Insilico Pharmacological Profiling of Endostemon Viscosus Bioactive Compounds Targeting MMP-9 for Wound Healing

Kavi Malar Surendran
Nirmala College for Women (Autonomous)
Saradha Maran
saradha.bio@gmail.com

Nirmala College for Women (Autonomous)
Sugandhi Pugazhendhi
Aarupadai Veedu Medical College and Hospital, Vinayaka Missions Research Foundation (Deemed to be University)

Research Article

Keywords: MMP-9, E.viscosus. essential oil, methanolic extract, Molecular docking, ADMET, wound healing

Posted Date: July 2nd, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6789028/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at In Silico Pharmacology on November 26th, 2025. See the published version at https://doi.org/10.1007/s40203-025-00487-2.

Abstract

Plants have been valuable sources of bioactive compounds with therapeutic potential. The exploration of phytochemicals in plant extracts and essential oils has gained attention due to their biological activities and medicinal properties. This study investigated the phytochemicals in methanolic extracts and essential oils by analyzing molecular docking and toxicological profiles of their bioactive and pharmacokinetic properties. Molecular docking using AutoDock Vina, integrated within PyRx version 0.8, was employed to dock the chemicals into MMP-9 protein 1L6J. Among these, the highest-scoring compounds underwent pharmacokinetics, receptor-ligand interactions, and binding affinity analysis after identification by GC-MS analysis. To prepare the grid boxes for docking, the active sites of MMP-9 were predicted using CASTp. The findings revealed that several active ingredients in the methanolic extract, particularly 3-fluoro-5-trifluoromethylbenzoic acid, exhibited significant affinity for MMP-9 and strong interactions, including hydrogen bonding with the key residue TYR 52. Conversely, most interactions formed by the essential oil compounds were hydrophobic. In addition to their biological activity, ADME prediction indicated that both the methanol extract and essential oil possessed adequate drug-likeness characteristics, such as high gastrointestinal absorbability and low permeability through the blood-brain barrier. Based on the toxicological predictions using AdmetSAR 1, the compounds were moderately toxic (class III); however, there were no indications of carcinogenic risk. The observed characteristics of bioactive chemicals derived from *E. viscosus* support their potential application in wound healing, suggesting that these compounds have favorable pharmacokinetic and safety profiles and exhibit a high MMP-9 inhibitory capacity.

Introduction

Plant-derived bioactive compounds have garnered significant attention in the field of therapeutic research due to their diverse biological activities and potential medicinal applications (Zou et al. 2021). These natural substances, extracted from various plant sources, exhibit a wide range of beneficial effects, including anti-inflammatory, antioxidant, antimicrobial, and neuroprotective properties (Gangwar et al. 2021). The growing interest in these compounds stems from their systemic pleiotropic effects, minimal side effects, and low toxicity, making them attractive candidates for natural therapeutic agents against various disease conditions. One area of particular interest is the potential application of plant-derived bioactive compounds in wound healing. The wound healing process involves complex interactions between various cellular and molecular components, including matrix metalloproteinases (MMPs). Among these, MMP-9 plays a crucial role in tissue remodeling and wound closure. However, excessive activation of MMP-9 can impede the healing process, especially in chronic wounds (Zhou et al. 2019; Lan et al. 2021). Therefore, the inhibition of MMP-9 has emerged as a promising strategy to enhance wound healing by reducing excessive tissue breakdown, decreasing inflammation, and restoring the balance between MMPs and their inhibitors. Recent studies have highlighted the potential of natural compounds in inhibiting MMP-9 activity. For instance, flavonoid-rich fractions from plants such as Glycyrrhiza glabra and Sophora japonica have demonstrated wound healing potential, as evidenced by molecular docking studies of their major constituents with wound healing-related proteins (Aly et al. 2023). These findings suggest that *in silico* pharmacological profiling of bioactive compounds from medicinal plants could be a viable approach for identifying potential MMP-9 inhibitors for wound-healing applications. In this context, the present study focuses on *Endostemon viscosus*, a plant species with potential medicinal properties. Despite the lack of specific information regarding this plant, the study aims to explore its phytochemical composition and evaluate the potential of its bioactive compounds as MMP-9 inhibitors for wound healing applications. The research employs a comprehensive approach, combining GC-MS analysis of methanolic and oil extracts, molecular docking studies, and ADMET property evaluations to assess the pharmacokinetic behaviour and drug-likeness of the identified compounds. By integrating these analytical approaches, this study seeks to provide a thorough understanding of the phytochemical composition, potential biological activities, and safety profile of *Endostemon viscosus*. The findings may contribute to the development of novel therapeutic agents and inform future investigations of the medicinal properties of this plant species, potentially advancing the field of natural product-based wound healing therapies.

Materials and Methods

Target protein Preparation

MMP-9

The crystallographic three-dimensional structure of human matrix metalloproteinase 9 (MMP-9), identified by PDB ID 1L6J, was sourced from the Protein Data Bank (http://www.rcsb.org/). The protein structure was elucidated and experimentally validated using X-ray diffraction at a resolution of 2.5 Å. Subsequently, the three-dimensional structure was processed using Biovia Studio, where ligands, co-ligands, heteroatoms, and water molecules were removed to create an appropriate environment for docking (Kundu et al. 2022). Further optimization of the protein structure was conducted using AutoDock Tools (PyRx), which involved the addition of charges, energy minimization, and conversion to the PDBQT format. These optimized structures were then used for subsequent analyses.

Active site prediction and grid generations

Active site prediction and grid generation require a precise assessment of the active site during docking. Potential binding sites for amino acid residues associated with the target protein were identified using CASTp. (2018), web service (Computed Atlas for Surface Topography of Proteins).

Ligand preparation and retrieval

Ligand preparation and retrieval involved selecting the top six compounds from the methanolic extract and six compounds from the essential oil out of 37 and 27 compounds identified by GC-MS, respectively (https://pubchem.ncbi.nlm.nih.gov/). The three-dimensional chemical structures were downloaded in

SDF format from the PubChem database of the National Center for Biotechnology Information, 2021. The methanolic extract ligands included Avenanthramide B (Pubchem CID: 10087955), 3-Amino-1-phenyl-2-pyrazolin-5-one (Pubchem CID: 77794), (4,6-Diamino-1,3,5-triazin-2-yl) cyanamide (Pubchem CID: 592015), Heptanoic acid, 4-methoxyphenyl ester (Pubchem CID: 579908), 3-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenylester (Pubchem CID: 91730956), and 3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy (Pubchem CID: 624494). The essential oil ligands comprised (1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl) cyclohex-2-enol (Pubchem CID: 13213649), caryophyllene oxide (Pubchem CID: 1742210), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (R) (Pubchem CID: 5325830), 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene (Pubchem CID: 10233), d-limonene (Pubchem CID: 440917), and methyleugenol (Pubchem CID: 7127).

Molecular docking of MMP-9

Auto Dock Vina (PyRx software version 0.8) was employed to conduct molecular docking between the prepared receptor and ligands (Dallakyan and Olson 2015). Utilizing default parameters, the macromolecule (1L6J) was docked against the top five ligands derived from the methanolic and essential oil extracts, with default settings applied. During the docking phase, the ligands were considered flexible, whereas the protein was treated as rigid. A grid parameter configuration file was developed using the binding amino acids analyzed from CASTp. Upon completion of the separate protein-ligand docking and retrieval of the PDBQT output files, binding affinities (kcal/mol) were recorded in a CSV file. It was concluded that the ligand exhibiting the strongest interaction possessed the highest negative binding energy.

Visualization and post docking analysis of MMP-9

The three-dimensional and two-dimensional interactions of the docked complexes were visualized and analyzed using the Biovia Discovery Studio Visualizer version 24.1.0.23298 (BIOVIA, Dassault Systemes, 2019). Various receptor-ligand interactions, including hydrogen bonding and hydrophobic interactions, were evaluated.

