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To cite this article: N A Mokhtar *et al* 2021 *IOP Conf. Ser.: Mater. Sci. Eng.* **1051** 012078

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Docking study for assessment of wound healing potential of isosakuratenin isolated from *Chromolaena odorata*: An *In-silico* approach

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Abstract. Wound healing is a complex and well-orchestrated biological process in all multicellular organisms in which normal wound healing consists of four major phases that are haemostasis, inflammation, proliferations and reepithelization. Abnormal wound healing is always associated with inefficient or miscarried transition during inflammation to proliferation phase. Wound healing potential of various natural extracts have been studied progressively in recent years. In this present study, isosakuratenin, a phytoconstituent previously reported to be isolated from the extracts of *Chromolaena odorata* are aimed at targeting essential proteins involved in wound healing process. Matrix Metalloproteinases (MMP) is a protein essential in wound healing. Therefore, the present study is aimed to evaluate the inhibitory effect of isosakuratenin on MMP as a potential therapeutic target for wound therapy. Isosakuratenin from *Chromolaena odorata* were studied based on their ability to interact with the targeted protein via molecular docking approach. Isosakuratenin showed binding affinity to four different classes of MMPs. The binding energy of these MMPs with isosakuratenin are -7.7 kcal/mol (MMP2), -6.8 kcal/mol (MMP3), -9.0 kcal/mol (MMP8) and -9.7 kcal/mol (MMP12). Isosakuratenin forms stronger interaction with MMP12 in which it forms two bonding at the active site of the protein and shows the most stable dock conformation. This results suggested that, among these four MMPs, isosakuratenin are best interacted with MMP12 and hence, could be used to visualized the potential of isosakuratenin as MMP12 inhibitor during wound healing process. This recent work provides meaningful insights in regards to the molecular structure interaction and requirement of the phytoconstituents from *Chromolaena odorata* for subsequent pharmaceutical formulation in catering the wound healing products demand.

1. Background

Skin, the human largest organ functions as a natural environment defense for human body. When the skin's integrity is compromised, either by acute or chronic wound, a series of dynamic biochemical processes will be initiated to restore the health of the skin known as wound healing. Normal wound



healing process usually characterized as four sequential but overlapping phases that are haemostasis (0-several hour after injury), inflammation phase (1-3 days), proliferation phase (4-21 days) and remodeling (21 days till up to several years) [1]. Deregulation in any of these steps will results in impaired healing which subsequently leads to health and socioeconomic burden. Wound that failed to progress through normal stages of healing (usually more than 6 weeks) would lead to pathological inflammation [2]. Persisting inflammatory cells, mostly neutrophils and macrophages, generate large amount of proinflammatory cytokines and highly proteolytic microenvironment at the wound site [3]. Matrix metalloproteinases (MMPs), are proteolytic and matrix degrading enzyme, abundantly found in the extracellular matrix (ECM) of the wound site. MMPs belong to endoproteases that depends on zinc for their activity [4]. Currently, there are at least 24 known MMPs in human and it is generally appreciated that MMPs play crucial roles in each stages of wound healing [5]. Gelatinases (MMP2), stromelysins (MMP3), collagenase (MMP8) and metalloelastase (MMP12) are among MMPs that are mainly involved in tissue repairing process [3], [5]. Under normal condition, MMPs are secreted in a balanced manner, but due to oxidative stress in the wound site, there are improper regulation in MMPs and endogenous regulatory process, in which subsequently leads to degradation of newly form ECM by MMPs, thus, delaying the wound healing process [6].

Concurrently, nutraceuticals had gained much interest in the treatment for various medical application. Many medicinal plants have been reported to possess compounds that are showing significant wound healing activity. *Chromolaena odorata*, a tropical weed, is a plant commonly known with various names in many countries in Africa and Asia. Many parts of the *C. odorata* plant were used in the indigenous system of medicine. *C. odorata* possess many secondary metabolites such as terpenes, alkaloids and glycosides [7]. Various studies had showed that the aqueous extract of the leaf contained flavonoids (salvigenin, sakuratenin, kaempferide, odoratin, betulenol, two chalcones, tamarxetin), essential oils (geyren, bornyl acetate and beta eubeden), saponin triterpenoids, tannins, organic acids and numerous trace substances [8]–[10]. Traditionally, fresh leaves or a decoction of *C. odorata* has been used throughout many part of the world for the treatment of leech bites, soft tissue wounds, burns, skin infections, rashes, diabetes and dentoalveolitis as well as insect repellent [11], [12]. It is agreed that the presence of abundant phytoconstients of this plant is responsible for *C. odorata* medicinal properties. However, less study had been conducted to identify how each phytoconstituents in *C. odorata* interact with mediators responsible in the wound healing process.

