Phytochemicals from Bangladeshi Medicinal Plants Potentially Targeting Non-Structural Protein 2/Non-Structural Protein 3 Chimera of Dengue Virus: An *in silico* study

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ABSTRACT

In Bangladesh, the incidence and death rate of patients suffering from dengue infection are escalating. To date, there is no effective antiviral against dengue virus. Treatment regimen mostly includes symptomatic management of the disease. In this study, we performed molecular docking with 36 phytochemicals extracted from Bangladeshi medicinal plants namely *Averrhoa carambola* L., *Curcuma longa* L., *Nyctanthes arbor-tristis* L. and *Zingiber officinale* against dengue virus non-structural protein 2/non-structural protein 3 (NS2/NS3) chimera. The NS2/NS3 complex functions simultaneously as helicase/protease and/or RNA-tri-phosphatase. The compounds calceolarioside A, γ-elemene, β-sitosterol, β-elemene and curcuminoid-D exhibited high binding affinity (ranging from -8.3 to -7.2 kJ/mol) towards the selected protein and their drug-likeness was confirmed via ADMET analysis. Hence, these compounds have the potential to inhibit viral replication as indicated by computational calculation. However, further *in vitro* and *in vivo* studies can be undertaken to confirm such inhibitory properties of the reported phytochemicals.

Key words: ADMET analysis, Bangladeshi medicinal plants, dengue virus, molecular docking, non-structural protein 2/non-structural protein 3 chimera, phytochemicals,

INTRODUCTION

Dengue infection has become a worldwide problem and this disease has affected an estimated 2.5 billion people with an approximate 25,000 deaths annually.[1,2] Dengue virus (DENV) is a member of the genus *Flavivirus* of family Flaviviridae containing +ssRNA (genome). The DENV genome has a size of 11 kb and encodes a polyprotein.[3,4] The polyprotein precursor is translated by ORF and is cleaved into 10 viral proteins including three structural and seven non-structural proteins.[5,6] It is translated as NH2-C-prM-E-NS1- NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH. Capsid (C), premembrane (pRM), envelope protein (E) are structural proteins and the rest are non-structural protein.[7,8]

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Non-structural protein 3 (NS3) is the second largest encoded non-structural DENV protein and has a crystal structure which consists of a chemo-trypsin-like fold with two β-barrels consisting of a conserved catalytic triad (His51-Asp75-Ser135) at its N-terminal, helicase and RNA tri-phosphatase domains at C-terminal.[9] For maximum enzyme activity, β-barrel region of NS3 protease forms a complex with non-structural protein 2 (NS2) which act shields hydrophobic residues of NS3. Any interference in NS2/NS3 complex function results into halting of infection by DENV. Therefore, NS2/NS3 protein is a reliable target for drug candidates to inhibit dengue virus.[10,11]

Medicinal plants contain a variety of phytochemicals such as alkaloids, coumarins, flavonoids, furyl compounds, limonoids, organosulfur compounds, peptides, polyines, polyphenolics, terpenoids, thiophenes and saponins.

Nyctanthes arbor-tristis, is known by the local name Night-flowering jasmine. Nyctanthes is native to South Asia and Southeast Asia It is used for different therapeutic purposes, *viz.*, anthelmintic, anti-inflammatory, antipyretic, laxative and sedative effect.[12] *Averrhoa carambola* belongs to the family Oxalidaceae. It is native to Southeast Asia. The plant has been used since ancient times for remedy of coughing, hangovers, diabetes and arthralgia.[13] *Curcuma longa* belongs to the ginger family called Zingiberaceae.[14,15] The plant has rhizomes under the ground. *C. longa* has been used as a remedy for hundreds of years in the traditional Indian and folk medicine with the intention to cure a large variety of illnesses, such as, infectious diseases, inflammation and hepatic, gastric, and blood disorders. *Zingiber officinale* also known commonly as ginger is a flowering plant whose rhizome is widely used as a spice.[16] It also has numerous beneficial health effects and thus utilized in traditional herbal medicine formulation. *Z. officinale* is a herbaceous perennial that grows pseudostems annually.

