In-Silico Screening of Phytocompounds of *Justicia Adhatoda* for Thrombolytic Activity

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Abstract: Background: The risk of thrombosis and its associated mortality is becoming a greater public health concern in both industrialized and developing nations. Clinical condition that is myocardial infarction, ischemic stroke, unstable angina, peripheral vascular diseases like deep vein thrombosis prioritise top in the list of thrombotic disorders. Conventional thrombolytic medications including alteplase, anistreplase, streptokinase and urokinase offer life risking adverse effects like haemorrhage, stroke, vascular dysfunction, hypertension, internal bleeding and so on. In order to overcome the undesirable side effects caused by the conventional thrombolytic therapy, there is ominous need of alternate complementary treatment from traditional medicine. Majority of the Siddha formulations comprise of some potential medicinal herbs which are found to be biocompatible and also have a wide safety window. *Justicia adhatoda* is one such novel herb which is investigated broadly by researchers till date, aspired by its unique pharmacological property rendered by the bioactive components present in it. Methods: Hence, the present research is aimed at in-silico screening of certain classes of alkaloids and flavonoids retrieved from *J. adhatoda* to explore the possible anti-thrombotic activity against the target human plasminogen activation loop peptide using AutoDock screening tool. Results and Conclusion: The computational analysis's findings led to the conclusion that the bioactive compounds present in *Justicia adhatoda* like Astragalin, Kaempferol, Vitexin, Vasicolinone and Adhatodine reveals significant binding affinity against target plasminogen. Thereby it was determined that these compounds may have promising anti-thrombotic efficacy due to their considerable binding affinity towards the target plasminogen.

Keywords: *Siddha, Thrombosis, Thrombolytic agents, Justicia adhatoda, bioactive components, Adhatodine, Anti-thrombotic activity*

1. INTRODUCTION

Platelet activation is a conventional and spontaneous process in the sequence of blood clotting which is mediated by complex extrinsic and intrinsic pathways. Signals innervated by the injured blood vessels aggravate the mode of platelet adhesion which in turn forms the network of platelet mesh upon stabilization which may form the clot which prevents excess blood loss [1]. Perhaps this event of spontaneous thrombosis without major vascular involvement surely calls for health issues which are collectively called as thrombotic disorders [2].

Due to extensive action caused by thrombolytic drugs (alteplase, anistreplase, streptokinase (SK), urokinase) in thinning the bloods there might be a possible risk of haemorrhage, stroke, hypertension, internal bleeding, vascular dysfunction etc [3]. By considering these serious undesirable effects caused by the conventional thrombolytic agents,

the search of exploring alternate remedies preferably from herbal origin grabs greater attention.

The Siddha system of medicine that uses herbal therapeutics has proven to heal wide range of diseases in humans for several centuries [4]. *Justicia adhatoda* is a novel medicinal herb which is largely investigated owing to its unique pharmacological property rendered by its bioactive components. It belongs to the family Acanthaceae. Geographically this class of species is widely distributed in the central zone of South Asia and the Indo-China region [5]. *J. adhatoda*

is indigenously utilized as a traditional medicine for treating various conditions including bronchial inflammation, blood disorders, leprosy, leucoderma, allergic asthma, vomiting, pyrexia, dementia, cardiac dysfunction, jaundice, oral infections, tumour, sexual and venereal disorders [6]. It is evident from researches that *J. adhatoda* has anti-microbial, anti-asthmatic, anti-histaminic, neurotropic, anti-inflammatory, anti-ulcer, antioxidant, cough suppressant, anti-tubercular and hepatoprotective activity [7].

Earlier studies documented the existence of rare bioactive flavonoid components in *J. adhatoda* some of which includes Astragalin (flavonoid), Kaempferol (tetrahydroxy flavone), and Vitexin (flavone glycoside). In addition to flavonoids, potential alkaloids present in *J. adhatoda* include Vasicoline, Vasicolinone, Vasicinone, Vasicine and Adhatodine [8]. A study on the thrombolytic activity of the leaf extracts has reported that there is significant percentage of clot-lysis and has concluded that further work has to be done to establish the thrombolytic activity of *J. adhatoda* and to develop it as a potential thrombolytic agent[9].Hence the objective of the current study is to use the AutoDock screening tool to conduct in-silico screening of these novel alkaloids and flavonoids extracted from *J. adhatoda* to investigate potential thrombolytic activity against the target human plasminogen activation loop peptide.