Molecular docking is the *in silico* approach where the protein and ligand are used to find the best interactions between them. Hence, the results from *in silico* studies could be used to find the relevant information before conducting *in vitro* and *in vivo* studies. This present study is conducted to understand and evaluate the interaction of isosakuratenin, a new flavanone found in *C. odorata* [13] leaf extracts with MMPs that are involved in the wound healing process.

2. Materials and Methods

2.1. Protein preparation

The three-dimensional structure of the selected MMPs were retrieved from RCSB database (<https://www.rcsb.org/>) in Protein Data Bank (PDB) format. The four selected MMPs are of Homo sapiens origin with PDB code of 1GEN (MMP2), 4DPE (MMP3), 5H8X (MMP8) and 2WO8 (MMP12).

2.2. Ligand Preparation

The three-dimensional structure of the isosakuratenin was retrieved from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The compound ID of isosakuratenin is 160481 with the molecular formula of $C_{16}H_{14}O_5$ and the molecular weight is 286.27 g/mol.

2.3. Active Site of MMPs

The active site is one of the essential criteria in docking studies where the ligand interacts with the protein's active site amino acid residues to give effective binding interactions. In the present study, the experimentally verified active site residues of the selected MMPs were considered based on the literature reports [14], [15].

2.4. Dock preparation.

The retrieved MMPs were processed using USFC Chimera 1.14. The addition chains present in the protein structure were removed and only single chain was used [16], [17]. During dock preparation, ions, ligands and water molecules present in each protein crystal structure were removed. Then, the charges and polar hydrogen bonds were added. These dock prepared proteins were used for further docking process. Similarly, the isosakuratenin structure was also dock prepared using UCSF Chimera 1.14.

2.5. Molecular docking process

Autodock Tools 1.5.7 was used for analysing the docking studies between MMPs and isosakuratenin. The dock prepared MMPs and isosakuratenin were reconstructed into PDBQT format. The original grid spacing of 0.375 Å was remodelled according to the active site residues and fitted into the active site cavity of protein molecules. Isosakuratenin was docked against all selected MMPs separately. The degree of interactions between isosakuratenin and selected MMPs are determined based on the calculation of free energy interactions. Molecule with lower values of free energy for binding to the receptor are considered to be ones with higher potential for interacting with the receptor [18]–[20]. Based on the affinity score, the best docking pose for each MMP was taken for further analysis. The best resulting conformation of isosakuratenin and MMP was visualized using Discovery Studio Visualizer (BIOVIA). The hydrogen bonds between active site residues of MMP and isosakuratenin were also noted for interaction analysis. A flow diagram for the whole molecular docking process is shown in **Figure 1**.

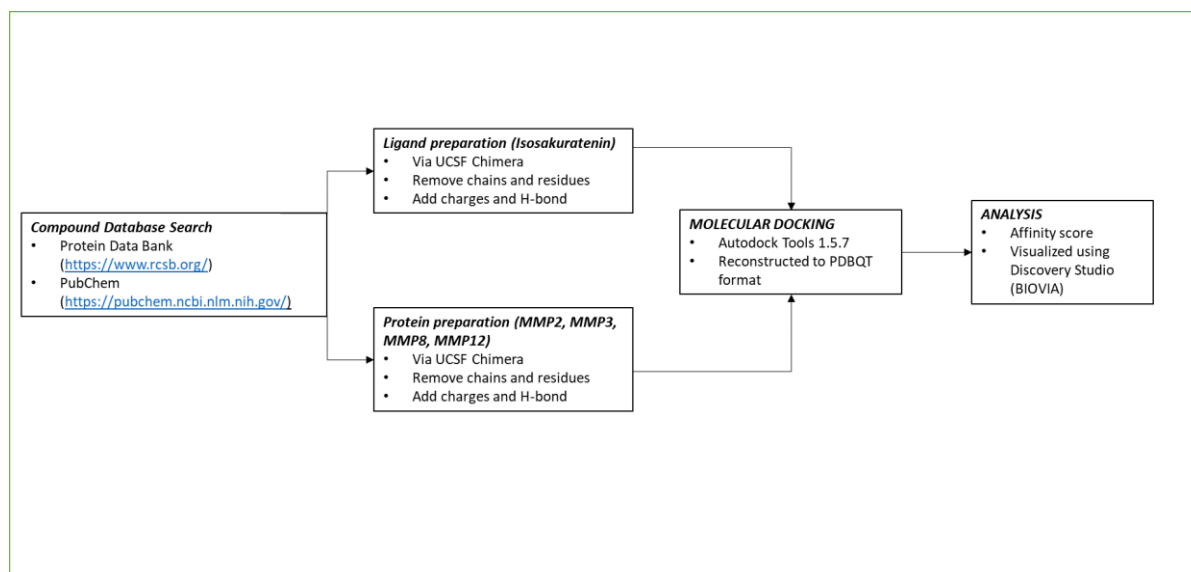


Figure 1. Flow diagram for molecular docking study.