Phytochemicals can be further developed for the purpose of producing lead compound to treat different types of diseases.[17] Phytochemicals not only perform defensive mechanism in plants but also mediate and reduce the severity of infections. These chemical entities provide their remedial utility by stopping viral entry and DNA/RNA replication against a wide range of viruses.

On the contrary to conventional methods of drug screening involving High Throughput Screening (HTS), virtual methods of drug screening is applied nowadays in order to obtain a speedy, cost-effective and efficient method of lead identification.[18] For this purpose, we selected medicinal plants from Bangladesh and their phytochemicals from the classes of alkaloids, coumarins, flavonoids, furyl compounds, limonoids, organosulfur compounds, peptides etc. Identifying phytochemical-based inhibitors against dengue virus proteins can pave the way to generate drug candidates to combat dengue viral infection. The objective of the current study is to identify effective chemical constituents, derived from Bangladeshi medicinal plants that can target NS2 / NS3 chimera of dengue virus. Since the protein complex has significant roleplay in the viral replication and survival mechanism, targeting it can successfully antagonize dengue viral infection inside host body.

MATERIALS AND METHODS

The overall step-by-step procedure of the study as depicted in figure 1, is described in the following sections.

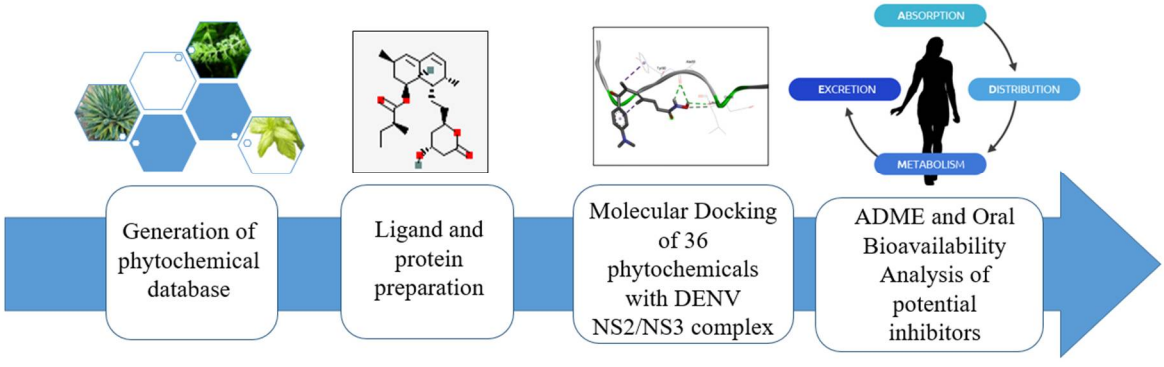

Figure 1. Methodology overview.

Generation of phytochemical database. To generate the database of phytochemicals, MPDB online database of Bangladesh medicinal plants was utilized. MPDB 2.0 database comprises more than 500 native medicinal plant from Bangladesh having information about active components, utilized parts, scientific, family and local names, and PubMed IDs of related articles. The aim of the database is to find

out the principal phytochemical agents, comprehend the underlying mechanism of these agents and discovering novel treatment methods for different diseases. The database organized the information regarding medicinal plants of Bangladesh into eight sections namely MPDB ID, scientific name, family name, local name, utilized part, ailment, active compound, reference (PMID).

In the database, several information regarding various Bangladeshi medicinal plants have been tabulated. Among the different tabulated information, one of the sections listed the main ailment for which the plant may be used. The keyword dengue fever was searched in the database. The database showed result for the plants that contained the keyword "dengue fever" in their ailment sections. Among 567 medicinal plants, four plants contained phytochemical that can be used to aid in dengue fever (Table 1). We targeted the main phytochemical constituents of these four plants namely *A. carambola*, *C. longa*, *N. arbor-tristis*, and *Z. officinale*.

Retrieval of structure data files of phytochemicals. The main phytochemical constituents of *A. carambola*, *C. longa, N. arbor-tristis*, and *Z. officinale* given in MPDB database were listed down. The compound name of each phytochemical was entered into PubChem Database to retrieve the structure data file of the chemical entity. The structure data files were further utilized for docking purpose. For *Z. officinale*, literature mining suggested that it contains 194 types of volatile oils, 85 types of gingerols, and 28 types of diarylheptanoids. Thus, 5 phytochemical constituents produced by *Z. officinale were selected* for molecular docking purpose. A total of 36 phytochemicals from four medicinal plants (*A. carambola*, *C. longa, N. arbor-tristis*, and *Z. officinale*) are used in the present study to identify effective small molecule against NS2/NS3 complex of dengue virus.

