 2024) the secreted aspartic protease from Candida albicans (**1ZAP**.pdb) (El-Assri et al. 2025) and the crystal structure of Bacillus cereus (**3U3W**.pdb) (Er-rahmani et al. 2024) Both Hesperetin and Gallic acid were first docked to the antibacterial protein responsible for the Staphylococcus aureus pathogenic strain, with binding energies of -8.2 and -5.01 kcal/mol, respectively, revealing one common hydrogen bond detected with the Ser1098 amino acid residue (A.A.R), more than one Pi-alkyl bond fixed with Phe1097 A.A.R as shown in **Figure 2**. Secondly, the candidate compounds were also docked to the targeted protein of the Escherichia coli pathogenic strain, with binding

energies of -6.07 and -4.73 kcal/mol, respectively, exhibiting similar intermolecular interactions, including two conventional hydrogen bonds created towards Asp37 and Asn23 A.A.Rs, more than one alkyl bond fixed with Val22 A.A.R, as displayed in **Figure 3**. Both small molecules were docked again to the secreted aspartic protease from Candida albicans strain, with binding energies of -5.48 and -4.63 kcal/mol, revealing three common hydrogen bonds created with Gln329, Asn269, and Ser182 A.A.Rs, as shown in **Figure 4**. Finally, the examined ligands were docked to the crystal structure of the Bacillus cereus receptor, with binding energies of -8.14 and -4.06 kcal/mol, respectively, revealing one common Pi-Sigma bond detected with Met272 A.A.R, in addition to one Pi-Alkyl bond formed with Lys87 A.A.R, and one conventional hydrogen bond created towards Asn230 A.A.R, as presented in **Figure 5**.

The molecular docking analysis of compounds Hesperetin) and Gallic acid against the anti-apoptotic protein BCL-2 (PDB ID: 6O0K) revealed distinct binding interactions within the BH3-binding groove of the protein with binding energies of -6.83 and -4.96 kcal/mol, respectively. Both compounds exhibited favorable binding orientations and interactions that support their potential as BCL-2 inhibitors, where the Hesperetin compound demonstrated a well-defined binding conformation, interacting with several key amino acid residues within the BH3-binding groove of BCL-2. Hydrogen bonds were observed with ALA100, ASN143, and TYR202, while additional stabilizing hydrophobic and π–π stacking interactions were formed with PHE104, VAL148, and ARG107. While Gallic acid displayed a different interaction profile, favoring a more polar binding environment. It formed several hydrogen bonds with SER117, HIS120, and LEU119, and engaged in strong electrostatic interactions with GLU160 and ARG164 via its carboxylic and hydroxyl groups, as shown in **Figure 6**.



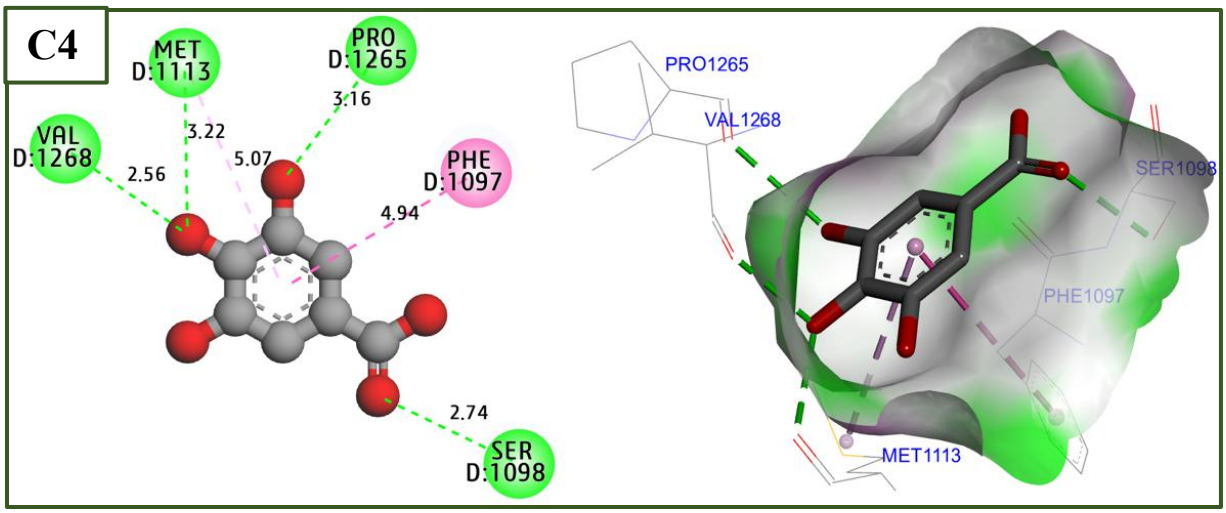
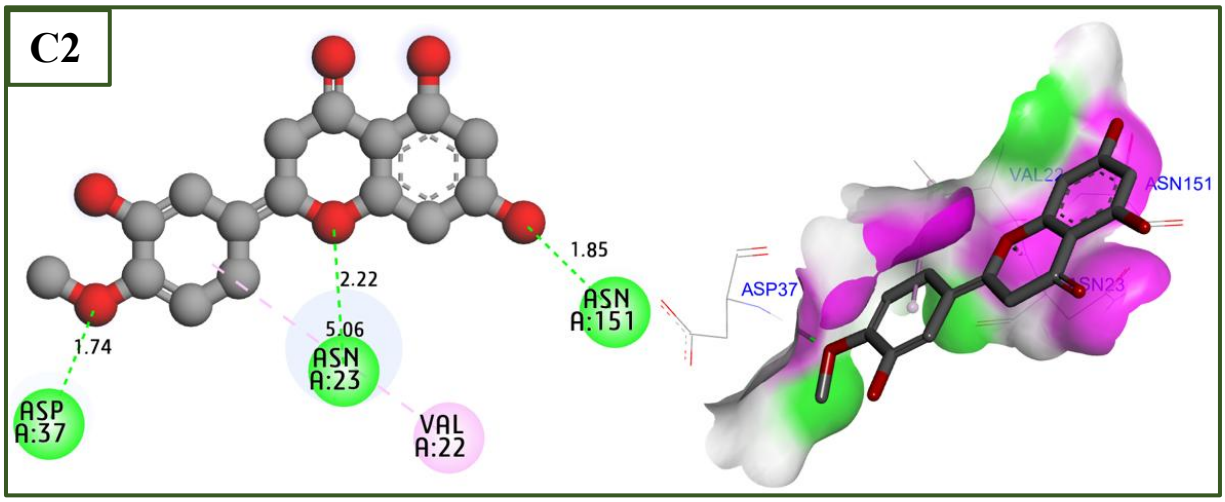


Figure 2. 2D and 3D views of docking results for C2 and C4 in complex with the crystal structure of S.A. gyrase protein (PDB ID of 5BS3).