3. Results and Discussion

3.1. Protein and ligand preparation

MMPs are group of enzymes involved in the wound healing process and four MMPs that are MMP2, MMP3, MMP8 and MMP12 were selected for the docking study. By default, these MMPs are regulated in a balanced manner, but under certain circumstances like chronic wound, MMPs activity were upregulated causing the degradation of newly form extracellular matrix at the wound site and delays wound healing. Isosakuratenin, is a new flavanone, compound found naturally in *C. odorata* leaf extracts was selected as the ligand for assessment of its interaction with selected MMPs responsible in wound healing process. The 3D structure of isosakuratenin and MMPs (MMP2, MMP3, MMP8 and MMP12) are shown in **Figure 2** and **Figure 3** respectively.

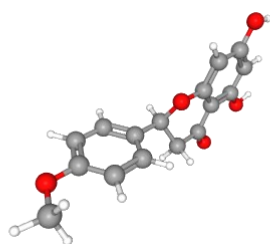
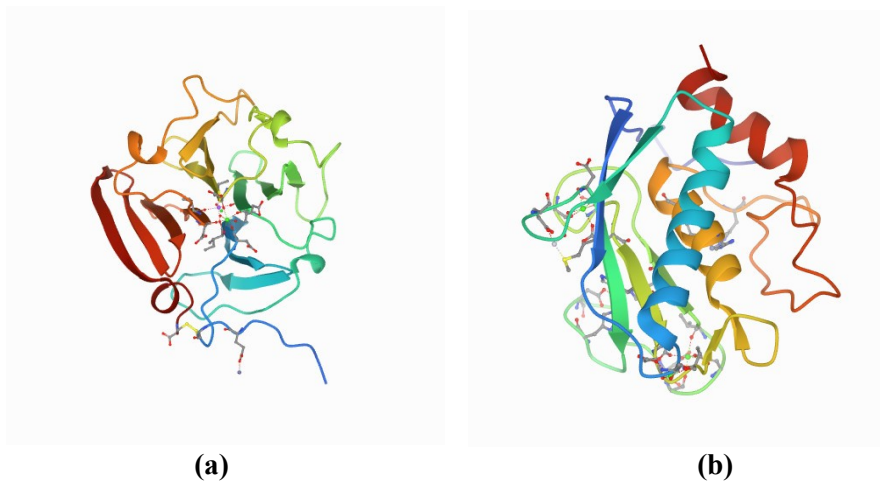


Figure 2. Structure of Isosakuratenin



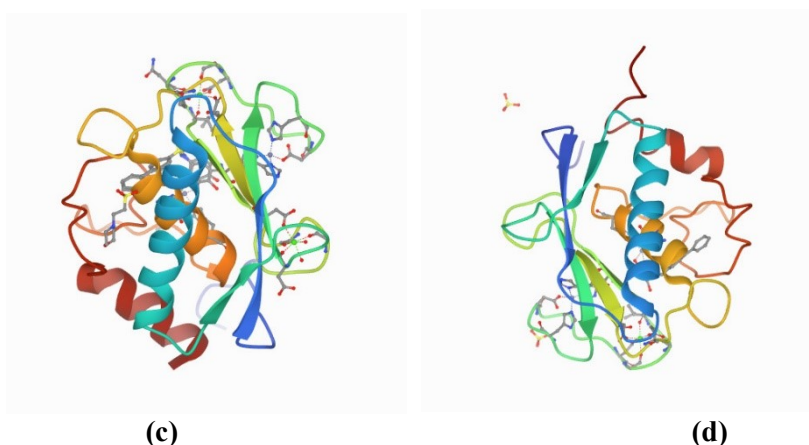


Figure 3. Structure of Matrix Metalloproteinases (MMPs) (a)MMP2, (b) MMP3, (c) MMP8 and (d) MMP12.

3.2. Docking interaction and analysis

The docking studies were done using AutoDock Vina. The prepared isosakuratenin was docked with all four selected MMPs. The best dock conformation between MMPs and isosakuratenin were predicted using the best binding affinity values and molecular interactions. The selected best poses were analysed for hydrogen and hydrophobic bond donor residues between the MMPs active site and isosakuratenin.

