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Molecular docking for thrombolytic activity of some isolated compounds from *Clausena lansium*.

Acoplamiento molecular para actividad trombólitica de algunos compuestos aislados de *Clausena lansium*.

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Abstract
Clausena lansium (Family- Rutaceae) is commonly known as wampee, is found in fallow lands throughout Bangladesh. Our aim of the study to performed molecular docking studies to identify potential binding affinities of the phytocompounds from *Clausena lansium*, namely Clausemarin B, Clausenaline C, Clausenaline E, Murrayanine, vanillic acid and Xanthotoxol for searching of lead molecule for thrombolytic activity. A wide range of docking score found during molecular docking by Schrodinger. Clausemarin B , Clausenaline C , Clausenaline E, Murrayanine , vanillic acid and Xanthotoxol showed the docking score -6.926, -4.041, -4.889 , -4.356, -3.007 and -5.816 respectively. Among all the compounds Clausemarin B showed the best docking score. So, Clausemarin B is the best compounds for thrombolytic activity, as it possessed the best value in Molecular docking. Further *in vivo* investigation need to identify the thrombolytic activity of isolated compounds from *Clausena lansium*.

KEYWORDS
Clausena lansium, thrombolytic activity, Molecular docking, Clausemarin B.

Resumen
La *Clausena lansium* (Familia- Rutaceae), comúnmente conocida como wampi o vampi, se encuentra en las tierras de barbecho o en terrenos baldíos en Bangladesh. Este estudio pretende hacer acoplamientos moleculares para identificar posibles afinidades de enlace de los fitocompuestos de Clausena lansium, específicamente Clausemarin B, Clausenaline C, Clausenaline E, Murrayanine, vanillic acid y Xanthotoxol en busca de la molecula principal de actividad trombolitica. El acoplamiento molecular realizado por Schrodinger ofreció un amplio rango de cocientes de acoplamiento que fueron para Clausemarin B , Clausenaline C , Clausenaline E, Murrayanine , vanillic acid and Xanthotoxol -6.926, -4.041, -4.889 , -4.356, -3.007 and -5.816 respectivamente. Entre todos los compuestos fue Clausemarin B el que mostró el mejor coeficiente de acoplamiento. Por tanto Clausemarin B es el más eficaz para actividad trombolitica. En el futuro serán necesarias investigaciones in vivo para identificar la actividad trombolitica de los compuestos aislados de *Clausena lansium.*

PALABRAS CLAVE
Clausena lansium,actividad trombolítica, acoplamiento molecular, Clausemarin B.

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INTRODUCTION

Blood clot formation has been a severe problem of blood circulation which are increasing at an alarming rate in the recent years. Thrombus or embolus upsets the blood stream by hindering the vein subsequently denying tissues of ordinary blood stream and oxygen. These results yield necrosis of the tissue in that area around there. Thrombin shaped blood clump from fibrinogen and is lysed by plasmin, which is enacted from plasminogen by tissue plasminogen activator (tPA)[(1)]. There are two parts to a thrombus: aggregated platelets that shape a platelet plug, and a lattice of cross-connected fibrin protein. The substance making up a thrombus is here and there called cruor. A thrombus is a sound reaction to damage planned to avoid dying, however, can be hurtful in thrombosis when clusters hinder blood course through solid veins. Mural thrombi are thrombi that adhere to the wall of a blood vessel. They happen in extensive vessels, for example, the heart and aorta, and can limit blood stream however, generally don't block it completely. They seem grey-red with rotating light and alternating light and dark lines es (known as lines of Zahn) which represents to speak to groups of fibrin (lighter) with entrapped white platelets and red platelets (darker).

Thrombolysis is the breakdown (lysis) of blood clots [(2)] by pharmacological means and commonly called clot-busting. It works by stimulating secondary fibrinolysis by plasmin through the infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin .Thrombolytic drugs are utilized to disintegrate clump and in the administration of thrombosis in patients.[(3)] Thrombolytic agents such as tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) [(4)] etc, are used all over the world for the treatment[(5)] , but their use is associated with hyper risk of haemorrhage [(6)], anaphylactic reaction and lacks specificity .In the context of the deficiencies comings of the accessible thrombolytic drugs,attempts are in advancement to make enhanced recombinant varieties of these pharmaceuticals [(7)]. Heparin and Aspirin are just properly gainful for acceleration of lysis and prevention of reocclusion, however, are protected. More particular thrombin inhibitors and antiplatelet agents are more competent, yet their safety stays to be confirmed [(8)].

In silico molecular docking technique plays an important role in the drug design and discovery to predict the conformations of each ligand molecule at the active site, hence the molecular docking study was carried out to predict the thrombolytic activity and results are reported.

