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Thrombolytic Activity, Drug Likeness Property and ADME/T Analysis of Isolated Phytochemicals from Ginger (*Zingiber officinale*) Using *In Silico* Approaches

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Abstract

This experiment has been carried out to observe the potential thrombolytic activity of naturally occuring phytochemicals in Ginger (*Zingiber officinale*) and to analyze their drug likeness property and ADME/T profile. Thrombolytic activity of Ginger has already been confirmed in laboratory experiment and this study focuses on the molecular interactions among four phytocompounds **(Isovanillin, Gingerol, Beta-sitosterol and 2,6-Dimethyl-2-octene-1,8-diol)** found in Ginger and Tissue Plasminogen Activator (tPA). Present experiment is largely based on computer-aided drug design protocol where the strength of interaction is described as binding energy function. Isovanillin exhibited better docking score, and so this compound might have greater thrombolytic activity than others. Moreover, Isovanillin also suggested sound drug likeness property and ADME/T profile which predicts its safeness for consumption in human body. But Beta-sitosterol violated Lipinski's rule of five and 2, 6-Dimethyl-2-octene-1,8-diol showed the lowest affinity of binding with tPA. However, further *in vivo* or *in vitro* study may be required to confirm the thrombolytic activity of Isovanillin.

Keywords

ADME/T, Ginger, Isovanillin, *In Silico*, Molecular Docking, Thrombolytic Activity

1. Introduction

1.1. Thrombosis and Its Treatment

Thrombosis generally refers to localized clotting of the blood which can occur in both arterial and venous circulation and has a great medical impact. In most of the developed country, the major cause of myocardial infarction (heart attack) and 80% of the stroke is attributed to acute arterial clotting [1]. Such complication eventually leads to death if not treated earlier. Thrombosis is usually caused by blood coagulation protein or platelet defect which leads to blockage of the circulatory vessel preventing the appropriate blood flow inside human body. However, beside heart attack and stroke, thrombosis can give rise to cardiac disability, stasis ulcers, loss of vision and some other manifestations [2]. The treatment of thrombosis involves antithrombotic drugs which specifically target the proteins involved in the coagulation cascade of human body. Administration of these drugs results in the binding of drug molecule with a target protein which then promotes the clot breakdown effectively. There are lots of antithrombotic drugs available in the market, which are effective in treating thrombosis in patients with cardiovascular diseases. However, some of the available treatments have been accused to cause severe bleeding upon administration [1] [3] [4].

Natural plant-derived compounds are being traditionally used as thrombolytic agents by the people of all ethnic groups around the world. Ginger is a common herb of Bangladesh which is commonly used as condiment in food and its thrombolytic activity has been proven in recent studies [5] [6]. Ginger is a rich source of Alkaloids, Tannins, Flavonoids, Saponins, etc. which encompasses greatest nutritional value for the consumers. The major phytochemicals of significant therapeutic importance of this plant include Gingerol, Shogaol, Paradol, Isovanillin, Beta sitosterol, Isohogaol, Gingerdione, etc. Thrombolytic activity of Ginger is assumed to be due to the presence of one or more of these compounds [7] [8].

1.2. Mechanism of Tissue Plasminogen Activator Action in Thrombolysis

Tissue Plasminogen Activator (tPA) is a 69 kD glycoprotein, an enzyme of serine protease family produced from the endothelial cells of the blood vessels which plays an important role in blood clot degradation [9]. The active site which is responsible for protease activity of tPA involves—His322, Asp371 and Ser478 in the backbone [10]. Basic function of tPA in human body is to cleave a zymogen called plasminogen at its Arg561-Val562 site to generate the active serine protease called plasmin which then acts as a molecular scissor to cut the cross-link of blood clot formed inside the blood vessel with fibrins down to fibrin degradation product (FDP) in a process called fibrinolysis [11] [12]. After being secreted from the endothelial cells, tPA first binds to the plasminogen presents on the fibrin of the blood clot and thus forms a ternary complex to-

gether. Immediately after complex formation tPA cleaves the plsminogen into plasmin which then carries out the fibrinolysis, a process that removes the blood clot (**Figure 1**) [13].

Different inhibitors inside the blood vessel confirm a controlled turnover between activated plasmin and inactivated plasminogen. tPA becomes rapidly inactivated by the Plasminogen Activator Inhibitor type I (PAI-I) after release from endothelial cell and activated plasmin action is again terminated by complex formation with *α*2-antiplasmin making the action very precise and short [11] [14].