Preparation of protein structure for molecular docking purpose. For the present study, NS2/NS3 protein complex of dengue virus was selected because of the significant functional roleplay of the protein in viral replication and survival success inside host body. NS2/NS3 protease three-dimensional structure was collected from the Protein Data Bank (PDB) using PDB-ID: 2M9P (http://www.rcsb.org/pdb). The X-Ray crystallography protein structure contained attached water molecules and heteroatoms. The structure was optimized by disposing of water molecules and heteroatoms attached within PDB. The protein macromolecule was cleaned using BioVia Discovery Studio Visualizer software. This cleaned structure was used as receptor for docking studies.

Molecular docking of dengue NS2/NS3 complex with phytochemicals. The structure data files (SDFs) of phytochemicals were retrieved from PubChem database. The cleaned protein structure of dengue NS2/NS3 complex with PDBID 2M9P was opened in PyRx software. The ligands were separately opened in PyRx. Then the ligands were minimized in terms of energy. The minimised ligands were selected and the AutoDock Vina wizard was used for docking of these ligands with target protein. The docked structure with protein was viewed by BioVia Discovery Studio for the interactions between protein and ligand.

ADMET analysis of phytochemicals. The absorption, distribution, metabolism and excretion (ADME) profile of the medicinal plants were detected by SWISSADME website. The drug-likeness properties of the plants' active components were determined by Lipinski filter using.[19] The following parameters were noted down form the webserver MW, iLogP, ESOL Class, GI absorption, BBB permeant, Lipinski violations, Veber violations, bioavailability score, lead-likeness violations and synthetic accessibility. The parameters required for analyzing toxicity of the top five molecules,

ProTox-11 webserver was utilized. Pro Tox-11 is a virtual lab which can predict toxicities of small molecules. The canonical smiles and Pubchem names of the compounds were individually entered into the web server

Oral bioavailability prediction. The oral bioavailability parameters depicted in illustration for

the top compounds were collected from SWISSADME. For this purpose, we used the SMILE IDs (retrieved from PubChem database) of the top compounds in SWISSADME webserver and generated bioavailability radar illustrations.

Table 1. List of Bangladeshi medicinal plants containing potential phytochemicals that may work against dengue fever.

MPDB ID	Botanical name	Family	Local name	Utilized part	Effective in	Active compound	Reference(s)
403	*Nyctanthes arbor*-tristis L.	Oleaceae	Sheuli, Shefali, Shefali Phool	Leaf, Leaves	Tuberculosis, Rheumatism, Gonorrhea, Bone Fracture, Fever, Helminthiasis, Waist Pain, Bone Fractures, Pain Due To Working Or Falling Down, Burning Sensations In The Scalp Or Head	β-Sitosterol, Calceolarioside A	12
409	*Averrhoa carambola* L.	Oxalidaceae	Kamranga	Leaf, Bark, Fruit	Fever, Helminthiasis, Absence Of Menstruation, Bone Fracture, Jaundice, Bleeding From Hemorrhoids	2-dodecyl-6-methoxycyclohexa-2,5 -diene-1,4-dione	13
550	*Curcuma longa* L.	Zingiberaceae	Holud, Kancha Holud	Rhizome, Leaf	Bleeding During Menstruation, Cold, Dengue Fever And Malarial Fever, Helminthiasis, Abscess, Common Cold, Liver Malfunction, Loss Of Appetite, To Increase Memory, Headache, To Improve Eyesight	β-Pinene, α-Phellandrene, Limonene, Eucalyptol, Terpinolene, Citronellal, 2-nonanol, Cis-α-bergamotene, Trans-α-bergamotene, β-Elemene, Terpinene-4-ol, γ-Elemene, (e)-β-farnesene, α-Terpineol, α-Zingiberene, β-Bisabolene, (e,e)-α-farnesene, β-Sesquiphellandrene, Ar-curcumene, Geraniol, Bisabolone, Ar-turmerol, α-Turmerone, Bisabolol, Germacrone, Curlone, Ar-turmerone, , Curcuminoid D	14,15
556	*Zingiber officinale*	Zingiberaceae	Ada	Rhizhome, Rhizome	Pain While Moving The Fractured Part, Gastrointestinal Problem, Bloating, Acidity, Skin Diseases, Passing Of Semen With Urine, Snake Bite, Asthma, Endocrinological Disorder Or Diabetes, Bone Fractures, Fever (Including Dengue and Malarial Fever), Helminthiasis, Abscess, Common Cold, Coughs And Mucus	194 types of volatile oils, 85 types of gingerols, and 28 types of diarylheptanoids	16

