Innovations

Docking Study for Assessment of Wound Healing Potential of Musa Acuuminata Extract and Invitro Wound Healing Activities

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Abstract : *In all mammals with multiple cells, wound healing is a complicated and well-orchestrated biological process that comprises of four key phases: hemostasis, inflammation, proliferation, and reepithelization. Improper or inefficient progression from the inflammatory to proliferative phases of wound healing is always present. In recent years, researchers have focused increasingly on the potential of several natural extracts to speed wound healing. The proteins involved in the wound healing process are the focus of this research, which uses a fresh bark extract from Musa acuminata. The purpose of this research is to examine the potential inhibitory impact of phytochemicals in the fresh extract on a panel of wound-healing proteins. Docking analysis with co-crystallized complexes of key proteins involved, including matrix metalloproteinase 9 (1GKC), transforming growth factor-β (1PY5), fibroblast growth factor (1BFB), vascular endothelial growth factor receptor (3HNG), and interleukin-6 (4CNI), our investigation unveils promising indications of wound-healing properties inherent to the fresh extract. The identity of active components within the extract was determined through the application of Liquid Chromatography-Mass Spectrometry (LC-MS). Further investigation utilized molecular docking techniques to assess the potential interactions of these active components with the aforementioned target proteins. Our docking analysis illuminates the binding affinity of Musa acuminata bark extract components, specifically 4-tert-butylphenyl 5-hydroxypentanoate, to the respective proteins, with binding energies of 7.1 kcal/mol for MMP-9, 7.3 kcal/mol for TGF-β, 4.7 kcal/mol for FGF-2, 6.7 kcal/mol for VEGF, and 5.2 kcal/mol for IL-6. These findings indicate a stable docked conformation of the proteins in association with 4-tert-butylphenyl-5-hydroxypentanoate molecules. In the context of wound healing, our research underscores the superior performance of Musa acuminata bark extract compared to the control group.*

Key words: *Musa acuminata bark extract, Docking studies, wound healing activities.*

Introduction:

The skin, being the body's largest organ, serves as a vital barrier against environmental factors. Wound healing is a dynamic and intricate biochemical process instigated to restore skin health following damage inflicted by acute or chronic wounds.[1] This restorative journey comprises four distinct yet interrelated phases: hemostasis (occurring within the initial hours after injury), inflammation, proliferation, and remodeling (spanning from days to years post-injury). Dysregulation of any of these phases can impede the healing process, leading to adverse effects on both health and economic well-being. Persistent wounds that exceed a six-week healing threshold may undergo pathological inflammation , characterized by an abundance of proinflammatory cytokines and a highly proteolytic microenvironment, primarily orchestrated by macrophages and neutrophils in close proximity to the wound site[1]. The intricate task of rebuilding damaged cutaneous and subcutaneous tissues involves a myriad of interconnected biochemical and cellular mechanisms, facilitated by the activation of numerous enzyme pathways. Challenges such as bacterial infections can manifest at any stage of this complex physiological process, with physiological conditions like rheumatoid arthritis, zinc deficiency, and diabetes posing additional obstacles.[2]

Traditional herbal remedies predominantly harness the potency of plant-derived natural ingredients, with minimal industrial intervention during extraction. This facet of medicine plays a pivotal role in global healthcare, boasting an estimated annual revenue of an astounding US$ 60 billion [World Health Organization Traditional Medicine Strategy].[3] Researchers have embarked on a quest to explore these plant-derived remedies for their potential wound-healing attributes [8]. This endeavor is driven by the presence of numerous life-sustaining elements within plants, which wield significant physiological effects. Due to their diverse array of active components and minimal side effects, alternative therapies utilizing phytochemicals and plant extracts are gaining prominence in the realm of wound healing. Alkaloids, carbohydrates, flavonoids, terpenes, and other phytochemicals have all exhibited wound-healing properties in various studies[6].

Musa acuminata, a species of banana native to Southeast Asia and classified under the genus Musa and family Musaceae, faces endangerment. It has long been a staple in folk medicine, applied to wounds, scrapes, and bruises with reported benefits such as wound soothing, pain reduction, and accelerated healing[12]. The broader spectrum of banana plants has been integral to alternative medicine for centuries, finding application in conditions such as ulcerative colitis, diarrhea, dysentery, diabetes, and even as burn dressings[15]. Banana leaves have been employed to treat

eczema [17], while the flowers have been used for dysentery and menorrhagia. The juice from the fruit's stem is effective against gastrointestinal disorders. This research explores the potential wound-healing properties of Musa acuminata using a freshly obtained extract.[4]

During wound healing, the production of inflammatory cytokines and inflammatory signal transduction pathways is downregulated, leading to a decrease in proinflammatory factors and an increase in antioxidative enzymes, among other responses. Enhanced expression of fibroblast growth factor and vascular endothelial growth factor is crucial for wound healing , as it promotes neovascularization and angiogenic pathways. Matrix metalloproteinases (MMPs), a group of zinc-dependent endoproteases, are highly concentrated in the extracellular matrix (ECM) surrounding a wound. Transforming growth factors from the TGF-β superfamily also play a pivotal role in tissue healing.[9]

Molecular docking investigations have long been employed to estimate the binding potential of small molecules, aiding in the elucidation of atomic interactions within the protein backbone. In this study, we analyzed the binding capabilities of active molecules derived from fresh Musa acuminata extract with their respective protein targets using molecular docking. Our docking analysis unveiled the potential wound-healing properties of the fresh extract, as evidenced by its interactions with co-crystallized complexes of matrix metalloproteinase 2 (PDB ID:1GKC), transforming growth factor-β (PDB ID:1PY5), fibroblast growth factor (PDB ID:1BFB), vascular endothelial growth factor receptor (PDB ID:3HNG), and interleukin-6 protein (PDBID:4CNI).[5]

Material and Methods

The identify and validate the active compounds within the fresh extract of Musa acuminata, we conducted a comprehensive set of qualitative phytochemical analyses. These analyses encompassed the determination of various phytochemical classes, including flavonoids, alkaloids, glycosides, saponins, tannins, phytosterols, and terpenoids.[6] According to the traditional medical practitioner from paruvadha hills(village), Thiruvannamalai district the fresh extract was subjected to invitro wound healing studies.

