Bangladesh Pharmaceutical Journal ***24****(2): 125-132, 2021 (July)*

Computer-aided Approaches to Support the Ethnopharmacological Importance of *Dillenia pentagyna* Roxb.: An *In silico* Study

Safaet Alam[1], Nazim Uddin Emon[2], Md. Sadman Hasib[3], Mohammad A. Rashid[3], Mahfuza Afroz Soma[1], Tanoy Saha[1] and Mohammad Rashedul Haque[3]

[1]Department of Pharmacy, State University of Bangladesh, 77 Satmasjid Road, Dhanmondi, Dhaka-1207, Bangladesh
[2]Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Chittagong-4318, Bangladesh
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

(Received: May 31, 2021; Accepted: July 2, 2021; Published (Web): July 15, 2021)

Abstract

Dillenia pentagyna Roxb., a deciduous and medium sized tree, is an ethnobotanically important species of several Asian countries including Bangladesh, Srilanka, and India. Various parts of this endangered tree including leaf, bark, root etc. are used in folkloric medicine systems for a very long time. It is locally used in the treatment of pain, wound healing, diabetes, diarrhea and other disease states. Molecular docking is an important technique to validate the exerted pharmacological actions of a plant or specific phytoconstituents by assessing the binding affinity and interaction pattern between small molecules and particular target receptors. Thus, to justify the notable ethnobotanical importances of *D. pentagyna*, an *in silico* study was designed employing selected phytochemicals *i.e.* isorhamnetin, lupeol, quercetin, kaempferol, and betulin isolated from this plant based on previous literature searches. The molecular docking revealed strong binding affinities (-4.8 to -12.9 kcal/mol) with the respective target proteins: 2OYE, 6COX, 4YK5, 5ZHP, and 1A5H. Besides, in accordance with the Lipinski's rules, all five selected compounds of *D. pentagyna* showed promising orally active drug-like characteristics. To recapitulate, the present study has been found parallel with the existing ethnobotanical significance of *D. pentagyna* as the source of pain, fever, thrombosis, and diarrhea management agent.

Key words: *Dillenia pentagyna*, Pyrexia, Pain, Diarrhea, Thrombosis, *In silico*, Molecular docking.

Introduction

Dillenia pentagyna Roxb., locally known as 'Mota karmal' is an important traditional plant with enormous ethnobotanical importance (Gandhi *et al*., 2013). It belongs to Dilleniacea family and mainly distributed in Asian regions (Gandhi *et al*., 2013). Plants from *Dillenia* genus also used to treat cardiac weakness, fever, septic sore, and traumatic injury (Khatun *et al.*, 2016). Among fockloric use of *D. pentagyna*, analgesic, anti-inflammatory, antidiarrheal, and antidiabetic actions are most important which is also used to treat bone fracture, piles, dysentery and other stomach diseases (Prasad *et al*., 2009; Dubey *et al*., 2009). Besides, bioactive secondary metabolites found from *D. pentagyna* can also exert anti-thrombotic action which support the thrombolytic potentials of this plant (Choi *et al.*, 2015; Gong *et al.*, 2020). Plants are always blessed with several kinds of phytochemicals which may attribute to their pharmacological actions (Alam *et al*., 2020a, Alam and Haque, 2020). Phytochemicals isolated from *D. pentagyna* attributed to wide ranges of bioactive secondary metabolites including flavonoids (kaempferol, quercetin, rhamnetin-3-glucoside, isorhamnatin, and naringenin-7 galactosyl (1-4) glucoside), terpinoids

Corresponding author: Mohammad Rashedul Haque; Email: haquemr@du.ac.bd
DOI: https://doi.org/10.3329/bpj.v24i2.54711

(betulin, mallic acid, lupeol, betunaldehyde, β-sitosterol, stigmasterol, and betulinic acid) (Gandhi *et al*., 2013). Among other phytochemicals naringenin 7-galactosyl glucoside, naringenin-41-O-β-D-xylopyranoside, dihydroquer-cetin 5-galactoside, α-L-rhamnopyranosyl-3-β-hydroxyl-lup20(29)-en-28-oic acid, and rhamnetin-3-glucoside are major (Gandhi *et al*., 2013).