RESULTS

Identification of Bangladeshi medicinal plants that may be effective in dengue viral disease. The number of dengue cases reported to WHO increased over 8 fold over the last twenty years, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032, affecting mostly younger age group. (https://www.who.int/). Phytochemicals extracted from medicinal plants of Bangladesh may be utilized to develop effective drug against dengue virus.[20]

At first, the plants that show cased beneficial therapeutic potential was searched for dengue fever. The terminology 'dengue' was entered in the search bar of the database. The database searched the ailment section of 567 plants using its default search mechanism. The database showed that four plants had the term dengue mentioned in their ailment sections. The plants are as follows: *A. carambola*, *C. longa*, *N. arbor-tristis* and *Z. officinale*. [12-16]

β-Sitosterol and calceolarioside A are constituents of *N. arbor-tristis*, 2-dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione is the constituent of *Averrhoa carambola* L., 2-nonanol, α-phellandrene, α-terpineol, α-zingiberene, (e)-α-farnesene, α-turmerone (e)-β-farnesene β-pinene, β-elemene, β-bisabolene, β-sesquiphellandrene, Ar-curcumene, ar-turmerone, bisabolone , citronellal, limonene, eucalyptol, terpinolene, *cis*-α-bergamotene, *trans*-α-bergamotene, terpinene-4-ol, γ-elemene, α-terpineol, α-zingiberene, (e)-α-farnesene, geraniol, bisabolone, germacrone, gurlone, curcuminoid D are constituents of both *C. longa* L. and *Z. officinale*. Alongside this, *C. longa* also contains eucalyptol. For *Z. officinale*, literature mining suggested that it contains 194 types of volatile oils, 85 types of gingerols, and 28 types of diarylheptanoids. Thus, we selected 5 phytochemical constituents produced from *Z. officinale* for molecular docking purpose.

Molecular docking of dengue NS2/NS3 complex with phytochemicals. We selected a functionally significant protein, non-structural protein 2/son-structural protein 3 complex was selected to carry out molecular docking studies. The NS2/NS3 protein complex is considered as the second largest non-structural dengue viral protein (Figure 2) which functions simultaneously as helicase, protease, and/or RNA tri-phosphatase.

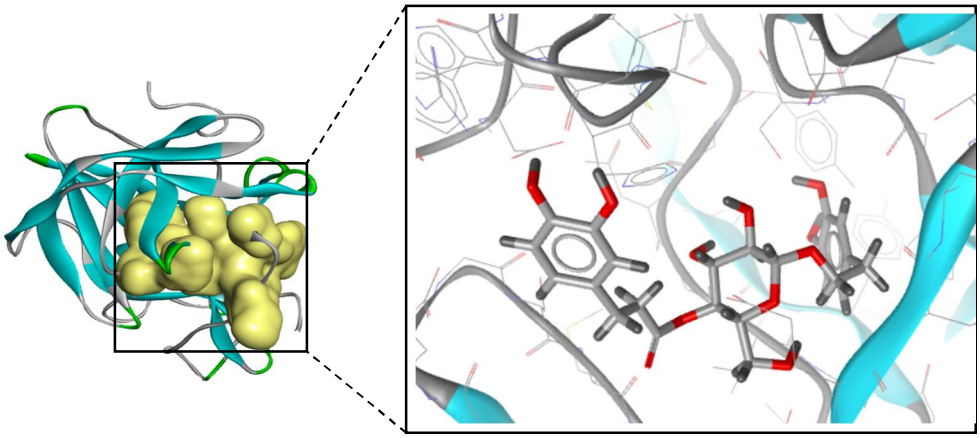

Figure 2. Three dimensional structure of DENV NS2/NS3 chimera (PDBID: 2M9P).