Pharmacokinetic properties analysis of MMP-9

The Swiss ADME web server, a freely accessible tool introduced in 2017, SwissADME. (2017) (http://www.swissadme.ch/) was employed to evaluate the ADME profile of each selected compound. This evaluation encompassed drug-likeness (as per Lipinski's rule of five), solubility profile, lipophilicity, pharmacokinetic properties, medicinal chemistry, and gastrointestinal absorption profile (GIT).

Toxicology properties prediction of MMP-9

Prior to the initiation of clinical studies, it is imperative to evaluate the safety profiles of these compounds. The use of in silico toxicity profile prediction offers advantages in terms of speed, accuracy, and accessibility, leading to significant savings in time, cost, and effort. The admetSAR1 (2012), a web-based tool available at http://lmmd.ecust.edu.cn/admetsar1, which is both user-friendly and cost-free, was employed to analyze the toxicity profiles and potential adverse effects of the selected four compounds. This server provides predictions on carcinogenicity, acute oral toxicity, rat acute toxicity (LD50), biodegradation, and the inhibition of human ether-a-go-go-related genes.

Results

Active site prediction and grid generation of MMP-9

CASTp analysis has delineated the functional binding regions of MMP-9, identifying critical active site residues: 34, 35, 36, 38, 39, 44, 47, 48, 51, 52, 92, 94–98, 182, 184–187, 209, 213, 215, 216, 232, 233, 242, 334, 338, 366–368, 370, 371, 387–393, 423–425 in Chain A, as illustrated in Table 1 and Fig. 1. A grid box was configured to encompass these binding sites for the subsequent docking analysis.

Table 1
Predicted actives for target protein (1L6J) for wound healing activity.

S.No	Surface area	Volume	Site Residues
1	841.248	1233.188	Chain A
			34,35,36,38,39,44,47,48,51,52,92,94,95,96,97,98,182,184,185,186,187,209,213,215,216,232,233,242,334,338,366,367,368,37

Molecular docking

The evaluation of bioactive compounds from *E. viscosus* has focused on assessing their potential as drug candidates. This study examined 37 compounds from the methanolic extract and 27 from the essential oil extract, applying Lipinski's Rule of Five and ADME analysis to determine their drug-likeness. This screening process is crucial in early-stage drug discovery as it helps identify compounds with favorable physicochemical properties that are likely to be orally active in humans. The analysis revealed that 14 compounds from the essential oil extract and 10 compounds from the methanolic extract met these criteria, suggesting their potential for further investigation as drug candidates.

Following the initial screening, the selected compounds underwent molecular docking studies against the 1L6J protein (Chain A) using PyRx software. This computational approach aims to predict the binding affinity and interaction modes between compounds and the target protein. The results of these docking studies, presented in Tables 2 and 3, provide insights into the potential effectiveness of these compounds as inhibitors or modulators of target proteins. Furthermore, Tables 4 and 5 offer a comprehensive list of compounds that not only exhibit drug-like properties but also demonstrate strong binding energies (below − 7 kcal/mol) in their interactions with the protein. This information is valuable for prioritizing compounds for further experimental validation and optimization of the drug discovery pipelines.

Table 2
GC-MS identified phyto constituents from methanolic extract of *E. viscosus* based on Lipinski's rule and ADME properties for wound healing

S.No	Compound Name	Lipinski's Rule of 5 (Ro5) violation	ADME based exclusion
1*	3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy	No	No
2*	P-Methoxyheptnophene	No	No
3*	Heptanoic acid, 4-methoxyphenyl ester	No	No
4*	3-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenylester	No	No
5*	Avenanthramide B	No	No
6*	3-Amino-1-phenyl-2-pyrazolin-5-one	No	No
7*	1-Methyl-1H-imidazole-4-carboxylic acid	No	No
8*	(4,6-Diamino-1,3,5-triazin-2-yl) cyanamide	No	No
9*	2-Furancarbodithioic acid, ethyl ester	No	No
10	Thiophene, 3-methyl	Yes (Low HBA)	low solubility
11	Methyl acetoxyacetate	Yes (Low MW)	Rapid Metabolism
12	Benzene, 1,2,3-trimethyl-	Yes (High Log P)	Low Bioavailability
13	Methoxyacetaldehyde diethyl acetal	Yes (High Log P)	Poor Absorption
14	Cyclopentanecarboxylic acid, 1-(2-ethyl-1-oxobutoxy)-2-oxo-, ethyl ester	Yes (High MW)	Poor Solubility
15	1-Nonene, 4,6,8-trimethyl-	Yes (High Log P)	Poor Permeability
16	5-[4-(Carboxyadamanthyl-3)-phenyl]-10,15,20-triphenyl-21H,23H	Yes (High MW, Log P)	Poor Absorption
17	Benzene,1-methyl-4(1,2,2-trimethylcyclopentyl)	Yes (High Log P)	Low Metabolism
18	Cycloocta-1,3,6-triene,2,3,5,5,8,8-hexamethyl-	Yes (High MW)	Poor Absorption
19	2H-1-benzopyran-2-one, 3,4-dihydro-4,4,6-trimethyl	Yes (High Log P)	Low Bioavailability
20	Tridecene,5-propyl	Yes (High Log P)	Poor Absorption
21	Heptadecane,3-methyl	Yes (High Log P)	Low Metabolism
22	Decane,5-propyl-	Yes (High Log P)	Poor Permeability
23	Heptacosane	Yes (High MW)	Low Solubility
24	Pentadecanoic acid, 14-methyl-, methyl ester	Yes (High Log P)	Poor Absorption
25	Phthalic acid, cis-hex-3-enyl,octadecyl ester	Yes (High MW, Log P)	Poor Bioavailability
26	3-Penten-2-one	Yes (High Log P)	Rapid Metabolism
27	2,4-Diamino-6-cyanamino-1,3,5-triazine	Yes (High MW)	Poor Absorption
28	Eicosane, 1-iodo	Yes (High MW, Log P)	Poor Solubility
29	Caprolactone oxime, (NB)-O-	Yes (High MW)	Poor Metabolism
30*	Nonane, 5-methyl-5-propyl-	No	No
31	1-Butyl-1-methyl-1,2,3,4-tetrahydroisoquinoline 2-(dimethylamino)	Yes (High Log P)	Low Permeability
32	4,4-bi-4H-pyran,2,2,6,6-tetrakis(1,1-dimethylethyl)-4,4-dimethyl-	Yes (High MW)	Poor Absorption
33	2,2,4,4-Tetrahydroxybenzophenone, tetrakis(tert-butyldimethylsilyl) ether	Yes (High MW, Log P)	Low Bioavailability
34	Phthalic acid, 4-nitrophenyl 2-propyl ester	Yes (High LogP)	Poor Solubility
35	Phenyl 4-[(Trimethylsilyl)amino] benzoate	Yes (High MW, Log P)	Poor Absorption
36	Perfluorobutyramide, N-allyl-N-(hept-2-yl)	Yes (High Log P),	Poor Permeability
37	l-Leucine, n-pentafluorobenzoyl-,pentadecyl ester	Yes (High MW, Log P)	Poor Absorption

HBA–Hydrogen Bond Acceptor, MW: molecular Weight, LogP– logarithm of the partition coefficient (P) Octanol–Water purification Coefficient, ADME– Absorption, Distribution, Metabolism and Excretion, Ro5–Lipinski's rule of five

Table 3
GC-MS identified phytoconstituents from essential oil of *E. viscosus* based on Lipinski's rule and ADME properties for wound healing