From the ancient era, herbal plants have become important sources of therapies to treat different ailments in all cultures and civilizations (Alam *et al.*, 2021c; Emon *et al*., 2020b). Folkloric usage of plant sources are important hints for the researchers to establish novel drug therapies from ethnopharmacologically important plant sources employing wet lab and dry lab experiments (Rudra *et al*., 2020). Phytochmecials isolated from medicinal plants have also offered lesser side effects than synthetic drugs. About 80% of therapeutic molecules are either a direct derivation or a modified form of the natural source (Maridass and De Britto, 2008). Thus, even after the establishment of synthetic therapies, the demand of herbal drugs has not been nullified.

Docking technique is a popular computational procedure in drug discovery to optimize targeted compounds and explore novel active molecules *via* virtual screening coupled with experimental binding interactions and affinities of small molecules (Parenti and Rastelli, 2012). Search algorithms have been used to study the free energy landscape and to discover the optimum ligand poses in molecular docking (Huang and Caflisch, 2011). Molecular docking can be a very nifty option to establish a preliminary concept of pharmacological actions exerted by a plant extract or isolated phytochemicals (Alam *et al*., 2021a). To develop and analyze drug designing of a newer molecule, execution of computer-aided drug discovery approaches and molecular docking have been proved as time-efficient procedure. Usually, structure-based technique involving ligand-receptor molecular docking is designed to estimate interaction affinities and binding patterns of the biological moieties with a particular target receptor. A convenient molecular docking should hold the potential to recognize the native ligand posture with the binding site of the three-dimensional protein structure coupled with physical connections (Guedes *et al*., 2014). Thus, it can be assumed that isolated phytochemicals from *D. pentagyna* can play the driving role which attributes to the pharmacological activities. In this study, we have performed an *in silico* study to justify and support the notable ethnopharmacological actions of *D. pentagyna* including analgesic, anti-inflammatory, antipyretic, antidiarrheal and thrombolytic activities using a computer aided approach (Alam *et al.*, 2020b).

Materials and Methods

Molecular docking

Protein preparation: 2OYE (cyclooxygenase-1), 6COX (cyclooxygenase-2), 4YK5 (microsomal prostaglandin E synthase 1), 5ZHP (muscarinic acetylcholine receptor) and 1A5H (tissue plasminogen activator) have been taken from RCSB Protein Data Bank (https://www.rcsb.org/structure) in PDB format for the screening of analgesic, anti-inflammatory, antipyretic, antidiarrheal and thrombolysis studies. Using Discovery Studio 2020, proteins have been separated from both water and heteroatoms. In order to prepare proteins, the gasteiger charge and nonpolar hydrogens were kept as default form. In addition, all proteins in UCSF Chimera were brought to the minimum energy level followed by further analysis, employing standard residues in AMBER ff14sB and other residues in gasteiger mode (Yang *et al*., 2012).

Ligand preparation: The structure of five phytocompounds of *D. pentaagyna* namely kaempferol (PubChem CID: 5280863), quercetin (PubChem CID: 5280343), isorhamnetin (PubChem CID: 5281654), lupeol (PubChem CID: 259846), betulin (PubChem CID: 72326) were collected from PubChem database (https://pubchem.ncbi.nlm.nih.gov/;). Besides, ibuprofen, indomethacin, loperamide and streptokinase were studied in order to compare and juxtapose the docking of the phytocompounds of

D. pentaagyna. The ligands were downloaded in 2DSDF format and curtailed by PyRx tool to figure out the best possible hit for the targets. The virtual screening software PyRx from MGLTools (https://ccsb. scripps.edu/mgltools/) was run in default format (Emon *et al*., 2020a).

Docking analysis: A semiflexible docking style was utilized for the docking study (Herowati and Widodo, 2014). Using PyRx AutoDock Vina program, PDB files of phytochemicals and proteins were obtained and later converted into PDBQT format. The rigidity of the protein and flexibility of the ligand were preserved by this study. The molecules of the ligand were given 10 degrees of freedom. AutoDock defines measures to automatically convert molecules into molecules of the pdbqt format, box sort, grid box formation, etc. The grid box was generated in the center of the box with an active location and sought out the binding affinity of selected phytoconstituents and receptors. Furthermore, to assess the best docking locations, BIOVIA Discovery Studio Visualizer 2020 has been accelerated. The PyRx AutoDock Vina was introduced (Herowati and Widodo, 2014).

