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IN SILICO PASS PREDICTION AND MOLECULAR DOCKING OF ISOLATED COMPOUNDS FROM *FLEMINGIA MACROPHYLLA* FOR THROMBOLYTIC EFFECT.

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ABSTRACT

This study aims to predict whether the isolated compounds from *Flemingia macrophylla* have thrombolytic effects, which were done by using two *in silico* tools PASS prediction and Molecular docking. Nine phytoconstituents namely beta-sitosterol, cajanin, flemichin E, flemiflavanone A, fleminone, genistein, genistin, kushenol E, neoraufurane, prunetin and stigmasterol were analyzed by the PASS prediction for their thrombolytic activity and found wide range of activity. Flemichin E was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.466) than Pi value (0.004). As a result, flemichin E had 116.5 ratio (Pa : Pi) value. A wide range of docking score found during molecular docking by CPI server. Beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin showed the docking score -7.9, -8.4, -8.8, -8.6, -8.0, -8.6, -8.7, -8.3 and -7.9, respectively. Data from the both *in silico* models showed similar values for the same compound, because flemichin E showed high value and beta-sitosterol, prunetin showed low value in both *in silico* models. All the data supported that flemichin E is the best compound for thrombosis management, as it possessed higher value both in PASS prediction and Molecular docking. After flemichin E, genistin showed well docking score (-8.6) and good prediction for thrombolytic effect in PASS prediction. Further *in vitro* and *in vivo* investigation need to identify whether flemichin E, genistin and other compounds have thrombolytic effect or not.

KEY WORDS: *Flemingia macrophylla*, PASS prediction, Molecular docking.

1. INTRODUCTION

Thrombosis is the fundamental pathophysiological process that brings about the acute coronary disorders such as deep vein thrombosis, strokes and heart attacks and they are the main causes of huge death in developed countries (Dewan and Das, 2013). This disease is characterized by the development of a blood clot (thrombus) in the circulatory system of the body due to the failure of homeostasis which leads to vascular blockage and while recovering causes fatal consequences, such as myocardial infarction, as well as death (Prasad *et al.*, 2006). Therefore, anticoagulation therapy is the basis of management, and the proper choice of thrombolytic drugs (Khan *et al.*, 2011). And searching of novel thrombolytic agents from plant source is a good practice. Because, many established drugs discovered from plant source.

Prediction of activity spectra for substances (PASS) is hosted by the V. N. Orechovich Institute of Biomedical Chemistry under the aegis of the Russian Foundation of Basic Research. The web based application predicts the biological activity spectrum of a compound based on its structure. It works on the principle that the biological activity of a compound equates to its structure. PASS prediction tools are constructed using 20000 principal compounds from MDDR database (produced by Accelrys and Prous Science). The database contains over 180000 biologically relevant compounds and is constantly updated (Hasanat *et al*, 2015).

Molecular docking has become an increasingly important tool for drug discovery. The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes (McConkey *et al.*, 2002). The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as pose) and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes, respectively, which will be discussed in the theory section (Meng *et al.*, 2011).

Flemingia macrophylla belonging to the family Fabaceae is a woody leguminous shrub. It is a native plant of sub-humid to humid region, thus it is naturally found in Asia, including

Bhutan, southern China, Cambodia, India, Indonesia, Laos, Myanmar, Malaysia, Nepal, northern Pakistan, Philippines, Sri Lanka, Thailand Vietnam and Chittagong district of Bangladesh. It has been cultivated and naturalized in Sub-Saharan Africa, Central and South America, and tropical Australia (Orwa *et al*, 2009). It is a multipurpose plant widely used in agriculture, crop improvement, fodder, dyes and for various therapeutic purposes. Locally, this plant is known as charchara (Bengali) (http:www.mpbd.info/plants/flemingia-macrophylla/); apa apa, hahapaan, pok kepokan (Indonesia); serengan jantan, beringan (Malaysia); cai duoi chon (Vietnam) (http://www.tropicalforages.info/key/Forages/Media/Html/Flemingia_macrophylla/). Leaves of *F. macrophylla* have very good thrombolytic activity (Jainul *et al.*, 2013). Many phyto constituents isolated from *F. macrophylla* (Lai *et al.*, 2013) and nine of them are beta-sitosterol, cajanin, flemichin E, flemiflavanone A, fleminone, genistein, genistin, kushenol E, neoraufurane, prunetin and stigmasterol.

The aim of the present study to predict whether the isolated compounds from *F. macrophylla* had thrombolytic effect, which was done by using two *in silico* tools PASS prediction and Molecular docking.

