Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 2Year 2024 Page 856-863

"Molecular Docking Analysis Of Alpha-Terpineol Against Matrix Metalloproteinases"

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Abstract

Objectives: The process of wound healing is crucial and complex within the human body, involving various enzymes. Increased concentrations of matrix metalloproteinases (MMPs) at the wound site lead to slowdown in the healing process. Small bioactive compounds produced from natural sources can be used to down-regulate or inhibit these over expressed MMPs. Alpha-terpineol, a mono-terpenoid alcohol with a monocyclic structure, is present in vitex negundo linn leaf, pemus boldus leaf, cajeput oil, coriander oil, and pine oil. It exhibits diverse biological uses, serving as an antioxidant, anticancer, anticonvulsant, antiulcer, anti-inflammatory, antihypertensive, and anti-nonciceptive agent. The work aims to use molecular docking to understand how alpha-terpineol binds to MMPs.

Materials and Methods: The MMPs was obtained from RCSB database and the alpha-terpineol was obtained from Pubchem. The Auto Dock software was used to create a molecular docking of alpha-terpineol activity on MMPs. Alpha-Terpineol binds to MMPs receptors. Auto Dock Vina was used to examine the binding box-related files, including a PDBQT file and PYMOL software was then used to analyze the receptor and ligand PDBPT files.

Result: Docking tests revealed that alpha-terpineol has a great binding affinity for all four MMPs tested. MMPs 3, 8, and 12 had free binding energies of around -9.1, -10.5, and -9.7kcal/mol, respectively.

Conclusion: Therefore, alpha-terpineol can be employed to regulate the function of matrix metalloproteinases and facilitate the process of wound healing.

KEYWORDS: Wound healing, Molecular docking, Alpha-terpineol, Matrix metalloproteinases.

INTRODUCTION:

Skin is the body's biggest and most vital part, protecting it from microbial invasion [1, 2]. Healthy skin is highly resilient, maintaining the ability to manage and regulate the influx and development of microorganisms effectively [3, 4]. A wound refers to whatever damage to the skin resulting in a loss of its structural integrity.

In general, a wound is a common occurrence [5]. A sequence of events contributes to wound healing [6]. Wound healing involves four events: Hemostasis, Inflammation, Proliferation, and Tissue remodeling [7, 8].
Several enzymes are present at the wound site, which aid in the healing process [9]. Matrix metalloproteinases (MMPs), also known as matrixins, are enzymes found in the extracellular matrix (ECM)[10,11]. MMPs are endoproteases that require zinc to activate [12, 13]. Humans have around 23 MMPs that are grouped into six classes. [14]. Certain MMPs play a role in tissue healing, such as gelatinase (MMP2), stromelysins (MMP3), collagenase (MMP8), and metalloelastase (MMP12) [15, 16]. Under typical circumstances, MMPs are released in a balanced state. However, when oxidative stress occurs at the wound site, there is a disruption in MMP regulation and an internal control mechanism breakdown, resulting in MMP-mediated degradation of newly generated tissue. As a result, the wound healing process is hindered, and persistent wound infections develop [17].
Numerous healing herbs contain substances that possess notable properties for healing wounds, and in contrast to synthetic medications, they exhibit fewer or no adverse effects [18, 19]. Terpineols, which are unsaturated monoterpenoid alcohols, are present in flowers such as freesia and narcissus, as well as herbs like lemon peel oil, oregano, marjoram, and rosemary. Terpineols have five frequent isomers: alpha terpineol, beta terpineol, gamma terpineol, delta terpineol, and terpinen-4-ol.□ Alpha-terpineol, a volatile monoterpenoid alcohol, plays a crucial role in the essential oils of various aromatic herbs like ocimum canum sims and origanum vulgare L, frequently utilized for medicinal purposes. Alpha-terpineol can be derived from vitex negundo linn leaf, petitgrain oil, cajeput oil, pine oil, coriander oil, and pemus boldus leaf. Alpha-terpineol proves to be of great value in the field of medicine. It possesses a pleasing fragrance reminiscent of lilacs and is commonly employed in perfumes, cosmetics, and aromatic fragrances. Alpha-terpineol has various biological applications, including antioxidant, anticancer, anticonvulsant, antiulcer, anti-inflammatory, antihypertensive, and pain -relieving properties. It also serves to enhance cutaneous absorption and exhibits pest-control characteristics. □lpha-terpineol has molecular formula C10H18O.p-menth-1-en-8-ol 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol is the IUPAC designation for □ - terpineol [20, 21, 22]. De Oliveria et.al, had studied the anti inflammatory property of □- terpineol on mice [23]. The study showed that it down-regulates TNF-□□□and IL-1-□□□Villegas et al studied the in-vivo wound healing property of □ - terpineol from Peperomiagalioides[24].
This work attempts to investigate the wound healing activity of □ - terpineol by docking it with MMP3, MMP8, and MMP12.
Molecular interaction analysis is an in silico method for discovering optimum interactions of a protein and a ligand. As a result, utilizing in silico research allows the identification of pertinent information before engaging in in vitro and in vivo experiments. This study aims to enhance comprehension of how alpha terpineol interacts with MMPs, potentially influencing the wound healing process.

