Egypt. J. Chem. **Vol. 68**, No. 8 pp. 577- 592 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Salvadora Persica(Miswak) Extract Inhibits Invasion of Oral Malignant Cells : In-vitro and In-silico Assessment

Marwa M. Ellithy[1*] and Abdulrahman M. Saleh[2]

[1]*Associate professor of oral pathology, Basic Dental science Department, Oral Research Institute, National Research Centre, Giza, Egypt.*

[2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo, Egypt*

Abstract

Background: Carcinogenesis is a multistep disease evolving from cells with unlimited proliferation, aborted death and acquired invasion potential. Intervention in each step may stop subsequent evolution. The most prevalent type of cancer in the oral cavity is squamous cell carcinoma (SCC). Mortality is due to invasion and spread of malignant cells to vital structures. Invasion is a crucial property gained by cancer cells via upregulation of a set of genes. In oral SCCs, there is an upregulation of matrix metalloproteinases (MMPs), particularly MMP-2, in stages of lymph node involvement. Research on MMP-2 expression in oral squamous cell carcinoma is, however, scarce. A significant part in dealing with cancer has been the use of medicinal plants. The majority of plant secondary metabolites' and their derivatives' uses have been focused on preventing cancer. Salvadora Persica known also as *Miswak* is a well reputable plant with known pharmacological impacts. Testing new anticancer drugs from natural sources for their cytotoxic action against human cancer cell lines (in vitro) or utilizing animals (in vivo) as model systems or in silico research led to the discovery of many of these compounds and their molecular mode of action. Therefore, documenting the molecular expression of MMP-2, an invasion protein, in OSCC is therefore of great value. **Results:** *Miswak* extract inhibited the production of MMP-2 protein in Hep-2 cells using ELISA assay. Molecular Docking proved that eight components of Salvadora Persica can bind efficiently to active sites in MMP-2 protein so inhibit its function. **Conclusion:** This work focuses for the first time on Miswak antiinvasive potential both in vitro and in silico. This research is a step for further preclinical and clinical applications trying to decrease the challenge of oral squamous cell carcinoma invasion and subsequent metastasis to surrounding as well as remote tissues.

Keywords: Salvadora persica, MMP-2, molecular docking studies, OSCC, alkaloids, flavonoids.

1. Introduction

The most common malignant epithelial tumor in the head and neck region is squamous cell carcinoma [20]. Like other cancers, oral squamous cell carcinoma (OSCC) is brought on by DNA mutations; it develops spontaneously but can get worse when exposed to various mutagens, including chemical, physical, and microbiological causes. About half of all oral cancers are caused by chewing betel nut in the region where chewing betel nut usage has expanded, 25% are caused by tobacco use (smoking, chewing, or both), 10%–15% are caused by micronutrient deficiencies, and 7–19% are caused by alcohol intake worldwide. Scleromatosis accounts for approximately 95% of mouth cancer cases [20]. Various DNA changes have the potential to convert oral keratinocytes, the source cell of OSCC, from a normal keratinocyte to a premalignant or potentially malignant keratinocyte. This transformation would increasein less constraint than usual, and develop cells on their own to create actual cancer [19]. Although they can originate from the oral movable tongue, oral squamous cell carcinomas more frequently originate from other anatomical sites inside the oral cavity and oropharynx [6].

Three phases can be distinguished in a tumor cell's invasion and metastasis: attachment, matrix disintegration, and movement. Invasion occurs only when the three stages come together. This process involves several extracellular matrix-degrading enzymes, such as matrix metalloproteinases (MMPs), cysteine proteinases, and serine proteinases, in conjunction with a number of tumor-host interactions. MMPs have a significant impact on the neoplasm's malignant behavior [20].

The extracellular matrix's constituent parts are all broken down by the zinc-binding endopeptidases known as matrix metalloproteinases (MMPs).

Collagenases (MMP-1, 8, 13, 18), gelatinases (MMP-2, 9), stromelysins (MMP-3, 10, 11), matrilysins (MMP-7, 26), membrane type MMPs (MMP-14, 15, 16, 17, 24, 25), and other enzymes make up the MMP family as a whole. The normal physiological functions of the mammary gland and postpartum uterus involution, wound healing, angiogenesis, and cervical dilatation are all dependent on them. However, excessive MMP activities have been linked to a number of diseases, including tumors, arthritis, periodontal diseases, liver cirrhosis, atherosclerosis, and cancer [34]. One important enzyme in the MMP family is MMP-2 [5]. It can cause tumor metastasis and spread by breaking down the basal membrane and extracellular matrix (ECM). Tumor and stromal cells are the primary sources of pro-enzyme MMP-2 secretion[26]. Additionally, it has been

*Corresponding author e-mail:moonlight20_7@yahoo.com; (Marwa Ellithy).

Received Date: 10 September 2024, Revised Date: 02 December 2024, Accepted Date: 07 December 2024

DOI:10.21608/EJCHEM.2024.319107.10371

documented that tumor cells can express MMP-2 toto break down type IV collagens, which in turn causes these tissue barriers to be destroyed, allowing tumor cells to invade and spread [27].
Recent studies have shown a favorable link between the propensity for tumor invasion and metastasis and the pattern of MMP expression. Consequently, MMPs have emerged as a primary target for drug discovery in the search for potent inhibitors [27].
MMP inhibitors serve a variety of biological purposes throughout the whole course of cancer growth. They are investigated in great detail as a possible target for cancer drugs.
Biphosphonate, peptidomimetic chemicals, nonpeptidic compounds, and derivatives of tetracycline are examples of synthetic MMP inhibitors that show promise in the treatment of arthritis and cancer [27].
Numerous synthetic MMP inhibitors have been investigated through preclinical research in living organisms. Despite the strong preclinical data that supported multiple clinical trials, there have been a lot of setbacks and little progress in recent years [9,35]. Preclinical research has mostly focused onthe function of MMPs during the initial phases of cancer (progression and metastases), when MMP inhibition appears to have the most impact. Regretfully, patients with advanced, metastatic cancer were the subjects of nearly all MMP inhibitor clinical trials, and regrettably, none of them were able to demonstrate any positive impact on patients [5,9,25]. A thorough review of the literature showed that Salvadora persica is a significant medicinal plant with a wide range of pharmacological effects. Pleiotropic qualities are inherent to it [2,11,24]. Scientific research has validated several of the traditional uses. The pharmacological activity of the plant's main constituents has been thoroughly investigated; the findings have shown that the plant possesses strong antimicrobial, anticonvulsant, analgesic, ace-inhibiting, antimycotic, hypolipidemic, antiplasmodial, antibacterial, antiulcer, topical medication, and locomotor properties. The plant's secondary metabolites, such as flavonoids, tannins, and alkaloids, are important in the treatment of terrible illnesses, cancer being the deadliest of them [3,13,30].
Research has indicated that petroleum ether extract significantly inhibits the growth of lung cancer cell line A549 and colon carcinoma cell line HCT116 [36].
The pharmaceutical industry has seen a greater rise in the use of rational medication design in recent years. Choosing a target protein molecule that is essential to a pertinent biological pathway is necessary for this strategy. By looking at the three-dimensional structure, it would be helpful to comprehend the specific interactions that the ligand has with the target receptor proteins. A fresh, fruitful platform for investigating contemporary computational techniques has been created by the quick advancements in computational chemistry and bioinformatics for pharmacological targets [10].
Salvadora Persica included intricate ingredients that weretough to comprehend using biological technologies and scant analyses. The molecular docking method was used to anticipate the ligand-protein interaction. Posing and scoring are the two primary phases in molecular docking calculations, which result in a prioritized list of potential complexes between ligands and targets. The binding site location that should be the focus of the docking calculations is often known.
Thus, the goal of the current work is to evaluate how different S.persica components docked with the MMP-2. This part would be utilized to prevent the invasion of OSCC and its subsequent metastases.

2. Methods

All experimental procedures were reviewed and approved by the MREC-NRC with a reference number: 03430424. All steps of the practical work adhere to the standards of the declarations of NRC.

3. Materials

Hep-2 cell line was purchased from Vacsera Biobank, Egypt.
An alcoholic extract of s.persica was purchased from Nawah Research Labs, Mokattam, Egypt, freeze-dried and kept at -21°C till use.
A 100 mM stock solution of s.persica extract was made in dimethyl sulfoxide (DMSO), and stored at -20℃. The final concentration of DMSO for all treatments was consistently less than 0.2%. Appropriate amounts of s.persica solution were added to the culture medium at the indicated concentrations.