2. MATERIALS AND METHODS

Protein-ligand docking

Auto dock virtual tools (Auto Dock version 4) were utilized for the purpose of lead identification that runs behind advanced computational algorithms which precisely forecasts the binding efficacy of the drugs under investigation. Investigations were conducted on Plasminogen activation loop with PDB id 4DCB to look at the binding affinity and interaction pattern of the lead molecules.

Protein preparation

Three dimensional orientation of the target protein of interest (Plasminogen activation loop with PDB id 4DCB) as represented in figure 1 was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB). The recovered protein construct was made to undergo surface optimization by eliminating native ligand moieties and subjected to cleavage of water molecules. With additional polar hydrogen atoms, Gasteiger charges were calculated and merged non polar and rotatable bonds were defined using AutoDock4 [10].

Ligand model preparation

With the use of ChemDraw sketch software, Two dimensional and three dimensional skeletons of selected ligands such as Astragalin, Kaempferol, Vitexin, Vasicoline, Vasicolinone, Vasicinone, Vasicine and Adhatodine were constructed. Physiochemical properties (Molar wt, Mol. formula, H bond donor, H bond acceptor) of each ligand molecule are listed in Table 1. Figure 2 & 3 represents the 2D and 3D structure of selected ligand molecules that were subjected to molecular docking analysis.

Docking simulations

A genuine licensed version of AutoDock 4 was used to run In-silico docking simulations. The efficacy of the lead molecules is determined by the molecular interactions between residual amino acids with the core functional groups.

Through the use of AudoDock 4, the three dimensional pharmacophores of the lead phytocomponents were virtually screened against the chosen protein target plasminogen activation loop peptide with PDB id 4DCB retrieved from RCSB. Docking grid were set with the pocket size measuring maps of 70×70×70 Å grid points and with 0.375 Å. Each docking calculation was set to run with 10 different cycles 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 [11], [12].

3. RESULTS

Docking calculation involves several crucial factors some of which includes spatial arrangement of the functional group, donor/ acceptor bonds, orientation of the pharmacophore skeleton over active site, mutual interaction of amino acids with functional moieties, minimisation of force fields, existence of water molecules around the site etc. Docking

score clearly depicts the extent of affinity and coverage of functional groups over the active pocket of the target receptor. The current studies have shown that amino acid 195 plays a critical role in the identification of the residues Arg561-Val562 of plasminogen activation loop. Thrombolytic agents are expected to occupy the residue 195 that mediates the cleavage of zymogen plasminogen at its Arg561- Val562.

In the present study the compound adhatodine ranks first with highest binding free energy -7.40kcal/mol followed by these compounds such as vitexin (-7.36), astragalin (-7.06), vasicoline (-6.51), vasicolinone (-6.24) ,kaempferol (-5.58), vasicinone (-5.41) and vasicine (-4.87) occupies other priority ranks as per the energy dominance level (Table 2).