It was found that calceolarioside A (CID5273566), γ-elemene (CID6432312), β-sitosterol (CID222284), β-elemene (CID6918391) and curcuminoid D (CID101341351) exhibited high binding affinity towards the selected protein complex. The binding affinities of all 36 compounds are shown in table 2.

Table 2. The binding affinities of phytochemicals with DENV NS2/NS3 complex.

Ligand (Pubchem ID)	Binding affinity (kJ/mol)
Calceolarioside A (CID5273566)	-8.3
γ-Elemene (CID6432312)	-7.9
β-Sitosterol (CID222284)	-7.7
β-Elemene (CID6918391)	-7.5
Curcuminoid D (CID101341351)	-7.2
Ar-Turmerol (CID5315469)	-6.2
Germacrone (CID6436348)	-6.2
Ar-Turmerone (CID160512)	-6
α-Curcumene (CID92139)	-5.8
Alpha-Turmerone (CID4632996)	-5.8
Alpha-Zingiberene (CID11127403)	-5.7
Beta-Bisabolene (10104370)	-5.7
Alpha-Farnesene (CID5281516)	-5.7
Beta-Farnesene (CID5281517)	-5.6
6-Gingerol (CID3473)	-5.6
3-Gingerol (CID71103175)	-5.6
Beta-Sesquiphellandrene (CID12315492)	-5.5
6-Gingerdione (CID162952)	-5.5
6-Shogaol (CID5281794)	-5.5
Cis-alpha-Bergamotene (CID6429303)	-5.4
Bisabolone (CID91753614)	-5.3
Zingerone (CID31211)	-5.3
Alpha-Terpineol (CID17100)	-5.2
Curlone (CID196216)	-5.2
6-Paradol (CID94378)	-5.2
Terpinolene (CID11463)	-5.1
Bisabolol (CID1549992)	-5.1
2-Dodecyl-6-methoxycyclohexa-2,5-diene-1,4-dione (CID193384)	-5.1
Alpha-Phellandrene (7460)	-4.9
Terpinen-4-ol (CID11230)	-4.9
Geraniol (CID637566)	-4.9
Limonene (CID22311)	-4.8
Eucalyptol (CID2758)	-4.7
Beta-Pinene (CID14896)	-4.5
2-Nonanol (CID12367)	-4.4
Citronellal (CID7794)	-4.4

As seen from the analysis represented in table 2, the binding affinities of the ligands range from -8.3 to -4.4 kJ/mol. The top compound with highest binding affinity is calceolarioside A with docking score -8.3 kcal/mol. The compound is an active component of *N. arbor tristis* L. The compound with second highest binding affinity is γ-elemene with docking score -7.9 kcal/mol. The compound is an active component of *C. longa* L. The compound beta-sitosterol has shown docking score of -7.7 kcal/mol. The compound is an active component of *N. arbor tristis* L.

The catalytic triad for NS2/NS3 complex is comprised of His51, Asp75 and Ser135. The interaction between protein macromolecule and atoms of top three ligands were studied using BioVia discovery studio. The interactions are visualized and the images of interactions are depicted in figure 3.

Molecular interaction between calceolarioside A (CID5273566) and DENV NS2/NS3 complex reveals that the phytochemical interacts with four amino acid residues ALA227, TRP144, LYS135 and LEU137. The analysis of molecular interaction of γ-elemene (CID6432312) and β-sitosterol (CID222284) with DENV NS2/NS3 complex shows that the biomolecule interacts with multiple amino acid residues.

ADMET analysis and oral bioavailability prediction of phytochemicals. The top compounds obtained from docking analyses were further investigated for absorption, distribution, metabolism and excretion (ADME) profiling and oral bioavailability filtering parameters. The ADME parameters of small biomolecules are important factors to consider before these can be further developed into drugs for use inside human bodies at useful concentrations. Different parameters such as; molecular weight, iLOGP, ESOL Class, lead-likeness violations, GI absorption, BBB permeant, bioavailability score, Lipinski violations, Veber violations, and synthetic accessibility are enlisted in Table 3. The estimated toxicity properties of top 5 compounds are enlisted in table 4.