3.1.Cell culture and maintenance:

Hep-2 cancer cells were cultured until the logarithmic growth phase. Then, 0.25% trypsin was used to digest the cells to prepare a single-cell suspension. The cells were seeded at 105 cells/well in a 24-well plate, and an RPMI-1640 culture medium (Gibco, Carlsbad, CA, USA) containing 10% FBS was added. The plates were cultured in a 37°C, 5% CO2 incubator. After 24 hours of culture, the culture medium was changed with RPMI-1640 culture medium containing S.persica extract at different concentrations (0, 100, 200, 400, 600, 800 μg for 24 h). After that, the culture supernatant was collected.

3.2.ELISA for MMP-2 :

The MMP-2 Enzyme-Linked Immunosorbent Assay (ELISA) technique is designed for the quantitative measurement of MMP-2 in cell culture supernatants, serum, and plasma (heparin). The ELISA kit is based on standard sandwich enzyme-linked immune-sorbent assay technology.
The culture supernatants were used for testing with ELISA kits (RAB0365-1KT Sigma-Aldrich Human MMP-2 Elisa Kit for serum/ plasma/ cell culture supernatant), following the manufacturer's instructions. To plot standard curves and to obtain calculation formulas, the absorbances at 450 nm were used as the y-coordinates, and the concentrations of the standards were used as the x-coordinates. The absorbances of the samples were substituted into the resulting formula to calculate the protein concentrations of MMP-2 in the cell culture supernatants.

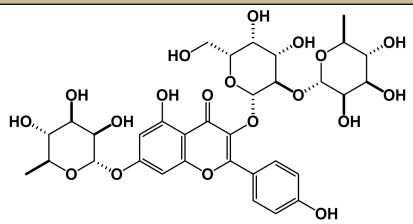
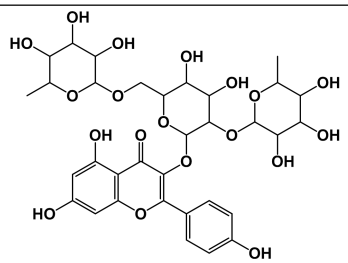
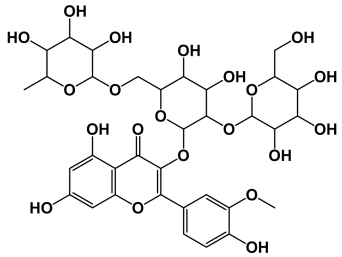
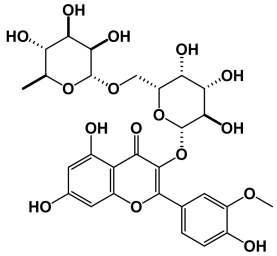
3.3.Statistical analysis:

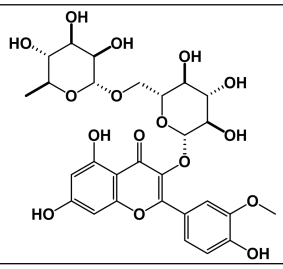
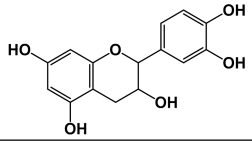
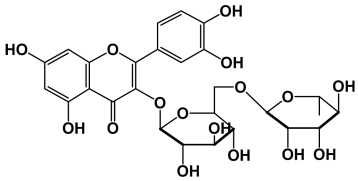
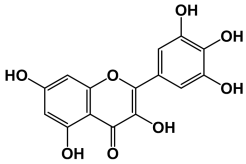
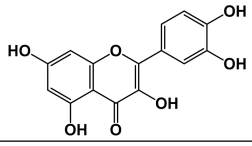
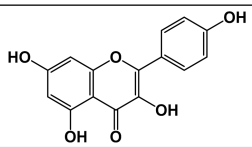
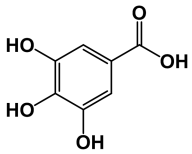
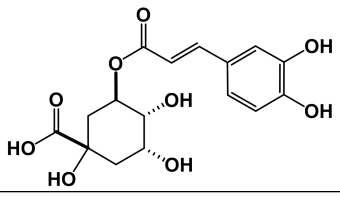
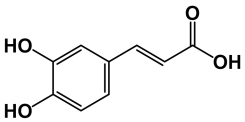
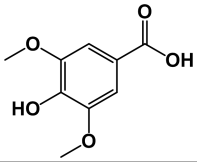
Statistical analysis was performed with SPSS 16 ® (Statistical Package for Scientific Studies), Graph pad prism & windows excel and presented in 2 tables and 2 graphs. Exploration of the given data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which revealed that data originated from non-parametric distribution. Accordingly, comparison between groups was performed by using Kruskal Wallis test followed by Dunn's multiple comparisons test for multiple comparisons. The significance level was set at $p \leq 0.05$.

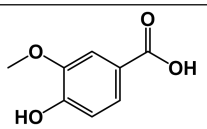
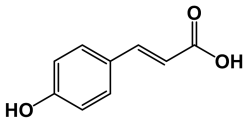
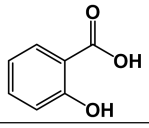
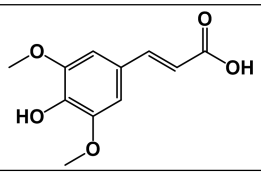
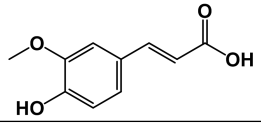
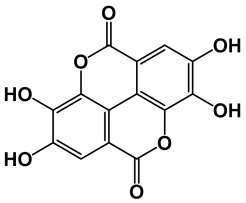
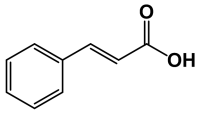
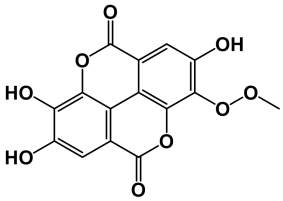
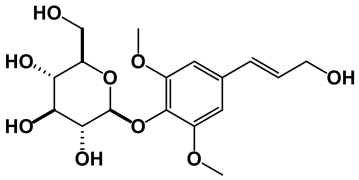
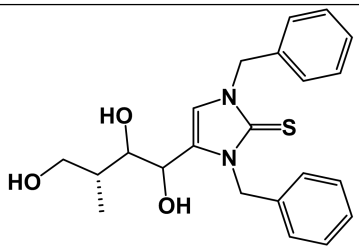
3.4.Molecular Docking:

The molecular docking was processed to evaluate the possible affinity of the tested compounds against metalloproteinase-2. The target proteins (code: 1hov) were obtained from the protein data bank [10]. At first, water molecules were removed from the complexes. Next, preparation options were used to prepare, and correct crystallographic disorders and unfilled valence atoms. Protein structure energy was minimized by applying CHARMM force fields. Hence, defining and preparing the pockets for the docking process. Using Chem-Bio Draw Ultra17.0, 2D structures of tested compounds were drawn and saved as SDF files, the saved files were opened, 3D structures were protonated, and 0.1 RMSD kcal/mole energy was minimized by the MMFF94 force field. Then, the minimized structures were prepared for docking via the ligand preparation tools. The docking process was carried out through the docking option using Autodock Vina software [28]. The receptor was held rigid while the ligands were allowed to be flexible. During the refinement, each molecule was allowed to produce twenty different poses with the proteins. Then docking scores (affinity energy) of the best-fitted poses with the active sites were recorded and 3D figures were generated by the Discovery Studio 2016 visualizer [8].

4. Results

serial	structure	Common name
	Flavonoids	
Compound 1		3-(((2S,3R,4S,5R,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-(((2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)oxy)-5-hydroxy-2-(4-hydroxyphenyl)-7-(((2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one
Compound 2		3-((4,5-dihydroxy-3-((3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-6-(((3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one
Compound 3		
Compound 4		5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-(((2S,3R,4S,5R,6R)-3,4,5-trihydroxy-6-((((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one

Compound 5		5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-((((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4H-chromen-4-one
6		Catechin
7		Rutin
8		Myricetin
9		Quercetin
10		Kaempferol
	Phenolic acids	
11		Gallic acid
12		Chlorogenic acid
13		Caffeic acid
14		Syringic acid

15		Vanillic acid
16		p-coumaric acid
17		Salicylic acid
18		Sinapic acid
19		Ferulic acid
20		Ellagic acid
21		Cinnamic acid
22		Methoxy ellagic acid
23		Syringin
	Alkaloids	
24		Persicaline

4.1.Quantitative analysis of MMP-2 activity inhibition

The effect of different conc. Of Salvadora persica extract on MMP-2 protein expression in Hep-2 cells:

Descriptive results (minimum, maximum, mean, and standard deviation) of different concentrations were presented in table (1) and figure (1,2).Comparison between all concentrations revealed highly statistical significant difference as P<0.0001.

