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IN SILICO PASS PREDICTION AND MOLECULAR DOCKING OF ISOLATED COMPOUNDS OF *ANDROGRAPHIS PANICULATA* FOR THROMBOLYTIC EFFECT

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ABSTRACT

This study aims to predict whether the isolated compounds from *Andrographis paniculata* have thrombolytic effects, which were done by using two in silico tools PASS prediction and Molecular docking. Six *phytoconstituents* namely 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide were analyzed by the PASS prediction for their thrombolytic activity and found wide range of activity. Neoandrographolide was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.557) than Pi value (0.021). As a result, neoandrographolide had 26.524 ratio (Pa: Pi) value. A wide range of docking score found during molecular docking by CPI server. 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14 deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide showed the docking score -7.2, -7.0, -7.0, -7.2, -7.4 and -9.0, respectively. Data from the both in silico models showed similar value for the same compound, because neoandrographolide showed high value and andrograpanin showed low value in both in silico models. All the data supported that neoandrographolide is the best compounds for thrombosis management, as it possessed higher value both in PASS prediction and Molecular docking. After neoandrographolide, isoandrographolide showed well docking score (-7.4) and good prediction for thrombolytic effect in PASS prediction.

Further in vitro and in vivo investigation need to identify whether neoandrographolide, isoandrographolide and other compounds have thrombolytic effect or not.

KEYWORD: deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide.

1. INTRODUCTION

Acute ischaemic stroke is a major cause of death and disability worldwide. Most strokes are due to blockage of an artery in the brain by a blood clot (ischaemic stroke) e.g. from the heart or neck arteries. Thromboembolic diseases are serious and life threatening. Despite the availability of antithrombotic drugs for the prevention and treatment of arterial and venous thrombosis, thrombotic diseases continue to be a major cause of death and disability worldwide (Kabir et al., 2015; Hasan et al., 2016; Uddin et al., 2016). Therefore, there remains a need for more effective therapies to combat these disorders (Gross and Weitz, 2008; Kader et al., 2016). Thrombolytic drugs derive from naturally-occurring enzymes that dissolve thrombus as part of the natural clotting cascade. Some are extracted from biological samples (e.g. urokinase, desmoteplase) and others are manufactured (e.g. recombinant tissue plasminogen activator (rt-PA), or recombinant pro-urokinase) (Wardlaw et al., 2014; Chowdhury et al., 2015; Tarek et al., 2015). And searching of novel thrombolytic agent 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 webbased 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 (Abul Hasanat, 2015; Kabir et al., 2016).

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; Islam et al., 2015). 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 (Dash et al., 2015; Dash et al., 2015). These two steps are related to sampling methods and scoring schemes, respectively, which will be discussed in the theory section (Meng et al., 2011).

Andrographis paniculata (Burm. f.) Nees (Acanthaceae) (*A. paniculata*, Chuanxinlian), native to Taiwan, Mainland China and India, is a medicinal herb with an extremely bitter taste used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection (Kligler et al., 2006; Negi et al., 2008). The aerial part of *A. paniculata* is commonly used in Chinese medicine. According to Chinese medicine theory, *A. paniculata* 'cools' and relieves internal heat, inflammation and pain and is used for detoxication (Mandal et al., 2001; Huang and Wu, 2002). Many phyto constituents isolated from *A. paniculata* (Chao and Lin, 2010) and six of them are 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide.

The aim of the present study to predict whether the isolated compounds from *A. paniculata* 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 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide 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 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide isolated from *A. paniculata* (Chao and Lin, 2010) 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 *A. paniculata*, 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; Al Noman et al., 2015; Kabir et al., 2016). 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 *phytoconstituents* namely 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide 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). Neoandrographolide was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.557) than Pi value (0.021). As a result, neoandrographolide had 26.524

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 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide for thrombolytic activity.