Then the fresh extract was further subjected to Liquid Chromatography-Mass Spectrometry (LC-MS) studies to facilitate compound identification. Subsequently,

the compounds identified through LC-MS were systematically investigated to assess their potential involvement for Docking studies in promoting wound healing.[8]

Invitro Wound Healing Activities

Cell Scratch Wound Healing Assay

The migration capabilities of L929 mouse fibroblasts were determined using in vitro cell scratch wound healing assay, which measures the expansion of a cell count on surfaces. A cell monolayer was formed by seeding cells at a density of 2x104 cells/mL in 48-well tissue culture dishes and cultivating them until nearly confluent. The linear wound was generated on the formed cell monolayer with a sterile 200-µL plastic pipette tip. Any cellular debris was removed by washing the cultured wells with PBS. After the incubation period of 24 hr, growth medium containing plant extracts (62 µg/mL) was added. Solvent such as methanol or water with no extract was added to the negative control wells. The inverted microscope is used to visualized the cells. For each replicate well, three representative digital images were taken at 0 (the beginning) and 24 h to calculate the relative migration of cells.[11]

By using imageJ software, the area between the scratch edges was measured. Firstly, contoured the borders of the cells, then calculated the pixel-free area between them. For every replicate well,the mean values were calculated from the three photographs taken from the same well. The closure rate was calculated with these values using the following formula:

$$S\ \text{closure rate} = [(\text{Area}_{t0} - \text{Area}_{t24})/\text{Area}_{t0}] \times 100,$$

Where, Area_{t0} = the calculated area value at 0 h and $\text{Area}_{t\,24}$ = the area value at 24 h. The experiment was performed in triple times and then, the mean values are used.

Protein preparation

Protein Data Bank (PDB) structured three-dimensional structures of the chosen proteins were acquired from the RCSB database (https://www.rcsb.org/). The five proteins chosen have the PDB codes 1GEN (MMP-9), 1PY5 (TGF-β), 1BFB (FGF), 3HNG (VEGFR), and 4CNI (IL-6 protein).

Ligand preparation

The ligands were selected for the study and their 3D structures were downloaded from the PubChem database in sdf format. The ligands was subjected to screening through an sowfter to determine it's physicochemical properties, including molecular weight, hydrogen bond donors, hydrogen bond acceptors, Lipophilicity, and molar refractivity [31,32.] The obtained properties was compared with Lipinskis rule of five[33,34].

Active sites of proteins

The active site plays a crucial role in docking studies, as it serves as the location where the ligand interacts with the amino acid residues of the proteins active site, leading to effective binding interactions. In the present investigation, the active site residues of the selected proteins MMP-9,TGF-β, VEGFR, and IL-6 were considered, based on literature reports that have experimentally validated their existence.[11-18]

Docking studies

The binding site of the protein targets are defined. Molecular Docking was performed using AutoDockVina.V.1.2.7 to study the interaction between the protein and ligand molecules. binding affinity of various ligands with protein were estimated.PDBQT reconstructions of the docked proteins and ligands were performed. Active site residues were used to reshape the grid and ensure a snug fit inside the protein molecule's active site cavity. All of the target proteins were docked individually with the active components. Free energy calculations were used to establish the strength of interactions between ligands and certain proteins. Higher potential for interaction with the receptor is attributed to molecules with lower values of free energy for binding to the receptor [29–31].