Table 1. Active site residues of selected MMPs

<i>MMP</i>	<i>Protein Data Bank Code</i>	<i>Active Site</i>
<i>MMP2</i>	1GEN	34,36,79,81,127,129,176,178
<i>MMP3</i>	4DPE	61,73,80, 81, 82, 83, 84, 85, 86,95,119,120,129,139, 140, 141,123,129
<i>MMP8</i>	5H8X	54, 73, 91, 112, 157, 167
<i>MMP12</i>	2WO8	56,68,75,76,77,78, 79, 80, 81,90,114, 115,124,134, 135, 136, 114, 115,118,124

3.2.1. Interaction between isosakuratenin and MMP2

Docking simulation of isosakuratenin ligand into MMP2 was visualized using Discovery Studio software as shown in **Figure 4a**. From this figure, it was found that isosakuratenin interacts with several amino acid residues of MMP2. The interaction between isosakuratenin and MMP2 (gelatinase) showed the best score of about -7.7 kcal/mol. The hydrogen bond and hydrophobic bond interactions between isosakuratenin and amino acid residues of MMP2 are listed in **Table 3**. However, based on this docking, no bonding was found at the MMP active site. The simulated interaction between isosakuratenin ligand and MMP2 receptor suggested that it could help in regulating the enzyme gelatinase and subsequently enhancing the amount of gelatin, collagen, elastin and fibronectin in the wound site as well as decreases the aggregation of platelets and pro-inflammatory factors so that the wound healing process could be escalated [17]. This finding is similar to a study conducted by Govindharaj et al [21].

3.2.2. Interaction between isosakuratenin and MMP3

MMP3 belongs to stromalysins subfamily, and it enhances the production of laminin, aggregan, gelatin and fibronectin in the wound site to accelerate the wound healing process. The hydrogen bonding formed between residues of MMP3 and isosakuratenin is shown in **Figure 4b** with interaction score of -6.8 kcal/mol. Based on **Figure 4b**, it can be seen that isosakuratenin interacts with MMP3 and forms hydrogen bonds and hydrophobic bonding with amino acid residues of MMP3 as listed in **Table 3**. Therefore, it can be considered that isosakuratenin might acted as a potential inhibitor molecule. The downregulation of MMP3 activity could reduce the activity of TGF- β and anti-inflammatory factors at the wound site and successively speed up the healing process [4].

3.2.3. Interaction between isosakuratenin and MMP8

MMP8 belongs to the collagenase 2 subfamily, which is involved in the degradation of collagen deposition in the extracellular matrix (ECM) [5]. Excess degradation of collagen Type I results in a decreases of newly formed ECM. Therefore, by inhibiting the activity of MMP8 at the wound site, uninterrupted collagen deposition could take place during the healing process. As can be seen in **Figure 3c**, isosakuratenin interacts with MMP8 via hydrogen bonding and hydrophobic bonding with amino acid residues as listed in **Table 3**. Isosakuratenin shown an interaction score of -9.0 kcal/mol with MMP8. Inactivation of MMP8 also leads to lowering the activity of chemokines in the wound site, which leads to reducing the inflammatory activity and promoting wound healing process [4].

3.2.4. Interaction between isosakuratenin and MMP12

MMP12 belongs to metalloelastase group of enzymes, which are essential for tissue repairing process. However, overexpression of MMP12 would results in infiltration of inflammatory cells and delays wound healing. Docking between isosakuratenin and MMP12 showed that isosakuratenin fitted into the active site of MMP12 as can be seen from **Figure 4d**. Based on this docking, it was found that isosakuratenin fit at the active site of MMP12 at sequence 115 (hydrophobic bonding) and 136 (hydrogen bonding). The list of hydrogen bonding and hydrophobic bond with respective amino acids residues of MMP12 are listed in **Table 3**. The binding affinity score of interaction between isosakuratenin and MMP12 is -9.7 kcal/mol. In addition to its ability to degrade fibrinogen interfering with blood clotting, MMP-12 is a potential regulator of angiogenesis, since it was demonstrated to be most efficient at producing angiostatin [4], [6]. Therefore, inhibition of MMP12 can be regarded as a potential treatment for wound healing.