Curcumin D does not violate any of the Lipinski rule of five. γ-Elemene, β-sitosterol and β-elemene violates one aspect of RO5. However, one violation of RO5 is acceptable and these compounds can be said to follow Lipinski rule. Curcumin D belongs to the ESOL class of soluble drug. The bioavailability

scores of the compounds range from 0.17 to 0.55. The synthetic accessibility score refers to how easily a molecule can be synthesized. The synthetic accessibility score for these top compounds range from 3.28 to 6.3. None of these phytochemicals are Blood Brain Barrier (BBB) permeant. In terms of bioavailability attributes, gamma- and beta-elemene behaved ideally with almost similar physicochemical properties and the rest of the three compounds appeared almost ideal except showing one or two deviations (Figure 4).

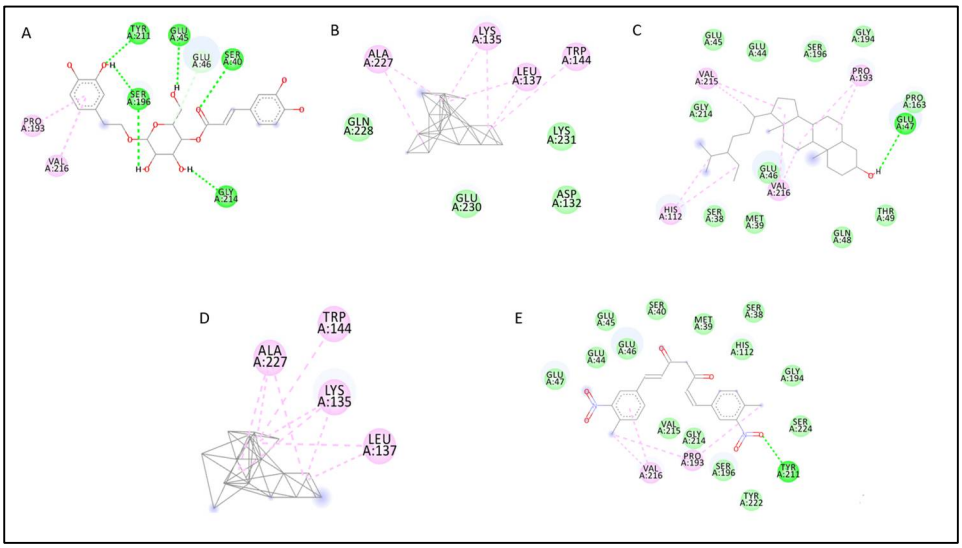

Figure 3. Molecular interaction between DENV NS2/NS3 complex and selected compounds. A. Calceolarioside A (CID5273566) B. γ-Elemene (CID6432312) C. β-Sitosterol (CID222284) D. β -Elemene (CID6918391) E. Curcuminoid D (CID101341351).

Table 3. The ADME properties of top 5 compounds.

Ligand	MW	iLogP	ESOL Class	GI absorp -tion	BBB permeant	Lipinski violations	Veber violations	Bioavailability Score	Lead-likeness violations	Synthetic accessibility
Calceolarioside A	478.45	2.19	S	Low	No	2	1	0.17	2	5.2
Gamma-Elemene	204.35	3.4	MS	Low	No	1	0	0.55	2	3.81
β-Sitosterol	414.71	4.79	PS	Low	No	1	0	0.55	2	6.3
β-Elemene	204.35	3.37	MS	Low	No	1	0	0.55	2	3.63
Curcuminoid D	394.38	2.11	MS	Low	No	0	0	0.55	3	3.28

S: soluble, MS: moderately soluble PS: poorly soluble

Table 4. The estimated toxicity properties of top 5 compounds.