Table (1): Descriptive results of control and different concentration, comparison between different concentrations using Kruskal Wallis test which revealed that there was a significant difference between them

	Minimum	Maximum	Mean	Standard Deviation	P value
Control Hep-2	3.13	5.62	4.22 a	0.76	0.0001*
100 µg/ml	3.11	5.52	3.91 a	0.89	
200 µg/ml	2.29	3.33	2.75 ab	0.36	
400 µg/ml	1.33	3.21	2.22 b	0.59	
600 µg/ml	0.17	1.24	0.74bc	0.36	
800 µg/ml	0.00	0.90	0.28 c	0.31	

***Significant difference as P<0.05.**

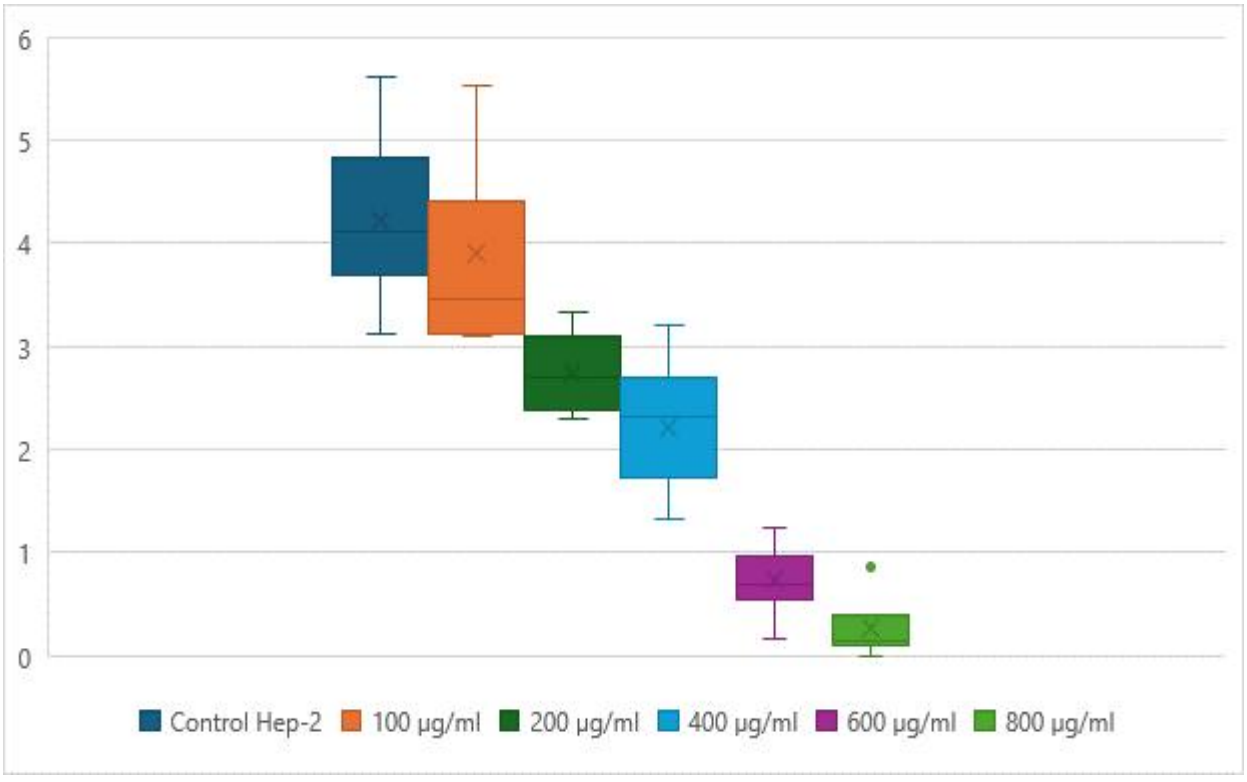


Figure 1: Boxplot representing different conc. Of Salvadora persica extract on MMP-2 protein expression in Hep-2 cells.

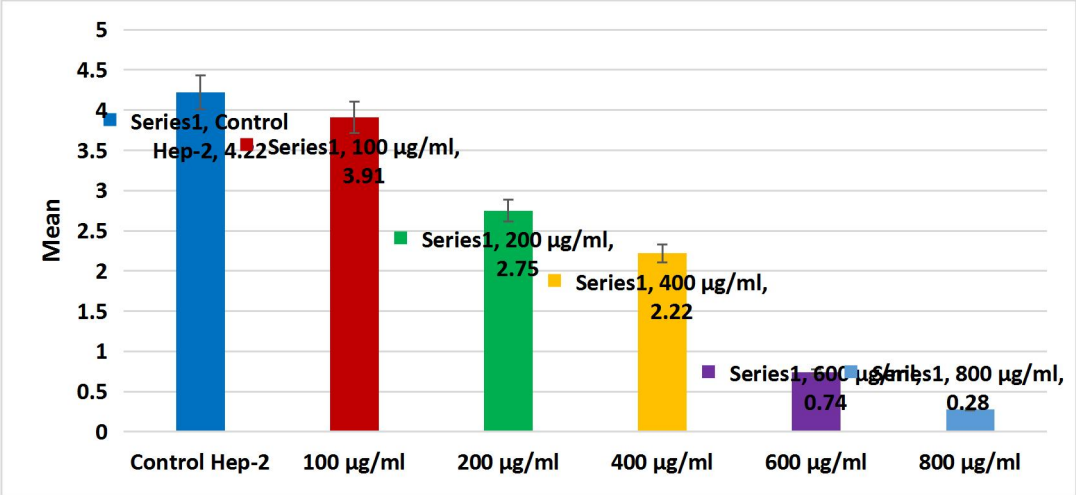


Figure 2: Bar chart representing the effect of different conc. Of Salvadora persica extract on MMP-2 protein expression in Hep-2 cells

Dunn's multiple comparisons test

Multiple comparisons revealed that control (4.22 ± 0.76), 100 µg/ml (3.91 ± 0.89), and 200 µg/ml (2.75 ± 0.36) were significantly the highest with insignificant difference between them, while 600 µg/ml (0.74 ± 0.36), and 800 µg/ml (0.28 ± 0.31) were significantly the least with insignificant difference between them, as presented in table 2.

Table (2): Dunn's multiple comparisons test:

Dunn's multiple comparisons test	Mean 1	Mean 2	Mean Diff.	SE of diff.	95.00% CI of diff.	Adjusted P Value
Control Hep-2 vs. 100 µg/ml	4.221	3.909	0.3121	0.2531	-0.5669 to 1.191	>0.9999
Control Hep-2 vs. 200 µg/ml	4.221	2.747	1.474	0.2	0.7793 to 2.169	>0.9999
Control Hep-2 vs. 400 µg/ml	4.221	2.224	1.998	0.3005	0.9540 to 3.042	0.0314*
Control Hep-2 vs. 600 µg/ml	4.221	0.7368	3.485	0.2602	2.581 to 4.388	<0.0001*
Control Hep-2 vs. 800 µg/ml	4.221	0.2805	3.941	0.2771	2.979 to 4.903	<0.0001*
100 µg/ml vs. 200 µg/ml	3.909	2.747	1.162	0.2947	0.1384 to 2.185	>0.9999
100 µg/ml vs. 400 µg/ml	3.909	2.224	1.686	0.3762	0.3792 to 2.992	0.0936
100 µg/ml vs. 600 µg/ml	3.909	0.7368	3.173	0.2799	2.200 to 4.145	0.0004*
100 µg/ml vs. 800 µg/ml	3.909	0.2805	3.629	0.2948	2.605 to 4.653	<0.0001*
200 µg/ml vs. 400 µg/ml	2.747	2.224	0.5239	0.1172	0.1168 to 0.9311	>0.9999
200 µg/ml vs. 600 µg/ml	2.747	0.7368	2.011	0.1716	1.415 to 2.607	0.0936
200 µg/ml vs. 800 µg/ml	2.747	0.2805	2.467	0.1356	1.996 to 2.938	0.0025*
400 µg/ml vs. 600 µg/ml	2.224	0.7368	1.487	0.2401	0.6527 to 2.321	>0.9999
400 µg/ml vs. 800 µg/ml	2.224	0.2805	1.943	0.1866	1.295 to 2.591	0.1826
600 µg/ml vs. 800 µg/ml	0.7368	0.2805	0.4563	0.1454	-0.04869 to 0.9612	>0.9999

***Significant difference as P<0.05.**

4.2.MMP-2 as a molecular target (Molecular Docking)

To evaluate the binding stabilities of S.persica components in the protein cavity of MMP-2, we first assessed the binding of a standard compound in the predefined active site of MMP-2 protein by using the Autodock software program. The active site of the MMP-2 protein consists of Zinc (Zn+2) and consensus(HEBGHXLGLXHS) of amino acids for the Zn+2 binding motif which are conserved throughout the MMPs and contain three histidine residues that are bound to Zn+2[21]. Previous reports have suggested that amino acids in the catalytic cavity play an important role in the binding of inhibitors in the protein cavity [15, 22, 23]. The protein cavity containing Zn+2 and important amino acids was selected for the docking of s.persica. Most of the compounds were found to dock into the active site of the MMP-2 protein cavity with a good fit.