Pharmacokinetics and toxicity measurement study

Utilizing the online tools (http://www. swiss-adme.ch/ and http://lmmd.ecust.edu.cn/ admetsar2/) ADME/T analysis was carried on in this study. All selected compounds' drug similarity profile, pharmaco-kinetics, and physiochemical charac-teristics were also evaluated based on Lipinski's rules (Zhang and Wilkinson, 2007; Lipinski *et al*., 1997).

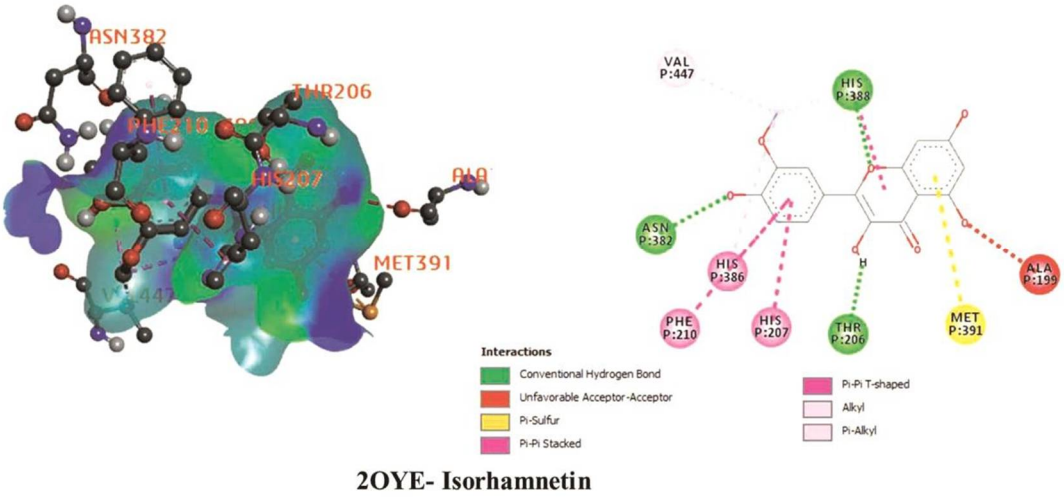
Results and Discussion

Molecular docking and ADME/T analysis of analgesic, anti-inflammatory, antipyretic, antidiarrheal and thrombolytic activities of selected phytoconstituents: This study demonstrated that the best docking score against cyclooxygenase-1 (PDB: 2OYE) is by isorhamnetin which is -12.9 (Table 1 and Figure 1). These compounds' docking scores were better than their respective commercially available marketized product. The ranking order based on the docking scores of analgesic effects against cyclooxygenase-1 was as followed: isorhamnetin > lupeol > quercetin > kaempferol > ibuprofen > betulin (Table 1 and Figure 1). The score of the docking interaction of the cyclooxygenase-2 (PDB: 6COX) and the selected compounds have been presented as follow: lupeol > betulin > kaempferol > isorhamnetin > quercetin > ibuprofen (Table 1 and Figure 2). In the antipyretic docking study, microsomal prostaglandin E synthase 1 (PDB: 4KY5) was docked against the selected metabolites and isorhamnetin secured the highest binding score (Table 1 and Figure 3). Isorhamnetin binds to the microsomal prostaglandin E synthase 1 receptor *via* a series of bonds: carbon-hydrogen bond (tyr80), pi-alkyl (val24) and scored highest binding affinity to the respective receptor In order to evaluate the possible antidiarrheal potentiality, all 5 specified metabolites within *D. pentagyna* were docked against muscarinic acetylcholine receptor (PDB: 5ZHP) where betulin showed the most promising activity with docking

Table 1. The docking score of screened phytochemical's binding at the active site of the selected proteins.