Compound	Hepatotoxicity	Carcinogenicity	Cytotoxicity	Predicted LD50	Toxicity class
Calceolarioside A	Inactive	Inactive	Inactive	5000mg/kg	5
Gamma-Elemene	Inactive	Inactive	Inactive	5300mg/kg	5
Beta-Sitosterol	Inactive	Inactive	Inactive	890mg/kg	4
Beta-Elemene	Inactive	Inactive	Inactive	5300mg/kg	5
Curcuminoid D	Inactive	Inactive	Inactive	2300mg/kg	5

Class I: fatal if swallowed (LD50 ≤ 5), Class II: fatal if swallowed (5 < LD50 ≤ 50), Class III: toxic if swallowed (50 < LD50 ≤ 300), Class IV: harmful if swallowed (300 < LD50 ≤ 2000), Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000), Class VI: non-toxic (LD50 > 5000)

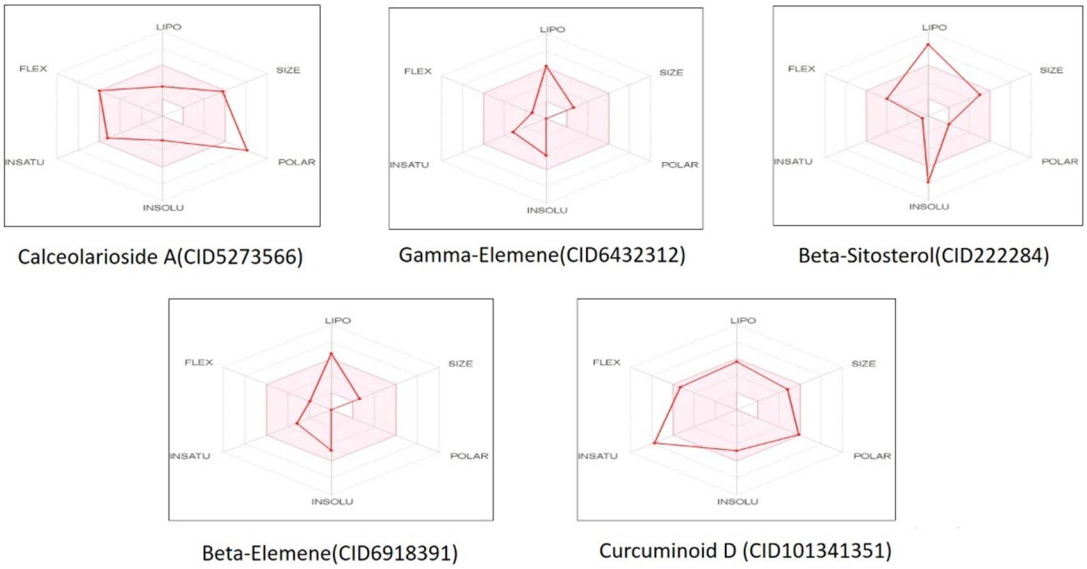

Figure 4. Bioavailability radar images of top 5 compounds.

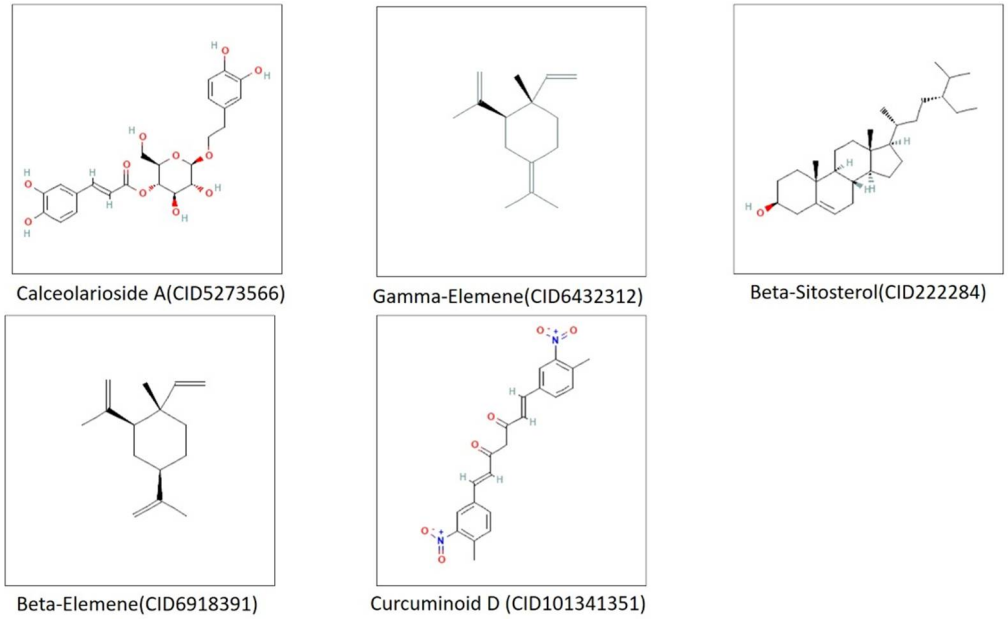

Figure 5. Chemical structures of top 5 compounds.