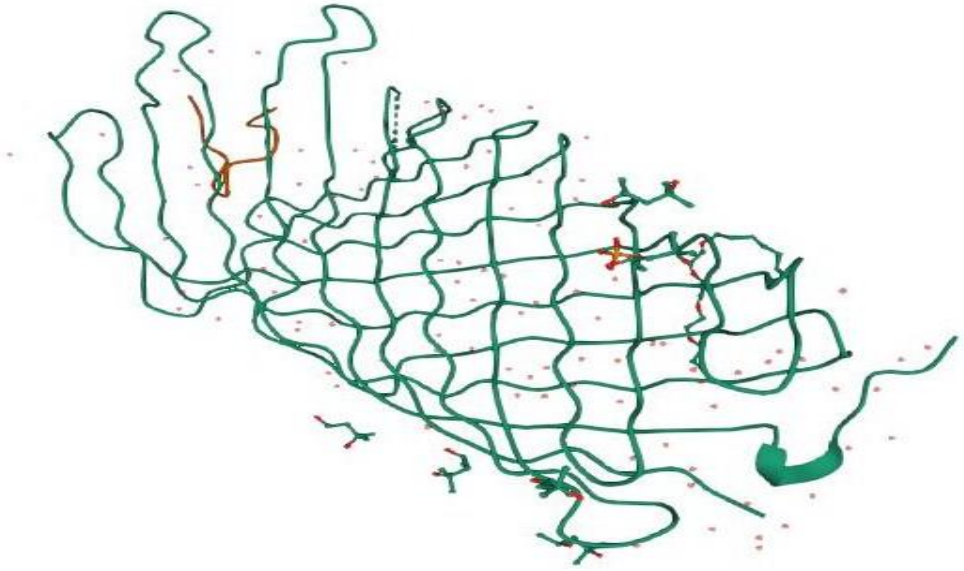

Figure 1: 3D crystalline structure of the enzyme target Plasminogen activation loop - PDB 4DCB

