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Thrombolytic effect of Siddha herbal formulation *Sikkanjar Manapagu* using in-Silico model

Research Article

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Abstract

Background: Thromboembolic disorders are one of the important causes leading to death. In the Siddha system of medicine, many drugs have been mentioned in the literature and their thrombolytic potential needs to be scientifically evaluated. Aim: The study aims to perform the In Silico computational studies of Phytoconstituents of Siddha formulation *Sikkanjar Manapagu* (SM) to evaluate its thrombolytic potential. Methods: Autodock program was used for the molecular docking studies of the retrieved phytoconstituents such as Zingiberene, Gingerenone-A, 6 Gingerol of *Zingiber officinale*, Menthol, Luteolin, Citronellol of *Mentha arvensis*, Eugenol, Limonene, Myrcene, and Linalool of *Citrus aurantium* against target protein Human Plasminogen Activation Loop Peptide - PDB 4DCB. Results: A total of ten compounds were screened, of these Zingiberene, Menthol, Citronellol, Eugenol, Limonene, Myrcene, and Linalool showed high binding against active amino acid residue 195. Conclusion: Based on further experiments and clinical trials, the formulation *Sikkanjar Manapagu* could be proved to be effective in thrombolytic treatment.

Key Words: Docking study, Thrombolytic, *Siddha formulation, Sikkanjar manapagu,* Human Plasminogen Activation Loop Peptide - PDB 4DCB.

Introduction

A thrombus is a solid mass formed in blood vessels from the components of blood and is colloquially called a "blood clot". Thrombosis occurs due to the imbalance in normal homeostasis in the circulatory system. It is a fatal disease and it can occur from the heart, arteries, and veins or its microcirculation, named cardiac thrombi, arterial thrombi, venous thrombi, and capillary thrombi based on its origin (1, 2). Thrombi are comprised of platelets, fibrin, erythrocytes, leukocytes, and extracellular traps of neutrophils (3). Unnecessary formation of thrombus may lead to serious consequences like embolism, ischemia, myocardial infarction, stroke, and so on (4). The fragments of thrombi can be separated off and may be passed to various locations via blood circulation. The broken piece of thrombus in the circulation is "embolus" which may obstruct the blood vessels and the condition is called as "Embolism". These obstacles hinder the supply of oxygen to the adjacent tissue, leading to the tissue's degradation and death. Based on the situation of thrombi or emboli, they may cause various diseases such as deep vein thrombosis (caused by venous thrombi), pulmonary embolism, arterial thrombosis, stroke, acute myocardial infarction (caused by cardiac thrombi), and retinal artery occlusion (5). An important event in the dissolution of the intravascular blood clot is plasminogen activation (6). Therefore, Plasminogen Activation Loop was chosen as the target for this study. The binding of phytocomponents with the core amino acid residue 195 plays a critical role in the recognition of the residues Arg561-Val562 of plasminogen. It has been found to have a similar pose in the mutant form. Thereby phytocomponents that bind with the amino acid 195 may be expected to medicate the cleavage of zymogen plasminogen at its Arg561-Val562. Further, these leads may be considered as potential thrombolytic agents. Thrombolytics are plasminogen activators, which are the substances used in the treatment of dissolving intravascular blood clots. Thrombolytics that are currently available are, Streptokinase, Urokinase, Streptokinase, Alteplase, Reteplase, Tenecteplase, Prourokinase, Anistreplase, etc. But there are chances of the occurrence of adverse reactions such as bleeding (most common), hypotension, angioedema, etc., while using these drugs (7). Because of these drawbacks of synthetic and recombinant thrombolytic drugs, the mergence of alternatives should be appreciated and also natural

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products may be good replacements to the currently used thrombolytic drugs (8). This research aims to analyze the thrombolytic potential of *Sikkanjar Manapagu* using an in-silico model. The drug *Sikkanjar Manapagu* is a syrup-based herbal preparation, mentioned in the Siddha literature. SM has *Zingiber officinale, Mentha arvensis, Citrus aurantium, Cicer arietinum, and Saccharum officinarum* (9).

Materials and methods

Ligand Preparation

The crystalline structure of the target protein Human Plasminogen Activation Loop Peptide - PDB 4DCB (Fig.1) was retrieved from the protein data bank. The protein clean-up process was done and essential missing hydrogen atoms were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

Methodology

Docking calculations were carried out for retrieved phytocomponents, Zingiberene, Gingerenone-A, 6 Gingerol of *Zingiber officinale* (10-11), Menthol, Luteolin, Citronellol of *Mentha arvensis* (12), Eugenol, Limonene, Myrcene, and Linalool of *Citrus aurantium* (13) against target protein Human Plasminogen Activation Loop Peptide - PDB 4DCB. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied (14-18).