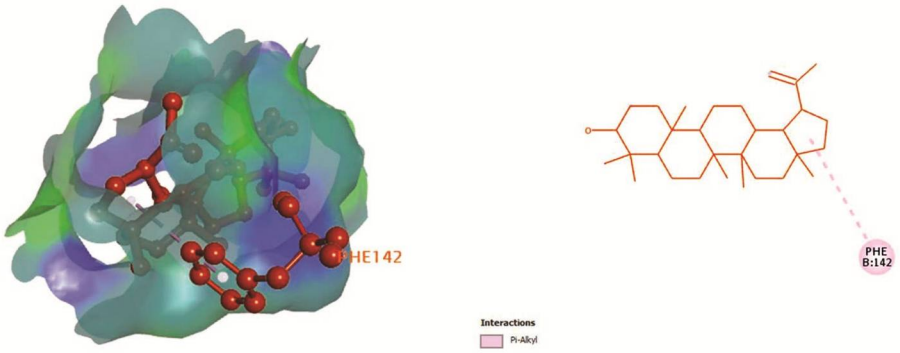
Compounds	Analgesic, Anti-Inflammatory and Anti-pyretic			Antidiarrheal	Thrombolytic
	2OYE	6COX	4YK5	5ZHP	1A5H
Kaempferol	-8.8	-8.7	-6.0	-6.2	-7.5
Quercetin	-9.0	-8.3	-5.9	-6.1	-7.1
Isorhamnetin	-12.9	-8.4	-6.9	-6.2	-7.2
Lupeol	-9.0	-10.4	-6.7	-7.9	-7.4
Betulin	-4.8	-9.1	-6.8	-11.8	-8.5
Standard drugs (Ibuprofen/Indomethacin/ Loperamide/Streptokinase)	-7.4	-6.4	-8.2	-7.1	-6.4

2OYE = cyclooxygenase-1, 6COX = cyclooxygenase-2, 4YK5 = microsomal prostaglandin E synthase 1, 5ZHP = muscarinic acetylcholine receptor, 1A5H = tissue plasminogen activator. Docking of standard drugs: ibuprofen against 2OYE and 6COX, indomethacin against 4YK5, loperamide against 5ZHP and streptokinase against 1A5H.



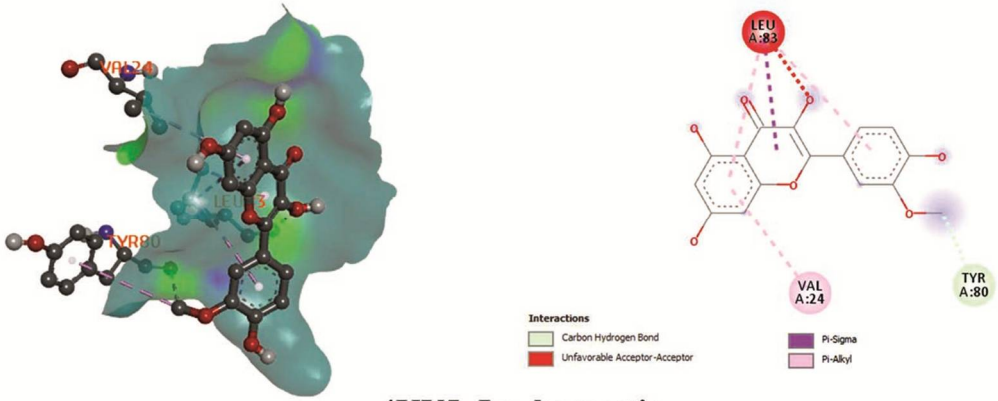
2OYE- Isorhamnetin

Figure 1. 3D and 2D representations of the best key interaction between cyclooxygenase-1 and selected phytochemicals (isorhamnetin).



6COX- Lupeol

Figure 2. 3D and 2D representations of the best key interaction between cyclooxygenase-2 and selected phytochemicals (lupeol).



4KY5- Isorhamnetin

Figure 3. 3D and 2D representations of the best key interaction between microsomal prostaglandin E synthase 1 and selected phytochemicals (isorhamnetin).