This study is based on the hypothesis that, binding of a ligand to tPA might provoke it to induce its protease activity which in turn may lead to more effective fibrinolysis causing blood clot to be removed more rapidly.

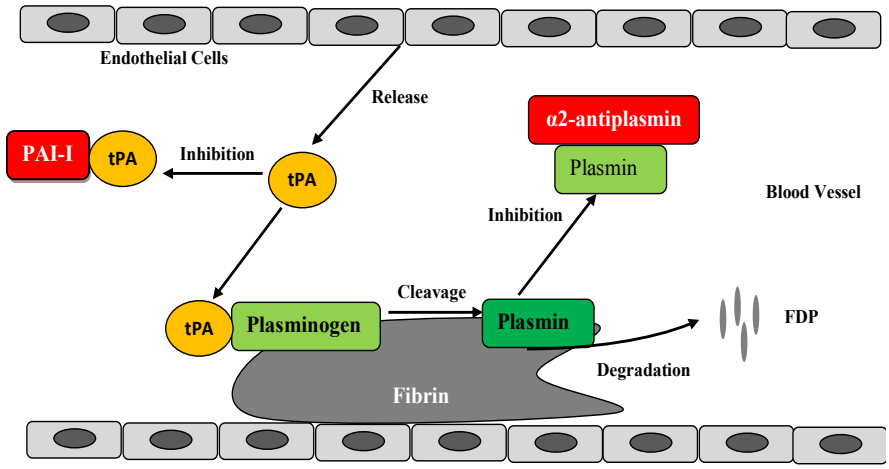

Figure 1. The mechanism of action of tPA in fibrinolysis inside the blood vessel. tPA releases from the endothelial cells and binds to the plasminogen embedded on fibrin where it cleaves inactivated plasminogen to plasmin. Plasmin then degrades the aggregated fibrins by its protease activity. The degraded products then release as fibrin degradation product (FDP) ultimately removing the blood clot. Both tPA and plasmin become inactivated immediately after their activation by forming complex with Plasminogen Activator Inhibitor type I (PAI I) and α2-antiplasmin respectively [9]-[14].

1.3. In Silico Docking Study and ADME/T Test

The advancement in virtual screening has enabled the *in silico* methods to identify and optimize lead efficiently. Recently some drugs proposed by the docking study have entered the clinical trial and some of them have already received FDA approval for marketing [15]. Molecular docking is a process which tries to define the native position, orientation and conformation of the ligand molecule within the active site of a large target molecule using computer-based programs. These programs usually function through calculation of energy grids and flexible generation of ligand binding "poses" [16]. Finally, they utilize energetic scoring algorithms to evaluate and rank compounds and poses in order to identify binding and non-binding ligands in the complex.

ADME/T test is the method which mimics the in vivo condition in order to describe the probable physicochemical properties like absorption, distribution, metabolism, excretion and toxicity inside the human body after administration [17] [18].

In this experiment, four compounds from Ginger-Isovanillin, Gingerol, Beta sitosterol and 2,6-Dimethyl-2-octene-1,8-diol (**Figure 2**) were analyzed for their potential interaction with tPA (**Figure 3**) in a search for best phytochemical with thrombolytic activity. Subsequently Drug Likeness properties and ADME/T profile of the selected ligand molecules were also analyzed. However, further *in vivo* and *in vitro* experiments are required to ensure the thrombolytic activity of phytocompounds from Ginger which was not carried out in this study due to the lack of facilities and funding.

2. Materials and Methods

Maestro Schrödinger Suite 2018, Discovery Studio Visualizer and ChemSketch were used as the analysis platform of this experiment. Ligand preparation, Grid generation and Glide docking and 2D representation of ligand receptor interaction (**Figure 4**) were obtained using Maestro Schrödinger Suite (Release: 2018-4), best possible poses of ligand-target interaction was obtained using Discovery Studio Visualizer (v19.1) (**Figure 5**) and the chemical structure of ligands were refined using ChemSketch (**Figure 2**) [19] [20] [21].

