Ind. J. Pharm. Edu. Res., 2025; 59(1s):s190-s197.
https://www.ijper.org

Original Article

Preclinical Evaluation of Thrombocytopenic Activity of Hydro-Alcoholic Extract of *Psidium guajava* L. Fruits on Wistar Rats with *in silico* Modelling on Dengue Virus Protease Inhibitors

Soumik Bhattacharjee[1,*], Abhik Si[2], Amit Kumar Halder[3], Sumit Nandi[1]

[1]Department of Pharmacology, Gupta College of Technological Sciences, Asansol, West Bengal, INDIA.
[2]Department of Pharmacology, Dr. B.C. Roy College of Pharmacy and A.H.S, Durgapur, West Bengal, INDIA.
[3]Department of Pharmaceutical Chemistry, Dr. B.C. Roy College of Pharmacy and A.H.S, Durgapur, West Bengal, INDIA.

ABSTRACT

Background: The prime objective of the investigation is to measure the capability of expanding the enumeration of platelet in rodent model utilizing hydro-alcoholic extract of *Psidium guajava* L. fruit in addition with *in silico* modelling on Dengue Virus Protease Inhibitors. **Materials and Methods:** Using a double maceration procedure with 30% water and 70% ethanol, the harvested fruit was extracted. Heparin-induced thrombocytopenic rats were used to assess the extract's thrombocytopenic action. Prednisolone was used as the standard medication and the animals were divided into five categories. The platelet count was performed using a hemocytometer. Additionally, bleeding duration was assessed and at last *in silico* studies has been carried out through several softwares to identify the targeting molecule. **Results:** Prednisolone, *Psidium guajava* L. low dosage and *Psidium guajava* L. high dose on the 14th day demonstrated the numbers of platelet 1732614, 874021 and 946224 respectively compared with toxicant control in the heparin-induced thrombocytopenic rat model. **Conclusion:** It is clear from the results above that *Psidium guajava* L. significantly increased the count of platelet when equated to the toxicant control. Thus, it can be said that *Psidium guajava* L. plant extract significantly induces thrombocytopenia and it is found that NS2-NB3 protease is the promising target for dengue virus and in future we will try to identify the quercetin or quercetin derivatives of *Psidium guajava* L. responsible for producing effectiveness against the said target.

Keywords: Heparin, *Psidium guajava L.*, Prednisolone, Haemocytometer, Platelet count, QSAR studies.

Correspondence:
Mr. Soumik Bhattacharjee
Assistant Professor, Department of Pharmacology, Gupta College of Technological Sciences, Asansol-713301, West Bengal, INDIA.
Email: soumiksubho1451996@gmail.com

Received: 28-06-2024;
Revised: 01-08-2024;
Accepted: 10-01-2025.

INTRODUCTION

In Dengue Hemorrhagic Fever (DHF), thrombocytopenia is a huge element. Thrombocytopenia is a typical hematologic condition that has a great many clinical signs. Low platelet counts can be the main indication of diseases like HIV and hepatitis C, or they can show the presence of perilous sicknesses such thrombotic micro-angiopathies. A platelet count of less than 150×103 per liter is viewed as thrombocytopenia. Diminished platelet count is brought about by diminished platelet combination and expanded platelet destruction. Different cycles might add to the improvement of thrombocytopenia in different sorts of thrombocytopenia, like essential Immunological Thrombocytopenia (ITP) and hepatitis C infection disease. Hence because of decrease in platelet count it is a conspicuous justification behind dengue and my work will do a point-by-point research on all elements as for dengue fever. *Psidium guajava* L. having name as Guava or its species belongs to the family Myrtaceae. *Psidium guajava* L. is a notable conventional restorative plant utilized in different native frameworks of medication due to its nutritional contents.[1-3]

Modern *in silico* modelling techniques, such as the utilization of QSAR or Quantitative Structure Activity Relationships, provide a feasible alternative for reducing benchwork and for more successfully investigating novel molecules during the evolution of materials. In order to create validated QSAR models that are effective against dengue virus and its likely targets, such as NS2B-NS3 proteases, as well as a exploration-based scheme for directing novel formulations, is the goal of this research.[4-6]

DOI: 10.5530/ijper.20250199

MATERIALS AND METHODS

Animal

Male and female Albino Wistar rodents weighing 150 g each were used in the investigation and housed in cages measuring 32x24x17 cm. The rodents were obtained from a licensed animal breeder and housed in an animal house at the Dr. B.C. Roy College of Pharmacy and A.H.S., Durgapur, West Bengal, under standard hygienic conditions (222°C, 65% relative humidity and a 12 hr light/dark cycle), with CPCSEA approval (BCRCP/IAEC/5/2022) and maintenance. They have unlimited access to tap water and industrial food pallets. Every trial took place between 10 am and 5 pm.

Plant Sample Authentication

After collection of fresh fruits from medicinal garden of Dr. B.C. Roy College of Pharmacy and A.H.S., Durgapur, West Bengal, a fruit sample has been sent to a botanist of a ayurvedic research institute at Mumbai for authentication and it has been authenticated as Guava fruit having Latin name *Psidium guajava* L. belonging to the family Myrtaceae and its authentication certificate (Authentication/ALARSIN/2021-22/215) is submitted along with this paper.