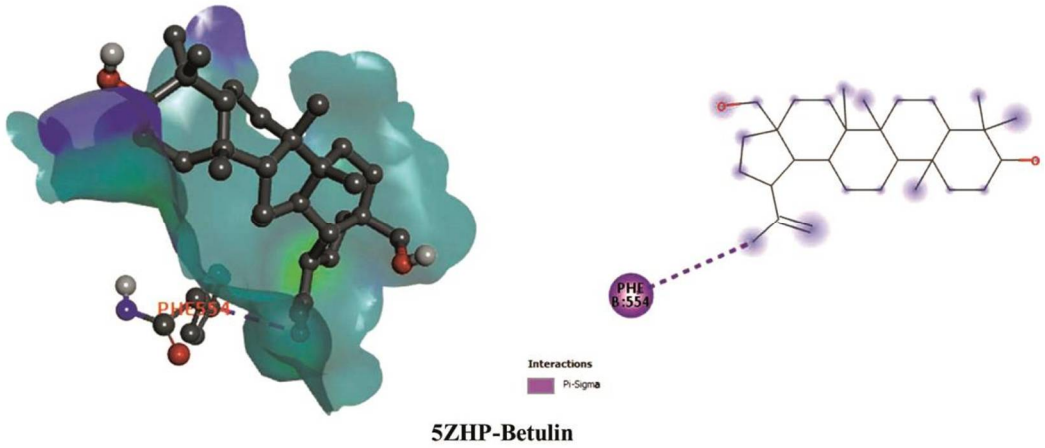


Figure 4. 3D and 2D representations of the best key interaction between muscarinic acetylcholine receptor and selected phytochemicals (betulin).

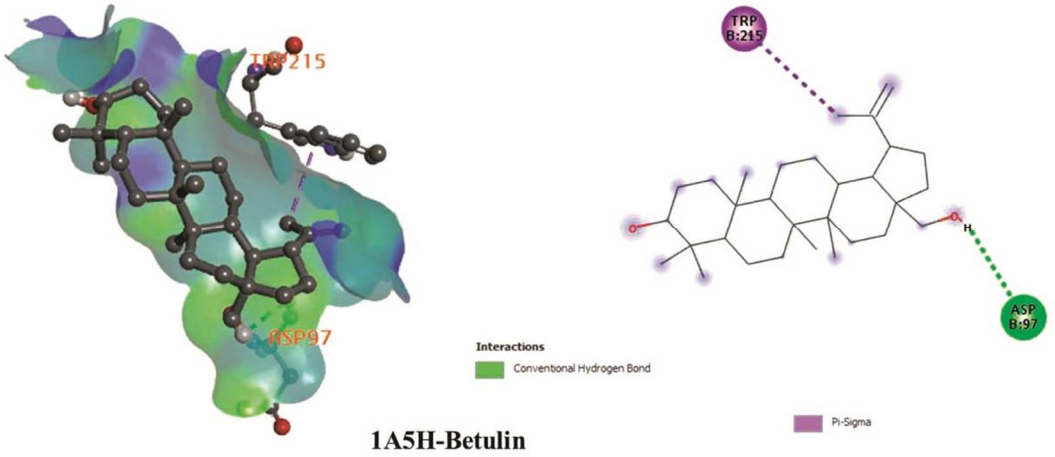


Figure 5. 3D and 2D representations of the best key interaction between tissue plasminogen activator and selected phytochemicals (betulin).

Table 2. Absorption, digestion, metabolism, excretion and toxicological (ADME/T) properties of the compounds for good oral bioavailability.

Compound name	PID	M.W	HBA	HBD	LogP	HIA	AM	CAR	PPB	AOT - kg/mol
Kaempferol	5280863	286.24	6	4	2.28	0.9881	0.7300	1.000	1.061	1.739
Quercetin	5280343	302.24	7	5	1.99	0.9833	0.9000	1.000	1.175	2.559
Isorhamnetin	5281654	316.26	7	4	2.29	0.9889	0.7300	1.000	1.259	2.84
Lupeol	259846	426.73	1	1	8.02	0.9930	0.7300	0.9714	1.017	3.852
Betulin	72326	442.73	2	2	7.00	0.9884	0.7500	0.9857	0.891	3.482