Fig.1: 3D- Structure of Human Plasminogen Activation Loop Peptide - PDB 4DCB

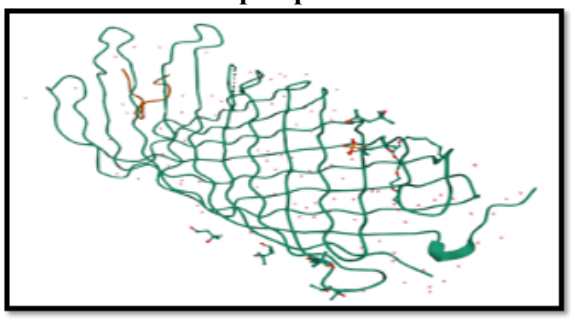


Fig.2: 2D and 3D Structure of Selected Ligands

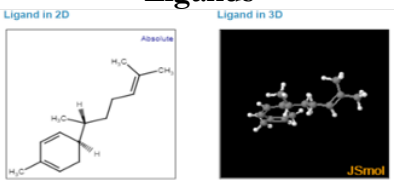
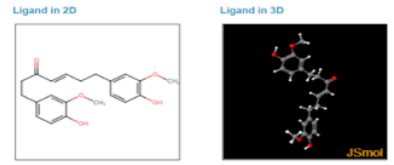
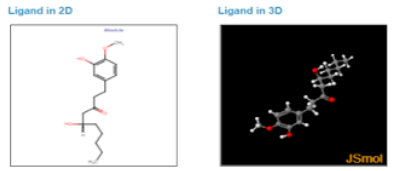
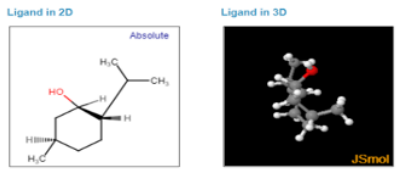
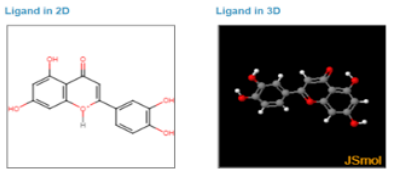
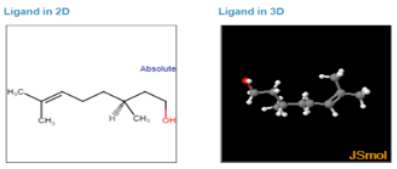
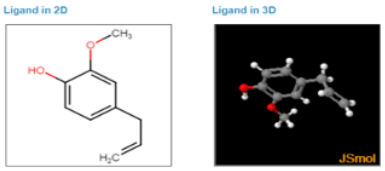
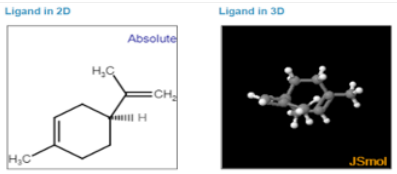
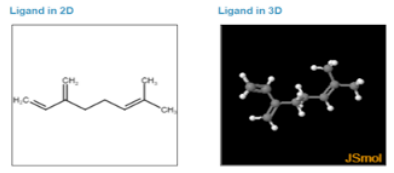
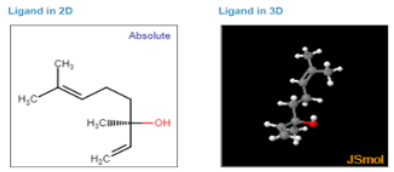
Ligands	2D and 3D structures of Ligands
Zingiberene	
Gingerenone-A	
6 Gingerol	
Menthol	
Luteolin	
Citronellol	
Eugenol	
Limonene	
Myrcene	
Linalool	

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Zingiberene	204.35 g/mol	$C_{15}H_{24}$	0	0	4
Gingerenone-A	356.4 g/mol	$C_{21}H_{24}O_5$	2	5	9
6 Gingerol	294.391g/mol	$C_{17}H_{26}O_4$	2	4	10
Menthol	156.26g/mol	$C_{10}H_{20}O$	1	1	1
Luteolin	286.24 g/mol	$C_{15}H_{10}O_6$	4	6	1
Citronellol	156.26 g/mol	$C_{10}H_{20}O$	1	1	5
Eugenol	164.2 g/mol	$C_{10}H_{12}O_2$	1	2	3
Limonene	136.23 g/mol	$C_{10}H_{16}$	0	0	1
Myrcene	136.238 g/mol	$C_{10}H_{16}$	0	0	4
Linalool	154.25 g/mol	$C_{10}H_{18}O$	1	1	4

Table 2: Summary of the molecular docking studies of compounds against Human Plasminogen Activation Loop Peptide - PDB 4DCB