Results and Discussion

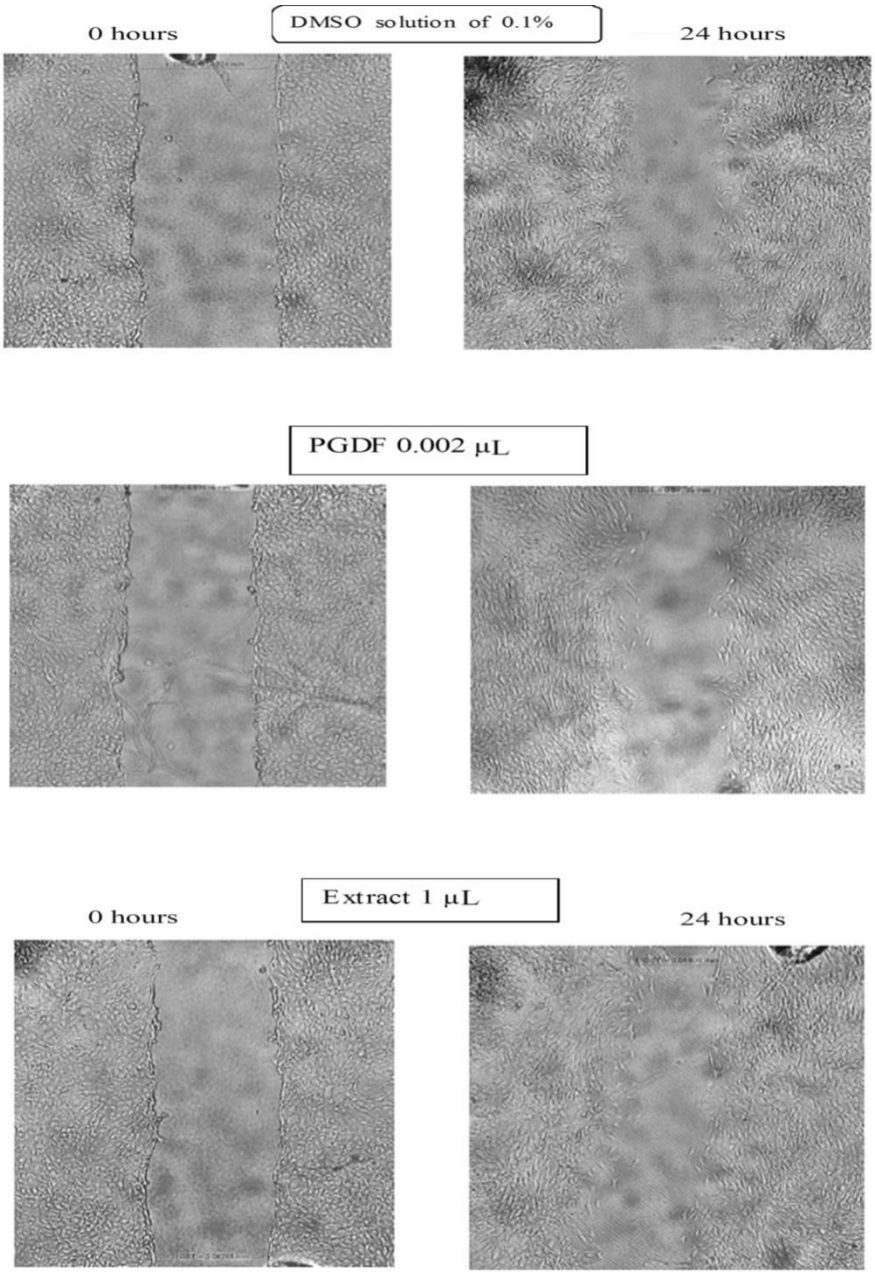
Invitro wound healing activities

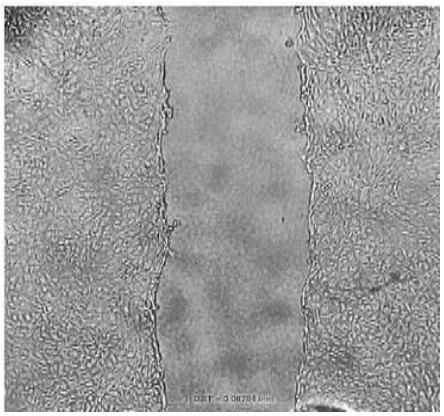
Spreading and migration capabilities of 3T3 L1 cells were assessed using scratch wound assay, which measures the expansion of a cellpopulation on surfaces as described by Fronza et al. (2009). The cells were seeded into 6-well tissue culture dishes in DMEM containing 10%FBS (Fetal Bovine Serum) and 2% penicillin and streptomycin. After the cell shave nearly formed a confluent cell monolayer, a linear woundwas generated in the monolayer using a sterile 100 µL plastic pipette tip. Any

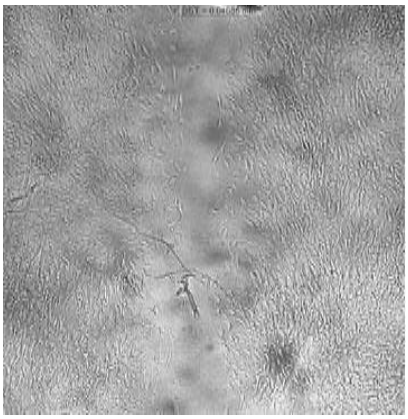
cellular debris was removed by washing the wells with phosphatebuffer saline (PBS). The medium (DMEM) used consisted of either dimethyl sulfoxide (<0.01% used as solvent control), platelet derived growth factor (PGDF; 0.002 μg/mL) (as positive control), or the crude extract (1μg/mL, 2μg/mL, and 3μg/mL). The cells were then incubated for 16 hr at 37°C with 5% CO_2. The scratched cell layers incubated under the different conditions were then photographed to estimate relative cell migration. The data were analyzed using Capture Pro Version 2.5 for Progres® microscope camera from Jenoptik Laser, Optik Systeme GmbH. The experiment was performed in triplicate.

The wound healing properties of the fresh extract by the comparison with the standard and platelet growth factor are tabulated figure 1.

Figure 1: Invitro wound healing activities of fresh extract of Musa acuminata.


DMSO solution of 0.1%
0 hours
24 hours
PGDF 0.002 µL
Extract 1 µL
0 hours
24 hours
0 hours
Extract 2 µL
24 hours

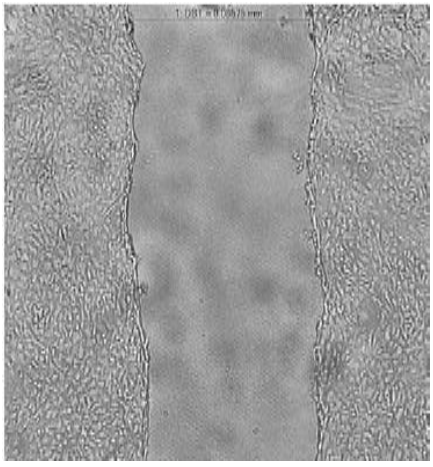


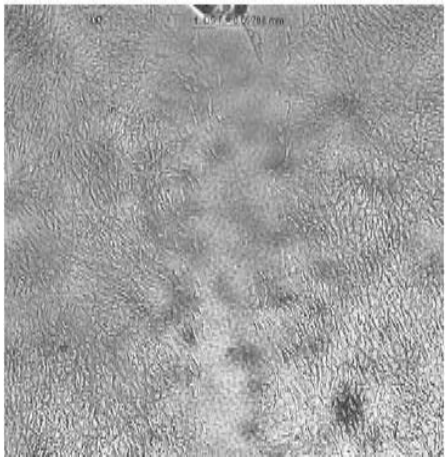


Extract 4µL

0 hours

24 hours





Treatment Group	**Concentration (µL)**	**Relative Cell Migration (mm) at 24hrs**			**Mean value**
		Exp 1	**Exp 2**	**Exp 3**	
0.1% DMSO	-	0.021	0.024	0.021	0.022
PGDF	0.002	0.026	0.026	0.024	0.025
Extract	1µL	0.051	0.053	0.051	0.051
	2µL	0.065	0.066	0.066	0.065
	4µL	0.079	0.078	0.077	0.078

The caputured relative cell migration photographs on the scratched cell layer was listed above, The first picture of DMSO solution of 1µg at 0 hour and 1µg at 24 hours which shows the cell migration activity. The secomd picture of extract of 1 µg at 0 hour and 1 µg at 24 hours shows the mild would closuring properties when compared to the 4 µg of extract. The third picture of the extract of 2 µg at 0 hour and 2 µg at 24 hours gives the effective would healing properties on the scratch cells. The forth picture of the extract of 4 µg at 0 hour and 4 µg at 24 hours shows the greater healing properties on the scratch would cells when compared to the other dose of the extract.