2. MATERIALS AND METHODS

2.1 Draw of the structures

The chemical structures of the beta-sitosterol, cajanin, flemichin E, flemiflavanone A, fleminone, genistein, genistin, kushenol E, neoraufurane, prunetin and stigmasterol were obtained from Pubchem compound repository (http://www.ncbi.nlm.nih.gov/pccompound). The structures were drawn using the Chem sketch package 11.0 belonging to the ACD Chem. Laboratory.

2.2 *In silico* Prediction of activity spectra for substances (PASS)

Prediction of phytoconstituents namely beta-sitosterol, cajanin, flemichin E, flemiflavanone A, fleminone, genistein, genistin, kushenol E, neoraufurane, prunetin and stigmasterol isolated from *Flemingia macrophylla* (Jainul *et al.*, 2013) for thrombolytic activity was done with the help of computer program, PASS (Prediction of activity spectra for substances). The software estimates predicted activity spectrum of a compound as probable activity (P_a) and probable inactivity (P_i). The prediction of activity is based on structure-activity relationship analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities. The values of P_a and P_i vary between 0.000 and 1.000.

Only activities with $P_a > P_i$ are considered as possible for a particular compound. If $P_a > 0.7$, the probability of experimental pharmacological action is high and if $0.5 < P_a < 0.7$, probability of experimental pharmacological action is less. If the value of $P_a < 0.5$, the chance of finding the activity experimentally is less, but it may indicate a chance of finding a new compound (Goel *et al.*, 2011; Khurana *et al.*, 2011; Tiwari *et al.*, 2011; Hasanat *et al*, 2015).

2.3 *In silico* Molecular docking

All the phytochemical structures of *F. macrophylla* need to upload in mol2 format with charges and hydrogens added. When a molecule submitted, The CPI server checks the format suitability and calculates the interaction profile of this drug towards all the targets in the database using DOCK6 (Ewing *et al.*, 2001; Luo *et al.*, 2011). Users can view the real-time progress online, and the page showing the current docking status of the uploaded drug will also be provided for bookmarking. It takes between 6 and 20 h to finish a one-molecule task and an email will be sent on completion. The outputs comprise the two following major elements:

(i) Library drugs which share similar (or opposite) interaction profile with the user's molecule, ranked by the similarity (or disparity) with known indications and ADR information, suggesting the underlying new indication and ADR of the user's molecule.

(ii) The candidate off-targets that tend to interact with the user's molecule. The server will visualize the drug-protein interactions, with amino acid residues around $6A^o$ of the molecule highlighted.

3. RESULTS

3.1 *In silico* PASS prediction

Nine phytoconstituents namely beta-sitosterol, cajanin, flemichin E, flemiflavanone A, fleminone, genistein, genistin, kushenol E, neoraufurane, prunetin and stigmasterol were analyzed by the PASS for their thrombolytic activity and results were used in a flexible manner. All the compounds showed greater Pa than Pi (Table 1). Genistein showed highest Pa for thrombolytic activity (Pa=0.666). But when ratio (Pa : Pi) value calculated, then it was clear that flemichin E was best for thrombolytic effect, though it had much bigger Pa value (0.466) than Pi value (0.004). As a result, flemichin E had 116.5 ratio (Pa : Pi) value. Pa and Pi values for different activities of examined phytoconstituents are presented in Figure 1 and ratio (Pa : Pi) value showed in Figure 2.

Table 1: PASS predictions of beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin for thrombolytic activity.

Phyto compounds	PASS predictions for thrombolytic activity		Ratio (Pa : Pi) value
	Pa	Pi	
beta-sitosterol	0.268	0.228	1.175
cajanin	0.152	0.146	1.041
flemichin E	0.466	0.004	116.500
flemiflavanone A	0.399	0.054	7.389
genistein	0.666	0.037	18.000
genistin	0.528	0.025	21.120
kushenol E	0.422	0.045	9.378
neoraufurane	0.229	0.041	5.5854
prunetin	0.168	0.120	1.400