PID= PubChem ID, M.W= Molecular weight, HBA= H-bond acceptors, LogP= partition coefficient (lipophilicity), HBD= H-bond donors, HIA = Human Intestinal Absorption, AM= Ames mutagenesis, CAR= Carcinogenicity (binary), PPB= Plasma protein binding, AOT= Acute Oral Toxicity.

score of -11.8 (Table 1 and Figure 4). Betulin binds to the muscarinic acetylcholine receptor *via* pi-sigma (phe554) bond.The docking ranks between the tissue plasminogen activator (PDB: 1A5H) and the analyzed compounds have displayed that betulin placed the major phytoconstituent followed by kaempferol, lupeol, isorhamnetin, quercetin and streptokinase (Table 1 and Figure 5).

ADME/T analysis has been also performed during this *in silico* study. It can be perceived from Table 2 that, lupeol and betulin have disrupted one of the five parameters of Lipinski's law. The partition coefficient (lipophilicity) of lupeol and betulin are slightly higher than the normal range (less than 5) (Lipinski *et al*., 1997). On the other hand, rest of the compounds have been fulfilled all the Lipinski criterias suggesting that these phytocompounds can be assumed as bioavailable (Lipinski *et al*., 1997).

Computational analysis or molecular docking is frequently used to estimate interactions between ligands and targets along with gaining the deeper insights into the biological actions of bioactive compounds (Alam *et al*., 2021b). It also offers further comprehensions into the potential mechanisms of action and binding manners within the enzyme binding pocket (Alam *et al.*, 2021a). Negative binding reveals strong favorable ligand-receptor complex interactions (Emon *et al*., 2021b). The molecular docking revealed that there were strong binding affinities with the respective target proteins. From these findings, by establishing promising interactions with target proteins, it can be assumed that the recognized compounds can attribute to antidiarrheal and COX inhibitory actions of the plant extract. The highest docking score showed by phytocompounds for 2OYE and 6COX were -12.9 and -10.4, respectively which are greater than standard drugs and supported the actions of *D. pentagyna* against fever, pain and inflammation. Besides, highest docking score for 5ZHP and 1A5H were -11.8 and -8.5, respectively and supported antidiarrheal and thrombolytic potentials.

According to the Lipinski's rules, all five compounds revealed promising orally active drug-like characteristics. It can now be assumed that the metabolites will possess good oral bioavailability and can be regarded as a potential lead compound and further study is recommended to evaluate associated toxicity concerns extensively. Since the drugs used for the fever, pain, inflammation, diarrhea, and thrombosis are not economical and accessible to the greater section of the society, and also showed noteworthy side effects, the application of this study may be a boon for them (Emon *et al.*, 2021a).

Conclusion

According to the previous literature review, it can be concluded that the *D. pentagyna* possesses an elevated amount of phytoconstituents that might be bioactive and may be the justification of its widely popular folkloric use. Molecular docking study performed in this study also support the ethnobotanical usage of this plant. It also ascertained its safety profile based on ADME/T analysis. Though further lab based research work is still recommended to find the long term safety and toxicity measurement along with establishment exact mechanism of actions responsible for displayed pharmacological actions.

Declarations

All authors have read the manuscript and approved it for submission. No part of the manuscript has been published before nor is any part of it under consideration for publication at another journal.

Conflict of Interest

The authors state they have no conflicting interests.

Funding

This work was carried out with the help of all contributors. The authors' have taken no grant or funding for this project.

Availability of data and materials

All the findings have been inserted to this manuscript.

Authors' contributions

SA and NUE conceptualized and designed the work. SA, NUE and MSH wrote the manuscript. SA and NUE performed the computationsl analysis. MSH, TS and MAS did the data curation. MAR and MRH critically evaluated the manuscript. MAR drafted the final manuscript. MRH supervised the total work.