Traditionally healthful plants have served to be efficient thrombolytic agents for ages because of their wealthy diversity of phytochemicals. *Clausena lansium* (Family- Rutaceae) is also known as wampee which is an evergreen tree 3–8 m tall . Its leaves are smooth and dark green. White flowers in late March are white, with four or five petals, about 3–4 mm in diameter. The fruit is oval, about 3 cm long and 2 cm in diameter, and contains two to five seeds that occupy ~40-50% of the fruit volume. The tree reaches a maximum height of 20 meters. It grows well in tropical or subtropical conditions and is susceptible to cold. Wampee trees grow well in a wide range of soil, but will grow best in rich loam.

The aim of the study to discover potent thrombolytic drug from *Clausena lansium* by *in silico* molecular docking process.

MATERIALS AND METHODS

Protein Preparation

Three-dimensional crystal structure of tissue plasminogen activator (PDB id: 1A5H) was downloaded in pdb format from the protein data bank [(9)]. After that, the structure was prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v10.1. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, selenomethionines were converted to methionines, and all waters were deleted. Using force field OPLS_2005, minimization was carried out setting maximum heavy atom RMSD (root-mean-square-deviation) to 0.30 Å.

Ligand Preparation

Compounds were retrieved from PubChem databases, i.e. clausemarin B (CID 101879359), Clausenaline C (CID 101879360), Clausenaline E (CID 86106588), Murrayanine (CID 96942), vanillic acid (CID 8468) and Xanthotoxol (CID 65090). The 3D structures for these were built by using Ligprep2.5 in Schrödinger Suite 2015 with an OPLS_2005 force field. Their ionization states were generated at pH7.0±2.0 using Epik2.2 in Schrödinger Suite. Up to 32 possible stereoisomers per ligand were retained.

Receptor grid generation

Receptor grids were calculated for prepared proteins such that various ligand poses bind within the predicted active site during docking. In Glide, grids were generated keeping the default parameters of van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS 2005 force field. A cubic box of specific dimensions centred around the centroid of the active site residues (Reference ligand active site) was generated for the receptor. The bounding box was set to 14 Å × 14 Å × 14 Å for docking experiments.

Glide Standard Precision (SP) ligand docking

To find out the accurate binding model for the active site of tubulin, molecular docking analysis was performed using ligand fit of GLIDE software from Schrodinger (http://www.schrodinger.com/). Molecular docking analysis was performed using crystal structure of plasminogen activator (PDB id: 1A5H). The structure of crystal structure of tissue plasminogen activator (PDB id: 1A5H) were obtained from Protein Data Bank (http://www.rcsb.org). The mechanism of ligand position is based on the fitting points. Fitting points are incorporated into the hydrogen bonding groups on the

ligand and the proteins. The ligand fit module [10] from GLIDE software was utilized to execute the molecular docking analysis, based on shape-based searching and Monte Carlo methods. At the time of docking, variable trials Monte Carlo conformation was applied where the number of steps depends on the number of rotatable bonds present in the compounds/ ligands. By default the torsion number is 2, the maximum minimizations steps are 300 and maximum successive failure is 110. During the docking process, the top ten conformations were engendered for each of the compounds after the minimization of the energy [11].

RESULTS

In silico Molecular docking analysis

In order to study the interaction of the compounds Clausemarin B, Clausenaline C , Clausenaline E, Murrayanine , vanillic acid, Xanthotoxol with 1A5H, we performed Glide docking analysis by Schrodinger suite v10.1, where among of these compounds 2-methylanthraquinone shows highest docking score shown in Table 1. The negative and low value of free energy of binding demonstrates a strongly favorable bond between 1A5H and Clausemarin B in most favourable conformations. The results of docking analysis were described in Table 1 and the docking figure showed in Figure 1.