Collection of sample

Fresh fruits were gathered and, softly cleaned with water to eliminate the undesirable particles and the good fruits were dried. The desiccated fruit was then extracted after being ground coarsely in a mixer grinder.

Plant extract

In order to use the same volume for both macerations, 70% ethanol and 30% water were divided into two portions and used to macerate the dried fruits twice. It required 72 hr to complete the double maceration process. The initial step required 48 hr and the second one 24 hr. Then the extract was gathered and stored in pressure desiccators to allow it to evaporate.

Exploratory phytochemical investigation of the extract

Ethanolic extract of *Psidium guajava* L. fruit were taken for phytochemical testing such as alkaloids, saponin, carbohydrates, flavonoids, glycosides and amino acids etc.

Acute oral toxicity studies

As stated by the Oppts (Office of prevention, pesticide and toxic substance) guidelines, the acute oral toxicity research was carried out.[7-10]

Statistical Analysis

For each treatment group, the experimental results were represented as mean SEM. Betwixt the data of the treated and control sets, the importance of exertion was evaluated using a one-way ANOVA analysis of variance succeeded by Dunnett's post parametric test. Statistics were deemed significant when $^{*}p<0.05$.

Dose preparation

To create a stock solution of 500 mg/kg, 7.5 mL of purified water was taken to dissolve 75mg of extract, while 3.75 mL of distilled water is taken to dissolve 37.5 mg of extract to create a 250 mg/kg stock solution. Heparin was then administered intravenously at a rate of 0.1 mg/100 g. (subcutaneous).

Drugs and chemicals

Ethanol (Banting Pharmaceuticals Pvt. Ltd.,), Diethyl ether, Distilled water, ReesEcker fluid, prednisolone (Wysolone 5 mg by Pfizer), Heparin (25000 I.U Injection from Gland Pharma Ltd.,).

Experimental design Model

Heparin instigated thrombopenic rat

The rodents were separated into four sets or groups and every group consists of six rats. To induce thrombocytopenia among the rodents of respective groups II, III and IV are served with heparin 0.1 mL/100 g subcutaneously for consecutive 3 days.

- **Group I:** Rats in these sets entertained with normal water across the experimental tenure. This set was appraised as normal or untreated batches.
- **Group II:** This batch was appraised as toxicant control set and entertained with heparin 0.1 mL/100 g subcutaneously for first consecutive 3 days daily.
- **Group III:** These was test group I which entertained extract solution *Psidium guajava* L. Low Dose (PGLD) 250 mg/kg, p.o. or orally for 13 days daily along with heparin 0.1 mL/100 g s.c. or subcutaneously for 1st 3 days.
- **Group IV:** These was test group II that entertained extract solution *Psidium guajava* L. High Dose (PGHD) 500 mg/kg orally for 13 days daily along with heparin 0.1 mL/100 g s.c. or subcutaneously for first consecutive three days.

Collection of blood samples done on day 1, 4, 7, 10 and 14 from respective groups of rodents and platelet evaluation done by haemocytometer. On 14th day, time of bleeding was obtained.

QSAR Modelling

Dataset preparation

The purpose of the current work is to develop validated and predictive 2D-QSAR or 2D-quantitative structure activity relationship models with Dengue protease inhibitors containing

Table 1: Mean platelet count with days.

Treatment classes and Dose (mg/kg)	Mean platelet count (cells per microliter) and Time interval in days				
	Day-1	Day-4	Day-7	Day-10	Day-14
Normal control	921445±29164	923343±13012	922657±15921	924115±17340	923005±16252
Toxicant control (Heparin, 0.1 mL/100 gm)	932657±32140	743056±18516	460983±8423	392565±18011	392105±19241
Standard Group (Prednisolone, 2 mg/kg)	928227±26013**	813315±21015***	1509516± 24022***	1500223± 32625***	1732614±22571***
Test group-I (PGLD, 250 mg/kg)	930616±16524***	727289±18562***	752176±15234**	831630±19654***	874021±17562***
Test group-II (PGHD, 500 mg/kg)	926520±18564***	820717±16321***	854117± 17524***	924021±19254***	946224±22541***

All values are mean±SEM, i=6, $^{**}p<0.01$, $^{***}p<0.001$ when experimental groups compared with normal control.

Table 2: Bleeding time of rats on day 14.

	Mean bleeding time (sec) on 14th Day				
Groups	Normal control	Toxicant control	Standard group	PGLD	PGHD
Time(sec)	90±5.71	178±11.32	79±5.81***	108±5.62***	117±5.2***

All values are mean±SEM, n=6, $^{***}p<0.001$ when experimental groups compared with normal control.