References

Alam, M.M., Emon, N.U., Alam, S., Rudra, S., Akhter, N., Mamun, M.M.R. and Ganguly, A. 2021a. Assessment of pharmacological activities of *Lygodium microphyllum* Cav. leaves in the management of pain, inflammation, pyrexia, diarrhea, and helminths: *In vivo*, *in vitro* and *in silico* approaches. *Biomed. Pharmacother*. **139**, 111644. DOI: https://doi.org/10.1016/j.biopha.2021.111644

Alam, S. and Haque, M.R. 2020. Phytochemical screening of *Colocasia gigantea* and *Colocasia affinis* (Family: Araceae) using ^{1}H-NMR and ^{13}C-NMR techniques. *BioRxiv*. DOI: https://doi.org/10.1101/2020.10.27.357590

Alam, S., Emon, N.U., Rashid, M.A., Arman, M. and Haque, M.R. 2020a. Investigation of biological activities of *Colocasia gigantea* Hook. f. leaves and PASS prediction, *in silico* molecular docking with ADME/T analysis of its isolated bioactive compounds. *BioRxiv*. DOI: https://doi.org/10.1101/2020.05.18.101113

Alam, S., Emon, N.U., Shahriar, S., Richi, F.T., Haque, M.R., Islam, M.N., Sakib, S.A. and Ganguly, A. 2020b. Pharmacological and computer-aided studies provide new insights into *Millettia peguensis* Ali (Fabaceae). *Saudi Pharm. J.* **28**, 1777. DOI: https://doi.org/10.1016/j.jsps.2020.11.004

Alam, S., Rashid, M.A., Sarker, M.M.R., Emon, N.U., Arman, M., Mohamed, I.N. and Haque, M.R. 2021b. Antidiarrheal, antimicrobial and antioxidant potentials of methanol extract of *Colocasia gigantea* Hook. f. leaves: evidenced from *in vivo* and *in vitro* studies along with computer-aided approaches. *BMC Complement. Med. Ther.* **21**, 1-12. DOI: https://doi.org/10.1186/s12906-021-03290-6

Alam, S., Sarker, M., Rahman, M., Afrin, S., Richi, F.T., Zhao, C., Zhou, J.R. and Mohamed, I.N. 2021c. Traditional herbal medicines, bioactive metabolites, and plant products against COVID-19: Update on clinical trials and mechanisms of action. *Front. Pharmacol.* **12**, 1248. DOI: https://doi.org/10.3389/fphar.2021.671498

Choi, J.H., Park, S.E., Kim, S.J. and Kim, S., 2015. Kaempferol inhibits thrombosis and platelet activation. *Biochimie* **115**, 177-186. DOI: https://doi.org/ 10.1016/j.biochi.2015.06.001

Dubey, P.C., Sikarwar, R.L.S., Khanna, K.K. and Tiwari, A.P. 2009. Ethnobotany of *Dillenia pentagyna* Roxb. in Vindhya region of Madhya Pradesh, India. *Nat. Prod. Radiance.* **8**, 546-548.

Emon, N.U., Alam, S., Rudra, S., Al Haidar, I.K., Farhad, M., Rana, M.E.H. and Ganguly, A. 2021a. Antipyretic activity of *Caesalpinia digyna* (Rottl.) leaves extract along with phytoconstituent's binding affinity to COX-1, COX-2 and mPGES-1 receptors: *In vivo* and *in silico* approaches. *Saudi J. Biol. Sci.* DOI: https://doi.org/10.1016/j.sjbs.2021.05.050

Emon, N.U., Alam, S., Rudra, S., Chowdhury, S., Rajbangshi, J.C. and Ganguly, A. 2020a. Evaluation of pharmacological potentials of the aerial part of *Achyranthes aspera* L.: *in vivo*, *in vitro* and *in silico* approaches. *Adv. Tradit. Med.*1-14. DOI: https://doi.org/10.1007/s13596-020-00528-5

Emon, N.U., Alam, S., Rudra, S., Riya, S.R., Paul, A., Hossen, S.M., Kulsum, U. and Ganguly, A. 2021b. Antidepressant, anxiolytic, antipyretic, and thrombolytic profiling of methanol extract of the aerial part of *Piper nigrum*: *In vivo*, *in vitro*, and *in silico* approaches. *Food Sci. Nutr.* **9**, 833-846. DOI: https://doi.org/10.1002/fsn3.2047