LC-MS analysis of fresh extract of Musa acuminata

The Liquid Chromatography-Mass Spectrometry (LC-MS) identified the following compounds are 4-Hexen2-one, 3-Bromopentane, 5-Ethyl-3-nonen-6-one, Aspirin methyl ester Benzoic acid, methyl ester Benzoic acid, 4-tert-Butylphenyl 5-hydroxy pentanoate, 4-Bromobutyric acid, 4-nitrophenyl ester, Benzamide, N, N-dioctyl-3-fluoro. The LC-MS report were shown in figure 2 and The identified compounds were shown in Table 2.

Figure 2: LC-MS analysis of fresh extract of Musa acuminate

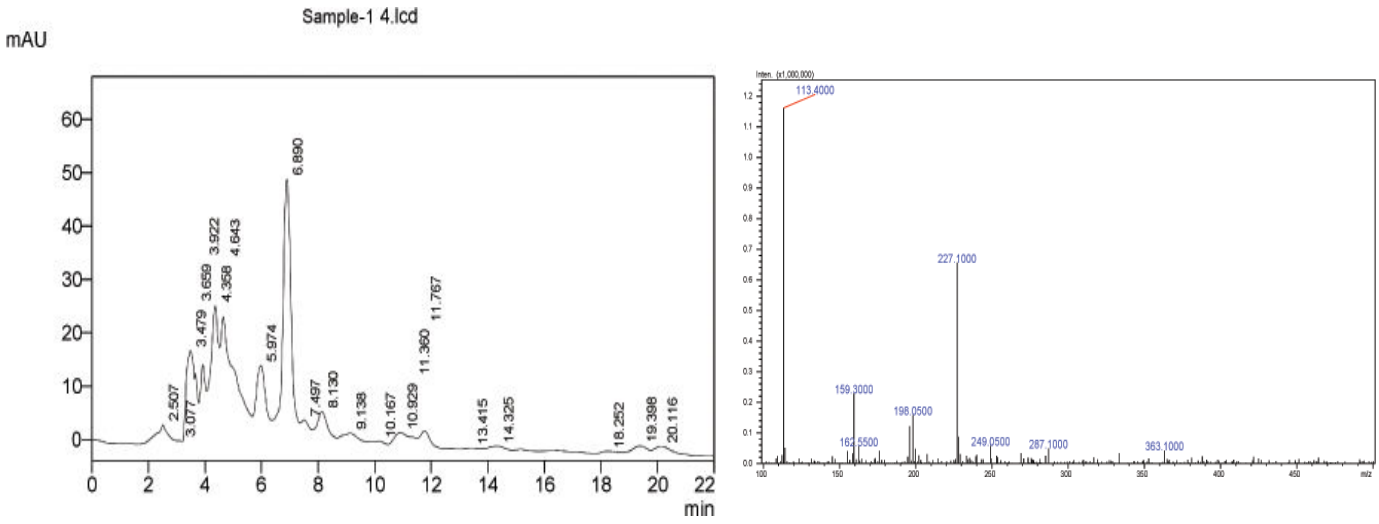
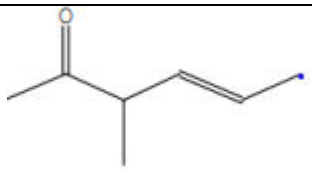
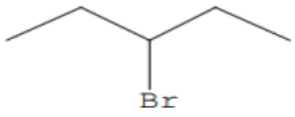
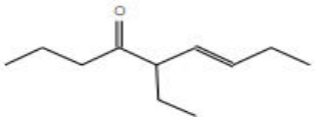
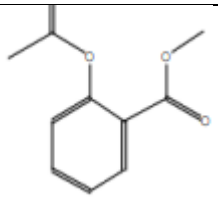
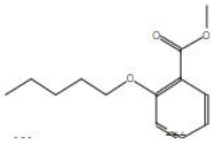
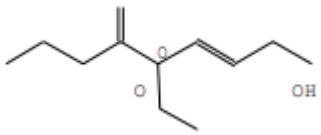
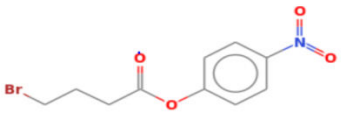
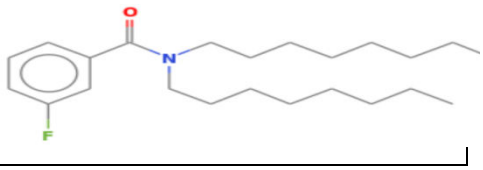
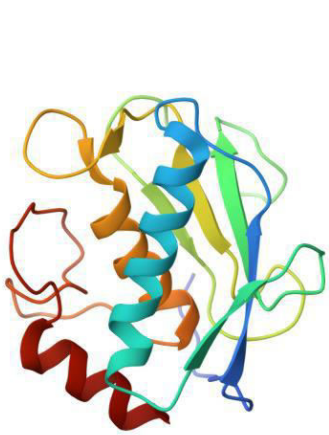