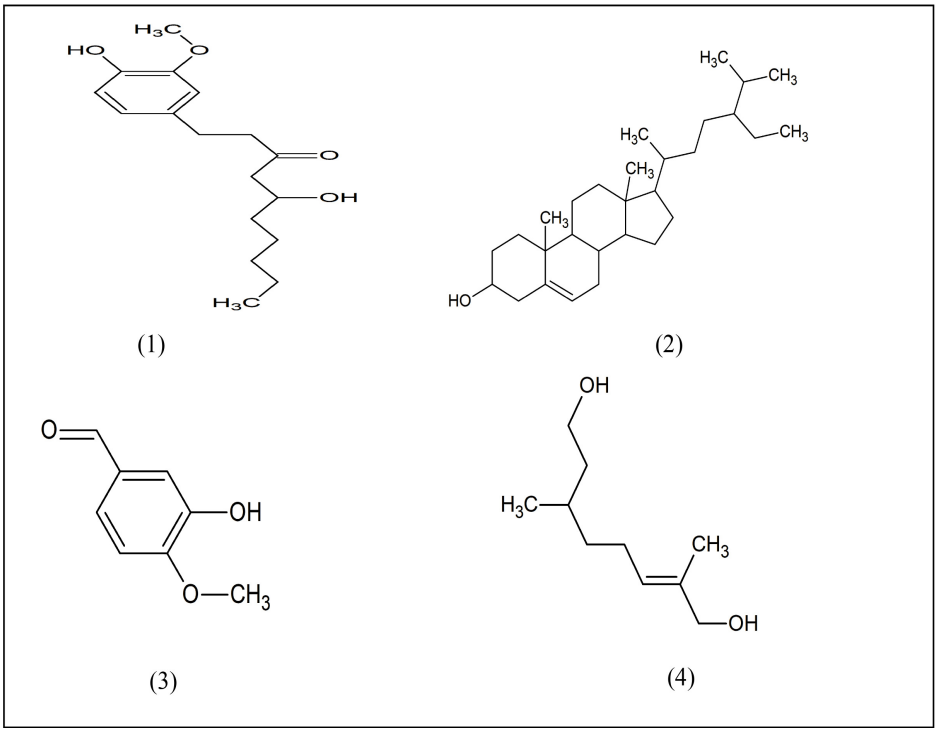

Figure 2. Chemical structures of (1) Gingerol (PubChem CID: 442793), (2) Beta-sitosterol (PubChem CID: 222284), (3) Isovanillin (PubChem CID: 12127) and (4) 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523).

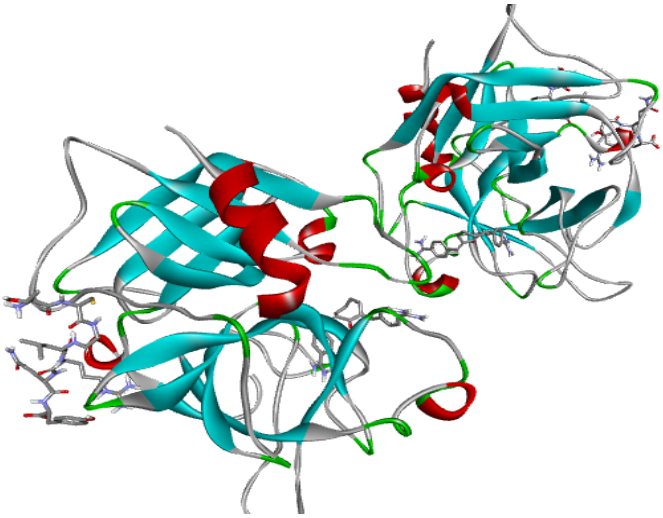

Figure 3. 3D representation of tPA (PDB ID: 1A5H) in its native ligand bound form. Four chains of tPA are represented in ribbon style and ligands are represented in stick style.

2.1. Protein Preparation

A three-dimension structure (**Figure 3**) of Tissue Plasminogen Activator (PDB Id: 1A5H) was downloaded in PDB format from protein data bank (http://www.rcsb.org). The protein was then prepared and refined using the Protein Preparation Wizard in Maestro Schrödinger suite. Bond orders were assigned and hydrogens were added to heavy atoms. All the water molecules were deleted and selenomethionines were converted to methionines. Finally, the structure was optimized and then minimized using Optimized Potentials for Liquid Simulations 2005 (OPLS_2005) force field. Minimization was carried out setting the maximum heavy atom RMSD (root-mean-square-deviation) to 30 Å and any remaining water less than 3 H-bonds to non water was again deleted during the minimization step.