S.No	Compound Name	Lipinski's Rule of 5 (Ro5) violation	ADME based exclusion
1*	3-Carene	No	No
2*	Beta-Phellandrene	No	No
3*	D-Limonene	No	No
4*	γ-Terpinene	No	No
5*	Linalool	No	No
6*	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,(R)	No	No
7	α-Cubebene	Yes (LogP > 5)	low aqueous solubility
8*	Methyleugenol	No	No
9	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	Yes (LogP > 5)	high hydrophobicity
10	1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl-,z,z,z	Yes (LogP > 5)	low permrability
11*	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7] decane-rel	No	No
12*	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	No	No
13*	Nerolidol 2	No	No
14*	Caryophyllene oxide	No	No
15*	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol	No	No
16*	αR-Turmerone	No	No
17*	Tetradecanoic acid	No	No
18	Isopimara-9(11),15-diene	Yes (LogP > 5)	high hydrophobicity
19	1-Napthalenepropanol, α-ethenyldecahydro-α,5,5,8a-tetramethyl-2-methylene	Yes (LogP > 5)	low water solubility
20	(4as,10aS)-7Isopropyl-1,1,4a-trimethyl-1,2,3,4a,5,6,9,10,10a-decahydrophenanthrene	Yes (LogP > 5)	high hydrophobicity
21	Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)	Yes (LogP > 5)	poor permeability
22	Trans-Kaur-16-ene	Yes (LogP > 5)	low metabolic stability
23	1-Naphthalenepropanol, α-ethenyldecahydro-[1α(R),2β,4aβ,8aα]	Yes (LogP > 5)	low absorption
24	Kaur-16-ene	Yes (LogP > 5)	high lipophilicity
25*	(2S,4aR,5S,8aR)-5-((S)-3-hydroxy-3-methylpent-4-methylenedecahydronaphthalen-2-ol	No	No
26	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-(1α,4aα,10aβ)	Yes (LogP > 5)	poor permeability
27	Heneicosane	Yes (LogP > 5), high MW	extremely poor solubility

MW – molecular Weight, LogP– logarithm of the partition coefficient (P) Octanol–Water purification coefficient; ADME – absorption, distribution, metabolism and Excretion, Ro5–Lipinski's rule of five.

Table 4
Binding affinity and hydrogen bond interactions of GC-MS identified bioactive compounds from methanolic extract of *E.viscosus* for wound healing

S.No	GCMS compounds	Binding affinity	No. of H bonds	H bonds	Hydrophobic interactions
1	10087955 Avenanthramide B	-7.5	4	MET 422, ARG 422, PRO 415, ALA 417	LEU 397, LEU 418, VAL 398, MET 422, ALA 417
2	77794 3-Amino-1-phenyl-2-pyrazolin-5-one	-7.7	3	ARG 424, PRO 430, GLU 416	VAL 398, MET 422, ALA417, LEU 397, LEU 418
3	592015 (4,6-Diamino-1,3,5-triazin-2-yl) cyanamide	-7.2	3	ARG 424, TYR 420, ALA 417	MET 422, LEU418, ALA 417, LEU 397, PHE 425, LEU 188, VAL 398, CYS 99
4	579908 Heptanoic acid, 4-methoxyphenyl ester	-7.5	2	HIS 401, ARG 424	LEU 418, LEU 397, MET 422, VAL 398, LEU 188, CYC 99, ALA 189
5	91730956 3-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenylester	-8.0	1	TYR 52	LEU 187, LEU 39, LEU 44, MET 94
6	624494 3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy	-7.5	1	TYR 52	LEU 44, LEU 187, MET 94, LEU 39

Table 5
Binding affinity and hydrogen bond interactions of GC-MS identified bioactive compounds from essential oil of *E.viscosus* for wound healing

S.No	GCMS compounds	Binding affinity	No. of H bonds	Interactions	Hydrophobic interactions
1	13213649 (1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol	-7.7	-	Van der Waals, alkyl, pi-alkyl	VAL 398, LEU 188, CYS99
2	1742210 Caryophyllene oxide	-7.5	-	Van der Waals, carbon hydrogen bond, alkyl	MET 94, LEU 187, LEU 39, LEU 44
3	5325830 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,(R)	-7.1	1	MET 94	ALA 417, LEU 188, VAL 398, LEU 397, MET 422, LEU 188
4	10233 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	-7	-	Van der Waals, alkyl	LEU 39, MET 94, LEU 187, LEU 44
5	440917 D-Limonene	-6.8	-	Van der Waals, alkyl, pi-alkyl	LEU 418, ALA 417, MET 422, LEU 397, VAL 398, LEU188, CYS 99
6	7127 Methyleugenol	-6.8	-	Van der Waals, pi-alkyl, alkyl, carbon hydrogen bond, pi-pi stacked, pi-sigma	ALA 417, MET 422, LEU 418, LEU 397, LEU188, VAL-398, CYC-99

The in silico analysis revealed significant binding affinities for compounds from both the methanolic extract and essential oil of *E. viscosus*. In the methanolic extract, 3-fluoro-5-trifluoromethylbenzoic acid demonstrated the highest binding affinity (-8.0 kcal/mol), interacting with TYR 52. Other compounds, such as 3-Amino-1-phenyl-2-pyrazolin-5-one, Avenanthramide B, and 4-methoxyphenyl ester of hexanoic acid, exhibited strong binding affinities and formed hydrogen bonds with key residues. These interactions, along with hydrophobic interactions involving residues like MET 422, ARG 422, and TYR 420, contribute to the stability of the ligand-protein complexes.

The essential oil compounds also showed notable binding affinities, with (1R, 4R) 6-Methylhept-5-en-2-yl-1-methyl-4-Cyclohex-2-enol exhibiting the highest at -7.7 kcal/mol. This compound interacted with VAL 398, LEU 188, and CYS 99 through various non-covalent interactions. Other compounds, including Caryophyllene oxide, D-Limonene, and Methyleugenol, primarily engaged in hydrophobic interactions with minimal hydrogen bonding. This suggests that the wound-healing potential of *E. viscosus* essential oil is predominantly based on hydrophobic interactions rather than hydrogen bonds, providing insight into the mechanism of action for these compounds.

The molecular docking analysis revealed intricate interactions between the bioactive compounds from both the methanolic extract and essential oils of *E. viscosus* with the target protein. Figures 2 and 3 demonstrate that compounds from the methanolic extract effectively occupied the binding pockets of the protein, engaging in multiple types of molecular interactions. These interactions include hydrogen bonding, which provides specificity and directionality to the binding, van der Waals forces that contribute to the overall stability of the complex, and hydrophobic interactions that play a crucial role in the binding affinity.

Similarly, Figs. 4 and 5 illustrate that compounds from essential oils also occupied the binding pockets, participating in the same types of molecular interactions.

However, a notable difference was observed between the two extracts. The bioactive compounds from the methanolic extract of *E. viscosus* exhibited a higher number of hydrogen bonds and stronger binding potential compared to those from essential oils. This enhanced binding profile suggests that the methanolic extract compounds may have a more pronounced effect on the target protein, potentially leading to more efficient modulation of its activity. These findings provide molecular-level support for the role of *E. viscosus* compounds, particularly those from the methanolic extract, in wound healing mechanisms. The stronger interactions observed with the methanolic extract compounds indicate their potential superiority in influencing the biological processes involved in wound repair, although further experimental validation would be necessary to confirm this hypothesis.

ADME (Absorption, Distribution, Metabolism, Excretion) properties of MMP-9

The methanolic extract and essential oil components of *E. viscosus* exhibited promising characteristics for wound healing applications, as demonstrated by in silico pharmacokinetics and drug-likeness analyses (Tables 6 and 7). The methanolic substances showed high gastrointestinal absorption (GI), with only one compound (3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy) capable of crossing the blood-brain barrier. These substances also inhibited key CYP enzymes (CYP2C9, CYP2D6, CYP1A2, CYP2C19, and CYP3A4) but were not substrates of P-glycoprotein. While meeting Lipinski's Rule of Five criteria for drug-likeness, some compounds presented Brenk alerts due to structural concerns.