Table 2: ligand chemical constituents in plant extract

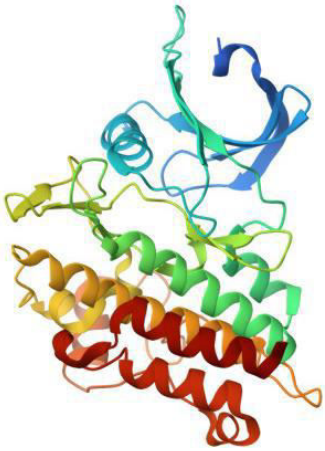
Molecular weight	Compound Name	Structure
113.400	4-Hexen2-one	
159.300	3-Bromopentane	
162.550	5-Ethyl-3-nonen-6-one	
198.050	Aspirin methyl ester Benzoic acid	
227.100	methyl ester Benzoic acid	
249.050	4-tert-Butylphenyl 5-hydroxy pentanoate	
287.100	4-Bromobutyric acid, 4-nitrophenyl ester	
363.100	Benzamide, N, N-dioctyl-3-fluoro	

Protein and ligand preparation

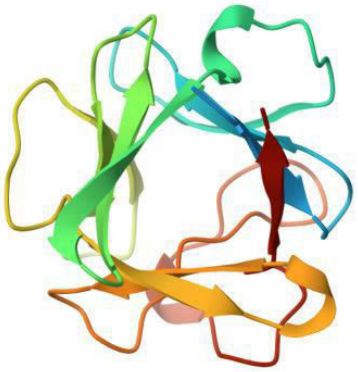
Matrix metalloproteinase 9 (PDB ID:1GKC), transforming growth factor-β (PDB ID:1PY5), fibroblast growth factor (PDB ID:1BFB), vascular endothelial growth factor receptor (PDB ID:3HNG), and IL-6 protein (PDB ID:4CNI) were selected for the docking study. The active constituents according to LCMS studies, present in the fresh extract of Musa acuminata was shown in Table 1. The constituents were selected as the ligand for assessment of its interaction with selected proteins in the wound healing process. The 3D structure of proteins are shown in Figure 3.



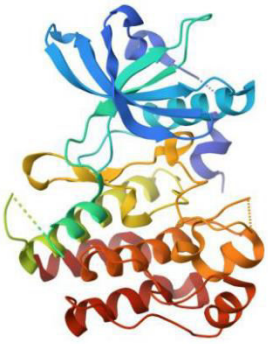
(a)



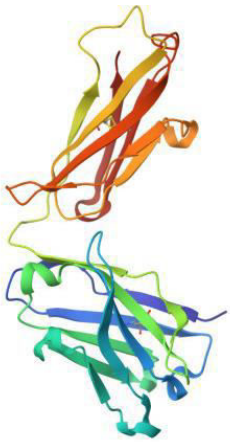
(b)



(d)



(e)



(f)

Figure 3: 3D structure of (a)1GEN (MMP-9), (b)1PY5 (TGF-β), (c) 1BFB (FGF), (d) 3HNG-VEGFR, and (e) 4CNI – (IL-6) protein

Ligands properties.

The ligands properties were predicted by using the Lipinski rules of five, such as Molecular mass, Hydrogen bond donor, hydrogen bond acceptor, LOGP, Molar refractivity, then the details are shown in table 3.

Table 3. Lipinski rules of five

Lipinskis rule	**Accepted values**	**Values for Ligands**							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Molecular mass	<500	98	150	168	194	136	250	287	245
Hydrogen bond donor	<5	0	0	0	0	0	1	0	0
Hydrogen bond acceptor	<10	1	0	1	4	2	3	4	2
High lipophilicity (LOGP)	<5	1.54	2.56	3.34	1.39	1.47	3.05	2.67	3.1873
Molar refracivity	40-130	30.11	33.29	53.12	49.09	37.78	71.71	61.75	58.69

A) 4-Hexen2-one, B) 3-Bromopentane, C) 5-Ethyl-3-nonen-6-one, D) Aspirin methyl ester Benzoic acid, E) methyl ester Benzoic acid, F) 4-tert-Butylphenyl 5-hydroxy

pentanoate, G) 4-Bromobutyric acid, 4-nitrophenyl ester, H) Benzamide, N, N-dioctyl-3-fluoro.

Active site prediction of proteins

The presents amino acid residues found in the active sites of selected proteins MMP-9, TGF-β, FGF, VEGFR and IL-6. Among these residues were Leu, Val, Tyr, Arg, His, Asn, Glu, Asp etc., were showen in Table 4. Which have been identified as key contributors to the binding interaction with the ligand molecule. These specific residues have shown strong affinity towards ligand binding with in the active site region.[11-15]

Table 4 active site residues present in selected proteins

Proteins	**Residues in the active site**	**Amino acids**	**Reference**
MMP9	188, 398, 213, 211,401, 405, 411, 402, 402, 421, 422, 189, 185,	Leu, Val, Gly, Ser, His, His, His, Glu, Gln, Pro, Met,Ala, Asp,	[11,12]
TGF-β	280,283, 231, 249, 245, 351, 340	Ser, His, Lys, Tyr, Glu, Asp, Leu	[13]
FGF	121, 102, 126, 27, 136, 135, 28,100, 107, 109, 99, 103, 113, 114	Arg, Asp, Lys, Lys, Lys, Glu, Asp, Ser, Arg, Arg, Glu, Tyr, Trp, Trp	[14,15]
VEGFR	48, 21,62, 56, 29, 70, 868, 1059, 1054, 885, 1046, 1028	Lys, Tyr,Asn, Ser, Trp, Gly, Lys, Tyr, Tyr, Glu, Asp, Asp	[16,17]
IL-6	93, 97, 143, 147, 150, 63, 144, 104, 60, 156, 157	Glu, Tyr, Thr, Leu, Lys, Asn, Asn, Arg, Asn, Gln, Trp	[18]

Docking interaction and analysis

Studies involving docking were conducted with the help of AutoDock Vina. All five target proteins were successfully docked with the prepared ligands. The optimal binding affinity values and molecular interactions were used to estimate the optimal dock configuration between proteins and ligands. The binding energies of ligands are shown in Table 5.