MATERIALS AND METHODS:

Protein preprocessing:
The 3D structures of the selected MMPs from Homo sapiens were obtained in PDB format from the RCSB database. The chosen MMPs and their respective PDB codes are MMP3 (2JNP), MMP8 (1A85), and MMP12 (1JIZ) [16].

Ligand preprocessing:
The 3D configuration alpha terpineol was obtained from the PubChem database, where it is identified by the chemical ID 17100. Alpha-terpineol has a molecular formula of C10H18O and molecular weight of 154.25g/mol. The physicochemical characteristics of alpha-terpineol, such as its molecular weight, count of hydrogen bond donors and acceptors and molecular refractivity, were examined in comparison to Lipinski's rule of five [25].

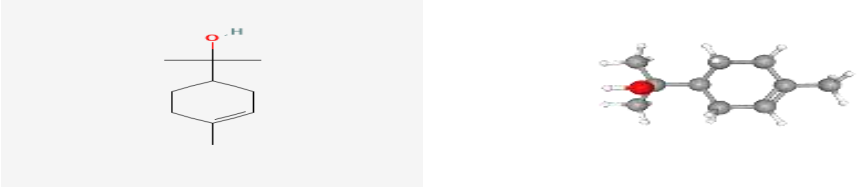
Active sites of MMPs:
One of the most significant requirements in docking studies is the active site. This is the analysis empirically validated active site residues from literature investigations for the selected MMPs (MMP3, MMP8, and MMP12), where the ligand engages with amino acid residues in the protein's active to facilitate successful binding interactions [27-29].

Molecular docking process:
□lpha-terpineol action on the MMP3, MMP8, and MMP12 Proteins Molecular Docking. Auto Dock software was used to build a molecular docking of □ - terpineol activity on MMP3, MMP8 and MMP12. Alpha-terpineol

and MMP3, MMP8, and MMP12 binding energies revealed that the former interacts with the MMP3, MMP8, and MMP12 receptors. Alpha- terpineol's 3D and 2D structures were acquired from the PubChem website [figure 1]. On the PDB platform, the MMP3, MMP8, and MMP12 proteins was also obtained. In PYMOL software, all three proteins were produced by adding polar hydrogen and eliminating solvent and organic. Auto Dock software was used to create the binding box of alpha-terpineol and all three MMPs. Auto Dock Vina was used to analyze the binding box-related files, and a PDBQT file was created [Table 3]. PYMOL software was then used to examine the receptor and ligand PDBPT files.

Table 1: Active site residues present in selected MMPs.

MMP3	Tyr, Leu, His, Ala.	[27]
MMP8	Asn, Ala, Tyr, Arg.	[28]
MMP12	Ala, Gly, Glu, His, LYS.	[29]



Structure 1: □lpha-terpineol

(a) (b) (c)

Structure 2: Matrix Metelloproteinases (MMPs) (a)MMP3**,** (b)MMP8, (c)MMP12.

RESULTS:

Table 2. Lipinski rule of five

Lipinski rule	**Acceptable value**	**Value for □ - terpineol**
H-bond donor	< 5	154.25
H-bond acceptors	< 10	1
Chemical mass (Da)	< 500	1
Molar refractivity	40-130	47.1
High lipophilicity (LOGP)	< 5	2.98

T ABLE3: (a)MMP3: Evaluate the MOE score for the MMP3 protein and the energy interaction with alpha-terpineol in kilocalories per mole.

MODE	AFFINITY (Kcal/mol)	DIST FROM RMSD L.B	BEST MODE RMSD U.B
1	-9.1	0.000	0.000
2	-9.0	1.586	2.706
3	-8.7	1.785	7.653
4	-8.5	1.905	7.737
5	-8.5	2.195	6.367
6	-8.4	1.781	2.292
7	-8.3	2.189	6.583
8	-8.3	1.050	2.291
9	-8.2	2.137	6.196

(b)MMP8: Evaluate the MOE score for the MMP8 protein and the energy interaction with alpha- terpineol in kilocalories per mole.