Table 6

In-Silico pharmacokinetics and drug-likeness of bioactive compounds from *E.viscosus* methanolic extract for wound healing activity

Parameters	Avenanthramide B	3-Amino-1-phenyl-2-pyrazolin-5-one	(4,6-Diamino-1,3,5-triazin-2-yl) cyanamide	Heptanoic acid, 4-methoxyphenyl ester	3-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenylester	3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy
Physiochemical properties						
MW (Da) g/mol	329.30	175.19	151.13	236.31	347.19	281.35
H-bond donors	4	1	3	0	0	0
H-bond acceptors	6	2	4	3	9	3
Rotable bonds	6	1	2	8	5	4
Lipophilicity						
Consensus Logp	1.89	0.78	-0.99	3.54	3.84	3.37
Water solubility						
Log S (ESOL)	-3.44	-1.43	-0.89	-3.63	-4.71	-3.75
Log S (Ali)	-4.60	-1.17	-1.85	-4.55	-5.48	-3.50
Pharmacokinetics						
GI absoorption	High	High	High	High	High	High
Bbb permeant	NO	No	No	No	No	Yes
P-gp substrate	No	No	No	No	No	No
CYP enzymes inhibitor	CYP2C9	-	-	CYP2D6	CYP1A2, CYP2C19, Cyp2C9	CYP1A2, CYP2C19, CYP3A4
Drug likeness						
Lipinski	0 V	0 V	0V	0V	0V	0V
Medicinal chemistry						
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenks	2 alerts: Hydroquinone, Michael acceptor	0 alert	2 alerts: cyanate aminonitrile thiocyanate, enamine	1 alert: phenol ester	3 alerts: nitro group, oxygen nitrogen single bond, phenol ester	0 alert

MW (Da)- Molecular Weight (Daltons), 0 V- 0 violations, Consensus LogP- Consensus Partition Coefficient (LogP), Log S (ESOL)- Logarithm of Water Solubility predicted by the ESOL model, Log S (Ali)Logarithm of Water Solubility predicted by Ali model, GI absorption – Gastrointestinal Absorption (High/Low), BBB permeant – Blood-Brain Barrier Permeability (Yes/No), P-gp substrate – Substrate of P-glycoprotein, related to drug efflux, PAINS – Pan-Assay Interference Compounds (alerts for potential false positives), CYP enzyme inhibitor – Cytochrome P450 Enzyme Inhibition.

Table 7
In-Silico pharmacokinetics and drug-likeness of bioactive compounds from *E.viscosus* essential oil for wound healing activity

Parameters	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl) cyclohex-2-enol	Caryophyllene oxide	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,(R)	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	D-Limonene	Methyl eugenol
Physiochemical properties						
MW (Da) g/mol	222.37	220.35	154.25	204.35	136.23	178.23
H-bond donors	1	0	1	0	0	0
H-bond acceptors	1	1	1	0	0	2
Rotable bonds	4	0	1	1	1	4
Lipophilicity						
Consensus Logp	3.79	3.68	2.60	4.12	3.37	2.58
Log S (ESOL)	-3.64	-3.45	-2.78	-3.43	-3.50	-2.61
Log S (Ali)	-4.40	-3.51	-3.36	-3.49	-4.29	-2.55
Pharmacokinetics						
GI absoorption	High	High	High	High	Low	High
Bbb permeant	Yes	Yes	Yes	No	Yes	Yes
P-gp substrate	No	No	No	No	No	No
CYP enzymes inhibitor	CYP2C9	CYP2C9, CYP2C19	-	CYP2C9, CYP2C19	CYP2C9	CYP1A2
Drug likeness						
Lipinski	0V	0 V	0V	Yes; 1 violation: MLOGP > 4.15	0V	0V
Medicinal chemistry						
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	0 alert
Brenks	1 alert: Isolated alkene	2 alerts: Three-membered heterocycle, isolated_alkene	1 alert: isolated alkene	1 alert: isolated alkene	1 alert: isolated alkene	1 alert: isolated alkene

MW (Da)- Molecular Weight (Daltons), Consensus LogP- Consensus Partition Coefficient (LogP), Log S (ESOL)- Logarithm of Water Solubility predicted by the ESOL model, Log S (Ali)Logarithm of Water Solubility predicted by Ali model, GI absorption – Gastrointestinal Absorption (High/Lsow), BBB permeant – Blood-Brain Barrier Permeability (Yes/No), P-gp substrate – Substrate of P-glycoprotein, related to drug efflux, PAINS – Pan-Assay Interference Compounds (alerts for potential false positives), CYP enzyme inhibitor – Cytochrome P450 Enzyme Inhibition.

The essential oil compounds similarly displayed favorable properties, with most exhibiting high gastrointestinal absorption except for d-limonene. All but one compound (1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene) (Lipinski violation MLOGP > 4.15)showed blood-brain barrier permeability. Several essential oil compounds inhibited specific CYP enzymes (specifically CYP2C9, CYP2C19, and CYP1A2), but none were P-glycoprotein substrates. Although no PAINS alerts were detected, some structures presented Brenk alerts due to solitary alkenes and three-membered heterocycles. Overall, both the methanolic extract and essential oil components of *E. viscosus* demonstrated favorable pharmacokinetic and drug-likeness properties, suggesting their potential for wound healing applications.

Toxicology properties of MMP-9

The methanolic extract and essential oil of *E. viscosus* have garnered attention for their wound-healing and antioxidant properties. To further assess their potential therapeutic applications, the bioactive compounds derived from these sources were subjected to carcinogenicity and acute oral toxicity evaluations using ADMESAR 1. The results, presented in Tables 8 and 9, provide valuable insights into the safety profile of these compounds. All evaluated compounds were classified as Class III in terms of wound healing activities, indicating moderate toxicity. This classification suggests that while these compounds may have some level of toxicity, they do not pose significant toxicological concerns, which is promising for their potential use in therapeutic applications.

Table 8
Toxicity Profile of Bioactive Compounds from Methanolic Extract of *Endostemon viscosus* for Wound Healing Activity

S.No	Compounds	Carcinogenicity	Acute oral toxicity
1	Avenanthramide B	No	III-Moderate toxicity
2	3-Amino-1-phenyl-2-pyrazolin-5-one	No	III-Moderate toxicity
3	(4,6-Diamino-1,3,5-triazin-2-yl) cyanamide	No	III-Moderate toxicity
4	Heptanoic acid, 4-methoxyphenyl ester	No	III-Moderate toxicity
5	3-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenylester	No	III-Moderate toxicity
6	3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy	No	III-Moderate toxicity

Table 9
Toxicity Profile of Bioactive Compounds from Methanolic Extract of *Endostemon viscosus* for Wound Healing Activity

S.No	Compounds	Carcinogenicity	Acute oral toxicity
1	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl) cyclohex-2-enol	No	III-Moderate toxicity
2	Caryophyllene oxide	No	III-Moderate toxicity
3	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,(R)	No	III-Moderate toxicity
4	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	No	III-Moderate toxicity
5	D-Limonene	No	III-Moderate toxicity
6	Methyl eugenol	No	III-Moderate toxicity

Furthermore, the evaluation revealed that none of the bioactive compounds exhibited carcinogenic properties, which is a crucial finding for their potential use in medical treatments. The majority of these compounds were found to be generally non-carcinogenic, further supporting their safety profile. The moderate levels of oral toxicity observed in these bioactive compounds from *E. viscosus* suggest that they may be suitable candidates for therapeutic applications. This combination of wound healing and antioxidant properties, coupled with a favorable toxicity profile, opens up possibilities for developing new treatments or enhancing existing ones using these natural compounds. However, further research and clinical trials would be necessary to fully establish their efficacy and safety for specific medical applications.

Discussion

The findings of this study highlight the potential of *E.viscosus* extract as a source of bioactive compounds with therapeutic applications. Through computational methods, researchers have identified compounds that meet drug-likeness criteria and exhibit promising binding affinities to the 1L6J protein. The analysis revealed that both methanolic and essential oil extracts contained compounds capable of interacting with the target protein through various non-covalent interactions, including hydrogen bonding, van der Waals forces, and hydrophobic interactions (Wang et al. 2022). Notably, the methanolic extract compounds demonstrated a higher number of hydrogen bonds and stronger binding potential than the essential oil-derived compounds, aligning with the understanding that hydrogen bonding significantly contributes to protein-ligand complex stability (Schiebel et al. 2018).