4Benzyloxyphenylglycine Residues. A dataset containing 79 4Benzyloxyphenylglycine derivatives as DENV protease inhibitors was collected from scientific literature.[11-14] The SMILES notations of these 79 compounds were first converted converted first to 2D structures by tool of MarvinView (MarvinView. Version 18.18.0; https://chemaxon.com/products/marvin, ChemAxon: Budapest, Hungary, 2010) and these structures were subsequently saved as 3D structures (in .sdf file). These 3D structures was then standardized by ChemAxon Standardizer tool utilizing the following options: (a) aromatize, (b) add explicit hydrogen atoms, (c) clean 3D, (d) clean 2D, (e) strip salts and (f) neutralize.[15-17] The experimental IC_{50} (μM) value of these amalgams were converted to pIC50 [i.e., -log10 (IC_{50}(M)] and these converted values were subsequently used as dependent parameter for fixing up the 2D-QSAR models.

Descriptor calculation

The 3D chemical structures of 79 DENV protease inhibitors were subjected to descriptors calculation using alvaDes cv.2.0.4 (https://www.alvascience.com/alvadesc/) under webserver of OCHEM.[18-20] For the evaluation of 3D descriptors, the framework of the amalgams were geometrically optimized using Corina.[21]

Model generation

The unbent interpretable 2D-QSAR models were created using SFS-QSAR tool, freely pervade from https://github.com/ncordeirfcup/SFS-QSAR-tool. In this tool, the dataset containing both dependent and independent parameters was first divided into a training set (80% of the modeling dataset) and a test set (20% of the modeling dataset) using three different dataset separation strategies namely (a) activity sorting (using starting point 2), (b) random division (using random seed 42 and 2) and (c) k-means cluster analyses (kMCA, using 5 clusters and random seed 2).[22]

Determination of statistical standard of the models

Several external and internal diagnostic statistical tools were utilized to compare the effectiveness of the various models of QSAR regression. Their internal statistical significance was evaluated using common validation criteria like R^2 (Eq. 1), R^2_A (Eq. 2), F-statistics (Eq. 3), the quit one out cross-validation R^2 (Q^2, Eq. 4). Similarly, the predictive capableness of the models was assessed by the following external validation parameters: R^2_{Pred} (Eq. 5).[23,24]

$$R^2 = 1 - \frac{\sum (Y_{Obs(Train)} - Y_{Calc(Train)})^2}{\sum (Y_{Obs(Train)} - \overline{Y}_{Training})^2} \quad (1)$$

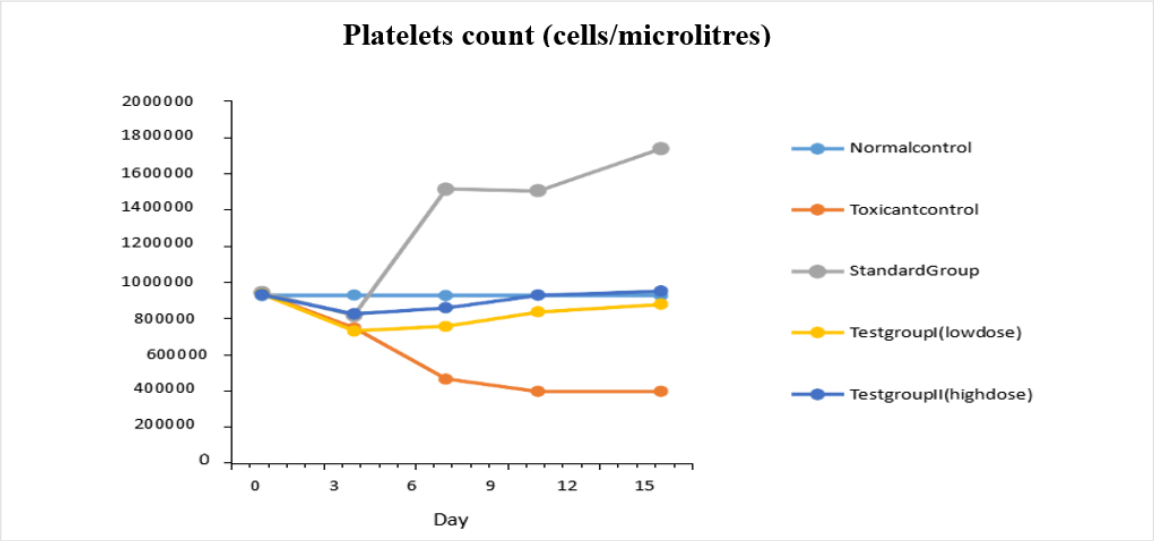

Figure 1: Mean Platelet Count With Days.