Phyto compounds	PASS predictions for thrombolytic activity		Ratio (Pa : Pi) value
	Pa	Pi	
5-hydroxy-7,8-dimethoxyflavone	0.208	0.063	3.302
14-acetylandrographolide	0.425	0.044	9.659
14-deoxyandrographolide	0.395	0.055	7.182
andrograpanin	0.217	0.184	1.179
isoandrographolide	0.548	0.022	24.909
neoandrographolide	0.557	0.021	26.524

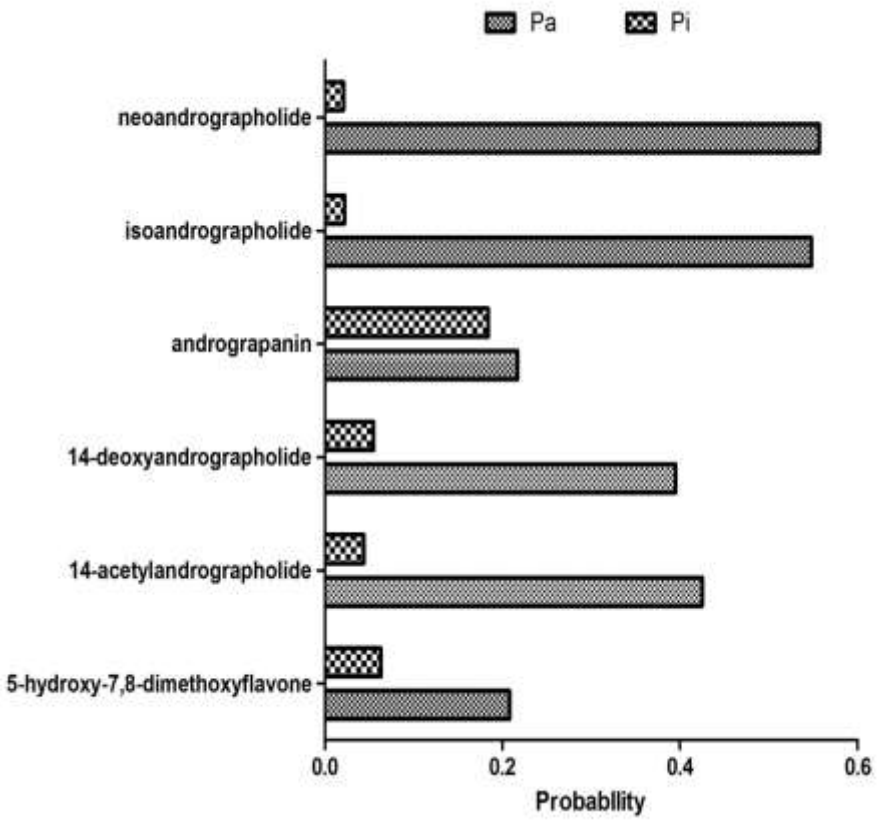

Figure 1: PASS predictions of 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide for thrombolytic activity.