This study is part of a broader trend in utilizing computational methods to screen plant-derived compounds for drug discovery. Similar studies have been conducted on various plant species including Gymnema sylvestre (Nganso Ditchou et al. 2024), Pleurotus ostreatus (Effiong et al. 2024), Wedelia trilobata (Gowtham et al. 2023), Teucrium polium (Noumi et al. 2020) and Caulerpa racemosa (Tassakka et al. 2023). These studies typically involve extracting compounds from plant materials, identifying them through techniques such as GC-MS or HPLC, conducting in silico molecular docking to predict binding affinities, and assessing drug-likeness properties through ADMET predictions. The consistent identification of promising compounds across these studies underscores the potential of plant-derived bioactive molecules for drug discovery and development.

The in silico analyses conducted to assess the pharmacokinetic profiles and drug-likeness of the identified compounds provided crucial insights into their potential as therapeutic agents. These analyses focused on the absorption, distribution, metabolism, and excretion (ADME) properties, as well as the potential interactions with key enzymes and transporters. The results indicated that many compounds from various plant extracts, including Moringa oleifera seed extract and *E. viscosus* methanolic extract, demonstrated good drug-likeness according to Lipinski's Rule of Five and Veber's rules (Shady et al. 2022). This suggests that these compounds possess favorable physicochemical properties for oral bioavailability and drug development.

Essential oil compounds, in particular, show promising characteristics, such as high gastrointestinal (GI) absorption and blood-brain barrier (BBB) permeability. Studies on essential oils from plants such as Satureja kitaibelii and Ptychotis verticillata revealed the presence of compounds such as p-cymene, limonene, geraniol, carvacrol, and borneol (Nikolova et al. 2025;Taibi et al. 2023). Similarly, compounds from guava essential oil exhibit good drug-likeness (Mandal et al. 2022). However, it is important to note that many of these compounds also showed potential inhibition of cytochrome P450 (CYP) enzymes, which could lead to drug-drug interactions. This highlights the need for a comprehensive evaluation of both the beneficial properties and potential risks when considering natural compounds for medicinal use, as exemplified by the study of antidiabetic flavonoids (Bitew et al. 2021).

The safety and efficacy of *E. viscosus* for wound healing and antioxidant applications have been established along with a broader trend in phytochemical research. Methanolic extracts and essential oils derived from various plants have shown significant potential in these therapeutic areas (Ramasamy et al. 2022). The bioactive compounds present in these extracts, including terpenoids, phenolics, and flavonoids, are primarily responsible for their medicinal properties (Bouyahya et al. 2022). These compounds often exhibit synergistic effects, which enhance their overall therapeutic potential.

The growing interest in plant-derived bioactive compounds stems from their promising therapeutic potential coupled with relatively low toxicity profiles. These characteristics make them attractive candidates for the development of new pharmaceutical products and natural remedies. The antioxidant properties of these compounds play a crucial role in wound healing by neutralizing free radicals and reducing oxidative stress in the damaged tissues. Additionally, many plant extracts possess antimicrobial properties that can prevent infections and promote faster wound closure. As research in this field progresses, more plant-derived compounds will likely be identified and characterized for potential applications in wound healing, antioxidant therapies, and other medical applications.

Conclusion

In conclusion, this study confirmed the therapeutic potential of *Endostemon viscosus* bioactive compounds, particularly those from the methanolic extract, in targeting MMP-9 for wound healing. The methanolic compounds exhibited strong binding interactions and hydrogen bonding, supporting their stability and efficacy. The ADME analysis demonstrated favorable pharmacokinetics, and toxicity assessments indicated moderate safety with no carcinogenic risk. These findings suggest that *E. viscosus* could serve as a natural source of bioactive agents for wound-healing applications, encouraging further experimental validation through in vitro and in vivo studies.

Declarations

Conflict of Interest declaration: None

Funding Statement:

None

Author Contribution

Author contributions: KMS and SM conceived the idea; KMS experimented; KMS analyzed the data; KMS, SM, and SP wrote the manuscript; KMS, SM, and SP edited the manuscript, and all authors approved the manuscript.

References

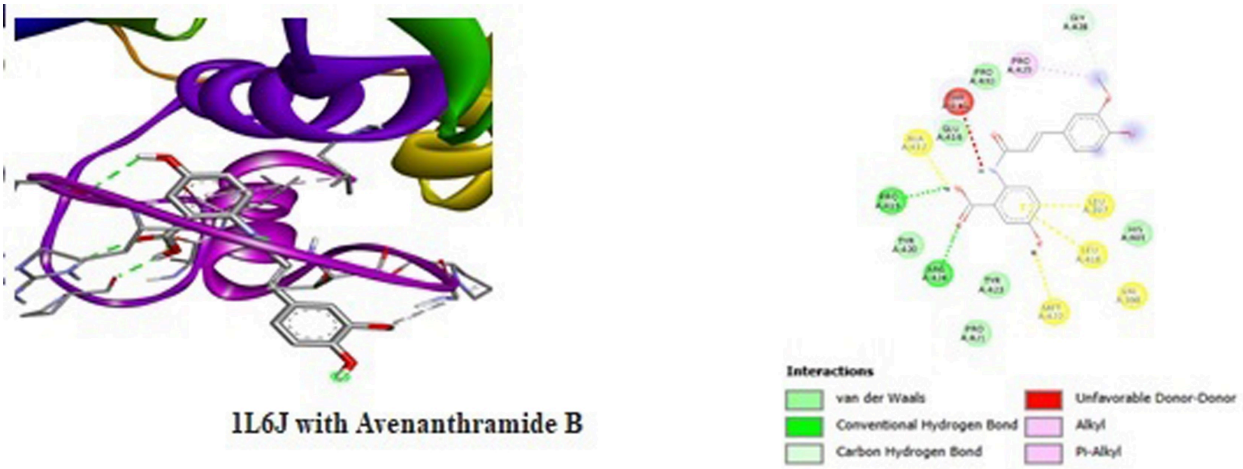
1. Aly SH, Elissawy AM, Mahmoud AMA, El-Tokhy FS, Mageed SSA, Almahli H, Al-Rashood ST, Binjubair FA, Hassab MAE, Eldehna WM, Singab AE-NB (2023) Synergistic Effect of Sophora japonica and Glycyrrhiza glabra Flavonoid-Rich Fractions on Wound Healing: In Vivo and Molecular Docking Studies. Molecules 28:2994. https://doi.org/10.3390/molecules28072994
2. Bitew M, Desalegn T, Demissie TB, Belayneh A, Endale M, Eswaramoorthy R (2021) Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. PLOS ONE 16:e0260853. https://doi.org/10.1371/journal.pone.0260853
3. Bouyahya A, Chamkhi I, Balahbib A, Rebezov M, Shariati MA, Wilairatana P, Mubarak MS, Benali T, El Omari N (2022) Mechanisms, Anti-Quorum-Sensing Actions, and Clinical Trials of Medicinal Plant Bioactive Compounds against Bacteria: A Comprehensive Review. Molecules 27:1484. https://doi.org/10.3390/molecules27051484
4. Dallakyan S, Olson AJ (2015) Small-Molecule Library Screening by Docking with PyRx. In: Hempel JE, Williams CH, Hong CC (eds) Chemical Biology. Springer New York, New York, NY, pp 243–250
5. Effiong ME, Bella-Omunagbe M, Afolabi IS, Chinedu SN (2024) *In silico* evaluation of potential breast cancer receptor antagonists from GC-MS and HPLC identified compounds in *Pleurotus ostreatus* extracts. RSC Adv 14:23744–23771. https://doi.org/10.1039/D4RA03832K
6. Gangwar V, Garg A, Lomore K, Korla K, Bhat SS, Rao RP, Rafiq M, Kumawath R, Uddagiri BV, Kareenhalli VV (2021) Immunomodulatory Effects of a Concoction of Natural Bioactive Compounds—Mechanistic Insights. Biomedicines 9:1522. https://doi.org/10.3390/biomedicines9111522
7. Gowtham HG, Ahmed F, Anandan S, Shivakumara CS, Bilagi A, Pradeep S, Shivamallu C, Shati AA, Alfaifi MY, Elbehairi SEI, Achar RR, Silina E, Stupin V, Murali M, Kollur SP (2023) In Silico Computational Studies of Bioactive Secondary Metabolites from Wedelia trilobata against Anti-Apoptotic B-Cell Lymphoma-2 (Bcl-2) Protein Associated with Cancer Cell Survival and Resistance. Molecules 28:1588. https://doi.org/10.3390/molecules28041588
8. Kundu P, Debnath SL, Devnath HS, Saha L, Sadhu SK (2022) Analgesic, Anti-inflammatory, Antipyretic, and *In Silico* Measurements of *Sonneratia caseolaris* (L.) Fruits from Sundarbans, Bangladesh. BioMed Res Int 2022:1405821. https://doi.org/10.1155/2022/1405821
9. Lan B, Zhang L, Yang L, Wu J, Li N, Pan C, Wang X, Zeng L, Yan L, Yang C, Ren M (2021) Sustained delivery of MMP-9 siRNA via thermosensitive hydrogel accelerates diabetic wound healing. J Nanobiotechnology 19:130. https://doi.org/10.1186/s12951-021-00869-6
10. Mandal AK, Paudel S, Pandey A, Yadav P, Pathak P, Grishina M, Jaremko M, Emwas A-H, Khalilullah H, Verma A (2022) Guava Leaf Essential Oil as a Potent Antioxidant and Anticancer Agent: Validated through Experimental and Computational Study. Antioxidants 11:2204. https://doi.org/10.3390/antiox11112204

