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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND DOCKING STUDIES OF *ANDROGRAPHIS PANICULATA* AGAINST DENGUE FEVER

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ABSTRACT

Objective: The objective of this study is to evaluate gas chromatography-mass spectrometry (GC-MS) analysis and molecular docking studies of ethanol extract of *Andrographis paniculata* (Burm.f.) against type 2 dengue virus (DEN2).

Methods: The ethanol extract of *A. paniculata* compounds was identified by GC-MS analysis. These compounds were further analyzed for their activities against NS2B/NS3 protease of DEN2 by molecular docking studies.

Results: The nine compounds obtained from the ethanol extract of *A. paniculata* were characterized and docked. Among these nine compounds, vor-diazepam,3-[[N-hydroxymethyl]aminocarbonyloxy], dasycarpidan-1-methanol, acetate (ester), ethanol, 2-(9, 12-octadecadienyloxy)-, (Z,Z), and Gibb-2-ene-1,10-dicarboxylicacid4a,7-dihydroxy-1-methylene-1,4a-lactone,10 methylester,(1a,4aa,4ba,10a) were found to show better interaction energetically and also showed crucial interactions with the active site of NS2B/NS3 protease. Thus, *A. paniculata*is suggested to be a medicinally important plant to cure dengue fever.

Conclusions: From the result, it can be concluded that the crude extract of *A. paniculata* compounds positively inhibits the activities of NS2B/NSB protease of DEN2, thus by preventing the dengue viral infection. This strategy reflects a logical progression for an early stage drug discovery which can be used to identify new drug candidates.

Keywords: *Andrographis paniculata,* Ethanol, Gas chromatography-mass spectrometry, Molecular docking, NS2B/NS3 protease, Dengue virus.

INTRODUCTION

Dengue virus (DENV) belongs to the Flaviviridae family and is mosquito-borne human pathogen that can cause dengue hemorrhagic fevers (DHF) [1]. Dengue is currently an endemic problem in more than 100 countries around the world. There has been an estimate of 50 million infections per year globally with more than 2.5 billion people (or 40% of the world population) at risk, and it is one of the significant causes of mortality, especially in the tropical and subtropical regions [2]. There are four serotypes of DENV (DEN1, DEN2, DEN3, and DEN4) which are transmitted by the *Aedes aegypti* or more rarely by *Aedes albopictus* mosquito with DEN2 being the most prevalent. The virus in the electron micrograph consists of 40–50 nm sphere, surrounded by a lipopolysaccharide envelope that contains the 11-kb single-strand positive-sense RNA genome [3]. This RNA genome is consisting of 10,723 nucleotides that encodes three structural proteins (capsid [C], premembrane [prM], and envelope [E]) and seven non-structural protein (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [4]. *Flavivirus* replication is dependent on the correct cleavage of this polypeptide and requires both host cell and viral protease and two-component protease NS2B/NS3 [5,6]. The NS3 protein is a multifunctional enzyme that contains a N-terminal protease domain (N-terminal ~179 amino acids) and a C-terminal helicase domain (residues ~180–618) which includes nucleoside triphosphate and 5′-RNA triphosphate activities [7,8]. Analysis of virus sequence alignments indicates the catalytic triad of DEN2 protease is His51, Asp75, and Ser135, which is typical of a serine protease family [9]. The N-terminal region of NS3 chymotrypsin-like serine protease binds to an NS2B cofactor which is required to cleave the polyprotein. The protease activity is dependent on association with a hydrophilic region of 40 amino acids of the NS2B protein, which acts as a cofactor and actively participates in the formation of the S2 and S3 subpockets in the protease active site [10,11]. This NS2B-NS3 protease complex is required for viral replication [12]. Hence, the NS2B-NS3 protease complex serves to be a target for the development of antiviral drugs. Although there has been effort taken by many research groups, no vaccines or effective antiviral therapeutics are currently available against DENV infections [13]. Thus, it serves as a promising target for DENV antiviral drug development [14].