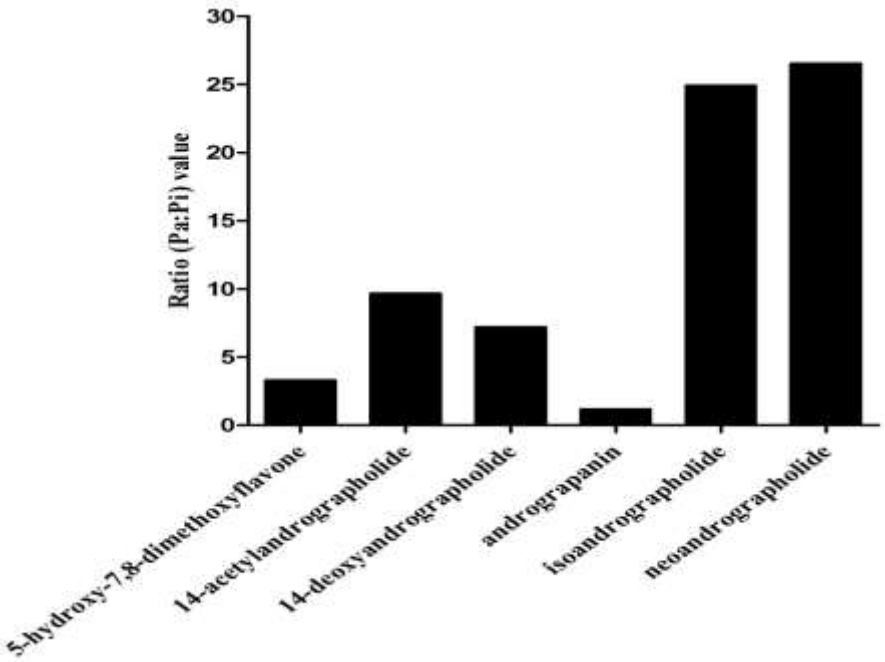

Figure 2: Ratio (Pa: Pi) value from PASS predictions of 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide for thrombolytic activity.

3.2 In silico Molecular docking

In the present study, molecular docking performed to identify the docking score of 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide 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. 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide showed the docking score -7.2, -7.0, -7.0, -7.2, -7.4 and -9.0, 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
5-hydroxy-7,8-dimethoxyflavone	-7.2
14-acetylandrographolide	-7.0
14-deoxyandrographolide	-7.0
andrograpanin	-7.2
isoandrographolide	-7.4
neoandrographolide	-9.0