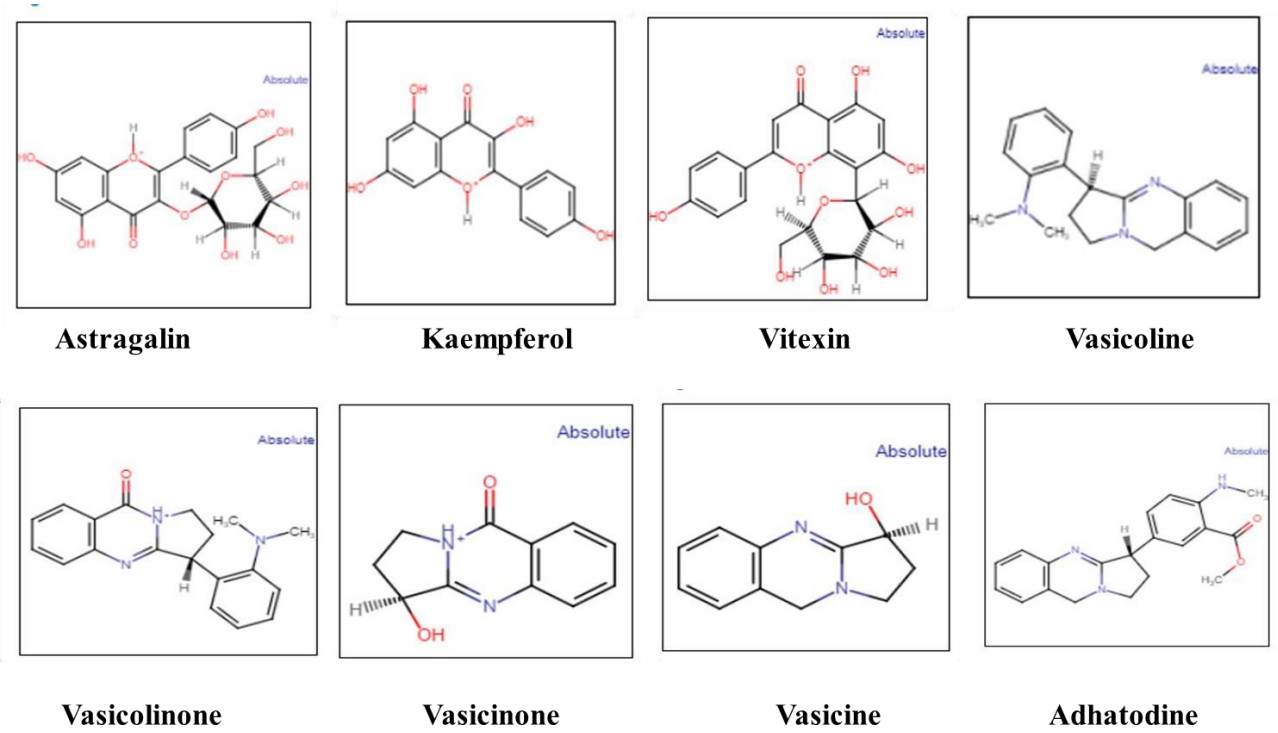

Figure 2: 2D structure of the Phytocompounds

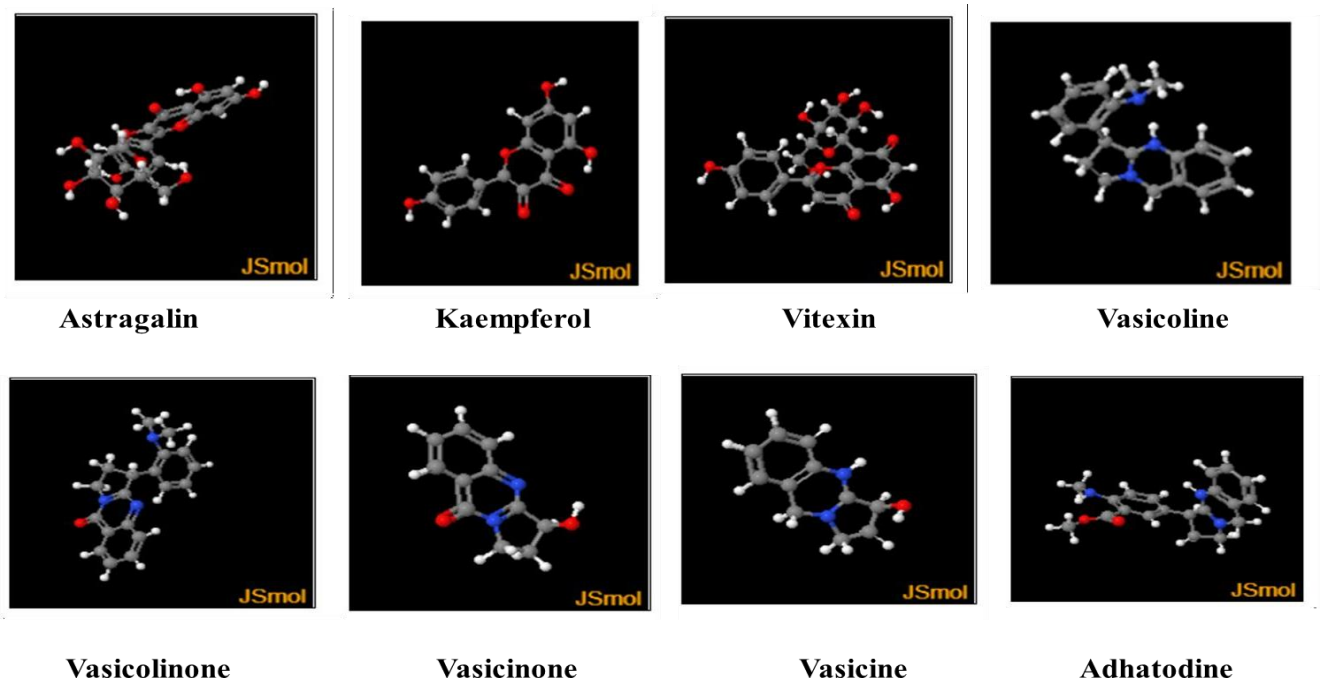

Figure 3: 3D structure of the Phytocompounds

