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COMPETITIVE MEASURES OF SOME PHYTOCONSTITUENTS FOR THROMBOLYTIC EFFECT: AN *IN SILICO* APPROACH

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ABSTRACT

This study aims to predict whether Podocarpusflavone A (isolated from *Podocarpus imbricatus*), Podocarpusflavone B (isolated from *Psilotum nudum*), Robustaflavone (isolated from *Selaginella sellowii*), Robustaflavone-7-methyl ether (isolated from *Podocarpus imbricatus*), Sciadopitysin (isolated from *Taxus cuspidata*) and β-sitosterol (isolated from *Rubus suavissimus*) have thrombolytic effects, which were done by using two *in silico* tools PASS prediction and Molecular docking. These six were analyzed by the PASS prediction for their thrombolytic activity and found wide range of activity. Podocarpusflavone A and Podocarpusflavone B was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.256) than Pi value (0.022). As a result, Podocarpusflavone A and Podocarpusflavone B both had 11.64 ratio (Pa : Pi) value. A wide range of docking score found during molecular docking by CPI server. Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol showed the docking score -9.5, -9.5, -9.2, -9.6, -9.9 and -7.9, respectively. Data from the both *in silico* models showed similar value for the same compound, because Podocarpusflavone A and B showed high value and β-sitosterol showed low value in both *in silico* models. All the dates showed that Podocarpusflavone A and B are the best compounds for thrombosis

management, as they possessed higher value both in PASS prediction and Molecular docking. Sciadopitysin also showed well docking score (-9.9) and probability of activity (0.243) for thrombolytic activity in PASS prediction. Further *in vitro* and *in vivo* investigation need to identify whether Podocarpusflavone compounds, Sciadopitysin and other compounds have thrombolytic effect or not and from them, which is best one for thrombosis treatment.

KEYWORDS: Isolated compound, PASS prediction, Molecular docking, Podocarpusflavone, Sciadopitysin.

1 INTRODUCTION

Thrombus developed in the circulatory system due to failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death (Lee, 1995). Thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinase, streptokinase etc. are used all over the world for the treatment of these diseases. In Asian countries, though Urokinase and streptokinase are widely used due to lower cost, (Mucklow; Collen, 1990) as compared to other thrombolytic drugs, their use is associated with hyper risk of hemorrhage (Rouf *et al.*, 1996) severe anaphylactic reaction and lacks specificity. Moreover, as a result of immunogenicity multiple treatments with SK in a given patient are restricted (Jennings). Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs (Nicolini *et al.*, 1992).

Herbal products are often perceived as safe because they are "natural" (Gesler, 1992). In Asian country, like china, Korea, India, Bangladesh, in recent years, there is increased research on traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. But for developing a new active pharmaceutical ingredient (API) from plant source is very costly. Because, huge investment need for drug development from plant, though many steps should follow in a scientific manner. So, we can do it by shorting the scientific investigation by using Bioinformatics tools. Since bioinformatics tools have long history to predict activity and mechanism for a biological effect, where PASS prediction and Molecular docking has well establishment and acceptance.

PASS (Prediction of Activity Spectra for Substances) predicts simultaneously several hundreds of biological activities. The biological activity spectrum of a compound presents all

compound's actions despite the difference in essential conditions of its experimental determination. Thus, "the biological activity spectrum" is defined as the "intrinsic" property of a compound depending only on its structure and physico-chemical characteristics (Poroikov and Filimonov, 2001). Prediction of this spectrum by PASS is based on SAR analysis of the training set containing more than 35,000 compounds which have more than 500 kinds of biological activity (http://www.ibmh.msk.su/PASS).

Molecular docking is a computational tool that predicts the binding site location and conformation of a compound when bound to a protein (Kitchen *et al.*, 2004; Warren *et al.*, 2006). This computational approach that 'dock' small molecules into the structures of macromolecular targets and 'score' their potential complementarily to binding sites are widely used in hit identification and lead optimization. Indeed, there are now a number of drugs whose development was heavily influenced by or based on structure-based design and screening strategies. Nevertheless, there remain significant challenges in the application of these approaches, in particular in relation to current scoring schemes. Here, we review key concepts and specific features of small-molecule–protein docking methods, highlight selected applications and discuss recent advances that aim to address the acknowledged limitations of established approaches (Kitchen *et al.*, 2004).