2.2. Ligand Preparation

The 2D conformations of Gingerol (PubChem CID: 442793), Beta-sitosterol (PubChem CID: 222284), Isovanillin (PubChem CID: 12127), 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523) were downloaded from PubChem (http://www.pubchem.ncbi.nlm.nih.gov). These structures were then prepared and processed using the LigPrep wizard of Maestro Schrödinger suite. Minimized 3D structures of ligands were generated using Epik2.2 within pH 7.0 +/− 2.0. Minimization was again carried out using OPLS_2005 force field which generated 32 possible stereoisomers for each of the compounds depending on available chiral centres of each molecule.

2.3. Receptor Grid Generation

Grid usually confines the active site to shortened specific area of the receptor protein for the ligand to dock specifically. In Glide, a grid was generated using

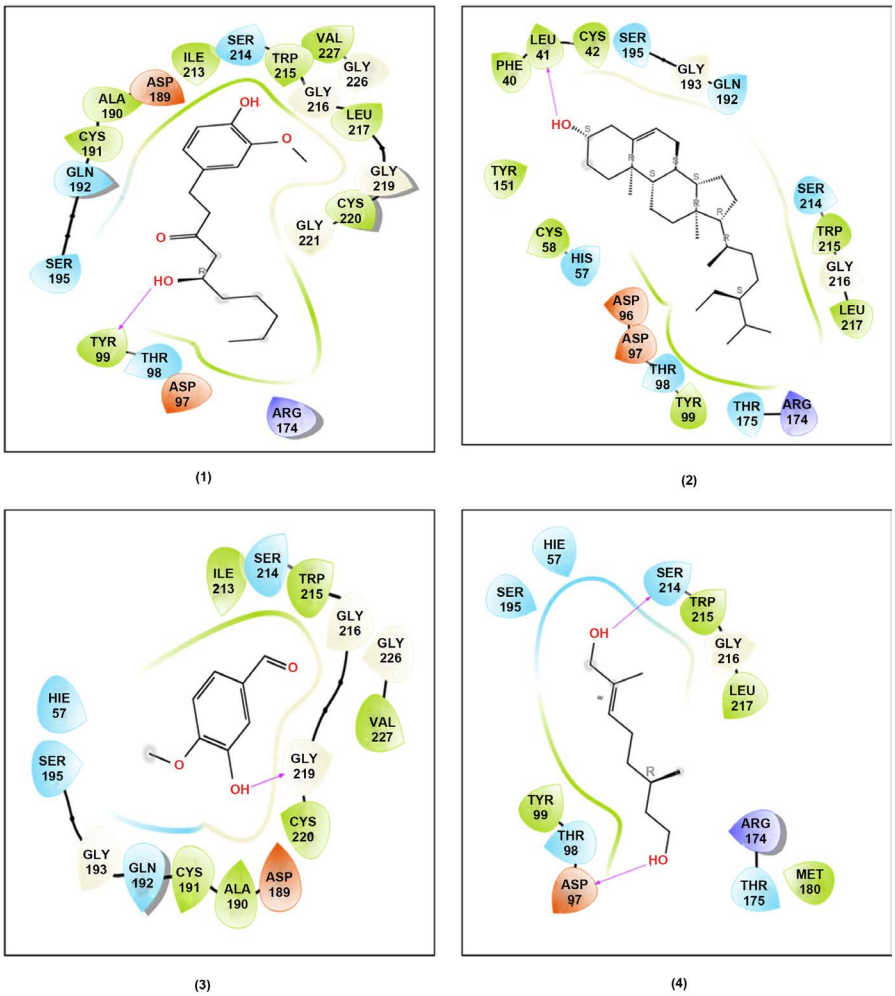

Figure 4. 2D representation of the best pose interaction between (1) Gingerol (PubChem CID: 442793), (2) Beta-sitosterol (PubChem CID: 222284), (3) Isovanillin (PubChem CID: 12127) and (4) 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523) and tPA (PDB ID: 1A5H). Color spheres indicates the residues type in the target: Red-Acidic (Asp, Glu), Blue-Polar (Ser, Thr, Gln, Asn, His), Green-Hydrophobic (Ala, Val, Leu, Ile, Tyr, Trp, Phe, Met, Cys, Pro), Purple-Basic (His, Lys, Arg), Darker gray-metal atom, Lighter gray-Other (Gly, water). Interactions are represented as color lines- Solid pink-H-bond in target, Dotted pink-H-bond between receptor and ligand, Green line-Pi-Pi stacking interaction, Orange-Pi-cation interaction. Ligands exposed to solvent are represented by grey sphere. The protein pocket for the ligand is marked with the color line according to the nearest atom. Interruption of line indicates opening of the pocket.

default Van der waals radius scaling factor 1.0 and charge cutoff 0.25 which was then subjected to OPLS_2005 force field. A cubic box was generated around the active site (reference ligand active site). Then the grid box dimension was adjusted to 14 Å × 14 Å × 14 Å for docking to be carried out.