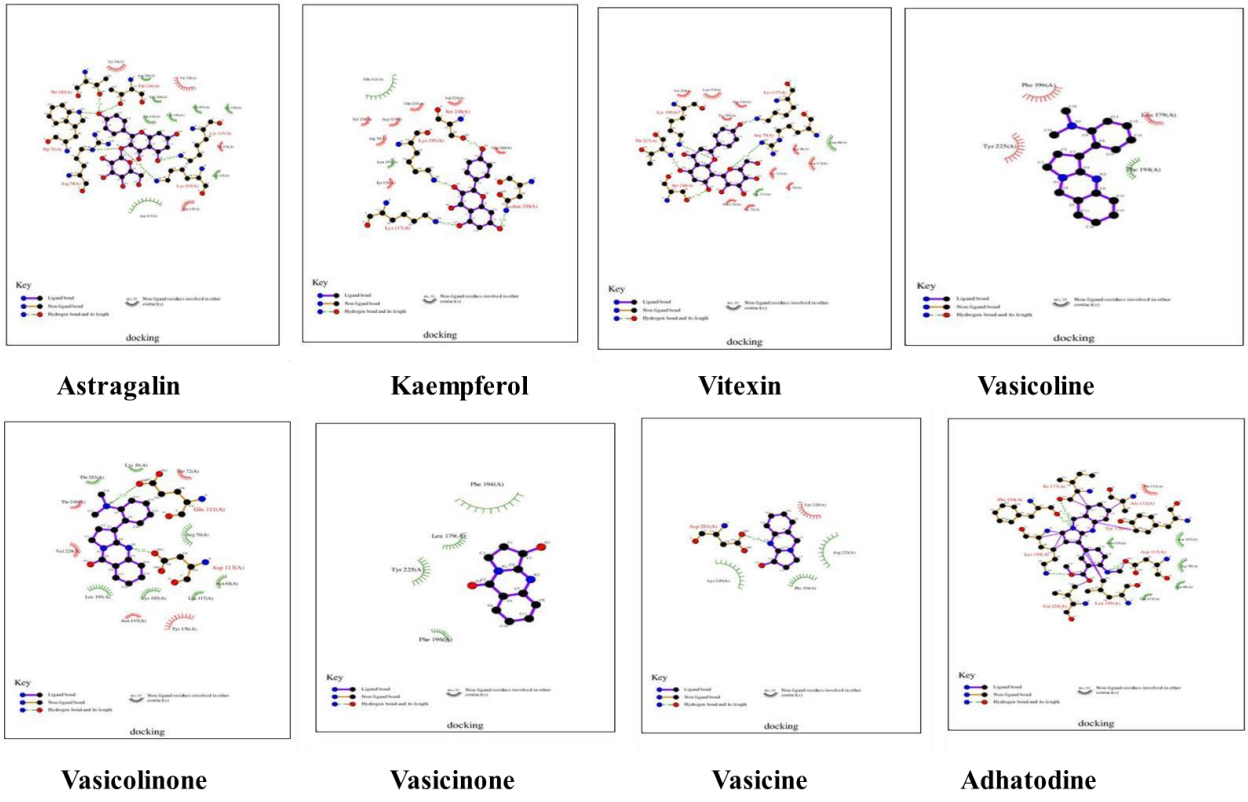

Figure 4. 2D amino acid interaction plot of phytocompounds against Plasminogen activation loop - PDB 4DCB