Emon, N.U., Kaiser, M., Islam, M., Kabir, M.F.I., Jamir, M., Uddin, M.A.J. and Islam, M.N. 2020b. Anxiolytic and thrombolytic investigation of methanol extract of *Piper nigrum* L. fruits and *Sesamum indicum* L. seeds. *J. Adv. Biotechnol. Exp. Ther*. ***3***, 158-164. DOI: https://doi.org/10.5455/jabet.2020.d121

Gandhi, D., Mehta, P. 2013. *Dillenia indica* Linn. and *Dillenia pentagyna* Roxb.: pharmacognostic, phytochemical and therapeutic aspects. *J. Appl. Pharm. Sci*. **3**, 134-142. DOI: 10.7324/JAPS.2013.31124

Gong, G., Guan, Y.Y., Zhang, Z.L., Rahman, K., Wang, S.J., Zhou, S., Luan, X. and Zhang, H., 2020. Isorhamnetin: A review of pharmacological effects. *Biomed. Pharmacother*. **128**, 110301. DOI: https://doi.org/10.1016/j.biopha.2020.110301

Guedes, I.A., de Magalhães, C.S. and Dardenne, L.E. 2014. Receptor–ligand molecular docking. *Biophys. Rev.* **6**, 75-87. DOI: 10.1007/s12551-013-0130-2

Herowati, R. and Widodo, G.P. 2014. Molecular Docking studies of chemical constituents of *Tinospora cordifolia* on glycogen phosphorylase. *Procedia Chem.***13**, 63-68. DOI: https://doi.org/10.1016/j.proche.2014.12.007

Huang, D. and Caflisch, A. 2011. The free energy landscape of small molecule unbinding. *PLoS Comput. Biol*. **7**, e1002002. DOI: https://doi.org/10.1371/journal.pcbi.1002002

Khatun, A., Rahman, M., Uddin, K.N., Ahsan, K., Shimu, S.N., Kobra, K., Shimu, S.A., Haque, W., Rahman, T., Jessy, T.H. and Akhter, F. 2016. Preliminary study on thrombolytic property of thirty six different extracts of eight Bangladeshi medicinal plants with folkloric relevance. *Orient. Pharm. Exp. Med.* **16**, 311-319. DOI: 10.1007/s13596-016-0247-5

Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **23**, 3-25. DOI: https://doi.org/10.1016/S0169-409X(96)00423-1

Maridass, M. and De Britto, A.J. 2008. Origins of plant derived medicines. *Ethnobot. Leaflet.* **2008**, Article 44. https://opensiuc.lib.siu.edu/ebl/vol2008/iss1/44

Parenti, M.D. and Rastelli, G. 2012. Advances and applications of binding affinity prediction methods in drug discovery. *Biotechnol. Adv.* **30**, 244-250. DOI: https://doi.org/10.1016/j.biotechadv.2011.08.003

Prasad, S.B., Rosangkima, G. and Rongpi, T. 2009. Studies on the ethnomedical value of *Dillenia pentagyna* Roxb. in murine model. *J. Pharm. Res.* **2**, 243.

Rudra, S., Sawon, M.S.U., Emon, N.U., Alam, S., Tareq, S.M., Islam, M.N. and Shakil, M. 2020. Biological investigations of the methanol extract of *Tetrastigma leucostaphylum* (Dennst.) Alston ex Mabb.(Vitaceae): *In vivo* and *in vitro* approach. *J. Adv. Biotechnol. Exp. Ther*. **3**, 216-224. DOI: https://doi.org/10.5455/jabet.2020.d127

Yang, Z., Lasker, K., Schneidman-Duhovny, D., Webb, B., Huang, C.C., Pettersen, E.F., Goddard, T.D., Meng, E.C., Sali, A. and Ferrin, T.E. 2012. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J. Struct. Biol.* **179**, 269-278. DOI: https://doi.org/10.1016/j.jsb.2011.09.006

Zhang, M.Q. and Wilkinson, B. 2007. Drug discovery beyond the 'rule-of-five'. *Curr. Opin. Biotechnol*. **18**, 478-488. DOI: https://doi.org/10.1016/j.copbio.2007.10.005