Table 5: Dock Binding energies of the ligands interact with proteins

S.No	Ligands	MMP-9 (1GKC)	TGF -β(1PY5)	FGF-2 (1BFB)	VGEF (3HNG)	IL-6 (4CNI)
		Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol
1	4-hexen2-one	-4.8	-4.4	-3.4	-4.9	-3.9
2	3-bromopentane	-4.1	-3.8	-2.7	-4.2	-2.8
3	5-ethyl-3-nonene-6-one	-5.5	-6.1	-4.0	-6.1	-3.9
4	aspirin methyl ester benzoic acid	-6.1	-6.7	-4.3	-5.6	-4.3
5	methylester benzoic acid	-6.5	-6.7	-4.7	-6.3	-4.3
6	4-tert-butylphenyl 5-hydroxypentanoate	-7.9	-7.3	-4.7	-6.9	-5.2
7	4-bromobutyric acid 4-nitrophenyl ester	-6.9	-6.7	-4.4	-7.0	-4.3
8	benzamide NN dioctyl 3 flouro	-7.2	-7.6	-5.0	-6.8	-4.9

Interaction between ligands with MMP-9 (1GKC)

The best possible ligand-MMP-9 Docking interaction were shown in Figure 4. Multiple metalloproteinase-9 (MMP-9) amino acid residues were identified as sites of ligand interaction in this diagram. Table 2 displays the ligand-MMP-9 interactions. Best docking energy was found for (-7.9 Kcal/mol) 4-tert-butylphenyl 5-hydroxypentanoate, (-7.2 Kcal/mol) NN-dioctyl-3-fluorobenzamide and (-6.9 Kcal/mol) 4-bromobutyric acid 4-nitrophenyl ester. By reducing platelet aggregation and pro-inflammatory factors, increasing wound-site gelatin, collagen, elastin, and fibronectin, and facilitating a more rapid healing process, a model interaction between a ligand and the MMP-9 receptor has been proposed[47].

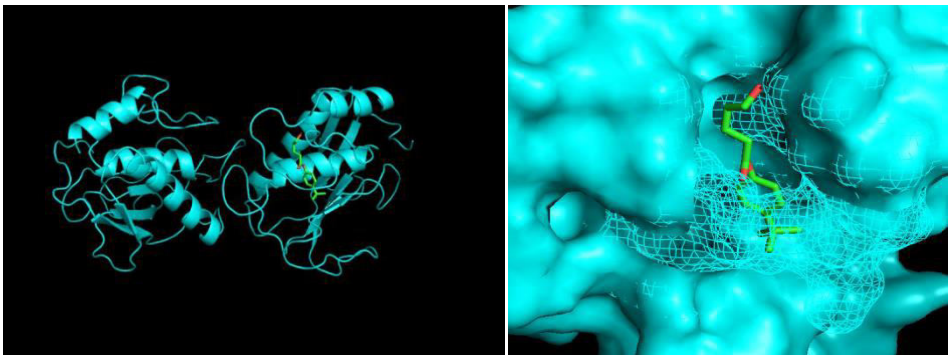


Figure 4: Best Scored Ligand (**4-tert-butyl phenyl 5-hydroxy pentanoate**) with MMP9 protein

Interaction between ligands with TGF-β (1PY5)

The best possible ligand-TGF-β Docking interaction were shown in Figure 5. Amino acid residues were identified as sites of ligand interaction in this diagram. Table 2 displays the ligand-MMP2 interactions. Best docking energy was found for (-7.3 Kcal/mol) 4-tert-butylphenyl 5-hydroxypentanoate, (-7.6 Kcal/mol) NN-dioctyl-3-fluorobenzamide and (-4.4 Kcal/mol) 4-bromobutyric acid 4-nitrophenyl ester. TGF-β inhibits the translation of collagenases, enzymes that degrade collagen and the extracellular matrix. By increasing TGF-β expression, the fresh extract of Musa acuminata promoted wound healing [48].

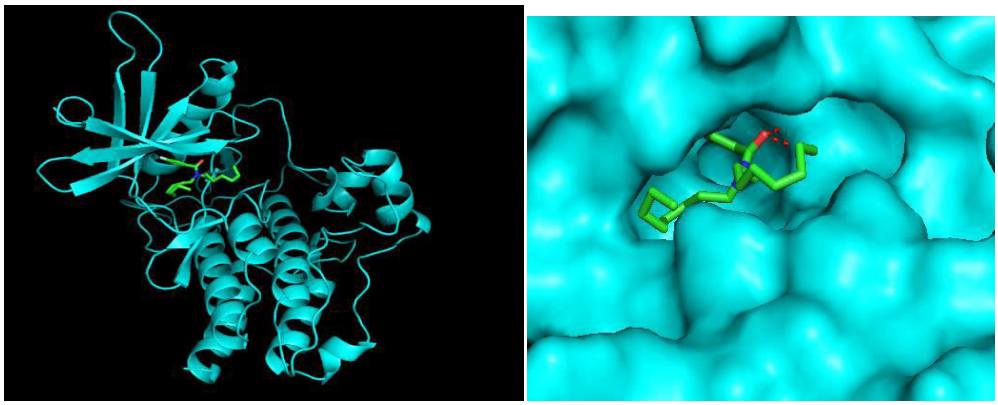


Figure 5: Best Scored Ligand (**Benzamide NN dioctyl 3 fluoro**) with **TGF-β** protein

Interaction between ligands with FGF-2 (1BFB)

The best possible ligand-FGF Docking interaction shown in Figure 6. Amino acid residues were identified as sites of ligand interaction in this diagram. Table 2 displays the ligand-FGF interactions. The best docking energy (-5.0 Kcal/mol) was

found for NN-dioctyl-3-fluorobenzamide. Fibroblast binding interaction increases wound tensile strength. By contracting, myofibroblasts cause the wound to close and the fluid to be released, which causes the epidermis to stratify. Complete wound healing is the result of matrix remodeling and compositional confirmation inside the wound [49].