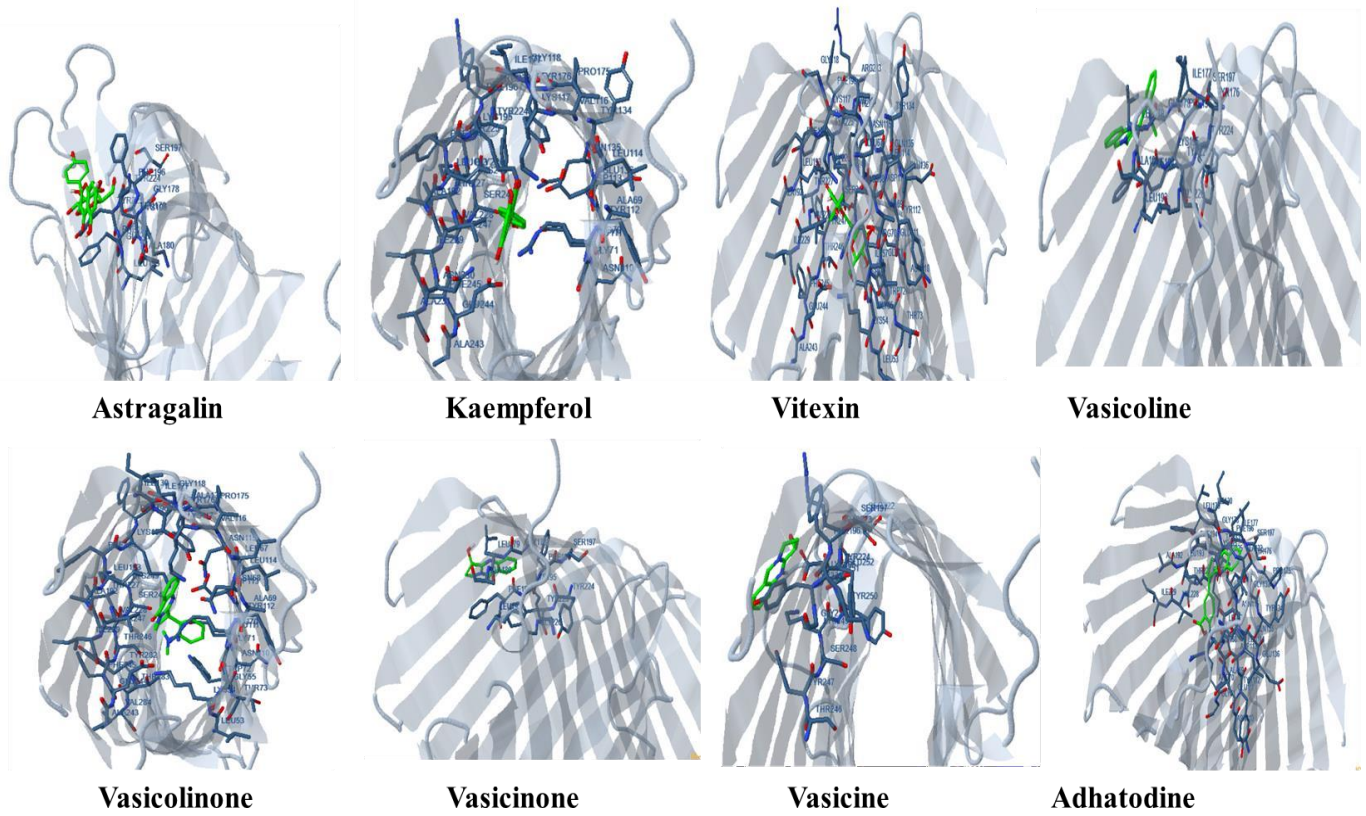

Figure 5. Docking pose of selected phytocompounds against Plasminogen activation loop- PDB 4DCB

Table 1. Physicochemical properties of Lead compounds

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Astragalin	448.4 g/mol	C21H20O11	7	11	4
Kaempferol	286.239 g/mol	C15H10O6	4	6	1
Vitexin	432.4 g/mol	C21H20O10	7	10	3
Vasicoline	291.4 g/mol	C19H21N3	0	2	2
Vasicolinone	305.4g/mol	C19H19N3O	0	3	2
Vasicinone	202.21 g/mol	C11H10N2O2	1	3	0
Vasicine	188.23 g/mol	C11H12N2O	1	2	0
Adhatodine	335.40g/mol	C20H21N3O2	1	4	4

Table 2. Summary of the molecular docking studies of the lead compounds against Plasminogen activation loop - 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
Astragalin	-7.06	6.68	-0.03	-6.10	489.71
Kaempferol	-5.58	81.70	-0.49	-5.93	488.39
Vitexin	-7.36	4.05	-0.01	-5.85	496.20

Vasicoline	-6.51	16.85	-0.06	-6.12	457.55
Vasicolinone	-6.24	26.69	-0.02	-6.87	527.36
Vasicinone	-5.41	108.21	-0.05	-5.71	453.83
Vasicine	-4.87	269.71	-0.12	-5.11	386.58
Adhatodine	-7.40	115.24	-0.06	-6.61	610.24

Table 3. Interaction of lead compounds with active site amino acid residue of Plasminogenactivation loop - PDB 4DCB