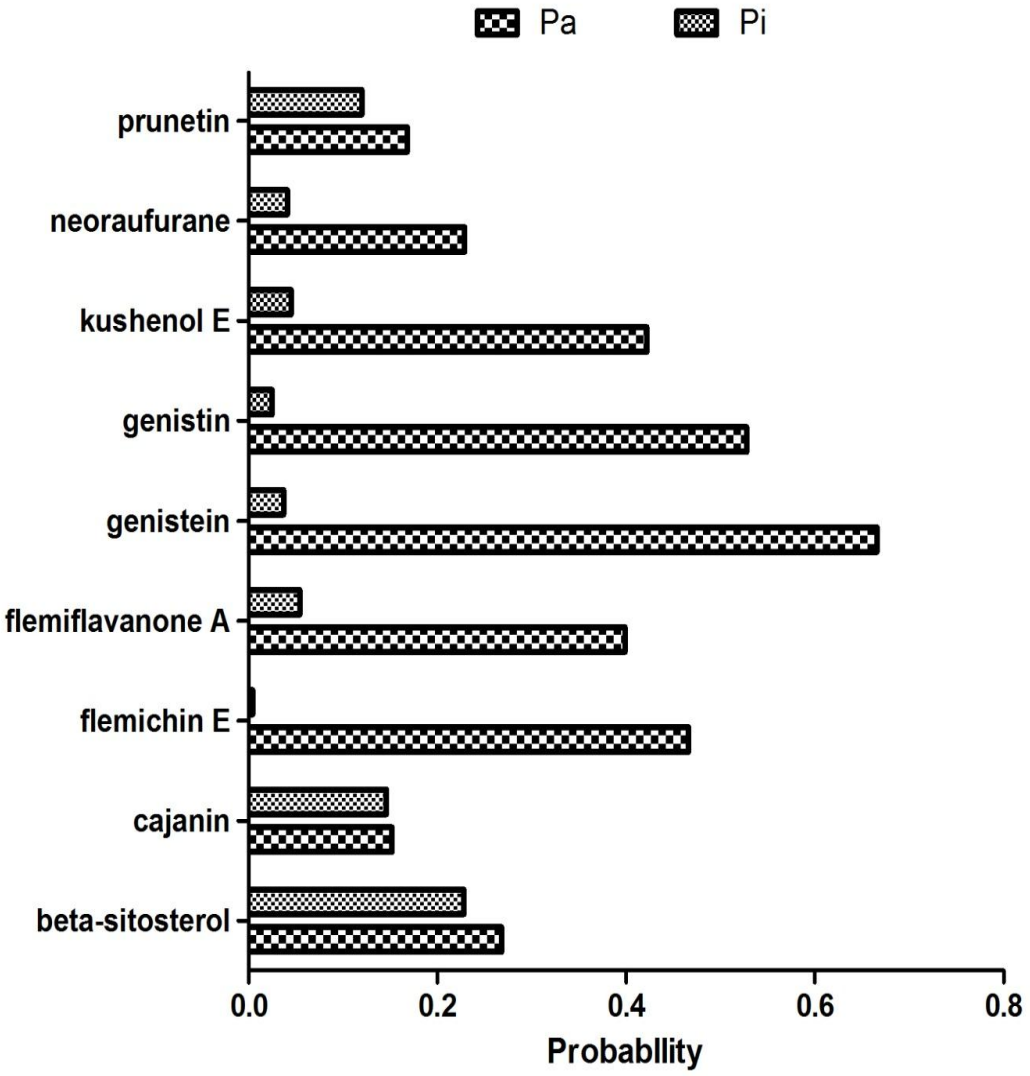

Figure 1: PASS predictions of beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin for thrombolytic activity.