The aim of the present study to predict whether Podocarpusflavone A (isolated from *Podocarpus imbricatus*) (Gu *et al.*, 1995), Podocarpusflavone B (isolated from *Psilotum nudum*)(Markham, 1984), Robustaflavone (isolated from *Selaginella sellowii*)(Rizk *et al.*, 2014), Robustaflavone-7-methyl ether (isolated from *Podocarpus imbricatus*) (Gu *et al.*, 1995), Sciadopitysin (isolated from *Taxus cuspidata*) (Choi *et al.*, 2006) and β-sitosterol (isolated from *Rubus suavissimus*) (Chaturvedula and Prakash, 2012) have thrombolytic effects, which were 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 Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol 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 phytoconstitutes namely Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol isolated from *Podocarpus neriifolius* (Xu *et al.*, 1993) for thrombolytic activity was done with the help of computer program, PASS (Prediction of activity spectra for substances). 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; Abul Hasanat, 2015).

2.3 *In silico* Molecular docking

All the phytochemicals structure of *Podocarpus neriifolius*, 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

Six phytoconstitutes namely Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol 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). Podocarpusflavone A and Podocarpusflavone B, both showed highest ratio (Pa : Pi) value (Pa : Pi = 11.64) for thrombolytic activity. Pa and Pi values for different activities of examined phytoconstitutes are presented in Figure 1 and Figure 2.

Table 1: PASS predictions of Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol for thrombolytic activity.

Phyto compounds	PASS predictions for thrombolytic activity		Ratio (Pa : Pi) value
	Pa	Pi	
Podocarpusflavone A	0.256	0.022	11.64
Podocarpusflavone B	0.256	0.022	11.64
Robustaflavone	0.185	0.094	1.97
Robustaflavone-7-methyl ether	0.182	0.100	1.82
Sciadopitysin	0.243	0.030	8.10
β-sitosterol	0.268	0.228	1.18

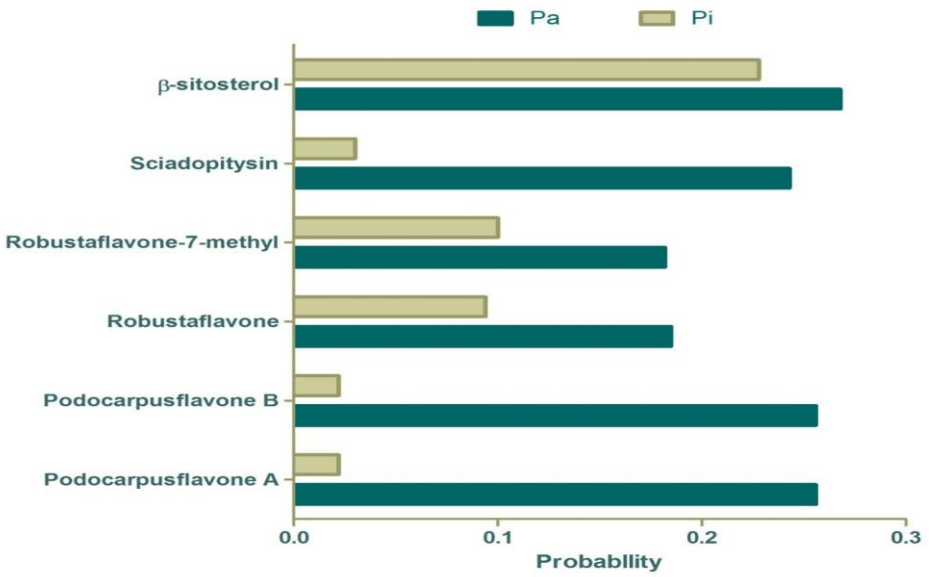

Figure 1: PASS predictions of Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol for thrombolytic activity.