Molecule	Interactions	Amino Acid Residue- Binding																	
Astragalin		54	70	72	111	113	115	117	135	176	191	193	195	228	230	244	246	281	283
	1	LYS	ARG	TRP	GLU	ASP	ASN	LYS	GLN	TYR	ASN	LEU	LYS	VAL	ASN	GLU	THR	ASN	THR
Kaempferol		70	111	113	117	135	176	193	195	230	244	248							
	1	ARG	GLU	ASP	LYS	GLN	TYR	LEU	LYS	ASN	GLU	SER							
Vitexin		54	56	68	70	72	111	113	115	117	135	193	195	224	228	244	248		
		LYS	ASP	ASN	ARG	TRP	GLU	ASP	ASN	LYS	GLN	LEU	LYS	TYR	VAL	GLU	SER		
	1													227					
														THR					
Vasicoline		179	194	196	225														
	0	LEU	PHE	PHE	TYR														
Vasicolinone		54	68	70	72	111	113	115	117	176	193	195	228	246	283				
	1	LYS	ASN	ARG	TRP	GLU	ASP	ASN	LYS	TYR	LEU	LYS	VAL	THR	THR				
Vasicinone		179	194	196	225														
	0	LEU	PHE	PHE	TYR														
Vasicine		196	223	225	249	251													
	0	PHE	ARG	TYR	LYS	ASP													
Adhatodine		68	70	111	113	115	131	132	135	176	177	193	194	195	228				
	1	ASN	ARG	GLU	ASP	ASN	THR	ALA	GLN	TYR	ILE	LEU	PHE	LYS	VAL				

4. DISCUSSION

Thrombolytic agents are a class of medications that are frequently prescribed for prevention of recurrent thrombogenic events in patients with the history of stroke and cardiovascular disorders. In the absence of vascular injury, unconditional thrombosis can have life threatening consequences like embolism, ischemia, heart attack, stroke, and so forth [13]. Any obstruction in the form of clot or plaques inside the blood vessels that supply blood to vital organs will end in infarction that in turn affects the physiological processes mediated by the organs. The ultimate pharmacology of thrombolytic drugs is to target the plasminogen activator thereby intended to convert plasminogen to plasmin which is a natural clot dissolver that dissolves the fibrin clot [14]. In order to overcome the undesirable side effects caused by the conventional thrombolytic agents there is dire need of alternative therapeutics.

Herbal therapeutics being safe and effective play a pivotal role in the process of new drug discovery [15]. Bioactive components present in the medicinal herbs are the key source in the development of anti-cancer agents, cardiovascular drugs, anti-microbial drugs, immune modulators etc [16]. Research hypothesises that nearly 30% of pharmaceuticals used globally are made of plant products [17].

Docking type simulation techniques advances the prediction and accuracy of new drug discovery. The process of lead identification and optimisation is further sped up to the next level with the help of Computational biology. Precise algorithm adopted by artificial intelligence actually minimises the chance of occurrence of error which in turn shifts the accuracy curve to the positive node [18].

In general, receptors (enzymes) are macromolecules made of sequential amino acid residues that are capable of mediating biological function. Active residues are involved in binding with either exogenous or endogenous ligands to arbitrate certain functions. Primary objective of the ligand is to competitively bind with these active residues thereby efficiently modulates the native action mediated through the enzyme or protein of interest [19], [20]. Result analysis of the present in-silico investigation signifies that the compounds such as astragalin, kaempferol, vitexin, vasicolinone

and adhatodine bind with active amino acid residue 195 that plays a critical role in the recognition of the residues Arg561-Val562 of the target plasminogen as represented in Table 3 and illustrated in Figure 4 & 5. With this novel action the phytochemicals present in the herb *Justicia adhatoda* may be expected to have wide therapeutic opportunity in the management of thrombosis as a viable thrombolytic alternative.

5. CONCLUSION

Advancement in the field of phytochemistry nourishes the research on finding alternate candidates based on its structural and functional moieties. Continued investigation on viable molecules will deliver newer insights and also accelerate the progress of research stepping ahead towards the identification of principle thrombolytic drugs with overwhelming efficacy and with minimal or no side effects. Based on the results of the computational analysis the bioactive compounds Astragalin, Kaempferol, Vitexin, Vasicolinone and Adhatodine present in *Justicia adhatoda* reveals significant binding against target plasminogen thereby it is concluded that these compounds may exert promising thrombolytic activity.