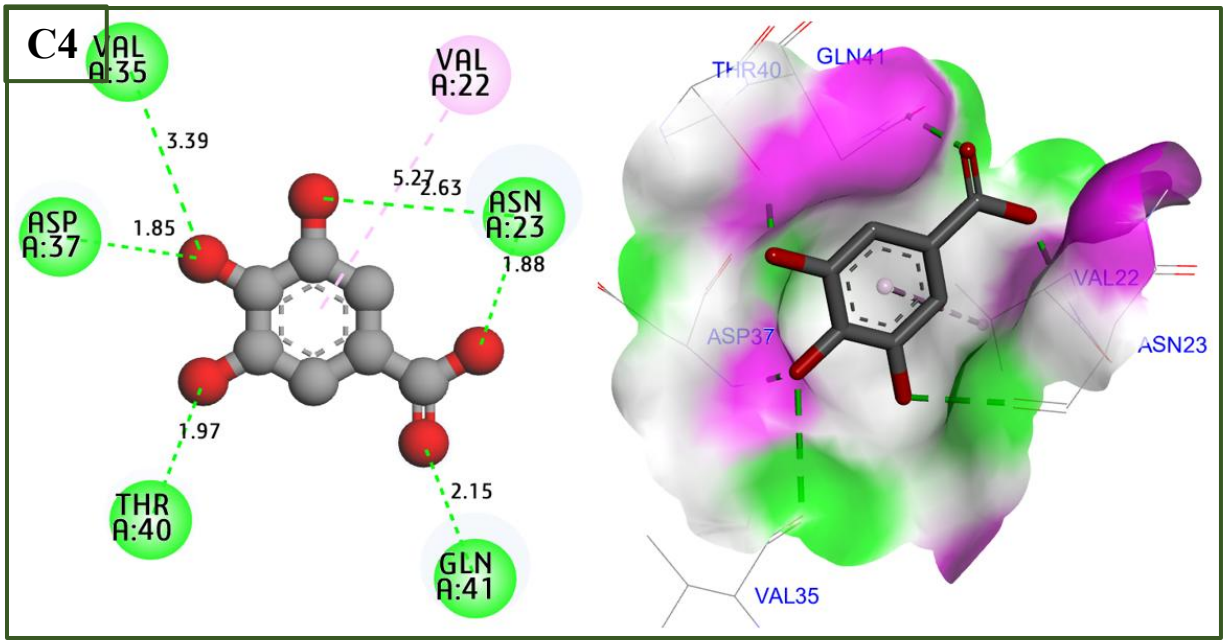
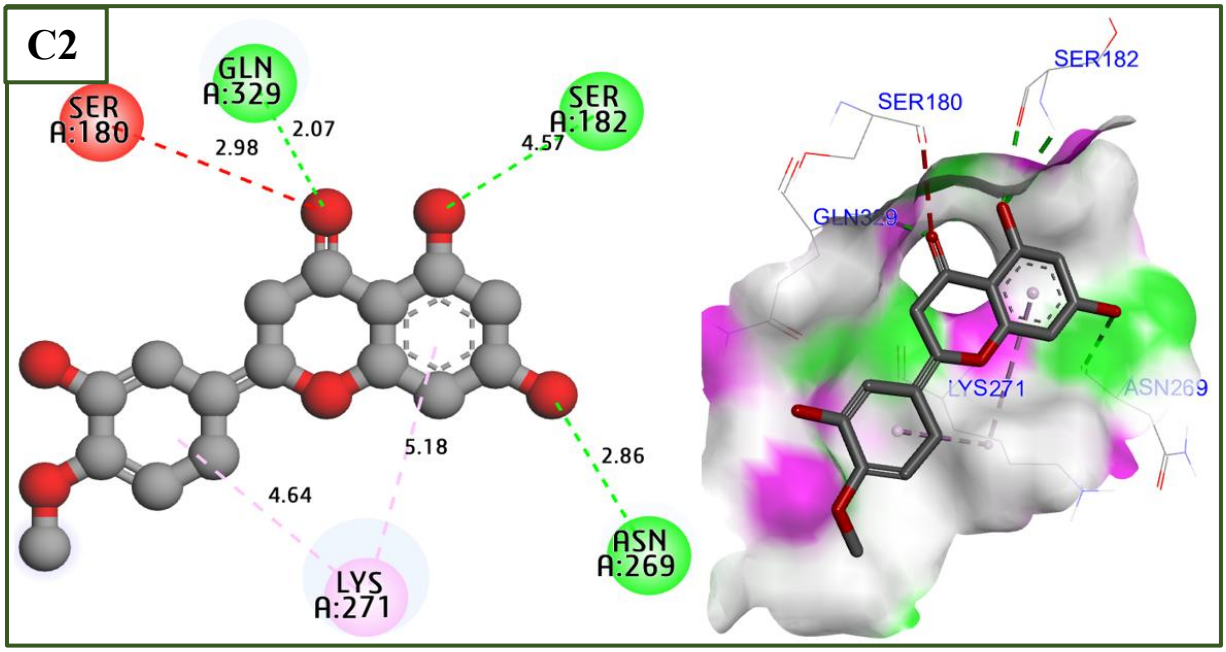


Figure 3. 2D and 3D views of docking results for C2 and C4 in complex with the crystal structure of the FimH lectin domain from Escherichia coli protein (PDB ID of 4XO8).



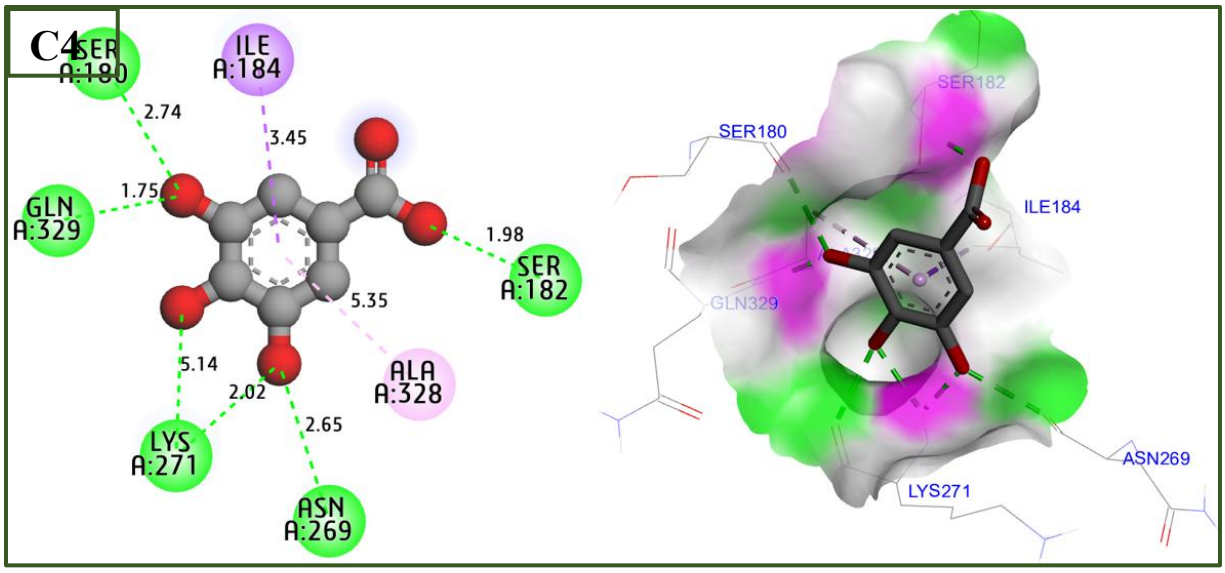


Figure 4. 2D and 3D views of docking results for C2 and C4 in complex with the secreted aspartic protease from Candida albicans protein (PDB ID of 1ZAP).