DISCUSSION

The evidence of the use of plants for medicinal purposes dates as far back as 60,000 years ago. Recently, the WHO estimated that 80% of people worldwide rely on herbal medicines for some aspects of their primary healthcare needs. Among 250,000 to 500,000 species of plants on earth, 35000 are used worldwide for medicinal purposes, 20000 medicinal plants are available in Indian subcontinent, some 3000 have shown potential in cancer, 1200 in diabetes and 2000 in pest control programs. Worldwide, mosquitos transmit disease to more than 700 million people. [20]

The binding affinity represents the affinity of the receptor macromolecule with the chemical structure of the ligand. Binding affinity is influenced by non-covalent intermolecular interactions such as hydrogen bonding, hydrophobic, Van der Waals forces, electrostatic interactions and between the two molecules. In addition, binding affinity between a ligand and its target molecule may be affected by the presence of other molecules. The docking score informs us of the tendency of the natural compound to bind to the NS2/NS3 complex and ultimately antagonize the viral activity. We found from our study that the compounds calceolarioside A (CID5273566), γ -elemene (CID6432312), β-sitosterol (CID222284), β-elemene (CID6918391) and curcuminoid D (CID101341351) exhibited high binding affinity towards the selected protein complex.

Lipinski's rule of five is also called Pfizer's rule of five. The rule of five (RO5) is a way to evaluate drug-likeness of a chemical compound. It can be used to understand if a certain chemical entity with biological activity has specific properties that would make it a potential orally active drug inside human bodies. Bioavailability is an estimate of the fraction of the initial dose of a drug that has access to either the site of action of the active ingredient or the fluid compartment from which the drug's targets have uninterrupted access. In terms of bioavailability attributes, gamma- and β-elemene behaved ideally with almost similar physicochemical properties and the rest of the three compounds appeared almost ideal except showing one or two deviations.[21]

Blood–brain barrier (BBB) endothelial cells make up a barrier which does not allow passage of solutes between blood and brain. Some BBB transport mechanisms mediate transcellular passage of solutes across the barrier either into or out of the brain. Drugs which are Blood Brain Barrier (BBB) permeant affects the central nervous system and may produce unwanted side effects. None of these phytochemicals are Blood Brain Barrier (BBB) permeant. The prediction of compound toxicities is a significant part of the drug design process. Computational toxicity estimations are faster than the determination of toxic doses in animal models. It can also help to reduce the amount of animal experiments.

ProTox-II incorporates molecular similarity, most frequent features, fragment propensities (fragment similarity based CLUSTER cross-validation) and machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, cytotoxicity, mutagenicity, hepatotoxicity, carcinogenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets[22]. The reported ligands show no hepatotoxicity or carcinogenicity.

CONCLUSION

The emergence of dengue viral infection among Bangladeshi population has raised serious concerns among health professionals and effective management protocol of the disease can be a solution to the concern that has arisen. Bangladeshi medicinal plants have long been used as source of therapeutic phytochemicals to aid in the ailment of several diseases. In the present study, computational drug discovery methodology has been exploited to search for effective natural biomolecule against dengue virus. In silico studies can reduce work and time-load by shortlisting the compounds with highest probability of binding with target macromolecule. The main constituents of the plants can be thoroughly checked for their efficacy against dengue virus to facilitate the development of new anti-viral medicine against dengue. The chemical constituents identified as potential inhibitors of dengue viral NS2/NS3 complex can be further studied in order to understand the probability of developing new drug candidates from these phytochemicals. Subsequent steps in the drug discovery methodology including laboratory based assays can be carried out in future to evaluate the efficacy of the reported ligands.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest**.**

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