Table 2. Binding energies of ligand-receptor from Autodock Vina

<i>Run</i>	<i>MMP2</i> (kcal/mol)	<i>MMP3</i> (kcal/mol)	<i>MMP8</i> (kcal/mol)	<i>MMP12</i> (kcal/mol)
1	-7.7	-6.8	-9.0	-9.7
2	-7.5	-6.7	-7.8	-8.4
3	-7.4	-6.7	-7.5	-8.4
4	-7.3	-6.7	-7.0	-8.3
5	-7.3	-6.7	-6.9	-7.9
6	-7.3	-6.6	-6.9	-7.3
7	-7.2	-6.6	-6.8	-7.2
8	-7.1	-6.6	-6.6	-7.0
9	-6.9	-6.4	-6.5	-6.9

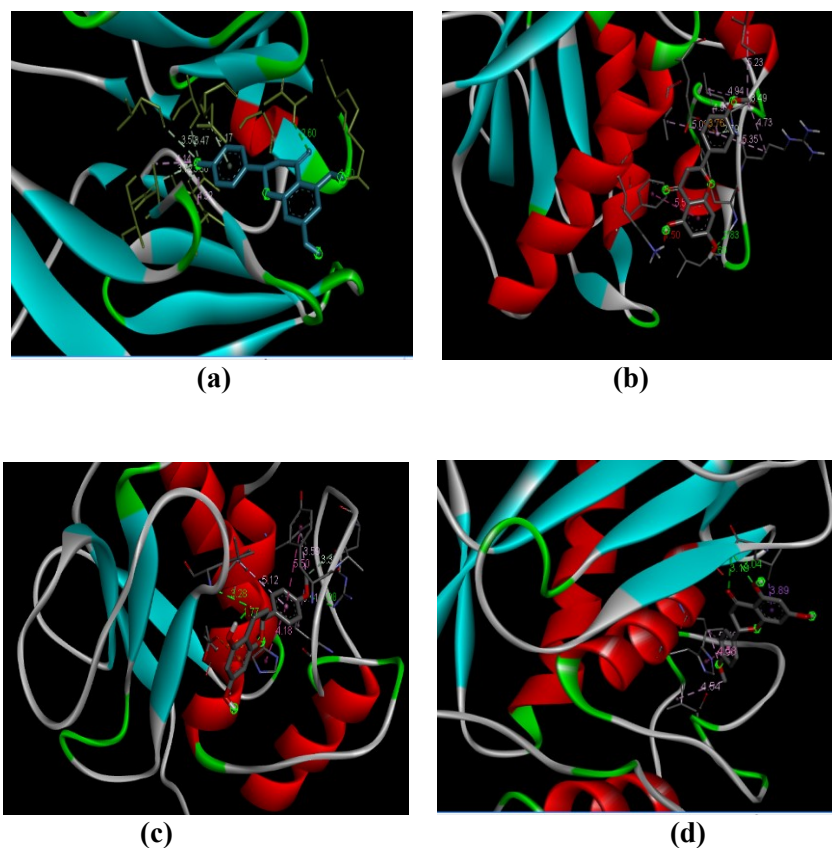


Figure 4. Receptor-Ligand interaction of isosakuratenin in the active site of MMPs (a) MMP2 (b) MMP3 (c) MMP8 (d) MMP12.

Table 3. Molecular interactions observed between Isosakuratenin and selected MMPs.

Receptor Protein	No of H bonds present	Residues form H bond	No of hydrophobic bond present	Residues form hydrophobic bond
MMP2	7	ILE 18 VAL 63 ALA 111 VAL 161 GLU 65 ASN 113 LYS 118	2	ALA 19 VAL 162
MMP3	3	LEU 147 PHE 150 LEU 152	4	VAL 41 PHE 109 ARG 151 ILE 157
MMP8	3	LEU 82 ALA142 ARG 143	3	VAL 115 LEU 114 LEU 135
MMP12	1	ALA 83	2	VAL 136 LEU115

4. Conclusion

In conclusion, from this docking study, isosakuratenin showed binding affinity to four different classes of MMPs. The binding energy of these MMPs with isosakuratenin are -7.7 kcal/mol (MMP2), -6.8 kcal/mol (MMP3), -9.0 kcal/mol (MMP8) and -9.7 kcal/mol (MMP12). Isosakuratenin forms stronger interaction with MMP12 in which it forms two bonding at the active site of the protein and shows the most stable dock conformation. This results suggested that, among these four MMPs, isosakuratenin are best interacted with MMP12 and hence, could be used to visualized the potential of isosakuratenin as MMP12 inhibitor during wound healing process. However, supplementary *in vitro* or *in vivo* study needs to be carried out to further evaluate the inhibitory potential of isosakuratenin in down regulating excess activity of these MMPs to promote faster wound healing.

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