GEOLOCATION INFORMATION – The study was conducted in Chennai, Tamil Nadu, India

ACKNOWLEDGEMENT - I wish to acknowledge my thanks to Sri Sairam Siddha Medical College & Research Centre, Chennai, Tamil Nadu, India and the Noble research solutions, Chennai, Tamil Nadu, India for their support.

FUNDING- No funds, grants, or other support was received

CONFLICTS OF INTEREST/COMPETING INTERESTS - The author declare that there is no conflict of interest to disclose

AVAILABILITY OF DATA AND MATERIAL- The author confirms that the data supporting the findings of this study are available within the article and in its supplementary materials

6. REFERENCES

[1]. Sanjeev P, Richa S, Anshu P. Overview of the coagulation system. Indian J Anaesth. 2014; 58(5): 515–523.
[2]. Mercy HP, Ahmad SH, Arman ZMS. Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. Int J Hematol Oncol Stem Cell Res. 2017; 11(4): 319–327.
[3]. Andrew B, Longting L, Mark WP. Review of Stroke Thrombolytics. J Stroke. 2013; 15(2): 90–98.
[4]. Thas JJ. Siddha medicine -Background and principles and the application for skin diseases. Clin Dermatol. 2008; 26(1): 62-78.
[5]. Ravi P, Elumalai A, Eswaraiah MC, Kasarla R. A review on Krishna tulsi, Ocimum tenuiflorum Linn. Int J Res Ayurved Pharm. 2012; 3(2): 291-293.
[6]. Hossain MT, Hoq MO. Therapeutic use of Adhatoda vasica . Asian J Med Biol Res. 2016; 2(2): 156-163.
[7]. Kapgate SM, Patil AB. Adhatoda vasica: a critical review. Int J Green Pharm. 2018; 11(04): S654-S662.
[8]. Jha DK, Panda L, Lavanya P, Ramaiah S, Anbarasu A. Detection and confirmation of alkaloids in leaves of Justicia adhatoda and bioinformatics approach to elicit its anti- tuberculosis activity. Appl Biochem Biotechnol. 2012 Nov; 168(5): 980-90.
[9]. Uddin, Mir & Mahmuduzzaman, Mohammad & Islam, Md & Parvin, Salma & Shahriar, Mohammad. (2013). PHYTOCHEMICAL SCREENINGS AND THROMBOLYTIC ACTIVITY OF THE LEAF EXTRACTS OF ADHATODA VASICA. The Experiment. 7. 438-441.
[10]. Stefano, F; Ruth, H.et al. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016, 11, 905–919.
[11]. Osterberg, F; Morris, G.M; Sanner, M.Fetal. Automated docking to multiple target structures: incorporation of protein mobility and structural water heterogeneity in AutoDock. Proteins. 2002, 46, 34-40.
[12]. Morris GM. Automated docking using a Lamarckian genetic algorithm and an empirical binding
[13]. Richard PW, Jack CS, Stephen EF. Antithrombotic and Thrombolytic Therapy for Valvular Disease. Chest journal. 2012;141:576-600
[14]. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. Blood Rev. 2015 Jan; 29(1):17-24.
[15]. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 2019 Nov 1;

9(11):258.
[16]. Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: An integrated approach. Ayu. 2012; 33(1):10-19.
[17]. Anwar AK, Ashfaq M, Nasveen MA. Pharmacognostic studies of selected indigenous plants of Pakistan. Pakistan: Pakistan Forest Institute, Peshawar NWFP; 1979:15–35.
[18]. Sliwoski G, Kothiwale S, Meiler J, Lowe EW Jr. Computational methods in drug discovery. Pharmacol Rev. 2013; 66(1):334-395.
[19]. Fukunishi Y, Nakamura H. Prediction of ligand-binding sites of proteins by molecular docking calculation for a random ligand library. Protein Sci. 2011; 20(1):95-106.
[20]. Kaistha SD, Sinha R. Homology modeling of phosphoryl thymidine kinase of enterohemorrhagic Escherichia coli OH: 157. Bioinformation. 2009; 3(6):240-243.

DOI: https://doi.org/10.15379/ijmst.v10i4.2414