MODE	AFFINITY (Kcal/mol)	DIST FROM RMSD L.B	BEST MODE RMSD U.B
1	-10.5	0.000	0.000
2	-10.4	1.530	2.446
3	-10.3	2.314	6.110
4	-10.0	2.006	2.564
5	-10.0	3.126	6.889
6	-9.0	3.302	6.464
7	-9.0	2.691	3.833
8	-8.9	2.955	6.676
9	-8.8	3.005	6.276

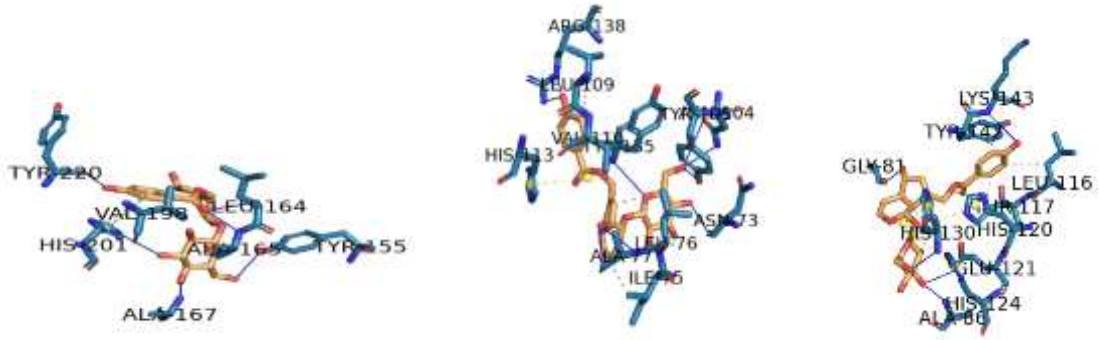
(c)MMP12: Evaluate the MOE score for the MMP12 protein and the energy interaction with alpha-terpineol in kilocalories per mole.

MODE	AFFINITY (Kcal/mol)	DIST FROM RMSD L.B	BEST MODE RMSD U.B
1	-9.7	0.000	0.000
2	-9.2	2.477	3.234
3	-8.7	3.845	7.597
4	-8.7	2.017	2.636
5	-8.7	3.265	7.535
6	-8.6	3.086	3.972
7	-8.6	3.207	9.073
8	-8.5	1.542	2.270
9	-8.4	2.983	7.775

Note. The reported bonding effectiveness is expressed in kcal/mol (energy), and RMSD values relative to the optimal mode are calculated using only mobile heavy atoms. Two RMSD measures, RMSD/l.b. (lower) and RMSD/l.c. (higher), are provided, with RMSD/u.b. (upper bound) varying in atom organization during distance computation. RMSD for root mean square deviation.

Table 4: The molecular interactions observed between alpha-terpineol and specific MMPs.

Binding protein	**No. of H bonds present**	**Amino acid residues that forms H bond**	**Length of H- bond (Å)**	**Binding energy (kcal/mol)**
MMP3	6	Tyr155 Leu164 Ala165 Ala167 His201 Tyr220	2.50 2.30 2.07 2.44 3.00 2.69	-9.1
MMP8	9	Asn73 Ala77 Ala77 Asn104 Asn104 Tyr105 Tyr135 Arg138 Arg138	2.70 2.99 2.23 2.58 3.35 1.97 2.92 2.81 2.83	-10.5
MMP12	5	Gly81 Ala86 Glu121 His130 Lys143	2.45 2.25 2.47 3.12 2.88	-9.7



(a) (b) (c)

Structure 3: Alpha-terpineol's hydrogen bonding interaction with MMPs' active site (a)MMP3, (b)MMP8, and (c)MMP12.

DISCUSSION:

Preparation of both protein and ligand:
Three MMPs (MMP3, MMP8, and MMP12) were selected for the molecular interaction analysis based on their roles in wound healing. Typically, these MMPs are regulated in equilibrium, but under certain circumstances such as persistent wounds, their activity rises, causing a declaration in the wound healing process.[30,31]. Alpha terpineol, a natural bioactive molecule discovered in vitex negundo linn, was employed as a ligand to explore its interaction with different MMPs [32]. Structures 1 and 2 show the 3D structures of chosen MMPs received from RCSB in PDB format, as well as the structure of alpha terpineol obtained from the PubChem database. Before the docking process, selected MMPs and alpha terpineol were docked using auto dock vina software. The dock preparation process comprises removing ions and ligands, adding hydrogen bonds, and adding Gasteiger charges.