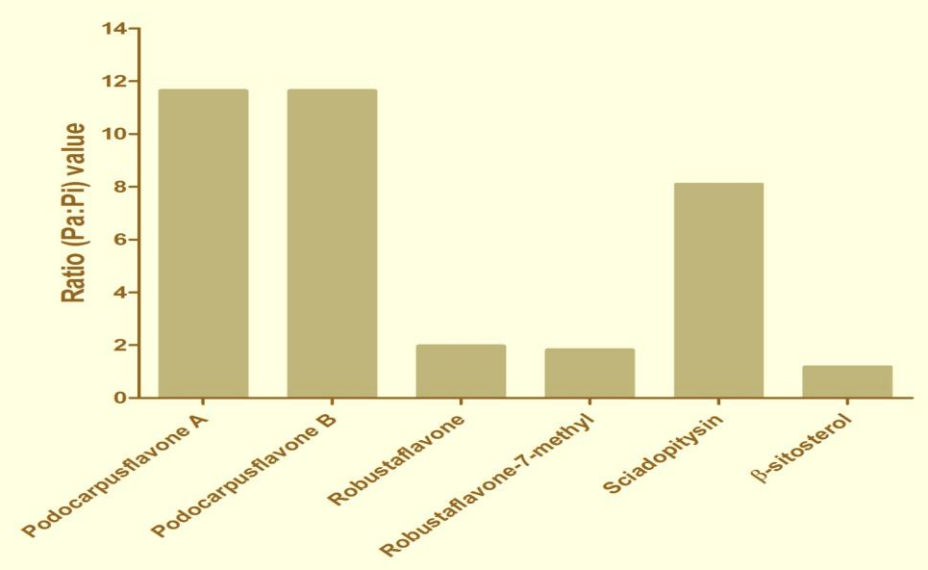

Figure 2: Ratio (Pa : Pi) value from PASS predictions of Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol for thrombolytic activity.

3.2 *In silico* Molecular docking

In the present study, molecular docking performed to identify the docking score of Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol towards tissue-type plasminogen activator (PDB code 1A5H), which is a protein involved in the breakdown of blood clots. A wide range of docking score found during molecular docking by CPI server. Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol showed the docking score -9.5, -9.5, -9.2, -9.6, -9.9 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
Podocarpusflavone A	-9.5
Podocarpusflavone B	-9.5
Robustaflavone	-9.2
Robustaflavone-7-methyl ether	-9.6
Sciadopitysin	-9.9
β-sitosterol	-7.9