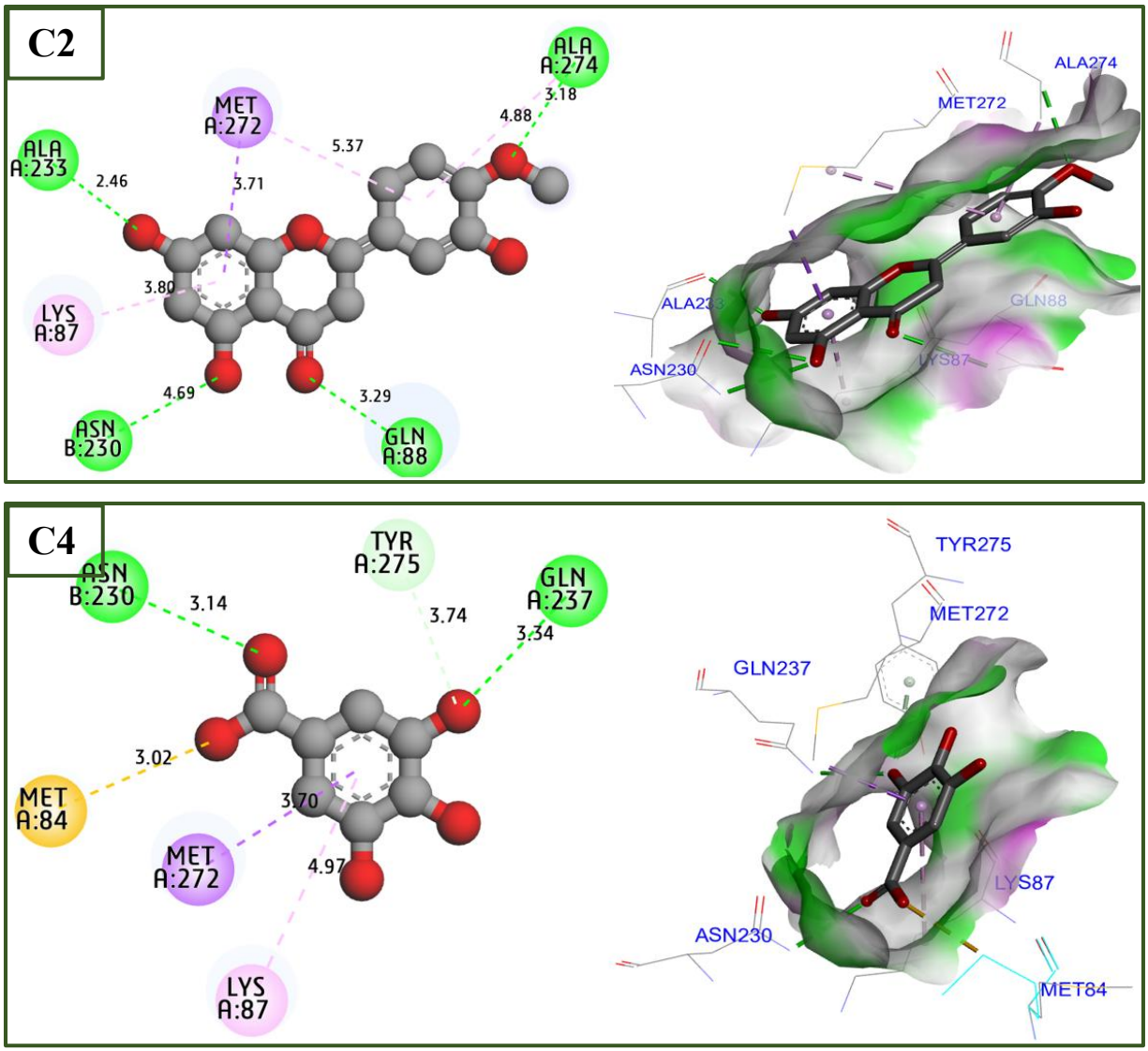


Figure 5. 2D and 3D views of docking results for C2 and C4 in the complex with the crystal structure of Bacillus cereus protein (PDB ID of 3U3W).