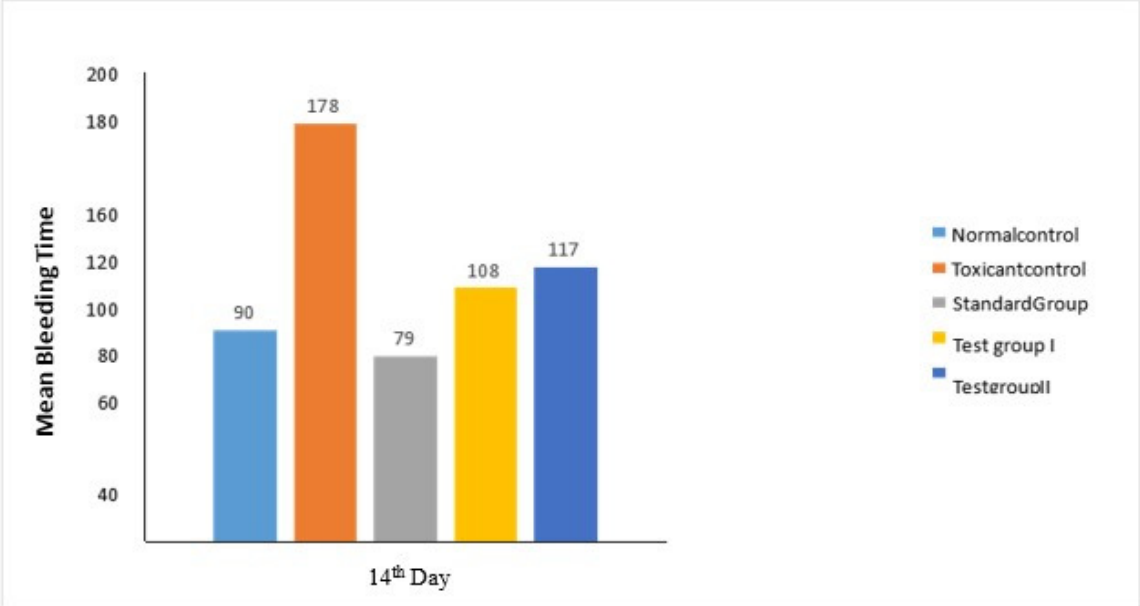

Figure 2: Mean Bleeding Time (sec).

$Y_{Obs\ (Train)}$ is the observed exertion of the training set compounds, $Y_{Calc\ (Train)}$ is the calculated exertion of the training set compounds, $\hat{Y}_{Train}$ is the mean observed exertion of the training set compounds.

$$R_A^2 = 1 - \frac{(1-R^2)(N-1)}{(N-k-1)} \qquad (2)$$

$R^2$is the squared correlation coefficient, N is the number of the compounds in the training set, k is the number of predictor variables used to derive the model

$$F = \frac{\sum (Y_{Calc(Train)} - \overline{Y}_{Training})^2 / p}{\sum (Y_{Obs(Train)} - Y_{Calc(Train)})^2 / (N-p-1)} \qquad (3)$$

N is the number of the compounds in the training set, p is the number of predictor variables used to derive the model

$$Q^2 = 1 - \frac{\sum (Y_{Obs(Train)} - Y_{Pdred(Train)})^2}{\sum (Y_{Obs(Train)} - \overline{Y}_{Training})^2} = 1 - \frac{PRESS}{\sum (Y_{Obs(Train)} - \overline{Y}_{Training})^2} \qquad (4)$$

$$R^2_{Pred} = 1 - \frac{\sum (Y_{Obs(Test)} - Y_{Pred(Test)})^2}{\sum (Y_{Obs(Test)} - \overline{Y}_{Training})^2} \qquad (5)$$

During generation of linear QSAR models, 1:10 ratio between the number of descriptor and number of training set data points was maintained. The best QSAR models will also be checked for inter-collinearity within its descriptors. Furthermore, *Y*-randomization test was carried out with 1000 runs to generate 1000 models with randomized response variables. To confirm

Table 3: Summary of results of the 2D-QSAR models obtained by varying dataset division techniques, scoring function (during feature selection) and types of descriptors.

Model	Dataset division	Scoring	Descriptor	Q2	R2Pred	Average
M01	KMCA (Cluster5, Seed2)	NMAE	alvaDes_2D	0.75	0.51	0.630
M02	KMCA (Cluster5, Seed2)	NMGD	alvaDes_2D	0.799	0.599	0.699
M03	KMCA (Cluster5, Seed2)	NMPD	alvaDes_2D	0.799	0.599	0.699
M04	KMCA (Cluster5, Seed2)	R2	alvaDes_2D	0.799	0.599	0.699
M05	Random (Seed42)	NMAE	alvaDes_2D	-2.37	0.518	-0.926
M06	Random (Seed42)	NMGD	alvaDes_2D	0.761	0.515	0.638
M07	Random (Seed42)	NMPD	alvaDes_2D	0.695	0.574	0.634
M08	Random (Seed42)	R2	alvaDes_2D	0.695	0.574	0.6345
M09	Random (Seed2)	NMAE	alvaDes_2D	0.687	-0.293	0.197
M10	Random (Seed2)	NMGD	alvaDes_2D	0.781	-0.137	0.322
M11	Random (Seed2)	NMPD	alvaDes_2D	0.774	-0.332	0.221
M12	Random (Seed2)	R2	alvaDes_2D	0.783	-0.114	0.3345
M13	AS(Startpoint2)	NMAE	alvaDes_2D	0.812	0.194	0.503
M14	AS(Startpoint2)	NMGD	alvaDes_2D	0.783	0.04	0.411
M15	AS(Startpoint2)	NMPD	alvaDes_2D	0.794	0.158	0.476
M16	AS(Startpoint2)	R2	alvaDes_2D	0.79	-0.061	0.3645
M17	KMCA (Cluster5, Seed2)	NMAE	alvaDes_3D	0.764	0.731	0.7475
M18	KMCA (Cluster5, Seed2)	NMGD	alvaDes_3D	0.798	0.732	0.765
M19	KMCA (Cluster5, Seed2)	NMPD	alvaDes_3D	0.831	0.833	0.832
M20	KMCA (Cluster5, Seed2)	R2	alvaDes_3D	0.831	0.833	0.832
M21	Random (Seed42)	NMAE	alvaDes_3D	0.736	0.508	0.622
M22	Random (Seed42)	NMGD	alvaDes_3D	0.844	0.652	0.748
M23	Random (Seed42)	NMPD	alvaDes_3D	0.844	0.652	0.748
M24	Random (Seed42)	R2	alvaDes_3D	0.847	0.678	0.7625
M25	Random (Seed2)	NMAE	alvaDes_3D	0.76	0.063	0.4115
M26	Random (Seed2)	NMGD	alvaDes_3D	0.846	0.218	0.532
M27	Random (Seed2)	NMPD	alvaDes_3D	0.846	0.218	0.532
M28	Random (Seed2)	R2	alvaDes_3D	0.844	0.102	0.473
M29	AS(Startpoint2)	NMAE	alvaDes_3D	0.822	0.495	0.6585
M30	AS(Startpoint2)	NMGD	alvaDes_3D	0.828	0.611	0.7195
M31	AS(Startpoint2)	NMPD	alvaDes_3D	0.848	0.77	0.809
M32	AS(Startpoint2)	R2	alvaDes_3D	0.848	0.77	0.809