Results from molecular docking demonstrated that S.persica's major components Chlorogenic acid, Methoxy ellagic acid, Syringin, persicalin, catechin, co-crystallized ligand (SC-74020), and myrecetin interacted with MMP-2 (Table 3). Therefore, the docking results suggested that s.persica exhibited anti-cancer effects by targeting the invasion pathways.

The binding mode of Chlorogenic acid, Methoxy ellagic acid, and Syringin against Metalloproteinase-2 exhibited binding energy equal to -7.20, -8.02, and -7.18 kcal/mol, respectively. Chlorogenic acid formed four hydrophobic π-interactions with Leu137, Ala139, His120, and Leu83, additionally, interacted with Zn166, Ala136, Ala84, and Gln121 by metal interaction and four hydrogen bonds with distances of 2.67, 2.12, 2.67 and 3.05 Å (Fig 3). while Methoxy ellagic acid interacted with His120, Tyr142, Leu83, Leu83, and Zn166 by nine hydrophobic π-interactions and metal interactions. On the other hand, five hydrogen bonds were observed with Ile141, Ala139, Thr143, Ala84, and Glu121 with distances of 2.85, 2.89, 3.05, 2.48, and 2.86 Å, respectively (Fig 4). Moreover, Syringin interacted by five hydrophobic π-interactions with His120, His130, Ala84, and Leu83. Additionally, formed three hydrogen bonds and metal interaction with Glu121, Thr143, Ala83, and Zn166 with distances of 3.08, 2.77, and 2.92 Å(Fig 5).

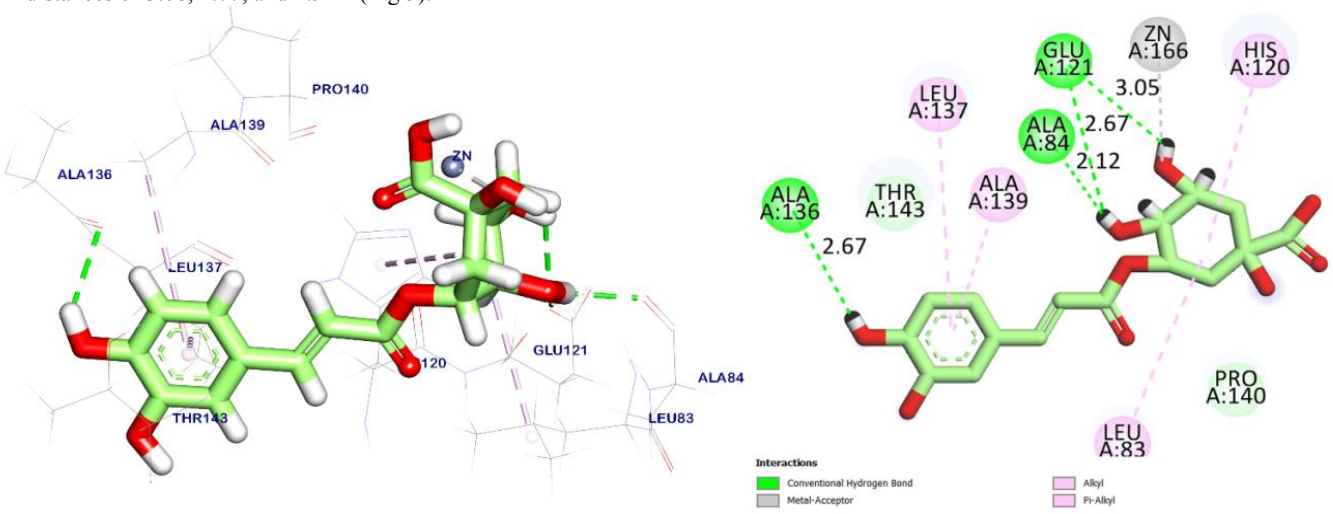


Fig 3: 3D and 2D figures of Chlorogenic acid against Metalloproteinase-2.

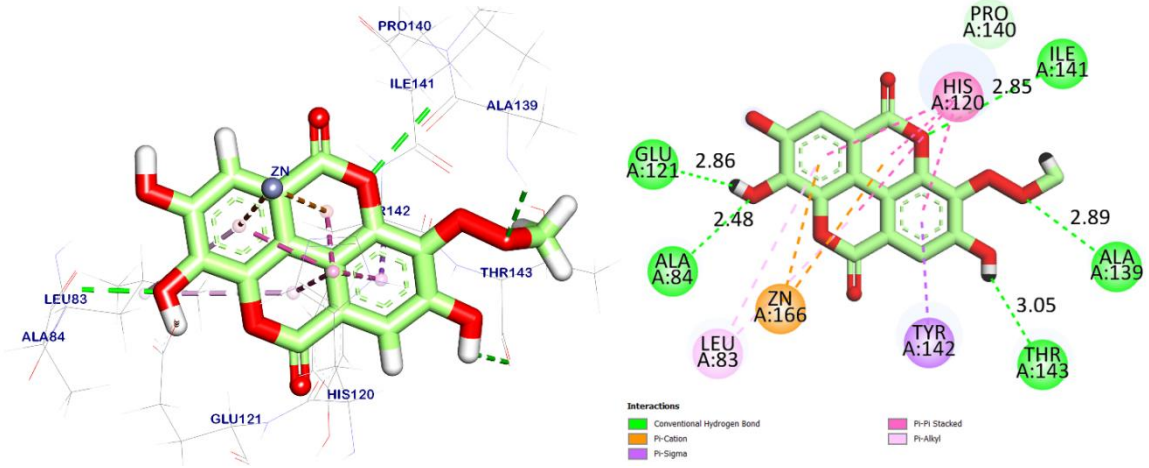


Fig 4: 3D and 2D of Methoxy ellagic acid against Metalloproteinase-2.

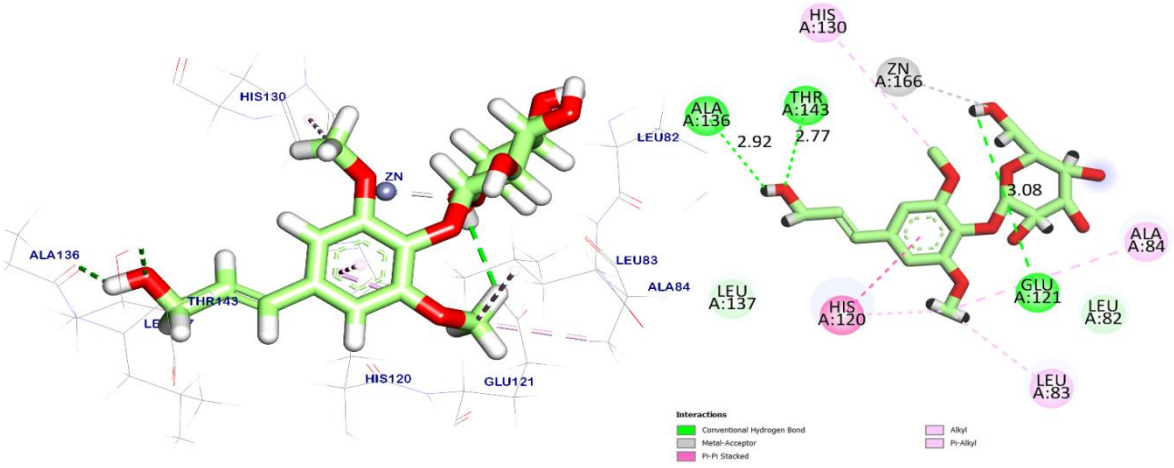


Fig 5: 3D and 2D of Syringin against Metalloproteinase-2.

The binding mode of Compound 1, and Compound 2 against Metalloproteinase-2 exhibited binding score equal to -7.04, and -8.63 kcal/mol, respectively. Compound 1 interacted with Pro140 by two hydrophobic π-interactions and supported by eight hydrogen bonds with Gln132, His130, Ala84, Glu121, and Ile141 with distances of 2.76, 1.97, 2.98, 2.12, 2.32, 2.57, 2.93, and 2.75 Å (Fig 5). On the other hand, Compound 2 interacted by seven hydrophobic π-interactions and metal interaction with His120, Leu84, Leu82, Gly81, and Zn166. moreover, Compound 2 formed five hydrogen bonds with Val117, Glu121, and Ala86 with distances of 2.51, 2.75, 2.39, 2.18, and 2.18 Å (Fig 6).