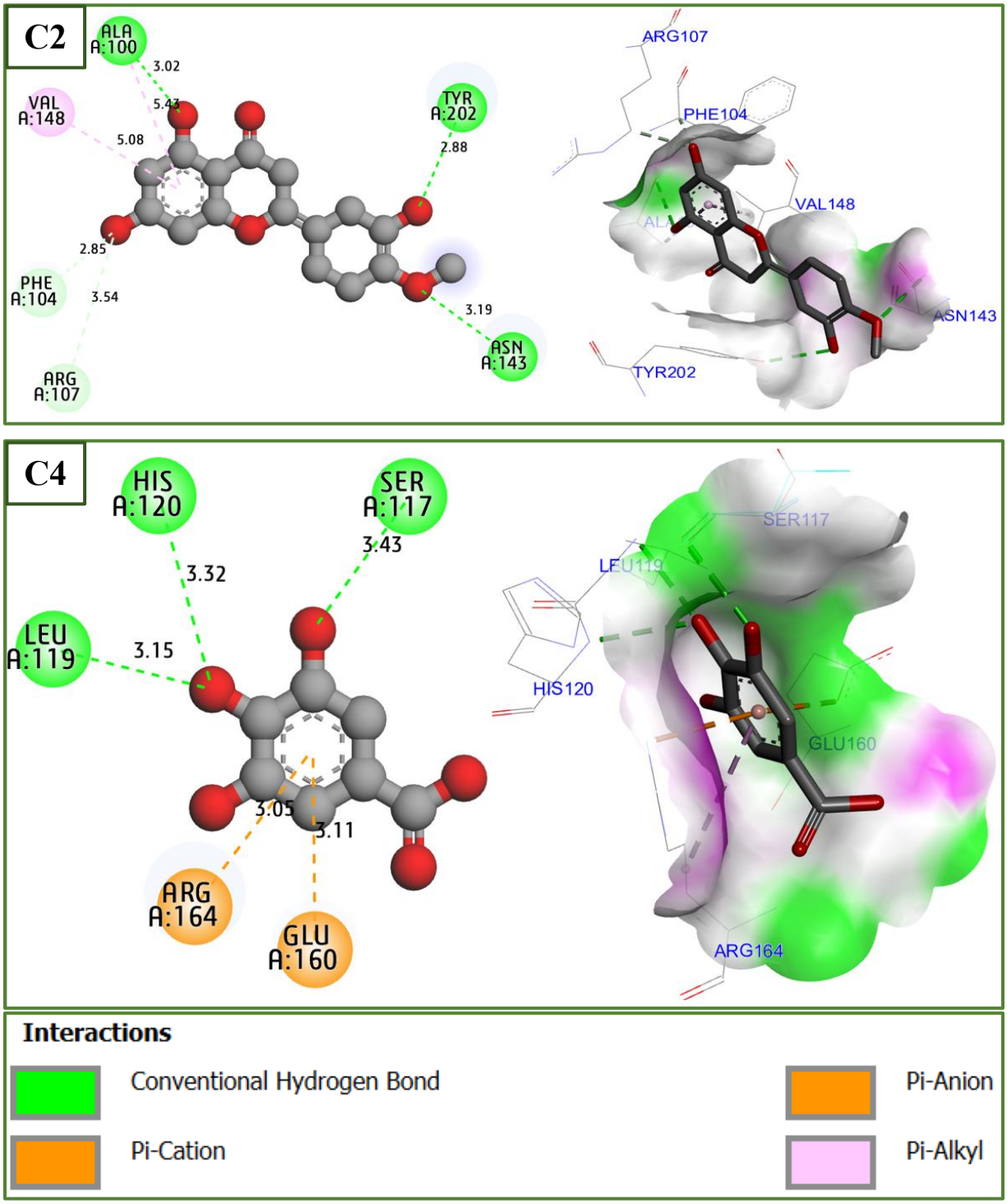


Figure 6. 2D and 3D views of docking results for C2 and C4 in the complex with the crystal structure of BCL-2 (PDB ID of 6O0K).

In-silico investigations play a crucial role in modern drug discovery and development by enabling the rapid and cost-effective assessment of compounds' pharmacokinetic properties and potential toxicity. These computational approaches, such as ADMET modeling and molecular docking, provide valuable insights into a molecule's behavior within biological systems, helping to prioritize the most promising candidates for further experimental validation. By predicting absorption, distribution, metabolism, excretion, and toxicity early in the research process, in-silico methods significantly reduce the risk of late-stage failures, accelerate the identification of lead compounds, and enhance the overall efficiency of therapeutic development. Through these investigations, we concluded that two compounds are predicted to be toxic, while Hesperetin (C2) and Gallic acid (C4) are predicted to be non-toxic. Both compounds demonstrated the ability to interact with antibacterial proteins targeting *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Bacillus cereus* pathogenic strains, exhibiting strong inhibition mechanisms supported by very low binding energies (in the range of kcal/mol). Notably, compound C2 consistently displayed lower binding energies compared to compound C4, indicating a stronger interaction with the targeted receptors. Both Hesperetin (C2) and Gallic acid (C4) showed promising inhibitory potential against the BCL-2 protein, engaging in key interactions within its binding groove. While C4 primarily formed hydrogen bonds and electrostatic interactions, C2 demonstrated a more favorable binding mode through multi-point anchoring with both hydrophobic and polar residues. This suggests that C2 may exhibit superior efficacy as a BCL-2 inhibitor due to its stronger and more diverse molecular interactions.

3.4. Antimicrobial activity of P. granatum peel

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the aqueous peel extract of *P. granatum* L. (aqE-PGP) were determined using the broth microdilution method. The results presented in Table 6, showed that the extract had strong antibacterial effects, particularly against Gram-positive bacteria. *Bacillus cereus* and *Staphylococcus aureus* were highly susceptible, with MIC values of 0.781 mg/mL and 1.56 mg/mL, respectively. In contrast, *Escherichia coli* showed greater resistance, with a higher MIC of 3.125 mg/mL.

The MBC values were consistent with the MIC results, further confirming the bactericidal properties of the extract, particularly against Gram-positive bacteria. These results suggest that *P. granatum* peel extract could be an effective antimicrobial agent, especially for treating Gram-positive bacterial infections.

Table 4. Antimicrobial effects of the aqueous leaf extract of *P. granatum* peel

	aqE-PGP		Chloramphenicol (µg/mL)
	MIC[a] (mg/mL)	MBC (MFC---- *C. albicans*) (mg/mL)	MIC(µg/mL)
E. coli	3.125	6.25	50
B. cereus	0.781	1.562	25
S. aureus	1.56	3.125	50
C.albicans	12.5	12.5	-

 MIC : Minimum inhibitory concentration (mg/mL) ; MBC : Minimum bactericidal concentration (mg/mL)

[a] Final bacterial density was around 10^6 CFU/mL.

Concerning the bacterial activity, our results demonstrate that the aqueous peel extract of *P. granatum* (*aqE-PGP*) exhibits notable antimicrobial activity, particularly against Gram-positive bacteria. The Minimum Inhibitory Concentration (MIC) values of 0.781 mg/mL for *Bacillus cereus* and 1.56 mg/mL for *Staphylococcus aureus* indicate potent antibacterial effects. In contrast, *Escherichia coli* displayed greater resistance with a higher MIC of 3.125 mg/mL. These findings align with previous studies that have reported varying degrees of antimicrobial activity of pomegranate peel extracts against different bacterial strains. For instance, a study by (Khan and Hanee 2011) found that pomegranate peel extracts exhibited high antimicrobial activity against *E. coli* and *S. aureus*, with inhibition zones of 21.8 mm and 23.7 mm, respectively. Similarly, a study by (Nasreddine et al. 2018) reported that pomegranate peel extracts had significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli*. These studies support our findings and suggest that pomegranate peel extracts possess broad-spectrum antimicrobial properties. The higher resistance observed in *E. coli* in our study is consistent with findings from other research. For example, a study by (Kupnik et al. 2021) found that pomegranate peel extracts exhibited varying degrees of antimicrobial activity against different bacterial strains, with *E. coli* showing higher resistance compared to *S. aureus*. This difference in susceptibility may be attributed to the structural differences between Gram-positive and Gram-negative bacteria, as well as the presence of efflux pumps and outer membrane barriers in Gram-negative bacteria that can limit

the penetration of antimicrobial compounds. The consistency between the MIC and Minimum Bactericidal Concentration (MBC) values further supports the bactericidal properties of the *aqE-PGP*, particularly against Gram-positive bacteria. In conclusion, the aqueous peel extract of *Punica granatum* shows promising antimicrobial activity, especially against Gram-positive bacteria. These findings suggest that pomegranate peel extract could be explored as a potential natural antimicrobial agent.

3.5. Cytotoxicity assay

In this study, we evaluated the cytotoxic potential of aqE-PGP on myeloma (P3) cell lines and splenocytes. The results, presented in Figure 7 and Table 5, indicate that *P. granatum* extract exhibited greater cytotoxicity toward splenocytes compared to P3 cell lines ($p < 0.001$). Specifically, the extract achieved a maximum inhibition of 71.12 ± 3.53% with an IC_{50} of 708.7 ± 49.46 µg/mL in splenocytes, whereas in P3 cell lines, the maximum inhibition was 63.95 ± 2.54%, with an IC_{50} of 950.3 ± 43.8 µg/mL.