11. Nganso Ditchou YO, Leutcha PB, Miaffo D, Mamoudou H, Ali MS, Amang À Ngnoung GA, Soh D, Agrawal M, Darbawa R, Zondegoumba Nkwengoua Tchouboun E, Meli Lannang A, Siwe Noundou X (2024) In vitro and in silico assessment of antidiabetic and antioxidant potencies of secondary metabolites from Gymnema sylvestre. Biomed Pharmacother 177:117043. https://doi.org/10.1016/j.biopha.2024.117043
12. Nikolova M, Lyubenova A, Yankova-Tsvetkova E, Georgiev B, Gavrilov G, Gavrilova A (2025) Satureja kitaibelii Essential Oil and Extracts: Bioactive Compounds and Pesticide Properties. Agronomy 15:357. https://doi.org/10.3390/agronomy15020357
13. Noumi E, Snoussi M, Anouar EH, Alreshidi M, Veettil VN, Elkahoui S, Adnan M, Patel M, Kadri A, Aouadi K, De Feo V, Badraoui R (2020) HR-LCMS-Based Metabolite Profiling, Antioxidant, and Anticancer Properties of Teucrium polium L. Methanolic Extract: Computational and In Vitro Study. Antioxidants 9:1089. https://doi.org/10.3390/antiox9111089
14. Ramasamy SP, Rajendran A, Pallikondaperumal M, Sundararajan P, Husain FM, Khan A, Hakeem MJ, Alyousef AA, Albalawi T, Alam P, Ali HM, Alqasim A (2022) Broad-Spectrum Antimicrobial, Antioxidant, and Anticancer Studies of Leaf Extract of Simarouba glauca DC In Vitro. Antibiotics 11:59. https://doi.org/10.3390/antibiotics11010059
15. Schiebel J, Gaspari R, Wulsdorf T, Ngo K, Sohn C, Schrader TE, Cavalli A, Ostermann A, Heine A, Klebe G (2018) Intriguing role of water in protein-ligand binding studied by neutron crystallography on trypsin complexes. Nat Commun 9:3559. https://doi.org/10.1038/s41467-018-05769-2
16. Shady NH, Mostafa NM, Fayez S, Abdel-Rahman IM, Maher SA, Zayed A, Saber EA, Khowdiary MM, Elrehany MA, Alzubaidi MA, Altemani FH, Shawky AM, Abdelmohsen UR (2022) Mechanistic Wound Healing and Antioxidant Potential of Moringa oleifera Seeds Extract Supported by Metabolic Profiling, In Silico Network Design, Molecular Docking, and In Vivo Studies. Antioxidants 11:1743. https://doi.org/10.3390/antiox11091743
17. Taibi M, Elbouzidi A, Ou-Yahia D, Dalli M, Bellaouchi R, Tikent A, Roubi M, Gseyra N, Asehraou A, Hano C, Addi M, El Guerrouj B, Chaabane K (2023) Assessment of the Antioxidant and Antimicrobial Potential of Ptychotis verticillata Duby Essential Oil from Eastern Morocco: An In Vitro and In Silico Analysis. Antibiotics 12:655. https://doi.org/10.3390/antibiotics12040655
18. Tassakka ACmar, Iskandar IW, Juniyazaki ABa, Zaenab S, Alam JF, Rasyid H, Kasmiati K, Sinurat E, Dwiany FM, Martien R, Moore AM (2023) Green algae Caulerpa racemosa compounds as antiviral candidates for SARS-CoV-2: In silico study. Narra J 3:e179. https://doi.org/10.52225/narra.v3i2.179
19. Wang C, Chao C, Yu J, Copeland L, Huang Y, Wang S (2022) Mechanisms Underlying the Formation of Amylose– Lauric Acid–β-Lactoglobulin Complexes: Experimental and Molecular Dynamics Studies. J Agric Food Chem 70:10635–10643. https://doi.org/10.1021/acs.jafc.2c04523
20. Zhou L, Ren M, Zeng T, Wang W, Wang X, Hu M, Su S, Sun K, Wang C, Liu J, Yang C, Yan L (2019) TET2-interacting long noncoding RNA promotes active DNA demethylation of the MMP-9 promoter in diabetic wound healing. Cell Death Dis 10:813. https://doi.org/10.1038/s41419-019-2047-6
21. Zou H, Li Y, Liu X, Wu Z, Li J, Ma Z (2021) Roles of plant-derived bioactive compounds and related microRNAs in cancer therapy. Phytother Res 35:1176–1186. https://doi.org/10.1002/ptr.6883