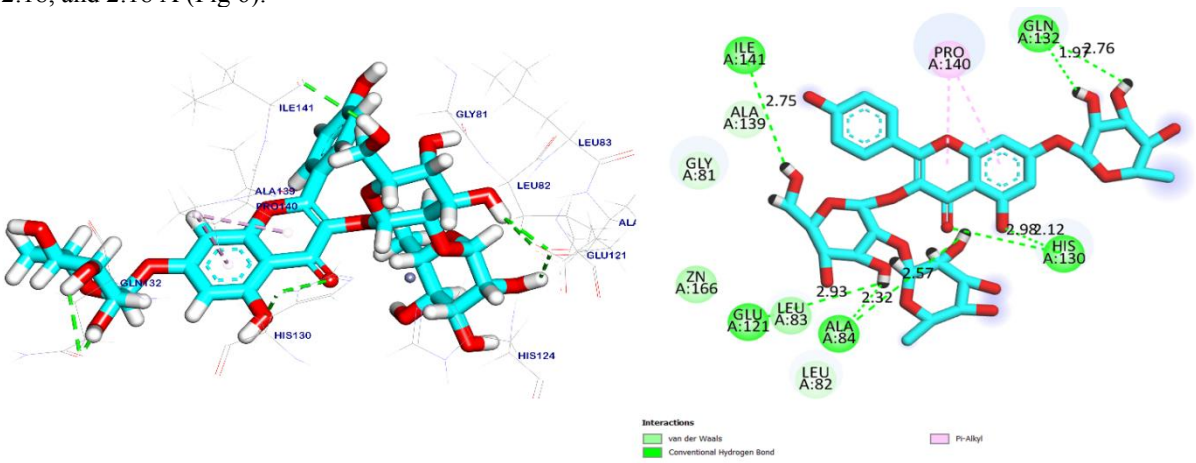


Fig 6: 3D and 2D figures of Compound 1 against Metalloproteinase-2.

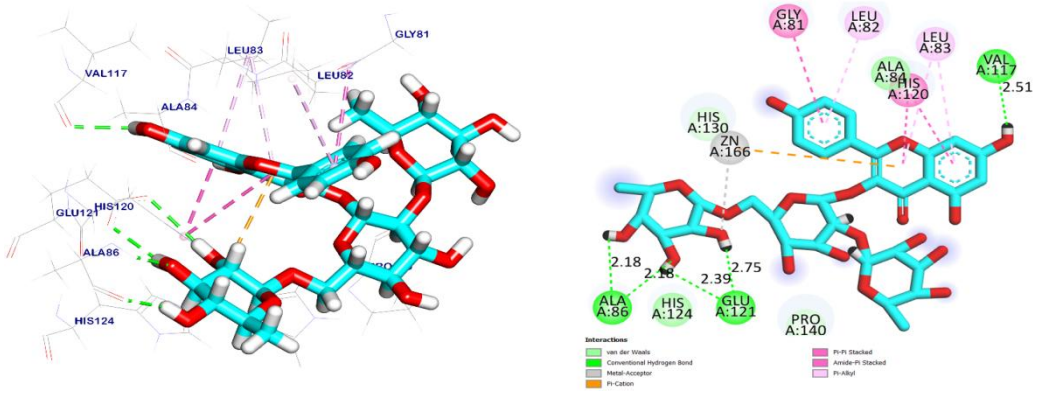


Fig 7: 3D and 2Dfigures of Compound 2 against Metalloproteinase-2.

The binding mode of Compound 4, Compound 5, Catechin and Myricetin against Metalloproteinase-2 exhibited binding energy equal to -8.30, -7.20, -6.80 and -7.40 kcal/mol, respectively. Compound 4 formed eight hydrophobic π-interactions with Leu83, His120, Ala139, Leu116, Leu137, and Ala84 , additionally, Compound 4 interacted with Zn166, Tyr142, Ala84, Glu121, and Ala136 by metal interaction and five hydrogen bonds with distances of 2.17, 1.98, 2.24, 2.82, and 2.10 Å (Fig 7). while Compound 5 interacted with His120, leu83, Val117, Tyr142, Leu116, and Tyr74 by seven hydrophobic π-interactions. Two hydrogen bonds and metal interaction were shown with Glu721, Val117, and Zn166 with distances of 2.54, and 2.77 Å, respectively (Fig 8). Moreover, Catechin and Myricetin showed the same pattern of interactions, both of them formed four hydrogenbonds with Ala136, Ala84, Thr143, Ala139, Gln121, and Val117 with distances range 1.84 to 2.76 Å. Additionally formed six hydrophobic π-interactions with His120, Leu83, Leu137, Leu116, Tyr142, and Zn166. (Fig 9, 10).

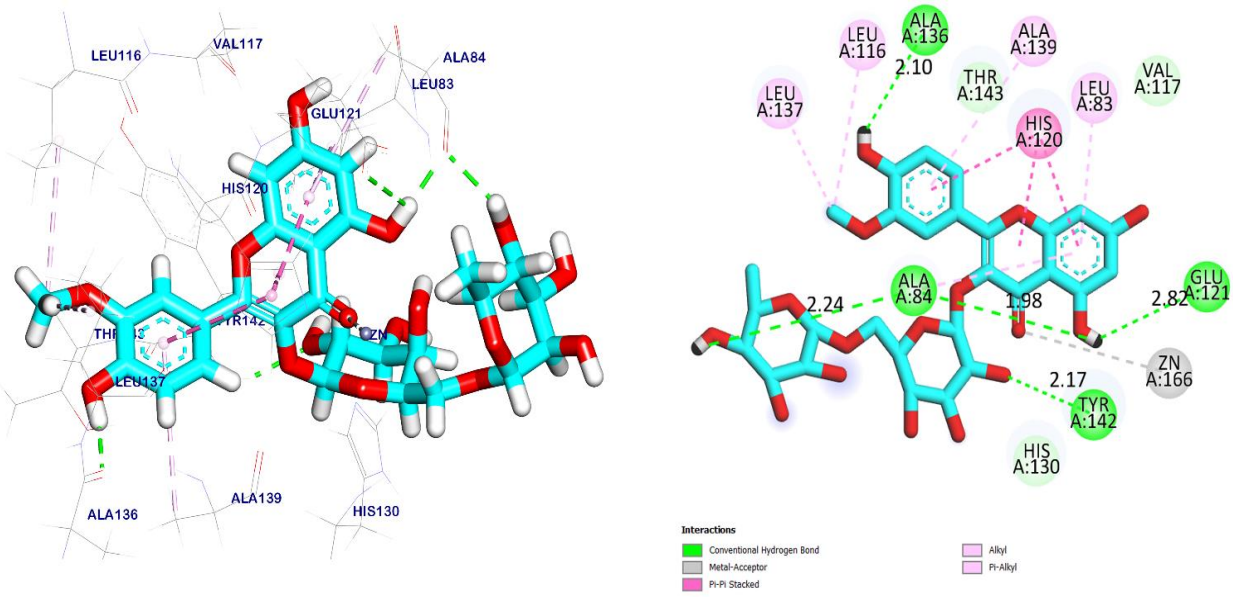


Fig 8: 3D and 2Dfigures of Compound 4 against Metalloproteinase-2.

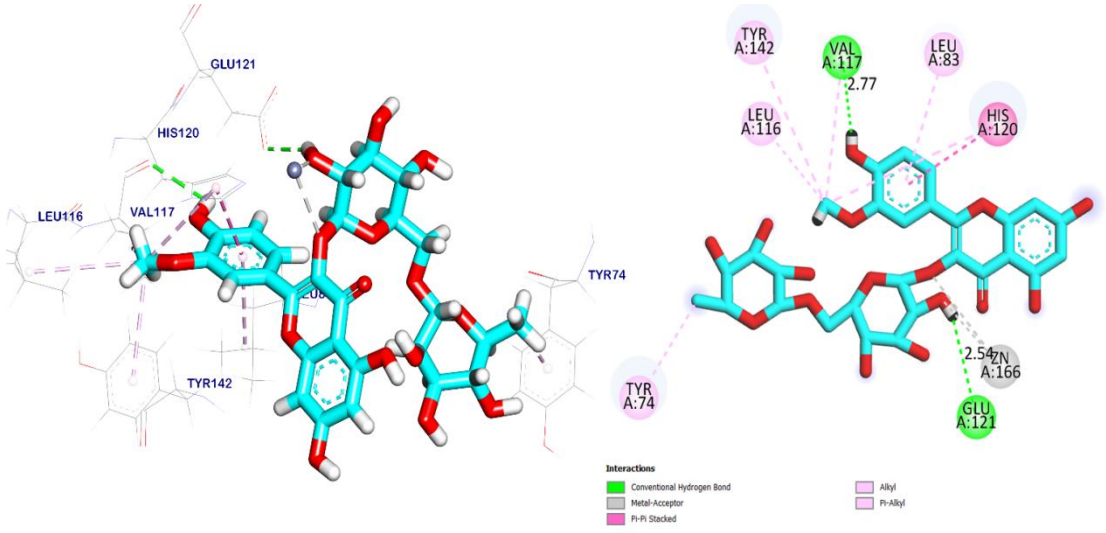


Fig 9: 3D and 2Dof Compound 5 against Metalloproteinase-2.