2.4. Glide Standard Precision (SP) Ligand Docking

SP adaptable glide docking was carried out using Glide in Maestro Schrödinger. The Van der waals radius scaling factor and charge cutoff were set to 0.80 and

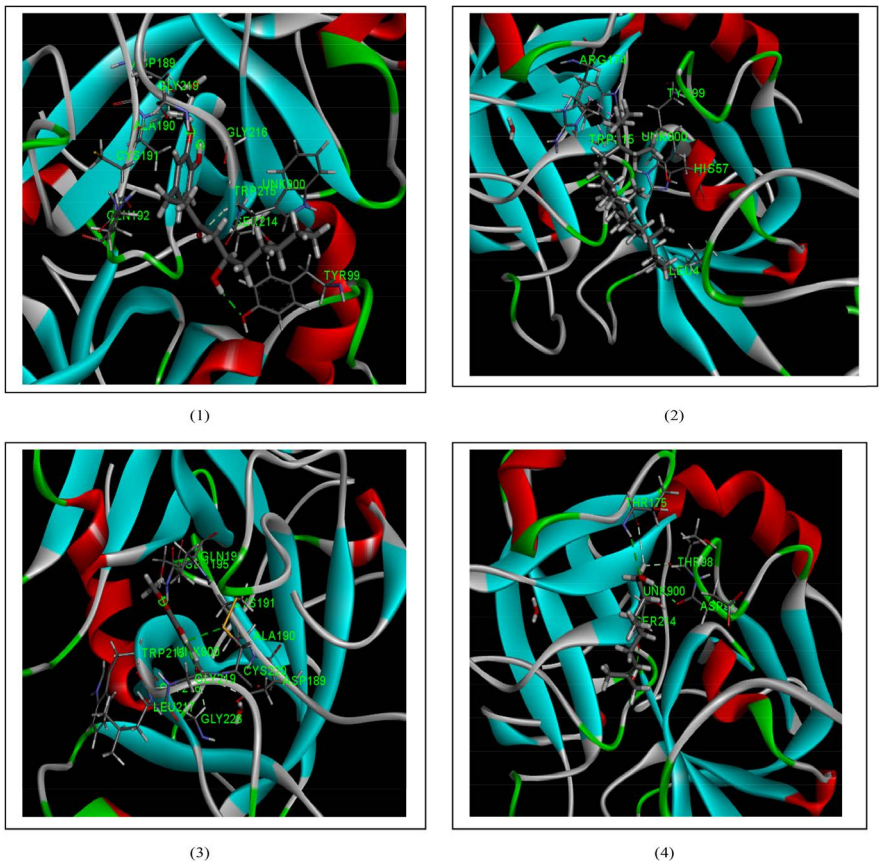

Figure 5. 3D representation of the best pose interaction, interacting residues between (1) Gingerol (PubChem CID: 442793), (2) Beta-sitosterol (PubChem CID: 222284), (3) Iso-vanillin (PubChem CID: 12127) and (4) 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523) and tPA (PDB ID: 1A5H). Interacting amino acid residues of target molecule are labeled in the diagram. UNK900 is the ligand. Dotted line depicts interaction between ligand and receptor molecule (Green-Hydrogen bond, White-Carbon bond, Pink-Alkyl bond). The ligands and interacting residues of the target are represented in stick style and other residues of target are represented as ribbon backbone.

0.15 respectively for all the ligand molecules. Final score was assigned according to the pose of docked ligand within the active site of the receptor. The ligand with lowest glide docking score was considered as the best ligand. The docking result is summarized in **Table 1**.

2.5. Ligand Based Drug Likeness Property and ADME/Toxicity Prediction

The molecular structures of every ligand were analyzed using SwissADME server (http://www.swissadme.ch/) to confirm whether they obey Lipinski's rule of five or not. Various physicochemical properties of ligand molecules were again calculated using ORISIS property explorer [21]. The result of drug likeness property analysis is summarized in **Table 2**.