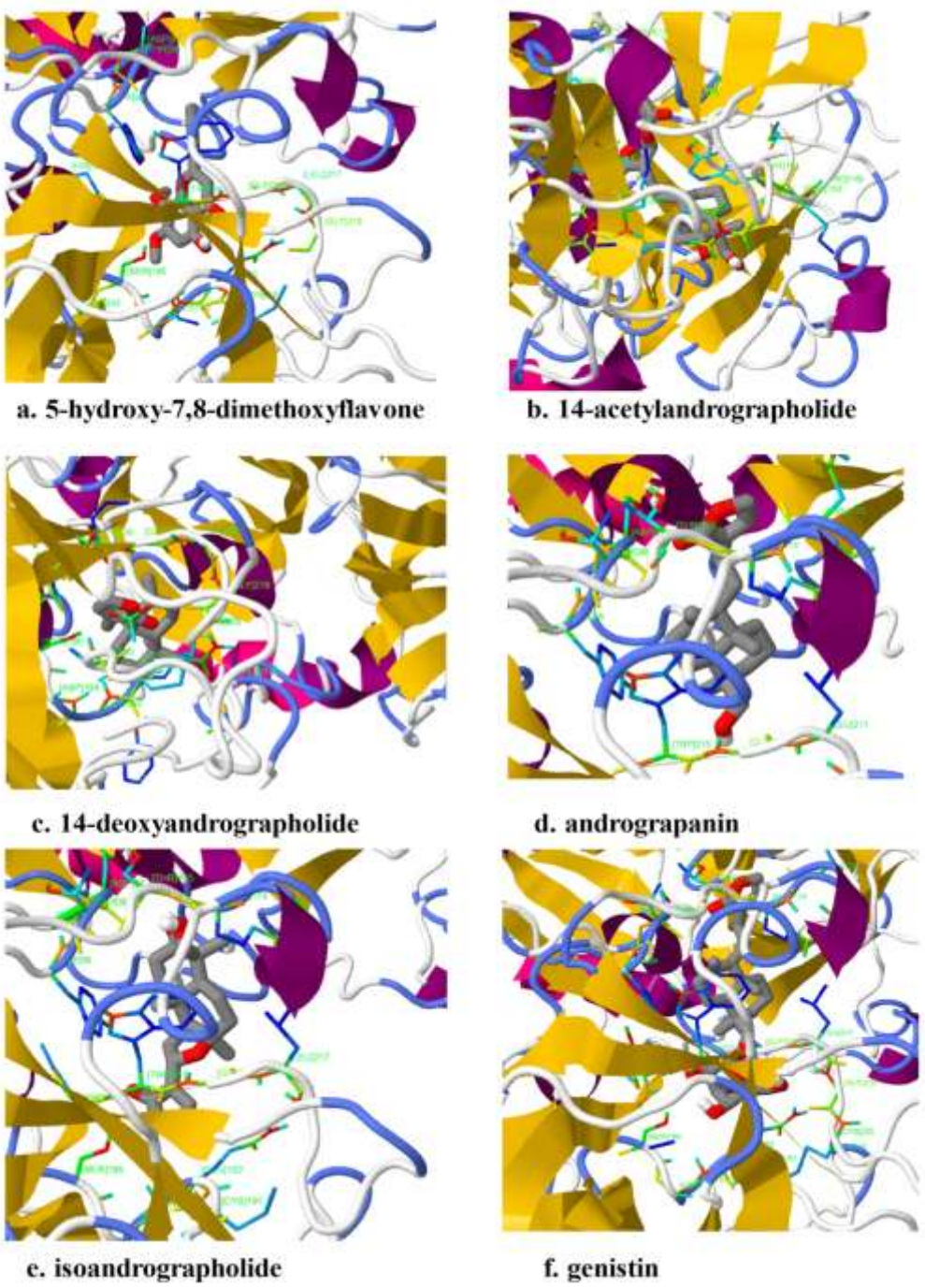

Figure 3: Molecular docking analysis of 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide with Tissue-type plasminogen activator complex obtained from docking.

4. DISCUSSIONS

The use of plants with medicinal purposes for the prevention and/or treatment of diseases is one of the most ancient forms of primary health care (Calixto, 2000). Plants produce several

secondary metabolites that present many important biological activities. So, finding a new thrombolytic agent with better effect and safety from plant source will be a great discovery. For, this purpose we used bioinformatics tools.

Several antithrombotic agents are available for the control and treatment of thrombotic diseases, but many are expensive and possess toxic effects. It is therefore necessary to search for safe, inexpensive, naturally occurring agents from plants and other natural sources. Though *A. paniculata* has well thrombolytic effect (Prakash and Manavalan, 2011), 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 *A. paniculata*.

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% A.V. P. Computer-Assisted Mechanism of Action Analysis of Large Databases, Including 250,000 Open NCI Database Compounds. Plant Resources 1998; 34(1): 61–64.. The result of prediction is presented as the list of activities with appropriate Pa and Pi ratio. Six *phytoconstituents* namely 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide were analyzed by the PASS prediction for their thrombolytic activity and found wide range of activity. Neoandrographolide was the best compound for thrombolytic effect from all the compounds, though it had much bigger Pa value (0.557) than Pi value (0.021). As a result, neoandrographolide had 26.524 ratio (Pa: Pi) value.

In molecular docking study, 5-hydroxy-7,8-dimethoxyflavone, 14-acetylandrographolide, 14-deoxyandrographolide, andrograpanin, isoandrographolide, and neoandrographolide showed the docking score -7.2, -7.0, -7.0, -7.2, -7.4 and -9.0, respectively towards tissue-type plasminogen activator. From all these phyto compounds, neoandrographolide exhibited best docking score (-9.0), which also possessed maximum thrombolytic effect prediction from PASS prediction. After neoandrographolide, isoandrographolide showed well docking score (-7.4) and good prediction for thrombolytic effect in PASS prediction.

From phyto compounds of *A. paniculata*, neoandrographolide and isoandrographolide may be competitive candidate for promising thrombolytic agent.

5. CONCLUSIONS

From the study it was found that, both in silico models showed similar value for the same compound, because neoandrographolide showed high value and andrograpanin showed low value in both in silico models. All the data support that neoandrographolide is the best compounds for thrombosis management, as it possessed higher value both in PASS prediction and Molecular docking. So, neoandrographolide and isoandrographolide may be competitive candidate for promising thrombolytic agent. Further in vitro and in vivo investigation need to identify whether neoandrographolide 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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