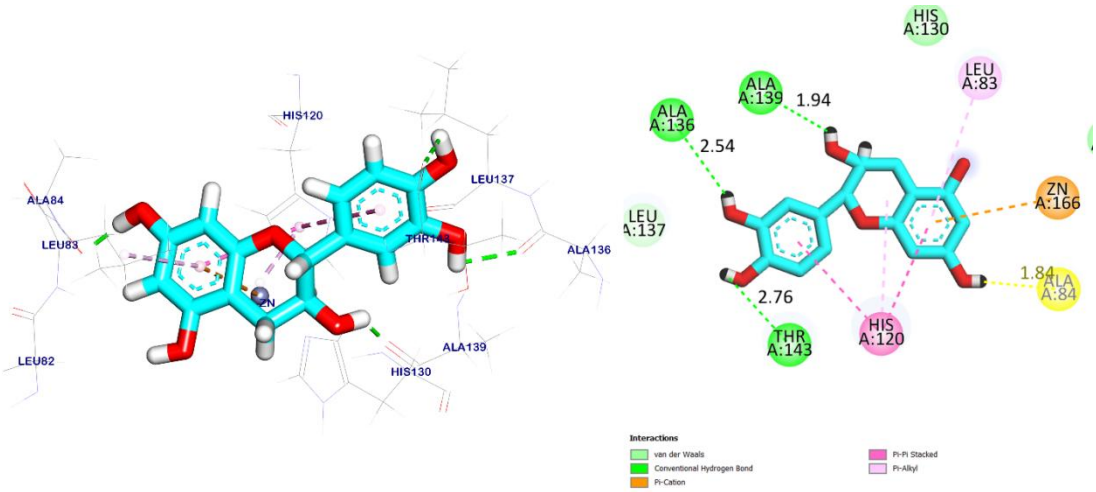


Fig 10: 3D and 2D of Catechin against Metalloproteinase-2.

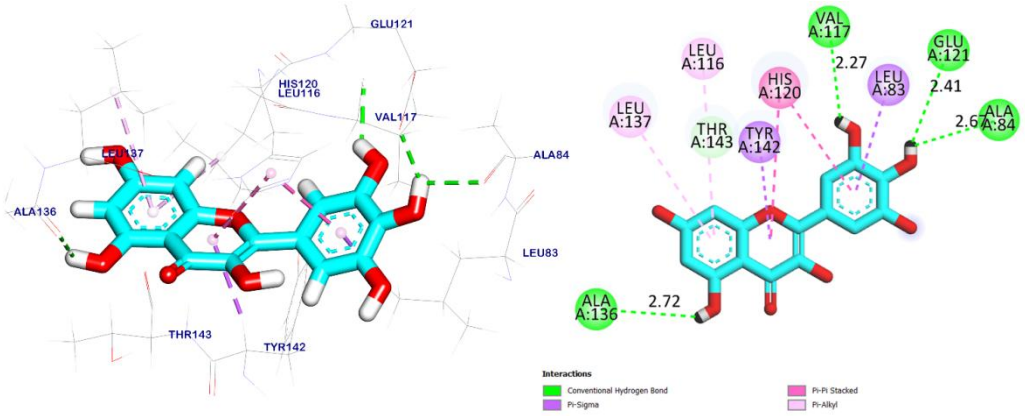


Fig 11: 3D and 2D figures of Myricetin against Metalloproteinase-2.

The binding mode of Persicaline exhibited binding score of -6.70 kcal/mol against Metalloproteinase-2. Persicaline interacted with His120, and Leu83 by two hydrophobic π-interactions. moreover, interacted with Tyr142, Leu83, Ala84 by four hydrogen bonds with distances of 2.92, 2.10, 2.09, and 1.80 Å (Fig 11).

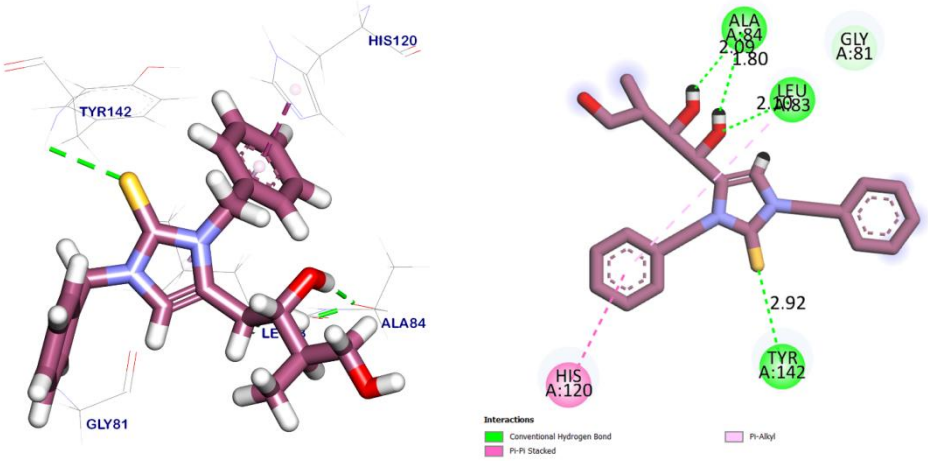


Fig 12: 3D and 2D figures of Persicaline against Metalloproteinase-2.

The co-crystallized ligand (SC-74020) as an inhibitor against Metalloproteinase-2 exhibited an affinity score of -8.21 kcal/mol. (SC-74020) formed seven hydrophobic π-interactions with His124, His130, Leu137, Leu150,and His120, Additionally, interacted with Leu83, Ala84, His130, Glu121 and Zn166 by four hydrogen bonds and metal interaction (attractive interaction) with distances of 1.76, 2.44, 2.10, and 2.98Å (Fig 12,13).

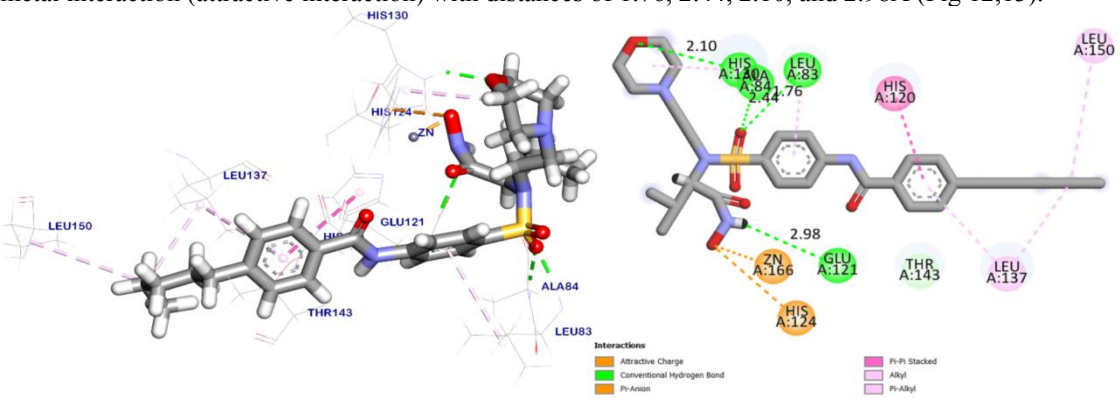


Fig 13: 3D and 2D figures of the co-crystalized ligand (SC-74020) against Metalloproteinase-2

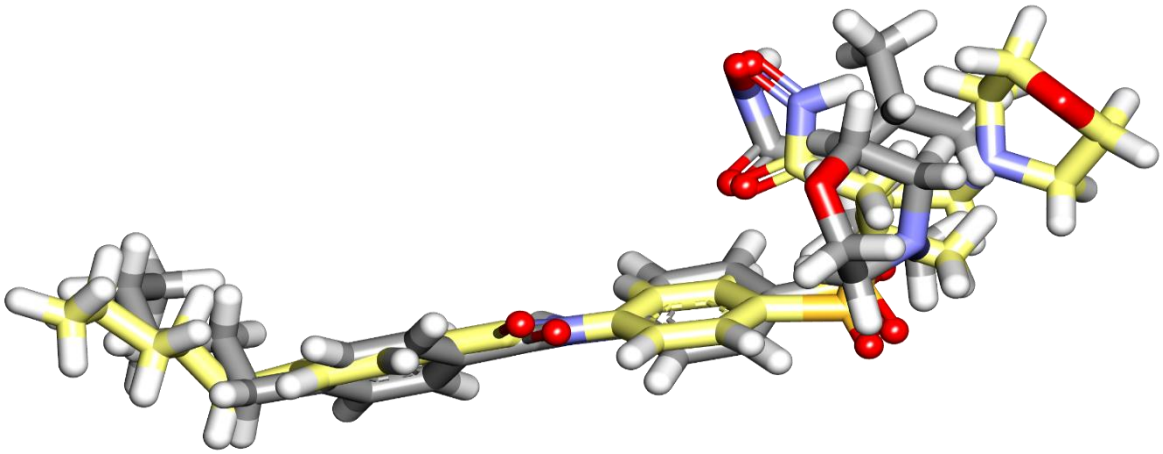


Fig 14: Superimposition of the co-crystalized ligand (SC-74020) yellow = original site, and gray= redocked pose with RMSD value = 1.01 Å

Table 3: Showing results of the molecular docking of natural compounds extracted from Salvadora persica against Metalloproteinase-2.