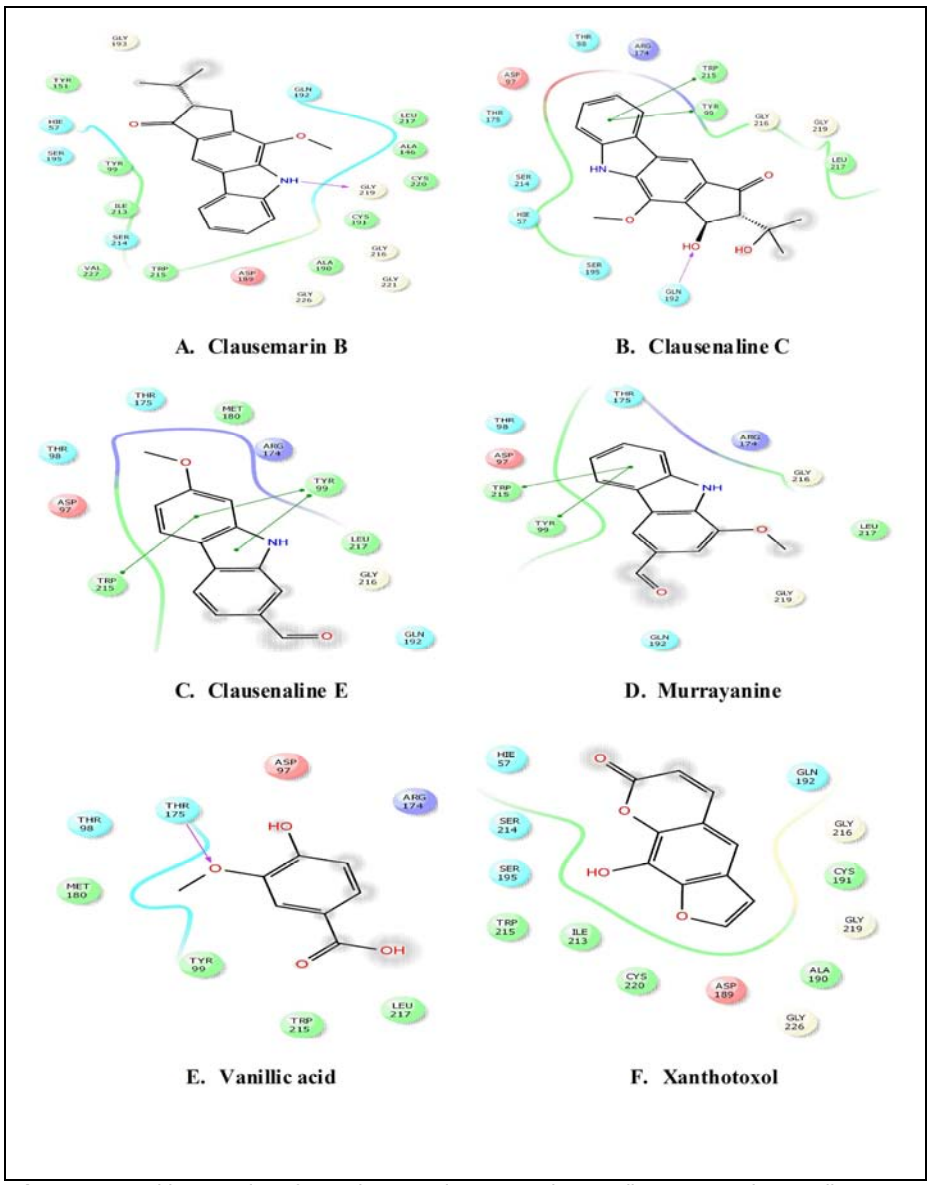

Figure 1. Docking results of A. Clausemarin B, B. Clausenaline C, C. Clausenaline E, D. Murrayanine, E. Vanillic acid, F. Xanthotoxol with tissue plasminogen activator (PDB: 1A5H).

Table 1. Docking results of Clausemarin B, Clausenaline C, Clausenaline E, Murrayanine, vanillic acid, Xanthotoxol with tissue plasminogen activator (PDB: 1A5H).

Compound Name	Docking Score	Glide emodel	Glide energy
clausemarin B	-6.926	-50.196	-37.136
Clausenaline C	-4.041	-35.522	-29.279
Clausenaline E	-4.889	-36.553	-27.331
Murrayanine	-4.356	-30.551	-23.553
vanillic acid	-3.007	-21.917	-18.854
Xanthotoxol	-5.816	-43.77	-30.627

DISCUSSION

Bangladesh is a great source for Phytomedicine. Phytomedicine has a long history of use for the prevention and treatment of human diseases. And many pharmaceuticals currently approved by the Food and Drug Administration (FDA) have origins to plant sources. A major role for phytoconstituents based on the reported immunomodulatory effects has emerged in recent times and has led to the rigorous scientific examination to determine efficacy and safety [(12)]. A number of plants source especially several fruits and vegetables have been studied for their supplements having anticoagulant, antiplatelet and fibrinolytic activity and there is evidence that consuming such food leads to prevention of coronary events and stroke [(13-16)]. Some of these plant products are modified further with recombinant technology [(17)] to make them more effective and site specific. A wide variety of phytochemicals has been shown to prevent certain chronic diseases, such as cancers and cardiovascular diseases, by mitigating or correcting cellular dysfunctions [(18)].

The aim of molecular docking is the accurate prediction of the structure of a ligand within the constraints of a receptor binding site and to correctly estimate the strength of binding. The binding mode of tissue plasminogen activator was investigated by doing computational analysis, glide docking. Both glide standard (SP) had been introduced. The results of docking analysis were described in Table 1 and the docking figure showed in Figure 1. Among all the compounds, Clausemarin B showed the well docking score, glide emodel and glide energy. Because the negative and low value of free energy of binding demonstrates a strongly favorable bond is preferable for best docking study. So the docking score between 1A5H and Clausemarin B in most favorable conformations.

CONCLUSION

From our study, we were found that *Clausena lansium* could be a great source for the thrombolytic drug. So, we can say that all the compounds except Clausemarin B give negative result. Only Clausemarin B was the best thrombolytic activity according to the docking score. On the other hand all the compounds have less thrombolytic activity then Clausemarin B. So, we do further *in vivo* investigation need to identify the thrombolytic activity of isolated compounds from *Clausena lansium*.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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