Compounds	Binding Free energy Kcal/ mol	Inhibition constant Ki µM (*mM) (**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Zingiberene	-5.86	50.77	-0.03	-6.92	527.87
Gingerenone-A	-6.07	35.35	-0.17	-7.33	623.33
6 Gingerol	-4.32	685.12	-0.10	-6.16	554.34
Menthol	-4.76	326.58	-0.24	-5.33	365.97
Luteolin	-5.28	135.69*	-0.34	-4.97	470.09
Citronellol	-4.81	297.00	-0.14	-6.49	410.22
Eugenol	-4.98	223.32	-0.23	-5.35	383.88
Limonene	-4.80	302.96	-0.04	-5.10	357.68
Myrcene	-4.89	261.69	-0.01	-6.02	402.24
Linalool	-5.26	138.51	-0.29	-6.67	403.70

Table 3: Amino acid Residue Interaction of Lead against Human Plasminogen Activation Loop Peptide - PDB 4DCB

Molecule	Interactions	Amino Acid Residue- Binding											
Zingiberene	1	19 SER	54 LYS	56 ASP	70 ARG	72 TRP	111 GLU	113 ASP	195 LYS	244 GLU	246 THR	248 SER	
Gingerenone-A	0	179 LEU	194 PHE	196 PHE	225 TYR	249 LYS	251 ASP						
6 Gingerol	0	179 LEU	194 PHE	196 PHE	223 ARG	225 TYR	251 ASP						
Menthol	1	56 ASP	68 ASP	70 ARG	113 ASP	115 ASP	117 LYS	193 LEU	195 LYS	228 VAL	230 ASN		
Luteolin	0	179 LEU	196 PHE	223 ARG	225 TYR	251 ASP							
Citronellol	1	54 LYS	70 ARG	72 TRP	111 GLU	113 ASP	195 LYS	224 TYR	228 VAL	246 THR	248 SER	281 ASN	
Eugenol	1	54 LYS	70 ARG	72 TRP	111 GLU	113 ASP	195 LYS	224 TYR	228 VAL	248 SER	281 ASN		
Limonene	1	54 LYS	70 ARG	72 TRP	111 GLU	113 ASP	195 LYS	224 TYR	228 VAL	246 THR	248 SER		
Myrcene	1	54 LYS	70 ARG	72 TRP	111 GLU	113 ASP	195 LYS	224 TYR	228 VAL	246 THR	248 SER		
Linalool	1	54 LYS	70 ARG	72 TRP	111 GLU	113 ASP	135 GLN	195 LYS	224 TYR	228 VAL	246 THR	248 SER	281 ASN

Results and discussion

Thrombolytic treatment plays a key role in the management of cardiovascular diseases. A total of ten bioactive lead compounds were retrieved from the herbs present in the formulation and they are known to be Zingiberene, Gingerenone-A, 6 Gingerol of *Zingiber officinale*, Menthol, Luteolin, Citronellol of *Mentha arvensis*, Eugenol, Limonene, Myrcene, and Linalool of *Citrus aurantium*. From reported data of the herb, the leads like Zingiberene, Menthol, Citronellol, Eugenol, Limonene, Myrcene, and Linalool bound with active amino acid residue 195 that plays a critical role in the recognition of the residues Arg561-Val562 of target plasminogen.

While comparing binding affinities (Table 2) of the Phyto compounds, it was found that Zingiberene showed the highest binding affinity of -5.86 Kcal/mol to amino acid residue 195. Linalool showed the second-highest binding affinity with the binding free energy

of-5.26Kcal/mol, followed by Eugenol, Myrcene, Citronellol, and limonene with binding energies -4.98 Kcal/mol, -4.89 Kcal/mol, -4.81 Kcal/mol, and -4.80 Kcal/mol respectively. Menthol has the lowest binding affinity (-4.76 Kcal/mol) to amino acid residue 195. The compounds Zingiberene, Linalool, Eugenol, Myrcene, Citronellol, Limonene, and Menthol shared one active site amino acid in common (Table 3). Though the compound Gingerenone-A has the highest binding energy among all the compounds, while considering the interactions, it did not show any interactions with the amino acid residue 195. Similarly, 6 Gingerol and Luteolin did not show any interactions with the amino acid residue 195. As a whole, considering its bonding affinity and number of interactions with the amino acid residue 195, seven possible inhibitors were identified against the target protein Human Plasminogen Activation Loop Peptide - PDB 4DCB.

Conclusion

Based on the results of the computational analysis, it was concluded that the bioactive compounds like Zingiberene, Menthol, Citronellol, Eugenol, Limonene, Myrcene and Linalool present in *Zingiber officinale, Mentha arvensis, and Citrus aurantium* of *Sikkanjar Manapagu* reveals significant binding against target plasminogen. Thereby it was concluded that these compounds may exert promising anti-thrombolytic activity.

Conflict of interest

Nil

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