that the model was developed by chance, a parameter named ${}^{c}R^{2}_{p}$ was calculated as:

$$ {}^{c}R^{2}_{p} = R \times \sqrt{R^{2} - R_{r}^{2}} $$

Where, R_{r}^{2} is the scrambled squared randomized scrambled correlation coefficient. The higher value of ${}^{c}R^{2}_{p}$ implies that the original model was not developed by chance.[25-28]

RESULTS

Extract preparation

A yield of 3.33% was obtained after the fruits of *Psidium guajava* L. were double macerated in 70% ethanol for two successive 72 hr periods.

Table 4: Descriptions of the descriptors appeared in the QSAR model.

Name	Description	Type	Sub-Block	2D/3D
ASP	Asphericity.	Geometrical	Shape indices.	3D
H6m	H auto correlation of lag6 /weighted by mass.	GETAWAY	H-indices.	3D
RDF135v	Radial Distribution Function - 135 / weighted by vander Waals volume.	RDF	Weighted by vander Waals volume.	3D
RDF010s	Radial Distribution Function - 010 / weighted by I-state.	RDF	Weighted by I-state.	3D
P_VSA_charge_12	P_VSA-like on partial charges, bin12.	P_VSA	Weighted by charge.	2D

Preliminary phytochemical investigation

Following a preliminary phyto-chemical analysis of the extract made from *Psidium guajava* L. fruits, carbohydrates, flavonoids, alkaloids, glycosides and saponins are present in the extract.

Acute oral toxicity studies

As per the guidelines of OPPTs, drug found to be risk free at 779000 I.U/kg body weight.

Heparin instigated thrombocytopenia in rat

Heparin is an anticoagulant drug that is typically utilized to treat thromboprophylaxis; however, because of how it works to sequester or clump platelets, it can have the side affect of thrombocytopenia. The rodents were divided into sets or groups and given prednisolone treatment in accordance with their group membership in the research, "Heparin instigated thrombocytopenia in rat model." From day 4 to day 14, PGLD (*Psidium guajava* L. Low Dose) and PGHD (*Psidium guajava* L. High Dose) increased the platelet count in a dose-dependent manner. On day 14, Prednisolone, PGLD (*Psidium guajava* L. Low Dose) and PGHD (*Psidium guajava* L. High Dose) all revealed platelet counts of 1732614, 874021 and 946224, respectively, as shown in Table 1 and Figure 1. When compared to the toxicant control, all three groups displayed a noticeably higher amount of mean platelet count. As shown in Table 2 and Figure 2, prednisolone, PGLD (*Psidium guajava* L. Low Dose) and PGHD (*Psidium guajava* L. High Dose) have demonstrated a critical rise on coagulating rate when contrasted and toxicant control.

QSAR Modelling

During model development a maximum of 5 descriptors were allowed. A total 32 models (M01-M32) were generated by varying different dataset division methods (AS/random/KMCA) and scoring functions (R^2/NMAE/NMPD/NMGD) and type of descriptor (2D or 2D+3D). Overall statistical quality of each model justified by internal validation parameter Q^2 and external validation parameter R^2Pred. The results are depicted in Table 3.

Note that M19 and M20, which are redundant models, afforded the most statistically significant QSAR models. The most predictive model (M19/M20) is presented below.

pIC_{50}=9.510(±0.676)-0.074(±0.015) P_VSA_charge_12+5.927(±0.566) ASP+0.111(±0.012) RDF135v-0.085(±0.011) RDF010s-2.052(±0.369) H6m

Ntr=62, R^2=0.866, R^2A=0.854, F(5,56)=72.08, Q^2LOO=0.831, Nts=17, R^2Pred=0.833, $^cR^2p$=?