Best Scored Ligand : **benzamide NN dioctyl 3 flouro**

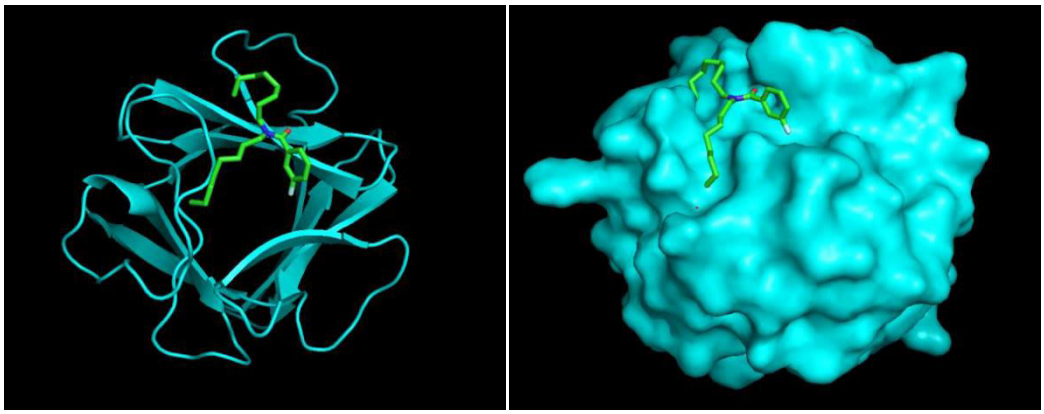


Figure 6: Best Scored Ligand (**Benzamide NN dioctyl 3 fluoro**) with **FGF** protein

Interaction between ligands with VGEF (3HNG)

The best possible ligand-VEGF Docking interaction are shown in Figure 7. Amino acid residues were identified as sites of ligand interaction in this diagram. Table 2 displays the ligand-VEGF interactions. The best docking energy (-7.0 Kcal/mol) was found for 4-bromobutyric acid 4-nitrophenyl ester. The second best energy (-7.0 Kcal/mol) 4-tert-butylphenyl 5-hydroxypentanoate, (-6.8 Kcal/mol) NN-dioctyl-3-fluorobenzamide. VEGF enhances wound healing by binding to two VEGF receptors that are expressed on vascular endothelial cells. These processes include the deposit of collagen and epithelialization. These results are consistent with the earlier findings that a fresh extract of Musa acuminata increased VEGF expression, leading to better wound healing[50].

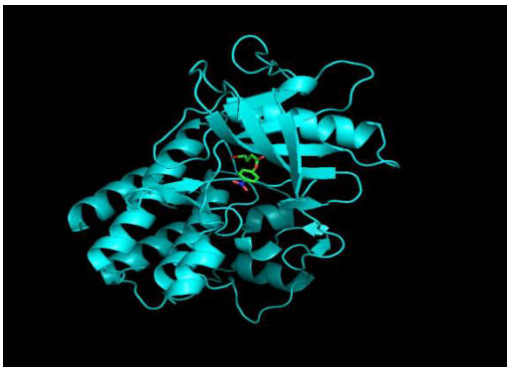


Figure 7: Best Scored Ligand (**4-bromobutyric acid 4-nitrophenyl ester**) with **VEGF** protein

Interaction between ligands with IL-6 (4CNI)

Figure 8 displays the best possible ligand-IL-6 docking interaction. Amino acid residues were identified as sites of ligand interaction in this diagram. Table 2 displays the ligand-IL-6 interactions. The best docking energy (-5.2 Kcal/mol) was found for 4-tert-butylphenyl 5-hydroxypentanoate. Second best energy (-4.9 Kcal/mol) NN-dioctyl-3-fluorobenzamide. The inhibitory activity shown in the fresh extract of Musa acuminata was potential wound-healing activity.

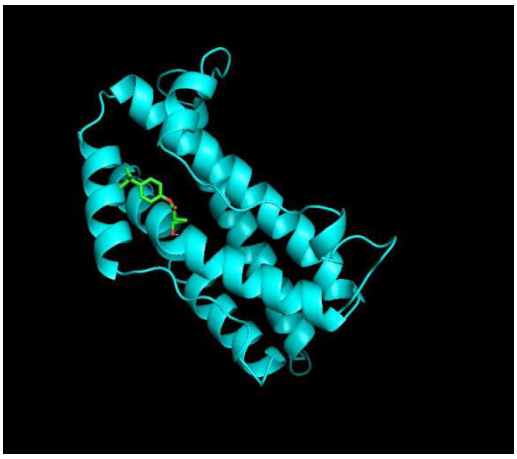
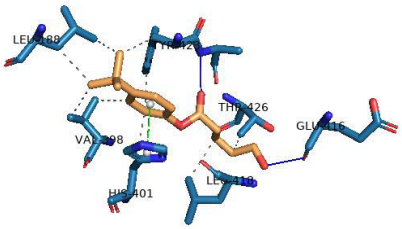
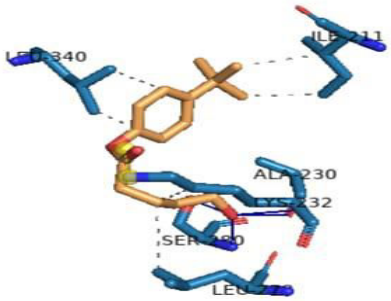