Many research groups worldwide are in search for antiviral therapeutic agents from natural plant products. However, this approach continues to furnish investigators with new and interesting findings. Several compounds from *Andrographis paniculata* (Burm.F.) wall. Ex Nees have shown inhibitory activity against dengue DEN2 protease. *A. paniculata* is an annual herbaceous plant and it belongs to family Acanthaceae (Acanthus). It is extensively cultivated in South Asian countries such as India and Sri Lanka [15]. In traditional medicine, *A. paniculata* is widely used to get rid of body heat, dispel of toxins from body. It prevents common cold, upper respiratory tract infections including sinusitis, and fever [16]. *A. paniculata* has been reported to exhibit various mode of biological activities *in vivo* as well as *in vitro*, namely, antibacterial, antiviral, anti-inflammatory, anti-HIV, anticancer, and immunomodulation [17-22]. Andrographolide is an extremely bitter substance extracted from the stem and leaves of *A. paniculata*. It is a major active constituent of *A. paniculata* [23]. In this study, we report on molecular docking studies of nine compounds identified from ethanol extract (Gas chromatography-mass spectrometry [GC-MS] analysis) against NS2B/NS3 protease of DEN2, using Glide XP module of Schrodinger 09. The aim of this study is to understand the interactions involved in binding of ethanolic compounds to NS2B-NS3 protease of DEN2 and also to gain insights into the experimental inhibition pattern. We propose that the information observed from this study will provide further understanding of the mechanism of the inhibition of NS2B-NS3 protease of DEN2and possibly enable researchers the design of antiviral drugs which inhibit DENV replication.

METHODS

Plant material

The leaves of *A. paniculata* were handpicked at the local area of Chennai in Tamil Nadu. Collected leaves were washed with distilled water and allowed to shade dry. The dried leaves were then mechanically grind and made into a fine powder [24].

Extraction

25 g of obtained powder was subjected to extract with 100% ethanol (500 ml) using Soxhlet extraction method. The crude extract was filtered, and excess of solvent was evaporated using rotary evaporator [25].

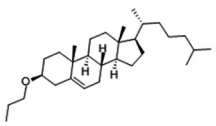
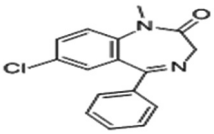
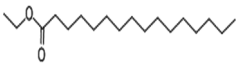
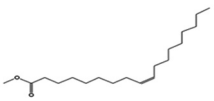
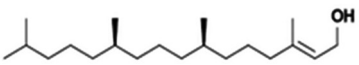
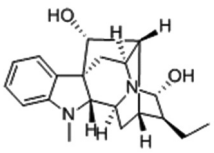
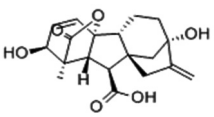
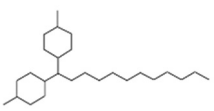
GC-MS analysis

The ethanolic extract of the leaves from *A. paniculata* was used for GC-MS analysis. This ethanolic extract was dissolved in HPLC grade methanol and subjected to GC-MS (JEOL GCMATE II). The sample was injected into a HP5 column (30 m length × 0.25 mm i.d × 0.25 µm film thickness), Agilent Technologies, consisting of 6890°N gas chromatography coupled with 5973 N mass spectrometer as detector [26]. For GC-MS detection, an electron ionization system was used with an ionization energy of 70eV. An inert gas, helium, was used as carrier gas and split ratio was 5:4. The injector temperature was set at 250°C and the column temperature at 280°C. The stepped temperature was programmed from 100°C for 2 min, then gradually increased to 280°C at 10°C/min, and kept there for 5 min. The total run time was 30 min. The diluted sample (1 µl) was injected manually in splitless mode. The MS scan range was from 35 to 1500 Da. The peaks were marked with retention time in the GC-MS (JEOL GCMATE II) of the ethanol extract of the leaves from *A. paniculata*. The extracts show the presence of nine compounds in the ethanolic extract as shown in Table 1.

Identification of components

The identification of chemical compounds in the crude extract was based on similarity of GC retention time and mass spectra (%) with the standards [27]. The mass spectra of the unknown components were compared with the spectrum of the known components stored in the National Institute of Standards and Technology (NIST) library. The details about their name, molecular formula, weight, and structure were ascertained from NIST library [28]. These compounds were further analyzed for their activities against NS2B/NS3 protease of DEN2 by molecular docking studies.