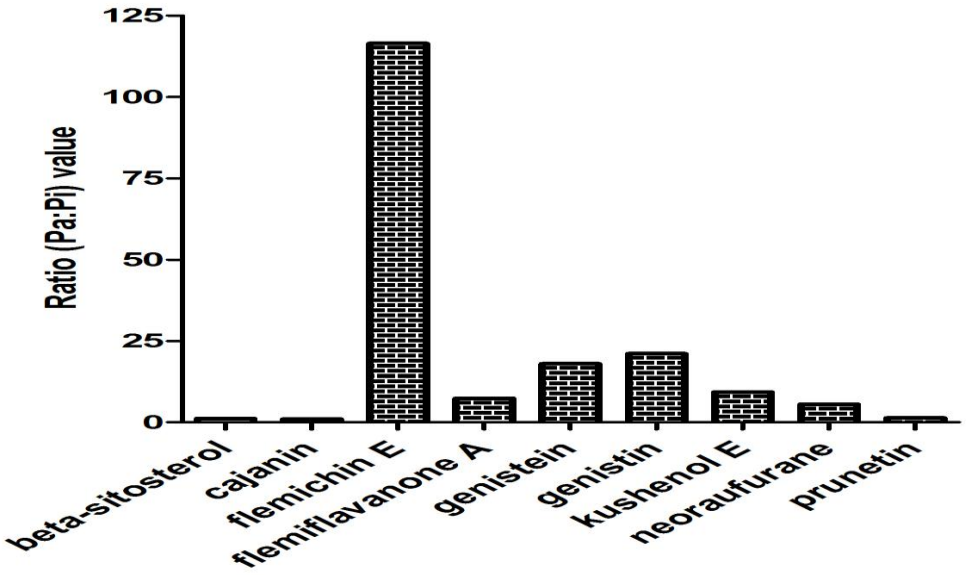

Figure 2: Ratio (Pa : Pi) values from PASS predictions of beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin for thrombolytic activity.

3.2 *In silico* Molecular docking

In the present study, molecular docking performed to identify the docking score of beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin towards tissue-type plasminogen activator, which is a protein involved in the breakdown of blood clots. A wide range of docking score found during molecular docking by CPI server. Beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin showed the docking score -7.9, -8.4, -8.8, -8.6, -8.0, -8.6, -8.7, -8.3 and -7.9, respectively. All the results presented in Table 2 and Figure 3.

Table 2: Docking results with interacting phyto compounds in the tissue-type plasminogen activator.

Compound name	Docking Score
beta-sitosterol	-7.9
cajanin	-8.4
flemichin E	-8.8
flemiflavanone A	-8.6
genistein	-8.0
genistin	-8.6
kushenol E	-8.7
neoraufurane	-8.3
prunetin	-7.9

Figure 3: Molecular docking analysis of beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin with Tissue-type plasminogen activator complex obtained from docking.

4. DISCUSSIONS

Thrombosis or blood clot formation is a critical event in which the damaged regions of the endothelial cell surface or blood vessel are blocked by the deposition of platelets, tissue factor and fibrin (Furie and Furie, 2008). In the formation process the major role is played by

platelets as the process of thrombosis is initiated when the activated platelets form platelets to platelets bonds. These activated platelets further bind to the leucocytes and bring them into a complex process of plaque formation and growth (Pantzar *et al.*, 1998). It is the thrombolytic agents that lyse clot by disrupting the fibrinogen and fibrin contained in a clot. Though *F. macrophylla* has well thrombolytic effect, we need to identify, which compound or compounds responsible for this activity. It is very costly to examine thrombolytic effect of all isolated compounds and that's why we used here *in silico* methods to predict the thrombolytic effect of phyto compounds of *F. macrophylla*.

In order to accelerate the research for potent natural products, computer-aided drug discovery program PASS was used to predict the biological activity. PASS prediction tools were constructed using 20000 principal compounds (Lagunin *et al.*, 2003) and about 4000 kinds of biological activity on the basis of structural formula with mean accuracy about 90% (NCI Database Compounds. Plant Resources 1998). The result of prediction is presented as the list of activities with appropriate Pa and Pi ratio. Nine phytoconstituents namely beta-sitosterol, cajanin, flemichin E, flemiflavanone A, fleminone, genistein, genistin, kushenol E, neoraufurane, prunetin and stigmasterol were analyzed by the PASS prediction for their thrombolytic activity and found wide range of activity. Flemichin E was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.466) than Pi value (0.004). As a result, flemichin E had 116.5 ratio (Pa : Pi) value.

In molecular docking study, beta-sitosterol, cajanin, flemichin E, flemiflavanone A, genistein, genistin, kushenol E, neoraufurane and prunetin showed the docking score -7.9, -8.4, -8.8, -8.6, -8.0, -8.6, -8.7, -8.3 and -7.9, respectively towards tissue-type plasminogen activator. From all these phyto compounds, flemichin E exhibited best docking score (-8.8), which also possessed maximum thrombolytic effect prediction from PASS prediction. After flemichin E, genistin showed well docking score (-8.6) and good prediction for thrombolytic effect in PASS prediction.

From phyto compounds of *F. macrophylla*, flemichin E and genistin may be competitive candidate for a promising thrombolytic agent.

5. CONCLUSIONS

From the study it was found that, both *in silico* models showed similar values for the same compound, because flemichin E showed high value and beta-sitosterol, prunetin showed low

value in both *in silico* models. All the data support that flemichin E is the best compounds for thrombosis management, as it possessed higher value both in PASS prediction and Molecular docking. So, flemichin E and genistin may be competitive candidate for a promising thrombolytic agent. Further *in vitro* and *in vivo* investigation need to identify whether flemichin E and other compounds have thrombolytic effect or not.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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