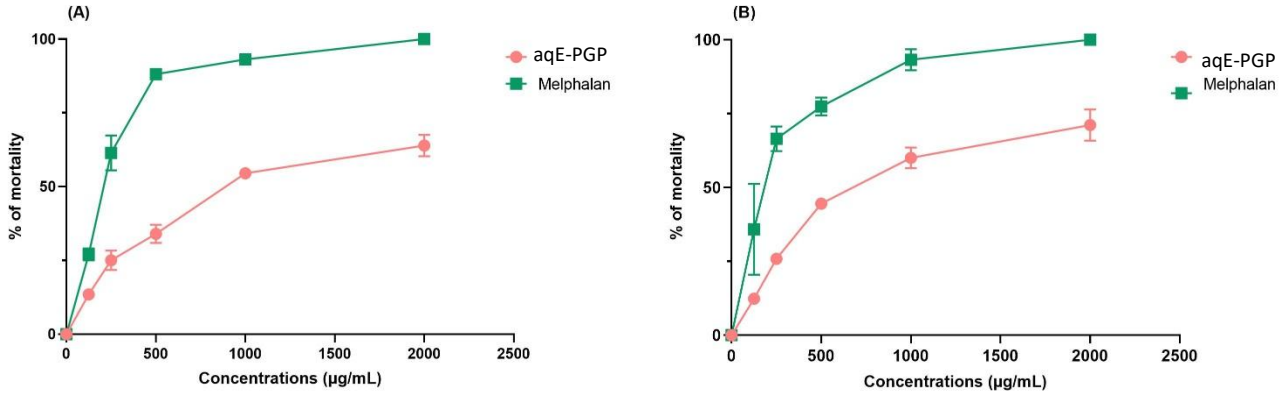


Figure 7. Evaluation of anticancer activity of *Punica granatum* on P3 cancer cell lines (A) and splenocytes (B) using MTT Assay.

Table 5. The cytotoxic effect of *P. granatum* on P3 cancer cell lines and splenocytes, expressed as IC_{50} (µg/mL).

	P. granatum IC_{50} (µg/mL)	Melphalan IC_{50}(µg/mL)
Myeloma (P3) cell lines	950.3 ± 43.8*	200.76 ± 11.95
Splenocytes	708.7 ± 49.46*	173.8 ± 27.4

Results are presented as mean ± SD (n=3). * $p < 0.0002$, P3 cell lines treated with *P. granatum* extract vs splenocytes treated with *P. granatum* extract by Ordinary one-way ANOVA test.

Evaluating the anticancer properties of medicinal plants is a key step in the search for natural therapeutic agents. This process is fundamental for identifying bioactive compounds with promising effects against different cancer types. It allows researchers to explore the mechanisms of action, cytotoxic effects, and overall biological activities of plant extracts. Such investigations not only deepen our understanding of how natural compounds interact with cancer cells but also support the development of innovative, plant-based treatments that could complement or improve current cancer therapies (Bajpai et al. 2024). In this study, we evaluated the cytotoxic potential of *P. granatum* extract on myeloma (P3) cell lines and splenocytes. Our findings demonstrate that *P. granatum* extract exhibits cytotoxic effects on both cell types, with a more pronounced effect on normal cells. The higher sensitivity of splenocytes to *P. granatum* extract might be attributed to differences in cellular metabolism and antioxidant defenses between normal and cancerous cells. Splenocytes, as normal immune cells, may be more vulnerable to oxidative stress and phytochemical-induced apoptosis compared to the more resilient, rapidly dividing P3 myeloma cells (Reuter et al. 2010). Furthermore, the UHPLC-MS/MS analysis of *P. granatum* extract reveals a diverse profile of bioactive polyphenols, including phenolic acids and flavonoids, which likely contribute to its cytotoxic effects on both myeloma (P3) cell lines and splenocytes. In fact, these compounds exert anticancer effects by triggering apoptosis in malignant cells by inducing oxidative stress, they may also induce cytotoxicity in normal immune cells due to their pro-oxidant activity at high concentrations (Upadhyay, Singh, and Vishwakarma 2024). Additionally, we can suggest that myeloma (P3) cells, often develop resistance mechanisms, such as upregulated antioxidant systems (e.g., glutathione, superoxide dismutase) and enhanced DNA repair pathways, which could contribute to their relatively higher IC_{50} values. This may explain why the P3 cells require (Pelicano, Carney, and Huang 2004). Finally, the cytotoxic effects of *P. granatum* extract on myeloma (P3) cells and splenocytes are likely mediated by its rich polyphenolic composition. While these compounds exhibit anticancer properties through oxidative stress induction, apoptosis, and inhibition of proliferation, their effects on normal immune cells, such as splenocytes, should be carefully considered. Further studies should focus on optimizing the selectivity of *P. granatum* extract for cancer cells while minimizing its impact on normal immune function.

Conclusion

This study demonstrates the significant pharmacological potential of the aqueous peel extract of *Punica granatum* L., collected from eastern Morocco. The UHPLC analysis revealed a high content of bioactive phenolic and flavonoid compounds, with hesperetin and gallic acid emerging as key constituents due to their notable biological activities. The extract exhibited strong antimicrobial effects, especially against Gram-positive bacterial strains, and showed selective cytotoxicity, indicating possible cell-type specificity. Additionally, *in silico* evaluations reinforced the therapeutic relevance of several identified compounds through their high binding affinity to antimicrobial and anticancer targets, including the BCL-2 protein. These insights support the potential use of pomegranate peel extract as a natural source for developing new antimicrobial and anticancer therapeutics. However, further research, particularly *in vivo* and clinical studies, is essential to confirm their efficacy and safety in practical medical applications.

Supplementary Materials: The following are available online, File S1: LC-MS/MS polyphenols analysis aqueous crude extract of *P. granatum* peel

Author contributions: FK: conceptualization, data curation, formal analysis, investigation, writing–original draft, and writing–review and editing. KE: methodology, resources, software. ME: methodology, resources, software. MAZ and CS: investigation, methodology, and writing–original draft. OE and MB: data curation, methodology, software. RC, FAC, and AAQ: funding acquisition, resources, writing–original draft, and editing. FZL: formal analysis, writing–original draft, conceptualization, writing–review and editing.

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Conflicts of Interest: The authors confirm that the research was carried out without any commercial or financial relationships that could be interpreted as a potential conflict of interest.

Data availability: All data generated or analysed during this study are included in this published article.