Target	Tested compounds	RMSD value (Å)	Docking (Affinity) score (kcal/mol)	Interactions		
				H.B	π-interaction	Metal
Metalloproteinase-	Chlorogenic acid	1.22	-7.20	4	4	1
	Methoxy ellagic acid	1.32	-8.02	5	9	1
	Syringin	0.91	-7.18	3	5	1
	Compound 1	1.78	-7.04	8	2	-
	Compound 2	1.45	-8.63	5	7	1

2*(MMP-2)*	Compound 4	1.15	-8.30	5	8	1
	Compound 5	1.38	-7.20	2	7	2
	Catechin	1.07	-6.80	4	5	-
	Myricetin	1.48	-7.40	4	6	-
	Persicaline	1.06	-6.70	4	2	-
	SC-74020	1.01	-8.21	4	7	1

5. Discussion

Cancer metastasis is a complex biological process that includes local tumor cell invasion, tumor cell entry into the bloodstream, tumor cell escapes from the bloodstream, and tumor cell colonization at distant locales. Extremely dynamic, the extracellular matrix (ECM) plays a role in several diverse cell activities, including adhesion, invasion, and migration, proliferation, differentiation, and death[32]. Zinc-dependent MMPs could take involvement in the breakdown of ECM and are crucial to the formation of tumors as well as the invasion and metastasis of cancer [12]. Yao et al. discovered that overexpression of MMP-2 and MMP-9 are independent indicators of early-stage gastric cancer metastasis [34].

The deadly cancer known as oral squamous cell carcinoma (OSCC) spreads quickly to other body organs. The two factors contributing to the high death rate from OSCC are lymph node metastases and a high recurrence rate [31]. Furthermore, alcohol intake and tobacco use are closely associated with oral cancer [25].

The most common treatments for OSCC include surgery, radiation therapy, and chemotherapy; however, the best course of action depends on the stage of the cancer. Even after receiving treatment, patients with OSCC still have a poor prognosis. Thus, preventing OSCC recurrence may be made easier by comprehending the molecular pathways that mediate migration and invasion.

Oral epithelial cells, hepatocytes, adrenal cortical cells, and renal tubular cells all produce MMP-2, a protease with gelatinolytic properties [15]. Its role in creating the tumor microenvironment is understandable because it promotes tumor invasion and metastasis by breaking down substances such as laminin, type IV collagen, and proteoglycans. It is mainly produced by fibroblasts and is activated by MMP-1 and MMP-14, among other MMPs [14]. Elevated levels of MMP-2 in saliva have been associated with various oral health conditions, including dental caries, according to scientific studies.

In relation to oral squamous cell carcinoma (OSCC), MMP-2 was detected in patients' saliva but not in healthy controls' saliva [14]. Its expression was correlated with a poor prognosis (low disease-specific survival, disease-free survival, and overall survival) according to secretome analysis from OSCC tissue samples [23].

Natural products, many of which are plant species, are widely used and gaining popularity due to their potential therapeutic benefits and lack of adverse effects. Multiple scientific papers have verified the strong anticancer properties of these conventional products [3]. S. persica is known to have a wide range of physiologically active phytoconstituents, such as fatty acids, steroids, alkaloids, flavonoids, sulfur compounds, and terpenoids. Key phytoconstituents with antiviral, antioxidant, and antimicrobial properties include persicaline, salvadourea, salvadoraside, and benzyl isothiocyanate [3].

The biological impact of S. persica and its constituents on the molecular expression of MMP-2 in oral cancer cells has not been the subject of any prior research. In this subject;we assayed the secreted MMP-2 using a quantitative Elisa assay. Hep-2 cells were first exposed to increasing concentrations of s.persica for 24 hours and then MMP-2 levels were assayed in the culture supernatants. Comparison between all concentrations revealed highly statistically significant difference (as $P<0.0001$) revealing a powerful inhibitory effect of s.persica extract on the expression of MMP-2 in Hep-2 malignant cells. This remarkable effect was dose dependent with the highest effect in a dose of 600 and 800 µg/ml.

Researchers looked at how different natural items affected the production of MMP-2 in cancerous cells negatively. Lu et al. used the MMP-2 assay to measure the impact of querecetin (Que) on the invasiveness of malignant cells. Que-shrank MMP-2 and MMP-9 levels show a connection between antimigratory and antiinvasive properties [21]. In a study by Zhao et al. [32]. MMP-2 and MMP-9 levels were also decreased when human oral cancer cells (HSC-6 and SCC-9) were treated with Que.

One phytoestrogen that slows the progression of cancer and carcinogenesis is kaempferol, or kae. It was demonstrated by Lin et al. that it decreased the MCF-7 breast cancer cell line's synthesis of proteins associated

with metastasis, such as MMP-2 and MMP-9 [18]. Research revealed that myrcetin inhibited the growth and migration of cancer in A549 cells, regulated the activities of MMP-2 and MMP-9, and decreased the creation of MMP-2 protein in colorectal cancer cells (COLO 205) [7,16].

Research conducted by Chandrashekar et al. has shown the function of baicalein (Bai) as an anticancer drug through a variety of pathways, such as controlling the production of MMP-2 and MMP-9 [4]. Yan et al. performed more research on the anti-proliferative characteristics of Bai in melanoma cell lines (A375 and SK-MEL-28). In cells treated with Bai, they found a notable decrease in MMP-2 expression [33].

The main challenge for any newly developed anticancer drug that demonstrates promise in terms of toxicity against cancer cell lines is identifying the molecular targets associated with the drug's biological activity. This holds true even for substances that are analogues of well-known and well-researched phytochemicals [1].

Human oral squamous cell carcinoma has been associated with matrix metalloproteinase-2 (MMP-2). To tackle the condition, it is therefore interesting to build novel MMP-2 inhibitors [17]. In this work, we examined MMP-2 as a potential protein target of S. persica. Consequently, we record the molecular docking characteristics of novel medicinal plant parts containing MMP-2 for further thinking. Alkaloids appear to be important chemical entities in the discovery of new drugs. Natural herb-derived alkaloids have antiproliferation and anti-metastasis properties against several cancer types. It seems that alkaloids have a special function during the cell cycle. In order to evaluate their efficacy against MMP-2 in OSCC, specific alkaloid compounds from a variety of medicinal plants are employed. Binding mode and molecular interaction analysis in the MMP-2 binding cavity were used to choose the top best-docked compounds with a higher affinity for the MMP-2 receptor (Table 3). The process by which unbound protein and ligand acceptors, as well as providers of hydrogen bonds, break their existing hydrogen bonds with water and create new ones in the protein-ligand complex is known as hydrogen bonding. Moreover Hydrogen-bonding formation could potentially be employed to improve the accuracy of binding energy measurement in addition to screening unnatural poses in docking.

In summary, MMP-2 breaks down the extracellular matrix (ECM) and basement membrane during tumor invasion and metastasis, allowing tumor cells to separate, invade, and spread and S.persica derivatives counteract this function. S.persica extract counteracts MMP-2 expression during cancer development.

6. Conclusions

The anti-cancer properties and chemical composition of S. persica have been identified through in-vitro and in-silico studies. The chemicals isolated from S. persica have therapeutic qualities and can inhibit invasion through MMP-2 downregulation. Computational techniques were used to demonstrate the bioactive components in S. persica that help prevent invasions. Furthermore, data illustrates the binding characteristics of substances from S. persica with MMP-2.

7. **Acknowledgements**

The authors gratefully acknowledge the National Research Centre, Egypt.

Conflict of Interest

The authors have no competing financial interests or personal interests to declare that are relevant to the content of this article or may influence it.

Funding

Not applicable

Authors contribution:

M.M.E. was responsible for the research design and conceptualization, and worked on the cell line A.M. handled the study's molecular docking component. Both of them were involved in gathering, analyzing, and interpreting data. M.M.E. was in charge of the final approval and ultimate accountability for the published work, as well as drafting and modifying the work for context and intellectual substance. The final manuscript was read and approved by both writers.