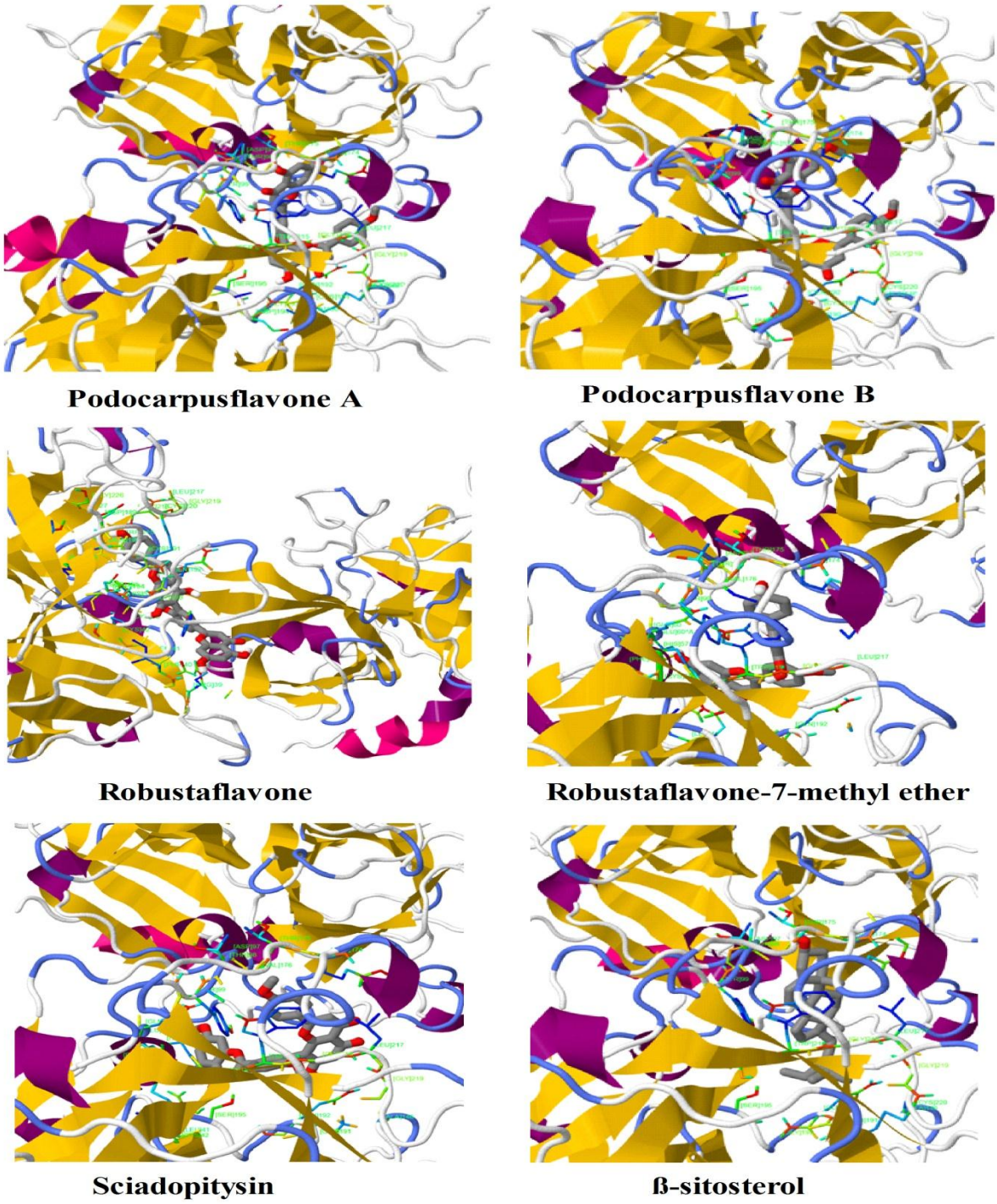

Figure 3: Molecular docking analysis of Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol with Tissue-type plasminogen activator complex obtained from docking.

4. DISCUSSIONS

Herbal preparations are used since ancient times to maintain health and regain healthy state of mind. Advances in phytochemistry and identification of plant compounds, which are

effective in curing certain diseases have renewed the interest in herbal medicines. About 30% of the pharmaceuticals are prepared from plants worldwide (Khan *et al.*, 1979). A number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke (Gillman *et al.*, 1995). There are several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology (Verstraete, 2000) in order to make these thrombolytic drugs more site specific and effective. Side effects related to these drugs have been reported that lead to further complications (Baruah *et al.*, 2006). Sometimes the patients die due to bleeding and embolism (Capstick and Henry, 2005). But, if a potent thrombolytic drug discover from plant resources with less side effect would be great discovery. Here, we used bioinformatics tools for predicting whether the examined phytoconstituents had any thrombolytic effect and how much.

Six phytoconstitutes namely Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol were analyzed by the PASS prediction for their thrombolytic activity and found wide range of activity. Podocarpusflavone A and Podocarpusflavone B was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.256) than Pi value (0.022). As a result, flemichin E had 116.5 ratio (Pa : Pi) value. Then, the choice is Sciadopitysin, because Sciadopitysin also showed well probability of activity (0.243) for thrombolytic activity in PASS prediction.

In molecular docking study, Podocarpusflavone A, Podocarpusflavone B, Robustaflavone, Robustaflavone-7-methyl ether, Sciadopitysin and β-sitosterol showed the docking score -9.5, -9.5, -9.2, -9.6, -9.9 and -7.9, respectively towards tissue-type plasminogen activator. From all these phyto compounds, Sciadopitysin exhibited best docking score (-9.9), which also possessed third maximum thrombolytic effect prediction from PASS prediction.

5. CONCLUSIONS

From the study it was found that, both *in silico* models showed similar value for the same compound, because Podocarpusflavone compounds showed high value and beta-sitosterol showed low value in both *in silico* models. All the data support that Podocarpusflavone compounds are the best compounds for thrombosis management, as it possessed higher value both in PASS prediction and Molecular docking. Sciadopitysin also showed well docking

score (-9.9) and probability of activity (0.243) for thrombolytic activity in PASS prediction. So, Podocarpusflavone compounds and Sciadopitysin may be competitive candidate for promising thrombolytic agent. Further *in vitro* and *in vivo* investigation need to identify whether Podocarpusflavone compounds, Sciadopitysin 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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