Reference

Abdul Qahir, Attiq-Ur-Rehman Kakar, Naqeebullah Khan, Samiullah, Abdul Hakeem, Rehana Kamal, and Fazal-Ur-Rehman. 2021. “The Antioxidant, Antimicrobial, and Clinical Effects with Elemental Contents of Pomegranate (Punica Granatum) Peel Extracts: A Review.” *Baghdad Journal of Biochemistry and Applied Biological Sciences* 2 (01): 21–28. https://doi.org/10.47419/bjbabs.v2i01.33.

Abid, Mouna, Héla Yaich, Salma Cheikhrouhou, Ibtihel Khemakhem, Mohamed Bouaziz, Hamadi Attia, and M A Ayadi. 2017. “Antioxidant Properties and Phenolic Profile Characterization by LC–MS/MS of Selected Tunisian Pomegranate Peels.” *Journal of Food Science and Technology* 54: 2890–2901.

Akhtar, Saeed, Tariq Ismail, Daniele Fraternale, and Piero Sestili. 2015. “Pomegranate Peel and Peel Extracts: Chemistry and Food Features.” *Food Chemistry* 174: 417–25.

Aleksandrova, Simona, Ralitza Alexova, Stela Dragomanova, Reni Kalfin, Ferdinando Nicoletti, Paolo Fagone, Maria Cristina Petralia, Katia Mangano, and Lyubka Tancheva. 2023. “Preventive and Therapeutic Effects of Punica Granatum L. Polyphenols in Neurological Conditions.” *International Journal of Molecular Sciences* 24 (3). https://doi.org/10.3390/ijms24031856.

Aloui, Mourad, Mohamed El fadili, Somdutt Mujwar, Sara Er-rahmani, Hatem A. Abuelizz, Mohammed Er-rajy, Sara Zarougui, and Menana Elhallaoui. 2024. “Design of Novel Potent Selective Survivin Inhibitors Using 2D-QSAR Modeling, Molecular Docking, Molecular Dynamics, and ADMET Properties of New MX-106 Hydroxyquinoline Scaffold Derivatives.” *Heliyon* 10 (19): e38383. https://doi.org/10.1016/j.heliyon.2024.e38383.

Bahmanzadegan, Atefeh, Hossein Tavallali, Vahid Tavallali, and Mohammad Ali Karimi. 2023. “Bioactive Compounds of Punica Granatum L. Wastes by High Performance Liquid Chromatography Analysis.” *Natural Product Research* 37 (14): 2432–36. https://doi.org/10.1080/14786419.2022.2041634.

Bajpai, Priyanka, Shazia Usmani, Rakesh Kumar, and Om Prakash. 2024. “Recent Advances in Anticancer Approach of Traditional Medicinal Plants: A Novel Strategy for Cancer Chemotherapy.” *Intelligent Pharmacy* 2 (3): 291–304.

Benchagra, L, H Berrougui, M O Islam, M Ramchoun, S Boulbaroud, A Hajjaji, and T Fulop. 2021. “Antioxidant Effect of Moroccan Pomegranate (Punica Granatum l. Sefri Variety)

Extracts Rich in Punicalagin against the Oxidative Stress Process. Foods 10 (9): 2219."

Borges, Gina, and Alan Crozier. 2012. "HPLC–PDA–MS Fingerprinting to Assess the Authenticity of Pomegranate Beverages." *Food Chemistry* 135 (3): 1863–67.

Brighenti, Virginia, Sebastiaan Frearick Groothuis, Francesco Pio Prencipe, Rachel Amir, Stefania Benvenuti, and Federica Pellati. 2017. "Metabolite Fingerprinting of Punica Granatum L.(Pomegranate) Polyphenols by Means of High-Performance Liquid Chromatography with Diode Array and Electrospray Ionization-Mass Spectrometry Detection." *Journal of Chromatography A* 1480: 20–31.

Çam, Mustafa, Necattin Cihat İçyer, and Fatma Erdoğan. 2014. "Pomegranate Peel Phenolics: Microencapsulation, Storage Stability and Potential Ingredient for Functional Food Development." *LWT-Food Science and Technology* 55 (1): 117–23.

Chraibi, Marwa, Mouhcine Fadil, Abdellah Farah, Sara Lebrazi, and Kawtar Fikri-Benbrahim. 2021. "Antimicrobial Combined Action of Mentha Pulegium, Ormenis Mixta and Mentha Piperita Essential Oils against S. Aureus, E. Coli and C. Tropicalis: Application of Mixture Design Methodology." *Lwt* 145: 111352.

Cruz-Valenzuela, Manuel Reynaldo, Rosa E. Ayala-Soto, Jesus Fernando Ayala-Zavala, Brenda A. Espinoza-Silva, Gustavo A. González-Aguilar, Olga Martín-Belloso, Robert Soliva-Fortuny, et al. 2022. "Pomegranate (Punica Granatum L.) Peel Extracts as Antimicrobial and Antioxidant Additives Used in Alfalfa Sprouts." *Foods* 11 (17). https://doi.org/10.3390/foods11172588.

Ed-Dahmani, Imad, Mohamed El Fadili, Ghizlane Nouioura, Fahd Kandsi, Yassine El Atki, Hatem A. Abuelizz, Raffaele Conte, et al. 2024. "Ferula Communis Leaf Extract: Antioxidant Capacity, UHPLC–MS/MS Analysis, and in Vivo and in Silico Toxicity Investigations." *Frontiers in Chemistry* 12 (January): 1–17. https://doi.org/10.3389/fchem.2024.1485463.

El-Assri, El Mehdi, Youssef El-Assri, Rajae El Brahimi, Mohamed El fadili, Asmae Baghouz, Hatem A. Abuelizz, Sara Er-Rahmani, Amal Lahkimi, and Abdelhak Bouia. 2025. "Molecular Characterization, Chemical Profile and Biological Properties of Essential Oils from Chamaemelum Nobile (L.) Flowers of Morocco: In Vitro and in Silico Studies." *Frontiers in Chemistry* 13 (February): 1–13. https://doi.org/10.3389/fchem.2025.1539872.

Elkoraichi, Ismail, Mounia Latif, Othman El Faqer, Rajaa Elaje, Abderrahmane Thoume, El

Mostafa Mtairag, Samira Rais, and Fouad Daoudi. 2022. “In Vivo and in Vitro Evaluation of Antioxidant, Antihemolytic, Acute Toxicity Effects and High-Performance Liquid Chromatography Analysis of an Aqueous Extract of Moroccan Punica Granatum L.” *Journal of Herbmed Pharmacology* 12 (1): 168–75.

Elouaddari, Anass, Abdelaziz Elamrani, Mouna Moutia, Nadia Oubrim, Noureddine Habti, and Jamal JamalEddine. 2019. “Chemical Composition and Evaluation of Antioxidant, Antimicrobial and Cytotoxic Activities of Moroccan Cladanthus Mixtus Essential Oil and Extracts.” *Journal of Essential Oil Bearing Plants* 22 (6): 1450–66.