The ADME/T test for each of the ligand molecules was carried out using an online based server admetSAR (http://lmmd.ecust.edu.cn/admetsar1/predict/) to

predict their different pharmacokinetic and pharmacodynamic properties including blood brain barrier permeability, human intestinal absorption, Cytochrome P (CYP) inhibitory capability, carcinogenicity, mutagenicity, Caco-2 permeability, etc. The result of ADME/T for all the ligand molecules is represented in Table 3.

3. Result

3.1. Binding Energy

All the ligand molecules docked successfully to the target molecule. Gingerol, Beta-sitosterol, Isovanillin and 2,6-Dimethyl-2-octene-1,8-diol docked with −3.767 Kcal/mol, −3.714 Kcal/mol, −6.848 Kcal/mol and 0.295 Kcal/mol binding energies respectively within the binding pocket of tPA (Table 1). Isovanillin interacted with the maximum number (11) of residues in the target molecule among all the ligands. On the contrary, Gingerol interacted with 9 residues, Beta-sitosterol interacted with 5 and 2,6-Dimethyl-2-octene-1,8-diol interacted with only 4 amino acid residues of the target molecule (Figure 5).

Gingerol and Beta-sitosterol formed 1 hydrogen bonds each with Tyr98 and Leu41 and 2.09 and 2.19 Å distance apart respectively from the corresponding amino acid residue of target molecule, Isovanillin also formed one hydrogen bond with Gly219 having distance of 2.03 Å. However,
2,6-Dimethyl-2-octene-1,8-diol formed two hydrogen bonds with two different residues-Asp97 (2.38 Å), Ser214 (2.17 Å), in the binding pocket backbone of tPA (Figure 4). None of the ligand molecules interacted with amino acids (His322, Asp371, Ser478) of the serine protease catalytic domain of tPA offering it to act freely.

3.2. Drug-Likeness Property

Gingerol, Isovanillin and 2,6-Dimethyl-2-octene-1,8-diol followed the Lipinski's rule of five without any violation with respect to molecular weight (acceptable range: <500), number of hydrogen bond donors (acceptable range: ≤5), number of hydrogen bond acceptors (acceptable range: ≤10), lipophilicity (expressed as LogP, acceptable range: <5) and molar refractivity (40-130) (Table 2) [22]. Beta-sitosterol violates Lipinski's rule of lipophilicity (logP 7.68). Gingerol possesses the highest topological polar surface area (66.76) and Beta-sitosterol possesses the lowest polar surface area (20.23) among all the ligand molecules. On the other hand, Isovanillin and 2,6-Dimethyl-2-octene-1,8-diol have moderate polar surface area—46.53 and 40.8. Beta-sitosterol exhibited extremely lowest LogS value (−7.90) and other three selected ligand molecules have similar LogS value within acceptable range. Isovanillin showed highest drug likeness score and Beta-sitosterol showed highest drug score. Other ligands exhibited almost similar drug likeness and drug score.

3.3. ADME/T Test

Result of ADME/T test of selected ligand molecules is summarized in Table 3.

All of them are capable of penetrating the blood brain barrier and are highly absorbed in human intestinal tissue. None of the ligand molecules showed P-glycoprotein inhibitory effect. All selected molecules are capable of permeating Caco2 cell lines. Isovanillin and 2,6-Dimethyl-2-octene-1,8-diol showed no inhibitory effect to Cytochrome P450 family of proteins but Gingerol is a potent inhibitor of CYP450 1A2. Isovanillin and 2,6-Dimethyl-2-octene-1,8-diol are readily biodegradable but Gingerol and Beta-sitosterol are not. None of the molecules showed AMES toxicity and carcinogenicity.

Table 1. Docking results of Gingerol (PubChem CID: 442793), Beta-sitosterol (PubChem CID: 222284), Isovanillin (PubChem CID: 12127) and 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523) against tPA (PDB ID: 1A5H).