The utmost absolute correlation (R^2) between any two of descriptors of the model was found as 0.45 that signifies that the model is devoid of high inter-collinearity and each descriptor of the model is thus unique. The high $^cR^2p$ of the model implies that the model is eccentric in nature and was not evolved by chance. The scatter plot observed vs predicted pIC_{50} activity is depicted in Figure 3.

After confirming overall statistical robustness and integrity of the QSAR model, the Applicability Domain (AD) of the model. The Williams plot is presented in Figure 3.

Next, we need to focus on five descriptors that appeared in the model and their significance towards determining the DENV protease inhibitory activity of the compounds. The benefaction of the descriptor in the model was determined by absolute standardized regression coefficients and as per these the descriptors of the model have following descending order of relative importance ASP>H6m>RDF135v>RDF010s>P_VSA_charge_12 (Figure 4).

The detailed description of these five descriptors is provided in Table 4. Significantly, four out if five descriptors of the models are of 3D type and it justifies the importance of such descriptors in characterizing DENV protease inhibitory potential of these compounds.

DISCUSSION

The hydro-alcoholic extract of *Psidium guajava* L. rich in quercetin shows increment in amount of SCF mRNA which can exaggerate IL-6 to induce TPO secretion by liver resulting an increment of the platelets number after administration thus it is a significant

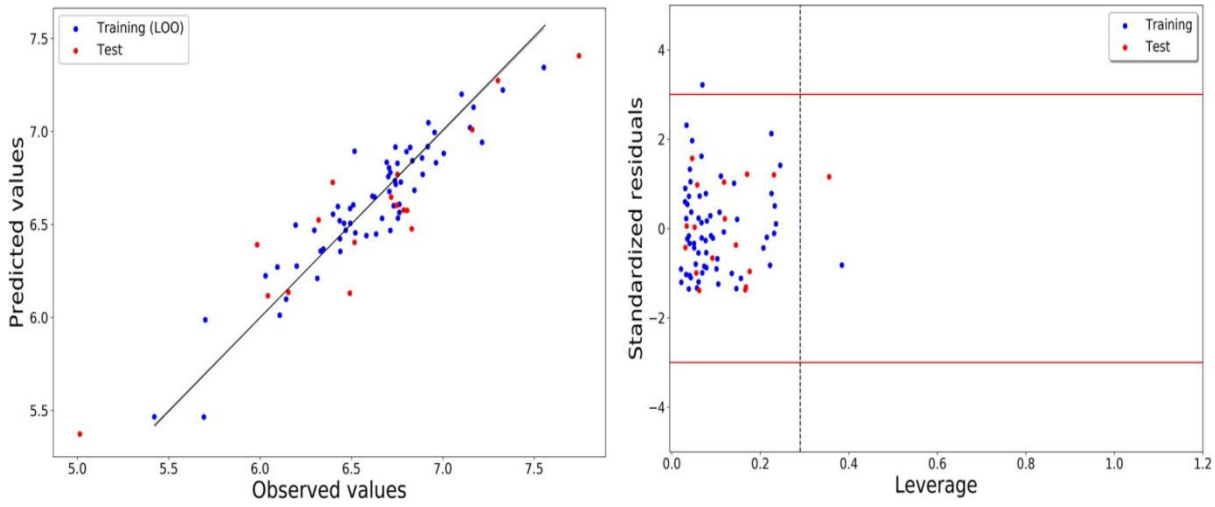

Figure 3: Scatter plot of the observed vs predicted pIC_{50} values (left) and Williams plot (right) of the most predictive QSAR model.

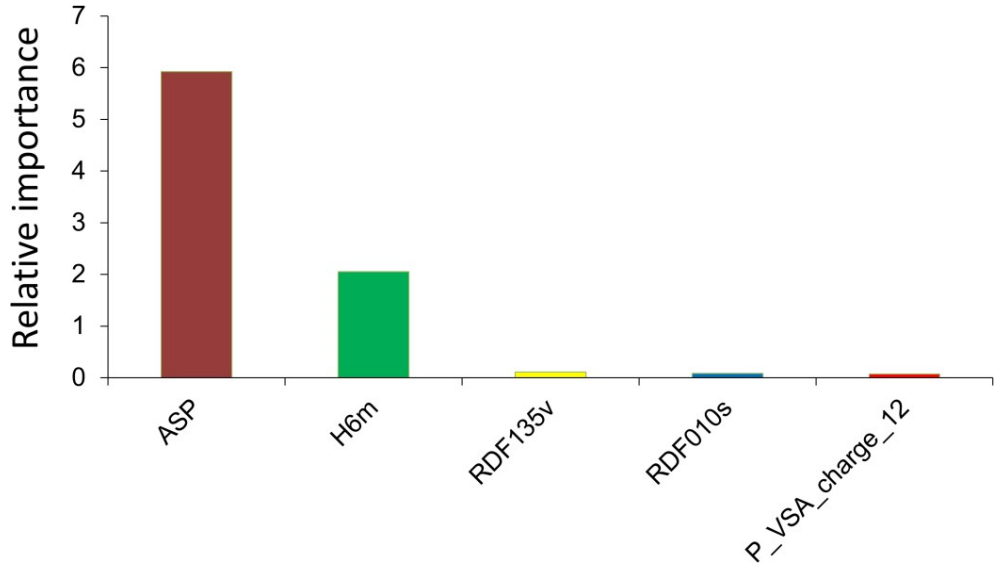

Figure 4: Descriptors of the model have following descending order of relative importance -ASP>H6m>RDF135v>RDF010s>P_VSA_charge_12.

remedy against thrombocytopenia. Assessment of the bleeding time in rats on day 14 has obviously provided a notion of platelet generation because the platelet number directly influences the clotting time and bleeding time. The bleeding duration has risen with heparin compared to the untreated control. The coagulation time has been significantly accelerated by prednisolone, PGLD (*Psidium guajava* L. Low Dose) and PGHD (*Psidium guajava* L. High Dose).