Er-rahmani, Sara, Francesco Trotta, Adrián Matencio, Badr Errabiti, Soumya El Abed, Hassan Latrache, and Saad Ibnsouda Koraichi. 2024. “Antimicrobial and Antiadhesive Activities of Secondary Metabolites against Bacillus Cereus Adhesion on PLA 3D Printing Material: ADMET Tox in Silico, Molecular Docking and Molecular Dynamic Analysis.” *Scientific African* 24: e02209.

fadili, Mohamed El, Mohammed Er-rajy, Mohnad Abdalla, Hatem A. Abuelizz, Sara Zarougui, Fadwa Mohammed Alkhulaifi, Nada F. Alahmady, Ashwag Shami, and Menana Elhallaoui. 2024. “In-Silico Investigations of Novel Tacrine Derivatives Potency against Alzheimer’s Disease.” *Scientific African* 23 (December 2023): e02048. https://doi.org/10.1016/j.sciaf.2023.e02048.

fadili, Mohamed El, Mohammed Er-rajy, Wafa Ali Eltayb, Mohammed Kara, Amine Assouguem, Asmaa Saleh, Omkulthom Al Kamaly, Sara Zarougui, and Menana Elhallaoui. 2023. “In-Silico Screening Based on Molecular Simulations of 3,4-Disubstituted Pyrrolidine Sulfonamides as Selective and Competitive GlyT1 Inhibitors.” *Arabian Journal of Chemistry* 16 (10): 105105. https://doi.org/10.1016/j.arabjc.2023.105105.

fadili, Mohamed El, Mohammed Er-rajy, Hamada Imtara, Mohammed Kara, Sara Zarougui, Najla Altwaijry, Omkulthom Al kamaly, Aisha Al Sfouk, and Menana Elhallaoui. 2022. “3D-QSAR, ADME-Tox In Silico Prediction and Molecular Docking Studies for Modeling the Analgesic Activity against Neuropathic Pain of Novel NR2B-Selective NMDA Receptor Antagonists.” *Processes* 10 (8). https://doi.org/10.3390/pr10081462.

fadili, Mohamed El, Mohammed Er-rajy, Hamada Imtara, Omar M. Noman, Ramzi A. Mothana, Sheaf Abdullah, Sara Zerougui, and Menana Elhallaoui. 2023. “QSAR, ADME-

Tox, Molecular Docking and Molecular Dynamics Simulations of Novel Selective Glycine Transporter Type 1 Inhibitors with Memory Enhancing Properties." *Heliyon* 9 (2): e13706. https://doi.org/10.1016/j.heliyon.2023.e13706.

Fadili, Mohamed El, Mohammed Er-Rajy, Mohammed Kara, Amine Assouguem, Assia Belhassan, Amal Alotaibi, Nidal Naceiri Mrabti, et al. 2022. "QSAR, ADMET In Silico Pharmacokinetics, Molecular Docking and Molecular Dynamics Studies of Novel Bicyclo (Aryl Methyl) Benzamides as Potent GlyT1 Inhibitors for the Treatment of Schizophrenia." *Pharmaceuticals* 15 (6). https://doi.org/10.3390/ph15060670.

fadili, Mohamed El, Mohammed Er-rajy, Somdutt Mujwar, Abduljelil Ajala, Rachid Bouzammit, Mohammed Kara, Hatem A. Abuelizz, Sara Er-rahmani, and Menana Elhallaoui. 2024. "In Silico Insights into the Design of Novel NR2B-Selective NMDA Receptor Antagonists: QSAR Modeling, ADME-Toxicity Predictions, Molecular Docking, and Molecular Dynamics Investigations." *BMC Chemistry* 18 (1): 1–22. https://doi.org/10.1186/s13065-024-01248-6.

Jeddi, Mohamed, Naoufal El Hachlafi, Mohamed El Fadili, Nesrine Benkhaira, Samiah Hamad Al-Mijalli, Fahd Kandsi, Emad M Abdallah, Zineb Benziane Ouaritini, Abdelhakim Bouyahya, and Learn-Han Lee. 2023. "Antimicrobial, Antioxidant, α-Amylase and α-Glucosidase Inhibitory Activities of a Chemically Characterized Essential Oil from Lavandula Angustifolia Mill.: In Vitro and in Silico Investigations." *Biochemical Systematics and Ecology* 111: 104731.

Kahramanoglu, Ibrahim, and Serhat Usanmaz. 2016. *Pomegranate Production and Marketing*. CRC Press.

Kandsi, Fahd, Raffaele Conte, Mohamed Marghich, Fatima Zahra Lafdil, Mohamed F Alajmi, Mohamed Bouhrim, Hamza Mechchate, Christophe Hano, Mohammed Aziz, and Nadia Gseyra. 2021. "Phytochemical Analysis, Antispasmodic, Myorelaxant, and Antioxidant Effect of Dysphania Ambrosioides (L.) Mosyakin and Clemants Flower Hydroethanolic Extracts and Its Chloroform and Ethyl Acetate Fractions." *Molecules* 26 (23): 7300. https://doi.org/10.3390/molecules26237300.

Kandsi, Fahd, Amine Elbouzidi, Fatima Zahra Lafdil, Nada Meskali, Ali Azghar, Mohamed Addi, Christophe Hano, Adil Maleb, and Nadia Gseyra. 2022. "Antibacterial and Antioxidant Activity of Dysphania Ambrosioides (L.) Mosyakin and Clemants Essential

Oils: Experimental and Computational Approaches." *Antibiotics* 11 (4). https://doi.org/10.3390/antibiotics11040482.

Karimi, Mahsan, Rohollah Sadeghi, and Jozef Kokini. 2017. "Pomegranate as a Promising Opportunity in Medicine and Nanotechnology." *Trends in Food Science & Technology* 69: 59–73.

Khan, Jahir Alam, and Sonali Hanee. 2011. "Antibacterial Properties of Punica Granatum Peels." *International Journal of Applied Biology and Pharmaceutical Technology* 2 (3): 23–27.

Khemakhem, Maissa, Youkabed Zarroug, Khaled Jabou, Sawsen Selmi, and Nabiha Bouzouita. 2021. "Physicochemical Characterization of Oil, Antioxidant Potential, and Phenolic Profile of Seeds Isolated from Tunisian Pomegranate (Punica Granatum L.) Cultivars." *Journal of Food Science* 86 (3): 852–59. https://doi.org/10.1111/1750-3841.15636.

Kiran, Saba, Asma Aslam, Asma Parveen, Muhammad Dilshad, and Shabbir Hussain. 2024. "Phytochemistry of Punica Granatum Fruit: Its Nutritional and Biological Potential." *Bioactivities* 2 (1): 57–73. https://doi.org/10.47352/bioactivities.2963-654x.220.

Kupnik, Kaja, Mateja Primožič, Katja Vasić, Željko Knez, and Maja Leitgeb. 2021. "A Comprehensive Study of the Antibacterial Activity of Bioactive Juice and Extracts from Pomegranate (Punica Granatum l.) Peels and Seeds." *Plants* 10 (8). https://doi.org/10.3390/plants10081554.