Compound Name	PubChem CID	Docking Score (Kcal/mol)	Residues Involved in Hydrogen Bonds, Distance (Å)	Interacting Residues of Target
Gingerol	442793	−3.767	Tyr99, 2.09	Gly219, Gly216, Trp215, Tyr99, Ser214, Ala190, Asp198, Cys191, Glu192
Beta-Sitosterol	222284	−3.714	Leu41, 2.19	Arg174, Tyr99, His57, Trp215, Leu41
Isovanillin	12127	−6.848	Gly219, 2.03	Ser195, Ala190, Asp189, Cys191, Gln192, Gly216, Gly219, Leu217, Trp215, Cys220, Gly226
2,6-Dimethyl-2-octene-1,8-diol	13498523	0.295	Asp97, 2.38; Ser214, 2.17	Ser214, Thr98, Asp97, Thr175

Table 2. Comparison of drug likeness properties of Gingerol (PubChem CID: 442793), Beta-sitosterol (PubChem CID: 222284), Isovanillin (PubChem CID: 12127) and 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523). Lipinski's rule of five: Molecular weight: <500, Number of H-bond donors: ≤5; Number of H-bond acceptors: ≤10; Lipophilicity (expressed as LogP): <5; and Molar refractivity: 40 - 130.

Drug Likeness Properties	Gingerol	Beta-Sitosterol	Isovanillin	2,6-Dimethyl-2-octene-1,8-diol
Molecular Weight	294.391	414.718	152.149	172.268
LogP	3.62	7.84	1.22	1.47
LogS	−2.96	−7.90	−1.66	−2.19
H-Bond Acceptor	4	1	3	2
H-Bond Donor	2	1	1	2
Molar Refractivity	83.11	129.77	41.09	52.26
Heavy Atoms	6	0	11	12
Polar Surface Area	66.76	20.23	46.53	40.8
Rotatable Bonds	10	6	2	6
Drug Likeness Score	−7.78	−4.48	−3.48	−7.82
Drug Score	0.40	0.13	0.18	0.23

Table 3. ADME/T properties of Gingerol (PubChem CID: 442793), Beta-sitosterol (PubChem CID: 222284), Isovanillin (PubChem CID: 12127) and 2,6-Dimethyl-2-octene-1,8-diol (PubChem CID: 13498523). BBB+: Capable of penetrating blood brain barrier; HIA+: Highly absorbed in human intestinal tissue; Caco-2+: Permeable through the membrane of Caco-2 cell lines; CYP450: Cytochrome P450.

Properties	Gingerol	Beta-Sitosterol	Isovanillin	2,6-Dimethyl-2-octene-1,8-diol
Blood-Brain Barrier	BBB+	BBB+	BBB+	BBB+
Human Intestinal Absorption	HIA+	HIA+	HIA+	HIA+
Caco-2 Permeability	Caco2+	Caco2+	Caco2+	Caco2+
P-glycoprotein Substrate	Substrate	Substrate	Non-Substrate	Substrate
CYP450 2C9 Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Non-Substrate
CYP450 2D6 Substrate	Non-Substrate	Non-Substrate	Non-Substrate	Non-Substrate
CYP450 3A4 Substrate	Substrate	Substrate	Non-Substrate	Non-Substrate
CYP450 1A2 Inhibitor	Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP450 2C9 Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP450 2D6 Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP450 2C19 Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP450 3A4 Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor	Non-Inhibitor
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
AMES Toxicity	Non AMES Toxic	Non AMES Toxic	Non AMES Toxic	Non AMES Toxic
Carcinogens	Non-Carcinogens	Non-Carcinogens	Non-Carcinogens	Non-Carcinogens
Biodegradation	Not Ready Biodegradable	Not Ready Biodegradable	Ready Biodegradable	Ready Biodegradable
Acute Oral Toxicity	III	I	III	III
Carcinogenicity (Three-Class)	Non-Required	Non-Required	Non-Required	Non-Required

4. Discussion

Natural plants are potent sources of secondary metabolites and other phytochemicals of great therapeutic value which play roles in treating numerous diseases. Different phytochemicals from different plants have already been demonstrated to take part in blood clot degradation both *in vitro* and *in vivo* [23] [24].

Aqueous extract of Gingerol has already been proven to have thrombolytic activity in rats [8] [25]. Four isolated compounds from Ginger were docked in this experiment and all the selected molecules docked successfully with the intended target protein strengthening suitable thrombolytic drug search.