QSAR modelling is also performed to establish a derivative or molecule of *Psidium guajava* L. which has effectiveness against NS2-NB3 protein present in DENV-2 and it is found that NS2-NB3 protease is the promising target for Dengue Virus (DENV-2) and infuture we will try to identify the quercetin or quercetin derivatives of *Psidium guajava* L. responsible for producing effectiveness against the said target.

CONCLUSION

The present study was undertaken to assess the form of thrombocytopenic activity of *Psidium guajava* L. So from the whole work I can conclude that the *Psidium guajava* L. has been extracted and phytochemical screening has undergone which shows the presence of phytochemicals like carbohydrate, glycosides, flavonoids, alkaloids, saponins and amino acids and its main constituent quercetin shows the platelet augmentation activity in thrombocytopenic condition and it also decreases the bleeding time by increasing clotting rate in comparison with toxicant control group.

In this current work, an attempt has been taken to develop linear interpretable 2D-QSAR models to predict DENV protease inhibition properties of compounds containing substituted benzyl and phenacyl ethers of 4-hydroxyphenylglycine. The IC_{50} of the 79 dataset compounds varied from 18 nM to 9695

nM and therefore, different structural attributes are found to play vital roles in determining the biological activity of these compounds. With 2D-QSAR we attempted to understand these crucial structural and/or physico-chemical features. Multiple QSAR models were generated by varying data-distributions, descriptor types and model generation techniques. The most predictive model provided satisfactory statistical significance with internal validation parameter Q^2 as 0.831 and external validation parameter R^2Pred as 0.832. Therefore, this model is capable of identifying crucial structural and/or physicochemical factors responsible for high protease inhibitory property of these compounds. The current work insinuated that relatively complex 3D structural characters are liable for predicting the activity of these compounds.

ACKNOWLEDGEMENT

In today's world full of competitions and rat races around, a race of existence prevails in the minds of the successful people. A project stands out as a bridge between the theoretical and the practical work. It was a matter of great pride that I was fortunate enough to have association with great personalities. I can remember their names only with profound gratitude and satiety. I would like to take this opportunity to thank the Almighty who has obviously guided me throughout in choosing between the right and the wrong and in making decisions.

Of all my facilitators, it was the magnanimity and support of my teacher and guide Professor Dr. Abhik Si, M. Tech, Ph.D., Associate Professor, Department of Pharmacology, Dr. B. C. Roy College of Pharmacy and Allied Health Sciences, Durgapur and Dr. Amit Kr. Halder, M-Pharm, Ph.D., Assistant Professor who has unconditionally encouraged me and provided me with bountiful resources and has enlightened me throughout my project work. His personal charm and professional eminency has inspired me a lot to put optimum efforts towards completing my project work.

I am also indebted to my parents who have brought me up with lots of love and care and also for their constant support, motivation and guidance throughout these days of my work.