Laaraj, Nassima, Mohamed Bouhrim, Loubna Kharchoufa, Salima Tiji, Hasnae Bendaha, Mohamed Addi, Samantha Drouet, et al. 2022. "Phytochemical Analysis, α-Glucosidase and α-Amylase Inhibitory Activities and Acute Toxicity Studies of Extracts from Pomegranate (Punica Granatum) Bark, a Valuable Agro-Industrial By-Product." *Foods* 11 (9). https://doi.org/10.3390/foods11091353.

Lafdil, Fatima Zahra, Abdelkhaleq Legssyer, Abderrahim Ziyyat, Ahmed Karim, Ali Berraaouan, Rachid Seddik, and Hassane Mekhfi. 2025. "Antithrombotic Activity of Punica Granatum, L (Pomegranate): Experimental Investigation and Exploration of Its Mechanism of Action on Primary and Secondary Hemostasis in Vitro and Ex Vivo." *Journal of Molecular Structure* 1321: 140195.

Lakka, Narasimha S., Chandrasekar Kuppan, Niroja Vadagam, Poornima Ravinathan, Kalyani Chepuri, and Sanjeeva R. Chinnakadoori. 2023. "Molecular Docking, in-Vitro Anticancer

Evaluation and ADME Profiling of 7-Oxo Midostaurin." *Journal of Molecular Structure* 1293 (April): 136159. https://doi.org/10.1016/j.molstruc.2023.136159.

Lebrazi, Sara, Kawtar Fikri-Benbrahim, Mohamed Jeddi, Abderrazak Aboulghazi, Zineb Benziane Ouaritini, Chaimae Moufakkir, Yassine Kharbach, Hicham Harhar, Abdelhakim Bouyahya, and Chaimae Najjari. 2025. "Investigating the Synergistic Action of Three Essential Oils for Antibacterial and Antioxidant Applications: A Comprehensive Framework Using Mixture Design, Artificial Neural Networks, and in Silico Methods." *Food Bioscience* 67: 106250.

Leesombun, Arpron, Ladawan Sariya, Jarupha Taowan, Chowalit Nakthong, Orathai Thongjuy, and Sookruetai Boonmasawai. 2022. "Natural Antioxidant, Antibacterial, and Antiproliferative Activities of Ethanolic Extracts from Punica Granatum L. Tree Barks Mediated by Extracellular Signal-Regulated Kinase." *Plants* 11 (17): 2258.

Mosmann, Tim. 1983. "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays." *Journal of Immunological Methods* 65 (1–2): 55–63.

Nasreddine, S, A Mcheik, MA Al-Seblani, H Shahrour, M Hammoud, and A Chokr. 2018. "Influence of the Extractive Method of Punica Granatum Peels in Its Antibacterial and Antibiofilm Activities Against Gram-Negative and Gram-Positive Bacteria." *Researchgate.Net* 4 (6): 133–41. https://doi.org/10.11648/j.jdmp.20180406.11.

Nouioura, Ghizlane, Mohamed El fadili, Azeddin El Barnossi, El Hassania Loukili, Hassan Laaroussi, Mohammed Bouhrim, John P. Giesy, et al. 2024. "Comprehensive Analysis of Different Solvent Extracts of Ferula Communis L. Fruit Reveals Phenolic Compounds and Their Biological Properties via in Vitro and in Silico Assays." *Scientific Reports* 14 (1): 1–19. https://doi.org/10.1038/s41598-024-59087-3.

Özcan, Mehmet Musa, Mohammed Saeed Alkaltham, Nurhan Uslu, and Ahmad Salamatullah. 2021. "Effect of Different Roasting Methods on the Bioactive Properties, Phenolic Compounds and Fatty Acid Compositions of Pomegranate (Punica Granatum L. Cv. Hicaz) Seed and Oils." *Journal of Food Science and Technology* 58 (6): 2283–94. https://doi.org/10.1007/s13197-020-04739-1.

Pelicano, Helene, Dennis Carney, and Peng Huang. 2004. "ROS Stress in Cancer Cells and Therapeutic Implications." *Drug Resistance Updates* 7 (2): 97–110.

Reuter, Simone, Subash C Gupta, Madan M Chaturvedi, and Bharat B Aggarwal. 2010. "Oxidative Stress, Inflammation, and Cancer: How Are They Linked?" *Free Radical Biology and Medicine* 49 (11): 1603–16.

Shahkoomahally, Shirin, Doosan Shin, Fariborz Habibi, Jeongim Kim, and Ali Sarkhosh. 2023. "Profiling Phenolic Compounds in Juice and Peel of Fourteen Pomegranate (Punica Granatum L.) Varieties Grown in Florida, USA." *Food Chemistry Advances* 2 (February). https://doi.org/10.1016/j.focha.2023.100225.

Singh, Balwinder, Jatinder Pal Singh, Amritpal Kaur, and Narpinder Singh. 2018. "Phenolic Compounds as Beneficial Phytochemicals in Pomegranate (Punica Granatum L.) Peel: A Review." *Food Chemistry* 261: 75–86.

Souhaili, Zineb, Hicham Mohammadi, Noureddine Habti, and Mohamed Faid. 2008. "Effet Létal de l'extrait Aqueux de l'algue Brune Marine (Cystoseira Tamariscifolia) Sur La Souris et Sur Les Cellules Tumorales Du Myélome Murin." *Afrique Science: Revue Internationale Des Sciences et Technologie* 4 (3).

Upadhyay, Prabhat K, Sonia Singh, and Vishal Kumar Vishwakarma. 2024. "Natural Polyphenols in Cancer Management: Promising Role, Mechanisms, and Chemistry." *Current Pharmaceutical Biotechnology* 25 (6): 694–712.

Wang, Dongdong, Cigdem özen, Ibrahim M. Abu-Reidah, Sridevi Chigurupati, Jayanta Kumar Patra, Jaroslaw O. Horbanczuk, Artur Józwik, Nikolay T. Tzvetkov, Pavel Uhrin, and Atanas G. Atanasov. 2018. "Vasculoprotective Effects of Pomegranate (Punica Granatum L.)." *Frontiers in Pharmacology* 9 (MAY): 1–15. https://doi.org/10.3389/fphar.2018.00544.

Zandonai, Rodrigo Hermes, Fabiana Coelho, Juliana Ferreira, Ana Karla Bittencourt Mendes, Maique Weber Biavatti, Rivaldo Niero, Valdir Cechinel Filho, and Ednéia Casagranda Bueno. 2010. "Evaluation of the Proliferative Activity of Methanol Extracts from Six Medicinal Plants in Murine Spleen Cells." *Brazilian Journal of Pharmaceutical Sciences* 46: 323–33.

Zhang, Chunping, Yibao Ning, Zhongqiu Zhang, Li Song, Huishen Qiu, and Heyi Gao. 2008. "In Vitro Antimicrobial Susceptibility of Streptococcus Suis Strains Isolated from Clinically Healthy Sows in China." *Veterinary Microbiology* 131 (3–4): 386–92.

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