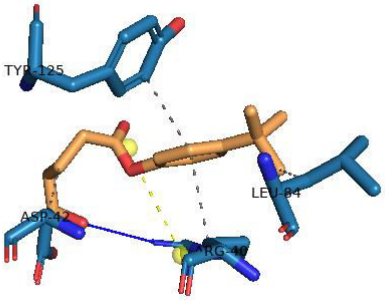
Figure 8: Best Scored Ligand (**4-tert-butylphenyl 5-hydroxypentanoate**) with **IL-6** protein



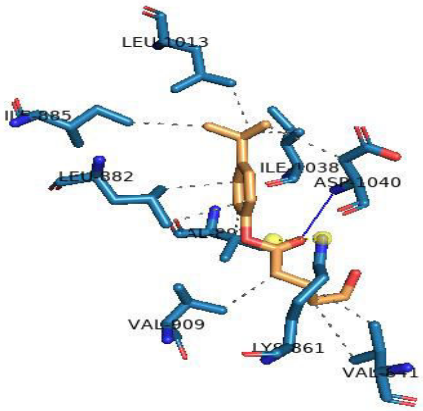
A)



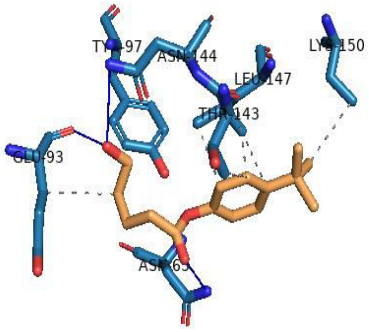
B)



C)



D)



E)

Figure 9. Interaction of ligands in the active site of proteins **A)** MMP-9, **B)**TGF, **C)**FGF, **D)**VGEF, **E)**IL6

Table 6. Molecular intraction observed between protein and 4-tert-butylphenyl 5-hydroxy pentanoate

Receptor protein	No. of hydrogen bonds present	Amino acid residues that form H bond	Length of H bond	Binding energy
MMP9	2	Thr 426 Glu 416 Leu 418 His 401 Arg 424 Tyr 423 Leu 188 Val 398	1.81 2.03	-7.9
TGF-β	4	Ala 230 Lys 232 Ser 280	2.34 3.13 2.69	-7.3
FGF	1	Tyr 125 Asp 42 Leu 84 Arg 40	2.54	-4.7
VEGFR	3	Val 909 Val 841 Lys 861 Asp 1040 Ile 1038 Leu 1013 Ile 885 Leu 882 Val 892	2.28	-6.9
IL-6	1	Tyr 97 Lys 150 Leu 147 Asn 144 Glu 93 Asn 63	3.40 2.13 2.71	-5.2

Table 7. Molecular intraction observed between protein and NN dioctyl 3 flouro benzamide

Receptor protein	No. of hydrogen bonds present	Amino acid residues that form H bond	Length of H bond	Binding energy
MMP9	2	Leu 187 Leu 188 His 190 Tyr 393 Leu 397 Tyr 423 His 401 His 411	2.21 2.33	-7.2
TGF-β	1	Ile 211 Ala 230 Lys 232 Leu 260 Leu 278 Tyr 282 Lys 337 Leu 340 Lys 232	2.58	-7.6
FGF	2	Arg 40 Asp 42 Tyr 125 Leu 127 Arg 40	3.39 1.93	-5.0
VEGFR		Ala 874 Thr 877 Glu 878 Ile 881 Leu 882 Ile 885 Val 891 Val 909 Leu 1013 Asp 1040		-6.8

		Leu 1043		
IL-6	1	Leu 92 Glu 95 Val 96 Glu 99 Thr 138 Pro 141 Asn 144 Ala 145 Leu 148	3.15	-4.9

Table 8. Molecular intraction observed between protein and 4-bromobutyric acid 4-nitrophenyl ester

Receptor protein	**No. of hydrogen bonds present**	**Amino acid residues that form H bond**	**Length of H bond**	**Binding energy**
MMP9	1	Val 398 His 401 Tyr 423 Arg 424	1.90	-6.9
TGF-β	1	Ile 211 Val 219 Ala 230 Leu 260 Ala 350 His 283	2.69	-6.7
FGF		Tyr 112 Tyr 112		-4.4
VEGFR		Val 841 Val 841 Val 892 Val 909 Val 909 Leu 1029		-7.0

IL-6	1	Val 96 Thr 138 Pro 139 Asn 144	 2.85	 -4.3

From this docking study, fresh Musa Accuminata Bark Extract shows the binding affinity to five various classes of proteins. Specially 4-tert-butylphenyl 5-hydroxypentanoate, NN dioctyl 3 flouro benzamide, and 4-bromobutyric acid 4-nitrophenyl ester compounds have better binding affinity in compare other ligands.

Conclusion

From this study, the fresh Musa Accuminata Bark Extract have better wound healing properties. From this docking study, Musa Accuminata Bark Extract shows the binding affinity to five various classes of proteins. The three ligands have excellent binding affinity compared with other compounds such as 4-tert-butylphenyl 5-hydroxypentanoate, NN dioctyl 3 flouro benzamide, 4-bromobutyric acid 4-nitrophenyl ester. Specially 4-tert-butylphenyl 5-hydroxy pentanoate ligands Binding energy -7.9 kcal/mol MMP-9 (1GKC), -7.3 kcal/mol TGF (1PY5), -4.7 FGF-2 (1BFB), -6.9 kcal/mol VGEF (3HNG), -5.2 kcal/mol IL-6 (4CNI. The 4-tert-butylphenyl 5-hydroxypentanoate compounds show the most stable dock conformation for the proteins. The 4-tert-butylphenyl 5-hydroxy pentanoate compounds have better binding affinity compared with other compounds. Finally we concluded Musa Accuminata Bark Extract showed better wound healing properties**.** In future studies, isolate the 4-tert-butylphenyl 5-hydroxypentanoate, and formulation, charactrization of nanogel, then the nanogl were subjected to invitro and invivo wound healing activities.

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