Molecular docking aims in the most accurate prediction about the binding between receptor and ligand molecules for potential lead discovery [26]. Molecular docking of this experiment suggests that all the ligand molecules may have thrombolytic activity since all of them docked successfully with the target molecule. The lowest binding energy of Isovanillin (−6.848 Kcal/mol) suggests the

most favorable interaction and as a result of higher affinity binding it occupies 9 interacting residues within the binding site of the target protein. Conversely, 2,6-Dimethyl-2-octene-1,8-diol showed the highest binding energy (0.295 Kcal/mol) and thus the lowest binding affinity among all the ligands and thereby it interacted with only 4 amino acids. Hydrogen bonds play a significant role between ligand and receptor molecule. It provides the stability, molecular recognition and specificity of the interaction [27]. All the selected ligand molecules formed almost similar number of hydrogen bonds with target molecule strengthening the ligand-receptor interaction.

Drug likeness properties are some specified chemical features of a compound which is considered as an integral part of lead discovery expenditure. Lipinski's rule of five helps in determining the chemical features which influence properties like membrane permeability and bioavailability of the drug inside biological system [28] [29]. Molecular weight and polar surface area or topological polar surface area (TPSA) influence the permeability of the drug molecule through the biological barrier inside the cell. Higher molecular weight and TPSA reduce the permeability and lower ones help in the increment of drug permeability. LogP is expressed in the context of lipophilicity and referred as the logarithm of partition coefficient of the candidate molecule in organic and aqueous phase. Lipophilicity affects the absorption of the candidate drug molecule inside the human body. Higher LogP is associated with lower absorption of the drug and lower value ensures a higher rate of absorption. LogS value influences the solubility of the candidate molecule and the lowest value is always preferred for the drug molecule under investigation. The number of hydrogen bond donors and acceptors outside the acceptable range again influences the ability of a drug molecule to cross membrane bilayer of cell. Increased number of rotatable bonds is related with oral bioavailability and it is assumed to be within 10 as acceptable range [30] [31]. In this experiment all the ligand molecules except Beta-sitosterol followed Lipinski's rule of five. The violation of Lipinski's rule of five by Beta-sitosterol might eliminate it as a thrombolytic drug choice or may be subjected it to further development.

In silico ADME/T test provides indication about the significant pharmacokinetic and pharmacodynamic property of a molecule prior to lead identification which reduces cost and time by reducing the rate of late-stage failure in a drug discovery approach. For approval as a commercial drug use, vigorous toxicity testing along pharmacokinetic and pharmacodynamic properties is required to be analyzed. *In silico* ADME/T allows these parameters to be checked quite accurately and easily which in turn drives the successful *in vitro* ADME/T screens [32] [33]. Blood brain barrier permeability becomes major concern when drugs target primarily the brain cells. Oral delivery system is the most commonly used route of drug administration and the administered drug passes through the digestive tract so it is appreciable that the drug is highly absorbed in intestinal tissue. P-glycoproteins embedded on the cell membrane facilitate the transport of many drugs and therefore its inhibition may affect the normal drug transport. *In*

vitro study of drug permeability test utilizes Caco2 cell line and its permeability to the intended candidate drug molecule reflects that the drug is easily absorbed in the intestine [34] [35].

Cytochrome P450 enzymes play a major role in drug interaction, metabolism and excretion inside the body. Inhibition of these enzymes may lead to the elevation of drug toxicity, slow clearance and malfunction of the drug compound [36]. Only Gingerol showed inhibitory effect toward CYP450 1A2 and no other inhibitory effect by other molecules was observed (**Table 3**). Considering all the parameters, Isovanillin again performed better in ADME/T test than any other ligand molecules of this experiment.

Molecular docking has been a fascinating approach nowadays in the designing of structure-based drug and it has already contributed to design many commercially available drugs. But still, there are some limitations with receptor flexibility, ligand preparations and scoring function algorithm which may lead to erroneous prediction. *In silico* ADME/T predictive tools are designed to assist *in vivo/in vitro* toxicological and pharmacological profiling of pharmaceutical compounds which in turn enables a better understanding of drug safety and liabilities. But less accuracy and low sensitivity sometimes may lead to faulty predictions also [16] [37] [38].

Finally, Isovanillin performed extraordinarily better than other ligand molecules in terms of every tests of this study which may strengthen its choice as a natural thrombolytic agent from Ginger. In addition to that, other molecules can also be investigated further since they also performed well in the docking experiment.

5. Conclusion

Gingerol could be a great natural source of thrombolytic drug since the current medication imposes problems with bleeding inside human body and other complications. Further laboratory experiments are required to confirm potential thrombolytic activity of Ginger by *in vivo* and *in vitro* study. Hopefully, this experiment will raise research interest among researchers about the thrombolytic agent from natural source.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

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