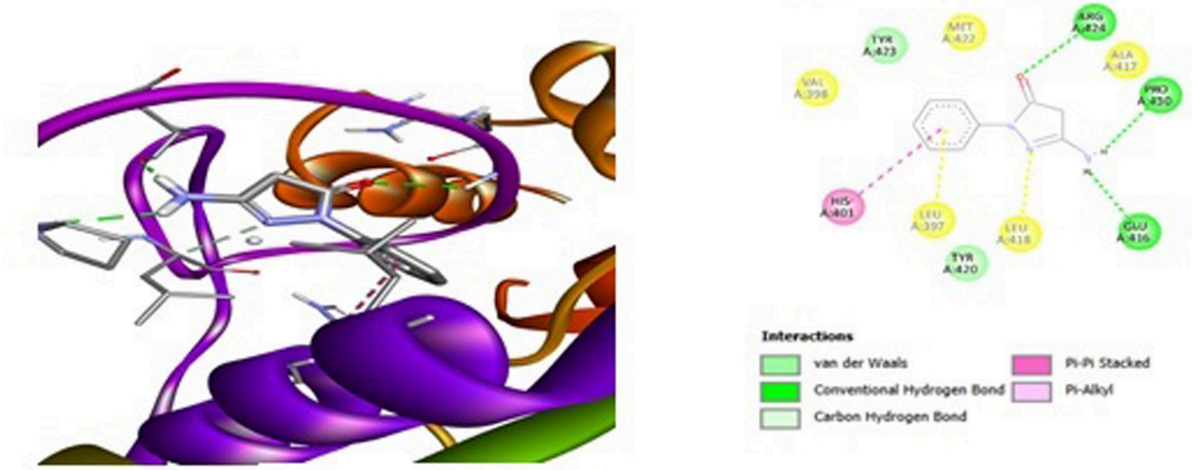
Figures

Figure 1

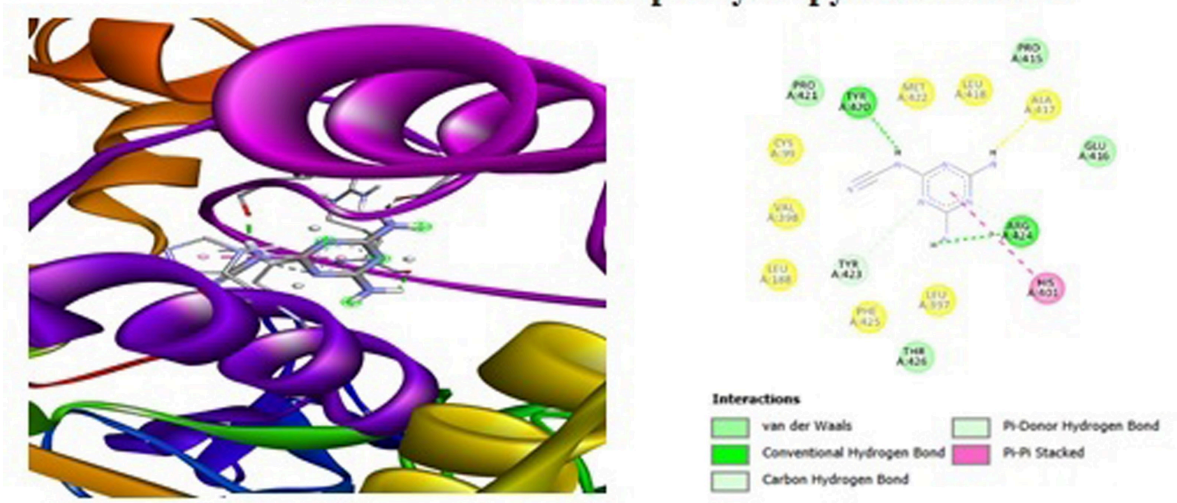
Active Site Predictions of Target Protein 1L6J for Wound



1L6J with Avenanthramide B



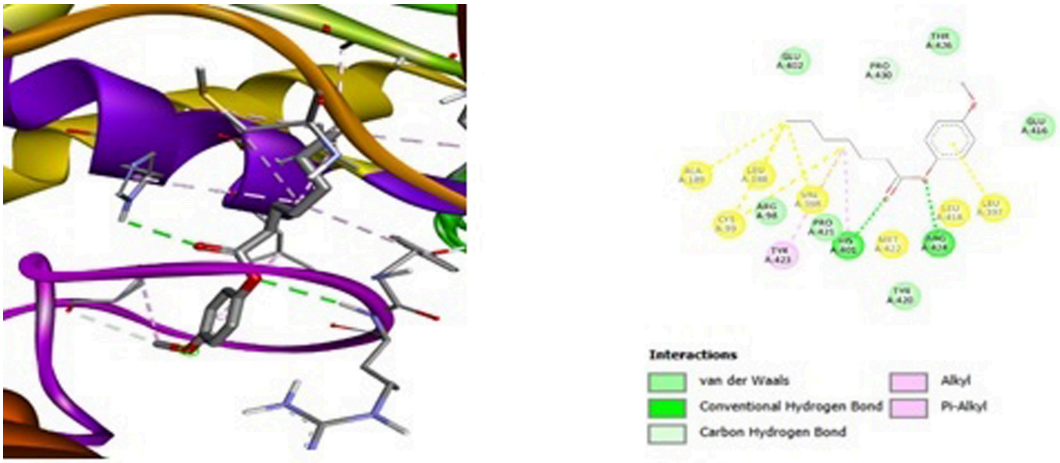
1L6J with 3-Amino-1-phenyl-2-pyrazolin-5-one



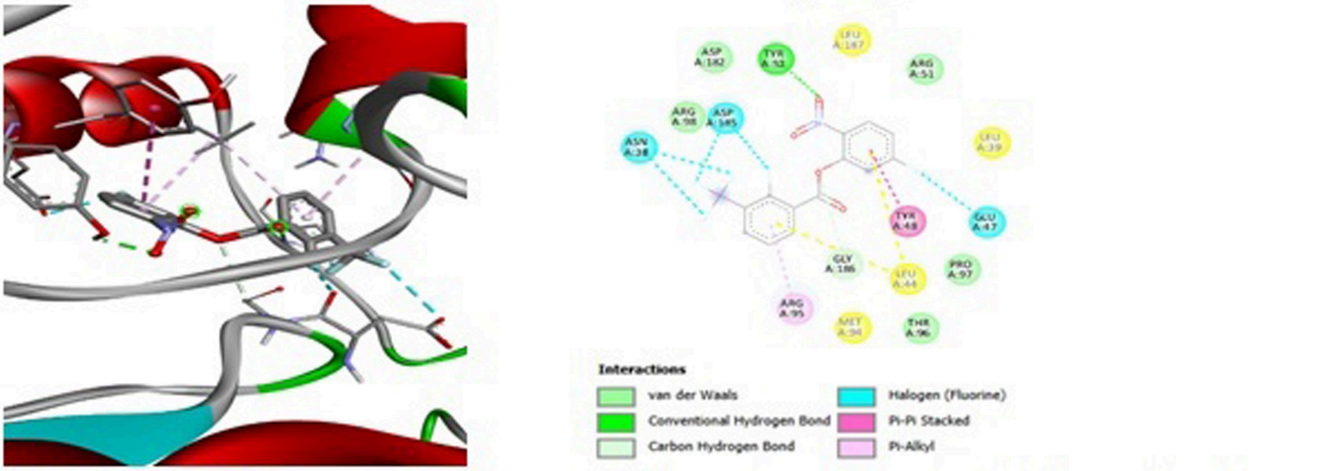
1L6J with (4,6-Diamino-1,3,5-triazin-2-yl) cyanamide

Figure 2

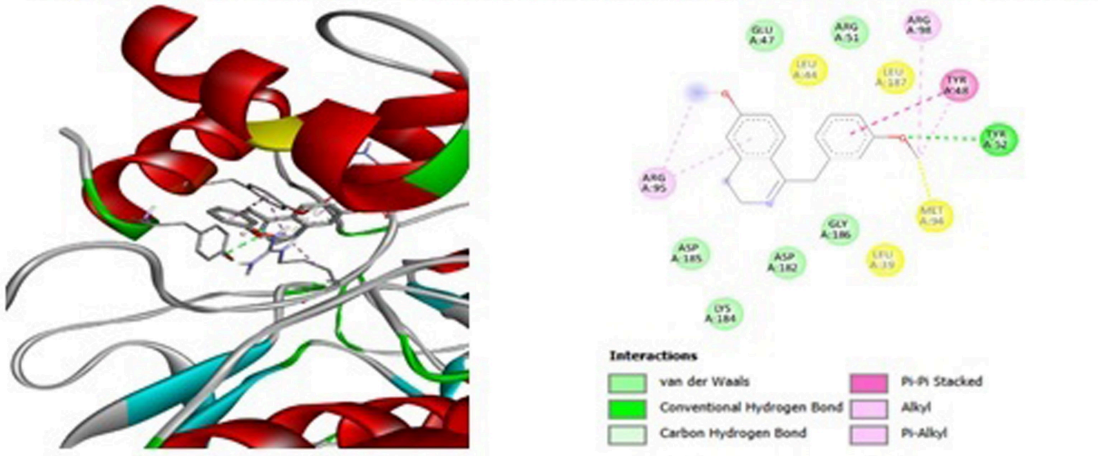
3D and 2D interactions of wound healing compounds from *E. viscosus* methanolic extract (GC-MS) with the target receptor