List of Abbreviations

SCC: Squamous cell Carcinoma
S.Persica: Salvadora Persica
OSCC: Oral Squamous Cell Carcinoma
MMPs: Matrix Metalloproteinases
ECM: Extracellular Matrix
DNA : Deoxy Ribo Nucleic Acid
DMSO :Dimethyl Sulfoxide
SPSS: Statistical Package for Scientific Studies
Mg: Microgram
H: Hour
Kcal: Kilo Calorie
MREC-NRC: Medical Research Ethics Committee- National Research Centre

NRC: National Research Centre
RPMI: Roswell Park Memorial Institute
ELISA: Enzyme-Linked Immunosorbent Assay
Å: Angstrom
ml: milliliter
mol: Mole
His: Histone
RMSD: Root Mean Square Deviation
MMFF 94: Merck Molecular Force Field

8. References

1. Aamir A, Afreen S, Kevin R, Vivek S, *et al.* 2015 Molecular docking and inhibition of matrix metalloproteinase-2. *Am J Transl Res.* **7** 298-308
2. Adilah N, Ramli N, Rahman Azli A, *et al.* 2023. The effect of Salvadora persica ethanolic extract on oral tissue healing in rats: An in vivo study. *Saudi Dent J.* **35** 663-667
3. Al-Dabbagh B , A Elhaty I , Murali C , *et al.* 2018. Salvadora persica (Miswak): Antioxidant and Promising Antiangiogenic Insights. American J. of Plant Sci. **9** 1228-1244
4. Chandrashekar N, Selvamani A, Subramanian R, *et al.* 2012. Baicalein inhibits pulmonary carcinogenesis-associated inflammation and interferes with COX-2, MMP-2 and MMP-9 expressions in-vivo. *Toxicol. Appl. Pharmacol.* **261** 10-21
5. Cheng X, Wang Q, Fang H, *et al.* 2008. Design,synthesis and preliminary evaluation of novel pyrrolidine derivatives as matrix metalloproteinase inhibitors. *Eur J Med Chem* **43** 2130-2139
6. Christopher D, Fletcher K, Krishnan U, *et al.* 2002. Pathology and Genetics of Tumours of Soft Tissue and Bone. *Int Agency Research on Cancer (IARC).* ISBN:9789283224136- 9283224132
7. Ci Y, Zhang Y, Liu Y, Lu S, *et al.* 2018. Myricetin suppresses breast cancer metastasis through down-regulating the activity of matrix metalloproteinase (MMP)-2/9. *Phytother. Res.* **32** 1373-1381
8. El Azab E 2023 Actinidia deliciosa Extract as a Promising Supplemental Agent for Hepatic and Renal Complication-Associated Type 2 Diabetes (In Vivo and In Silico-Based Studies). Inter. J.Molecular Sci. **24** 13759
9. Fathima J, Selvaraj J, Sivabalan V, *et al.* 2021. Molecular docking of alkaloid compounds with the matrix metalloproteinase-2. *Bioinformation.* **31** 206-211
10. Feng Y 2002. Solution structure and backbone dynamics of the catalytic domain of matrix metalloproteinase-2 complexed with a hydroxamic acid inhibitor. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics.* **1598** 10-23
11. Fridlender M, Kapulnik Y and Koltai H 2015 Plant derived substances with anti-cancer activity: From folklore to practice. *Front Plant Sci.* **6** 799
12. Gialeli C, Theocharis A and Karamanos N 2011 Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. *J. FEBS* **278** 16-2
13. Hammad H, Al-Qaoud K, Hammad M, *et al.* 2014. Effects of salvadora persica extract on DOK oral epithelial dysplasia and oral cancer cell lines. *Trop J Pharm Res.* **118** 195-196
14. Hoffmann C, Vacher S, Sirven P, *et al.* 2020 MMP-2 as an independent prognostic stratifier in oral cavity cancers. *Oncoimmunology* **9** 1754094
15. Jobin P, Butler G and Overall C 2017 New intracellular activities of matrix metalloproteinases shine in the moonlight. *Biochim. Biophys. Acta Mol. Cell Res.* **1864** 2043-2055
16. Ko C, Shen S, Lee T, *et al.* 2005 Myricetin inhibits matrix metalloproteinase- 2 protein expression and enzyme activity in colorectal carcinoma cells. *Mol. Cancer Ther.* **4** 281-290
17. Li W, Saji S, Sato F, *et al.* 2013 Potential clinical applications of matrix metalloproteinase inhibitors and their future prospects. *Int. J. Biol. Marker.* **28** 117-130
18. Lin C, Chen P, Chen M, *et al.* 2013 Kaempferol reduces matrix metalloproteinase-2 expression by down-regulating ERK1/2 and the activator protein-1 signaling pathways in oral cancer cells. *PLoS ONE* **8** e80883
19. Liotta L,Tryggvason K and Garbisa S 2002 Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* **284** 67-68
20. Liu L. 2004 The function of MMP-2&9 in oral squamous cell cancer's invasion and metastasis. *Foreign Medicine (Stomatology)* **31** 20.
21. Lu J, Wang Z, Li S, *et al* 2018 Quercetin inhibits the migration and invasion of HCCLM3 cells by suppressing the expression of p-Akt1, matrix metalloproteinase (MMP) MMP-2, and MMP-9. Medical science monitor: *Int. J. Clin. Exp. Med. Res.* **24** 2583
22. Maskos K 2005 Crystal structures of MMPs in complex with physiological and pharmacological inhibitors. *Biochimie* **87** 249-263
23. Matuszczak E, Cwalina I, Tylicka M, *et al.* 2020 Levels of Selected Matrix Metalloproteinases-MMP-1, MMP-2 and Fibronectin in the Saliva of Patients Planned for Endodontic Treatment or Surgical Extraction. *J. Clin. Med.* **9** 3971
24. Mohany M, Al-zharani M, Nasr F, *et al.* 2023 Persicaline, an alkaloid from *Salvadora persica*, inhibits proliferation and induces apoptosis and cell-cycle arrest in MCF-7 cells. *Open Chemistry* **21** 20220302
25. Pemberton M. 2018 Oral cancer and tobacco: developments in harm reduction *Br. Dent. J.*

26. Radhakrishnan N, Christina I and Vallivitan K 2015 Molecular docking analysis of selected clitoria ternatea constituents as matrix metalloproteinases (mmp 2 & mmp 9) inhibitors. *Rasayan J.Chem.* **14** 659- 64
27. Roy A. 2021 Molecular docking analysis of compounds from Andrographis paniculata with EGFR. *Bioinformation.* **17** 23-28
28. Saleh A. 2023 Design, synthesis, in silico studies, and biological evaluation of novel pyrimidine-5-carbonitrile derivatives as potential anti-proliferative agents, VEGFR-2 inhibitors and apoptotic inducers. *RSC advances.* **13** p. 22122-47
29. Santos M, Marques SM,Tuccinardi T, *et al.* 2006 Design,synthesis and molecular modeling study of iminodiacetyl mono hydroxamic acid derivatives as MMP in- hibitors. *Bioorg. Med. Chem.* **14** 7539-7550
30. Sardari F, Kazemi M, Heiranizade M, *et al.* 2015 Anti-inflammatory and cytotoxicity effects of Salvadora persica extract on jurkat t-cells J. Microbiology, Biotech, Food Sci. **4** pp. 379-382
31. Su C, Chen M, Hung W, *et al.* 2018 Functional variant of CHI3L1 gene is associated with neck metastasis in oral cancer *Clin. Oral Invest.* **36** 55-57
32. Takai K, Weaver V and Werb Z 2011. Extracellular matrix degradation and remodeling in development and disease Cold Spring *Harb. Perspect. Biol.* **3** 67-69
33. Yang X, Zhang C, Li Y, *et al.* 2021 Baicalein restrains proliferation, migration, and invasion of human malignant melanoma cells by down-regulating colon cancer associated transcript-1. *Braz. J. Med. Biol.* **54** e8934.
34. Yao Z, Yuan T, Wang H, *et al.* 2017 MMP-2 together with MMP-9 overexpression correlated with lymph node metastasis and poor prognosis in early gastric carcinoma. *Tumour biology.* **39** 1-8
35. Zapico J, Serra P, Garcia-Sanmartin J, *et al.* 2011 Potent "clicked" MMP2 inhibitors: synthesis, molecular modeling and biological exploration. *Org Biomol Chem.* **9** 4587-4599
36. Zhao J, Fang Z, Zha Z, *et al.* 2019 Quercetin inhibits cell viability, migration and invasion by regulating miR-16/HOXA10 axis in oral cancer. *Eur. J. Pharmacol.* **847** 11-18
37. Zhao L, Niu H, Liu Y, *et al.* 2018 LOX inhibition downregulates MMP-2 and MMP-9 in gastric cancer tissues and cells. *J Cancer.* **20** 6481-6490
38. Zijl F, Krupitza G and Mikulits W 2011 Initial steps of metastasis: cell invasion and endothelial transmigration. *Mutat. Res.* **728** pp. 23-34