I express my sincere thanks to all my friends and classmates and to all the others for their help during my project work. I extend my thanks and gratitude to my college Dr. B.C. Roy College of Pharmacy and AHS, Durgapur. I am at a loss of words to express my thanks although my heart is still full of favours received from every person.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. J ClinInvest.1966;45(5): 645-57.
2. Mourao, M.P. *et al.* Thrombocytopenia in patients with dengue virus infection in the Brazilian Amazon. Platelets 2007;18,605-612.
3. Honda, S. *et al.* Increased phagocytosis of platelets from patients with secondary dengue virus infection by human macrophages. Am. J. Trop. Med. Hyg. 2009;80,841-5.
4. Mital Kaneria, Sumitrachanda, Phytochemical and Pharmacognostic Evalution of leaves of *Psidium guajava* L. (Myrtaceae), Pharmacognosy Journal, 2011;3(23):41-5.
5. El-Mahmood MA, The use of *Psidium guajava* Linn. in treating wound, skin and soft tissue infections, Scientific Research and Essay,2009;4(6): 605-11.
6. Rathish N, Sumitra C, *in vitro* antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains, Brazilian Journal of Microbiology, 2007;38:452-8.
7. Kamath JV, Nair Rahul, Ashok Kumar CK, Mohana Lakshmi S, *Psidium guajava* L: A review, International Journal of Green Pharmacy, 2008;2(1):9-12.
8. BalaS A. *Psidium guajava*. In: some ethno-medicinal plants of the savannah regions of West Africa: Description and phytochemicals. Triumph publishing company limited, Kano, Nigeria. 2006;(Vol.II):21-56.
9. Nadkarni KM, Nadkarni AK. Indian Materia Medica-with Ayurvedic, Unani- Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies. Popular Prakashan Private Ltd., Bombay, India; 1991:142-49.
10. Heinrich M. Plants as antidiarrhoeals in medicine and diet. Proceedings from a Joint Meeting of the Society for Economic Botany and the International Society London, Royal Botanic Gardens, Kew, UK; 1998:17-30.
11. Leonti M, Vibrans H, Stiche O, Heinrich M, Ethnopharmacology of the Popoluca, Mexico: An evaluation. J. Pharmacy Pharmacol, 2001;53:1653-69.
12. Gutierrez RM, Mitchell S, Solis RV, *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology, J Ethnopharmacol, 2008;117(1):1-27.
13. Deo A, Satri NV, Purification and characterization of polygalacturonase inhibitory proteins from *Psidium guajava* Linn. (Guava) fruit, Plantscience, 2003;164:147-56.
14. Smith RM, Siwatibau S, Sesquiterpene hydrocarbons of Fijian guavas, Phytochemistry, 1975;14:2013-15.
15. Cumming JG, Davis AM, Muresan S, Haeberlein M, Chen H. Chemical predictive modelling to improve compound quality. NatRevDrugDiscov. 2013;12:948-62.
16. Falgout, B.; Pethel, M.; Zhang, Y. M.; Lai, C. J. Both Nonstructural Proteins NS2B and NS3 are Required for the Proteolytic Processing of Dengue Virus Non structural Proteins.J.Virol.1991;65: 2467-75.
17. Shiryaev, S.A.; Strongin, A.Y. Structural and Functional Parameters of the Flaviviral Protease: A Promising Antiviral Drug Target. Future Virol. 2010;5:593-606.
18. Erbel, P.; Schiering, N.; D'Arcy, A.; Renatus, M.; Kroemer, M.; Lim, S. P., *et al.* Structural Basis for the Activation of Flaviviral NS3 Proteases from Dengue and West Nile Virus. Nat. Struct. Mol. Biol. 2006;13:372-3.
19. Skorenski, M.; Sienczyk, M. Viral Proteases as Targets for Drug Design.Curr.Pharm. Des.2013;19: 1126-53.
20. Cregar-Hernandez, L.; Jiao, G.-S.; Johnson, A. T.; Lehrer, A. T.; Wong, T. A. S.; Margosiak, S.A. Small Molecule Pan-Dengue and West Nile Virus NS3 Protease Inhibitors. Antiviral Chem. Chemother.2011;21: 209-18.
21. Mueller, N. H.; Yon, C.; Ganesh, V. K.; Padmanabhan, R. Characterization of the West Nile Virus Protease Substrate Specificity and Inhibitors. Int. J. Biochem. Cell Biol.2007,39,606-14.
22. Shiryaev, S. A.; Ratnikov; Boris, I.; Chekanov; Alexei, V.; Sikora, S.; Rozanov, *et al.* Cleavage Targets and the D-Arginine-based Inhibitors of the West Nile Virus NS3 Processing Proteinase. Biochem. J.2006;393: 503-11.
23. Wu, H.; Bock, S.; Snitko, M.; Berger, T.; Weidner, T.; Holloway, S., *et al*. Novel Dengue. Virus NS2B/NS3 Protease Inhibitors. Antimicrob. Agents Chemother. 2014;59:1100-9.
24. Liu, H.; Wu, R.; Sun, Y.; Ye, Y.; Chen, J.; Luo, X., *et al*. Identification of Novel Thiadiazolo acrylamide Analogues as Inhibitors of Dengue-2 Virus NS2B/NS3 Protease. Bioorg. Med.Chem.2014;22: 6344-52.
25. Schüller, A.;Yin, Z.;BrianChia, C.S.;Doan, D.N.P.;Kim, H.K.;Shang, *et al.* Tripeptide Inhibitors of Dengue and West Nile Virus NS2B-NS3 Protease. Antiviral Res.2011;92: 96-101.
26. Hammamy, M.Z.;Haase, C.;Hammami, M.;Hilgenfeld, R.;Steinmetzer, T. Development and Characterization of New Peptidomimetic Inhibitors of the West Nile Virus NS2B-NS3 Protease. ChemMedChem. 2013;8:231-41.
27. Lim, S.P.; Shi, P. Y. West Nile Virus Drug Discovery. Viruses 2013;5:2977-3006.
28. Lim, S. P.; Wang, Q. Y.; Noble, C. G.; Chen, Y. L.; Dong, H.; Zou, B., *et al.* Ten Years of Dengue Drug Discovery: Progress and Prospects. Antiviral Res.2013;100: 500-19.

Cite this article: Bhattacharjee S, Si A, Halder AK, Nandi S. Preclinical Evaluation of Thrombocytopenic Activity of Hydro-Alcoholic Extract of *Psidium guajava* L. Fruits on Wistar Rats with *in silico* Modelling on Dengue Virus Protease Inhibitors. Indian J of Pharmaceutical Education and Research. 2025;59(1s):s190-s197.