1L6J with Heptanoic acid, 4-methoxyphenyl ester



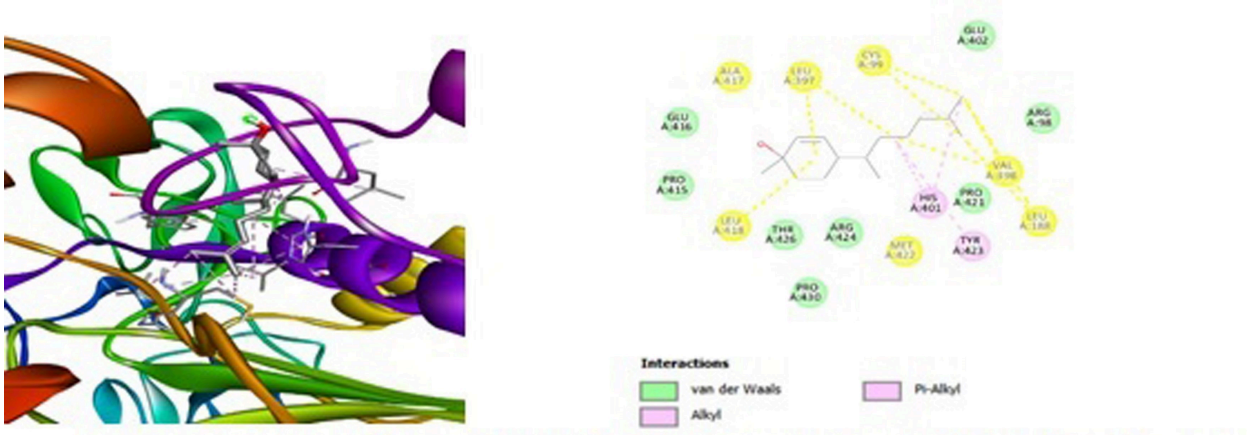
1L6J with 33-Fluoro-5-trifluoromethylbenzoic acid, 2-nitro-5-fluorophenylester



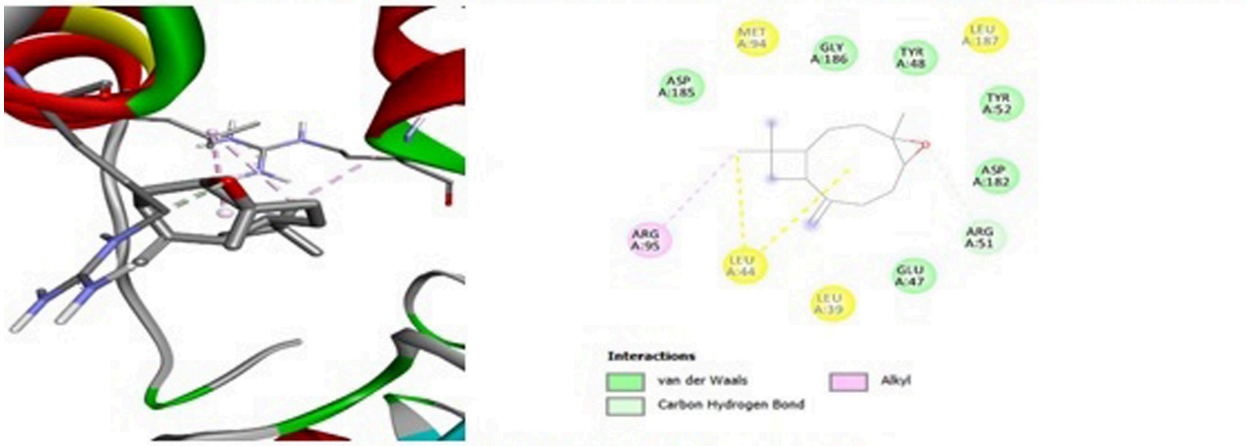
1L6J with 3,4-Dihydroisoquinoline,1-[3-methoxybenzyl]-6-methoxy

Figure 3

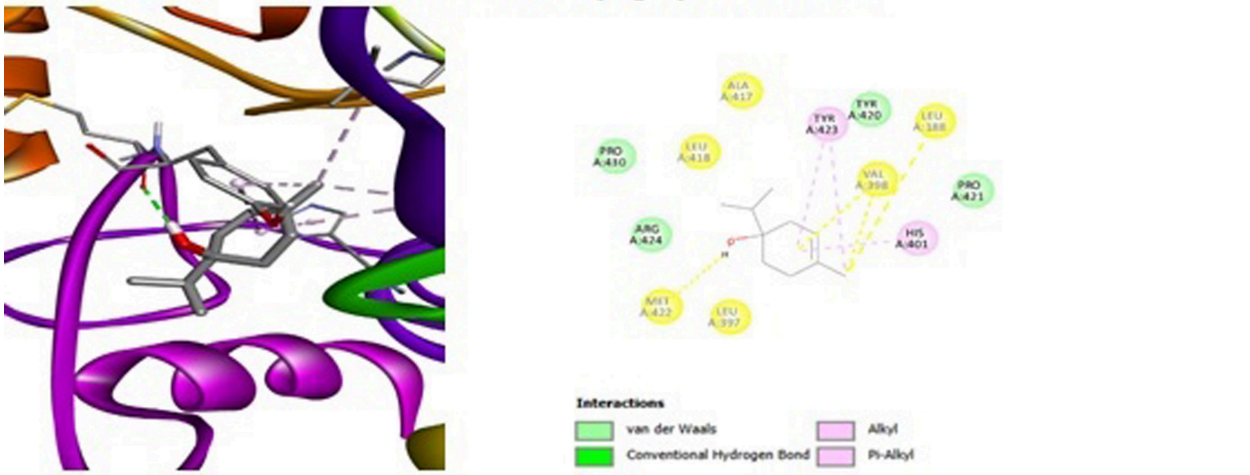
3D and 2D interactions of wound healing compounds from *E. viscosus* methanolic extract (GC-MS) with the target receptor



1L6J with (1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol



1L6J with Caryophyllene oxide



1L6J with 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,(R)

Figure 4

3D and 2D interactions of wound healing compounds from *E. viscosus* essential oil extract (GC-MS) with the target receptor

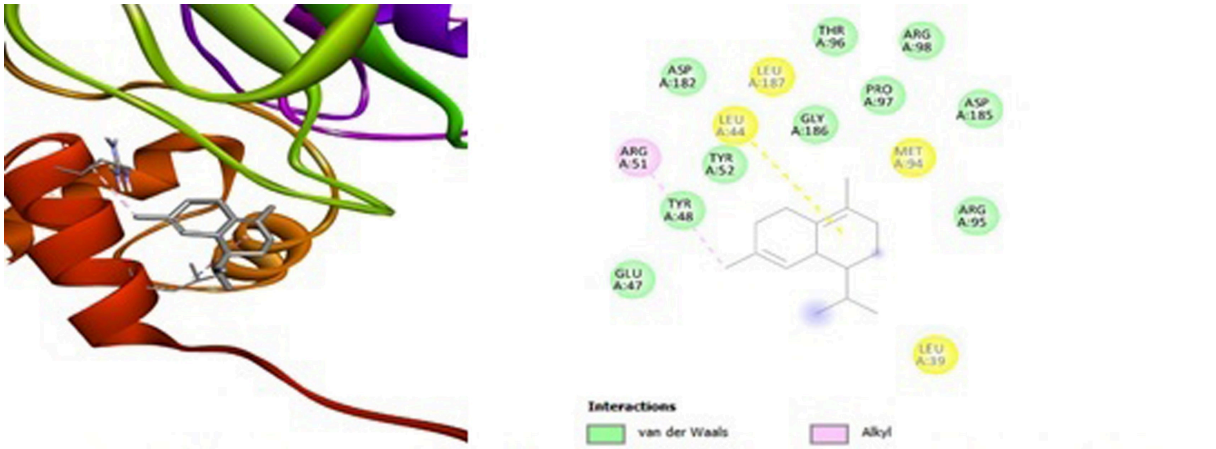


Figure 5

3D and 2D interactions of wound healing compounds from *E. viscosus* essential oil extract (GC-MS) with the target receptor

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.jpg