Characteristics of the ligand:
Alpha-terpineol's ligand properties were assessed using Lipinski's rule of five, with the findings outlined in Table 2. The results showed alpha terpineol satisfies all five criteria [33].

Forecasting of active sites in MMPs:
Table 4 displays the amino acids residues identified within the active sites of MMP3, MMP8, and MMP12. Some of the active site residues were TYR, LEU, ALA, ASN, GLU, and so on. These residues have a more powerful binding affinity with the ligand molecule.

Docking interaction and analysis:
Auto dock vina was used for the docking studies. Three dock prepared MMPs were docked with dock prepared - terpineol. The RMSD score and binding affinity values were employed to predict the optimal interactions in the middle of MMPs and alpha terpineol. The favorable orientations were employed to identify residues acting as hydrogen bond donors and evaluate the corresponding bond distances within the active sites of MMPs and alpha terpineol.

Interaction between alpha-terpineol and MMP 3:
MMP3 is a member of the stromelysins subfamily and promotes the synthesis of gelatin, laminin, fibronectin, and aggrecan at the wound site, thereby accelerating the healing process. Structure 3a illustrates the hydrogen bonds formed among the active site residues of MMP3. Alpha terpineol utilizing PyMOL Ver: 2.5.5. Table 4 shows the bond length and hydrogen bonding residues. It revealed an interaction score of around - 9.1 kcal/mol between MMP3 and - terpineol out of 9 poses. Structure 3a shows that alpha terpineol binds to MMP 3's active site pocket, forming hydrogen bonds with interacting amino acid residues. As a result, alpha-terpineol can be thought of as a possible inhibitor molecule. It has the ability to block MMP 3 (stromelysin) action, reducing the presence of TGF and anti inflammatory elements in the vicinity of the wound site influences its activity.

Interaction between alpha-terpineol and MMP 8:
MMP8 is a member of collagenase 2 subfamily and plays a role in the degradation of extracellular collagen deposits [34]. Excessive breakdown of collagen type I reduces wound healing. As a result, reducing MMP 8 activity at the wound site is critical for increasing collagen deposition [35]. Structure 3b depicts the hydrogen bonding interaction between MMP8 and alpha-terpineol utilizing PyMOL Ver: 2.5.5. Structure 3b demonstrates that alpha-terpineol fits into the active site pocket, with MMP 8 showing the greatest affinity score of about -10.5kcal/mol out of 9 positions. As a result, □ - terpineol may have superior inhibitory effect against MMP 8. Table 4 shows the bond lengths and hydrogen bonding residues. Controlling collagenase activity at the wound site can reduce collagen breakdown and boost the accumulation of gelatin, aggrecan, and fibronectin. Suppressing MMP8 activity diminishes chemokine effects and cell migration to wound area, thereby mitigating inflammation and facilitating the healing process.

Interaction between alpha terpineol and MMP 12:
MMP 12 is an enzyme in the metalloelastase family that is necessary for tissue healing. Elevated expression of MMP 12 leads to the entry of the incendiary cells and hinders the timely progress of wound healing [36]. The docking analysis between alpha-terpineol and MMP12 indicated that alpha terpineol effectively occupied the active site of MMP12. The interaction was visualized using PyMOL Ver: 2.5.5 and displayed in Structure 3c.Table 3 displays the length and number of hydrogen bonding residues in MMP12's active site. Structure 3c shows that alpha-terpineol exhibits a bonding effectiveness of -9.7 kcal/mol to the active site pocket of MMP 12 across 9 poses. It inhibitory characteristics are anticipated to reduce MMP12 activity in the wound area, resulting in increased buildup of elastin, gelatin, collagen, fibronectin, vitronectin, proteoglycan, and angiogenesis, thereby accelerating the wound healing process.

CONCLUSION:

Alpha-terpineol is a possible inhibitory molecule, according on molecular docking studies between alpha-terpineol and MMPs. According to the interaction results, alpha-terpineol penetrates the active sites of MMPs and engages in hydrogen bond interactions with amino acid residues located in the active site. Alpha-terpineol was also demonstrated to have affinity for all three MMPs. In vivo experiments will be conducted in the future to assess alpha-terpineol's inhibitory potential. To enhance faster wound healing, reduce elevated activity of particular MMPs in the chronic wound location.

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