Table 1: Compounds from the ethanolic extract of andrographolide used for docking

Compound name	IUPAC name	Molecular formula	Molecular Weight (g/mol)	Structure of the compound
1	2,2,4-Trimethyl-3 (3,8,12,16-tetramethyl-heptadeca -3,7,11,15-tetraenyl) -cyclohexanol	$C_{30}H_{52}O$	428.73	
2	Vor-diazepam, 3-[[N-hydroxymethyl] aminocarbonyl oxy]	$C_{16}H_{13}CLN_2O$	284.74	
3	Ethanol, 2-(9, 12-octadecadienyloxy)-, (Z, Z)-	$C_{20}H_{38}O_2$	310.51	
4	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	
5	Octadecenoic acid, methyl ester	$C_{19}H_{34}O_2$	294.47	
6	Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol)	$C_{20}H_{40}O$	296.53	
7	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326.43	
8	Gibb-2-ene-1,10-dicarboxylic acid 4a, 7-dihydroxy-1-methylene-1,4a-lactone, 10-methyl ester,(1a, 4aa, 4ba, 10a)	$C_{19}H_{22}O_6$	346.37	
9	Cyclohexane, 1,1'-dodecylidenebis[4-methyl-	$C_{26}H_{50}$	362.67	

Experimental section

Receptor three-dimensional (3D) structure

The 3D structure of DEN2 NS2B/NS3 protease was downloaded from the protein data bank (PDB ID: 2FOM), in which the crystal structure was solved at 1.5 Å resolution. Protein preparation and refinement studies were performed on NS2B/NS3 protease using protein preparation module and the energy was minimized was done using default constraint of 0.3 Å RMSD and OPLS 2005 force field. (Schrödinger suite). The distances of the catalytic triad (i.e. between the carboxyl oxygen of Asp75 and His51 as well as the hydroxyl of Ser135 and imidazole ring of His51) were calculated to ensure that they have structural parameters close to the published results [29]. Ligand structures were built using Maestro v9.1 and geometrically minimized using OPLS_2005 force field by LigPrep module of Maestro 9.1 (Schrödinger suite) [30]. LigPrep produces a minimized low-energy 3D conformation of each input structure with various ring conformations, ionization states, and tautomer using various criteria including molecular weight and types of functional groups present.

Receptor grid generation

Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Ligand docking cannot be performed until the receptor grids have generated.

Docking method

Two types of docking methods have been used for molecular docking:

- Glide docking (grid-based ligand docking with energetics).
- Induced fit docking.

Glide docking

Nine compounds of ethanol extract were docked into the active site of the NS2B/NS3 protease (PDB: 2FOM) using glide with XP precision (Schrödinger). This methodology regards the structure of the protein as a rigid body but treats the ligand as a conformationally flexible molecule. Glide XP docking results of these nine compounds docked against NS2B/NS3 protease are shown in Table 2.

Induced fit docking

From glide docking results, we have further selected four ligands based on their glide energy and docking score. The selected ligand molecules were further docked against the P1 pocket with the catalytic triad (His51, Asp75, and Ser135) of NS2B/NS3 protease using induced fit docking of Schrodinger package. During docking, the ligands were optimized using OPLS or MMFF force field, thus changing its conformation to find the best fit that can closely fit to the catalytic triad and P1' pocket of NS2B/NS3 protease. The binding affinity of each protein and ligand complex was reported, and it is shown in Table 3.

RESULTS AND DISCUSSION

DENV is an important insect-borne pathogen with significant impact on global health. The viral NS2B/NS3 protease which mediates processing of the viral polyprotein precursor is therefore an important determinant of virus replication [31]. GC-MS analysis of ethanol extract showed nine compounds. Among these nine compounds, some of the compounds have already been reported with various activities. In particular, phytol is a diterpene, a member of the group of branched-chain unsaturated alcohols [32]. It has been reported to have antimicrobial, antioxidant, anti-inflammatory, anticancer, diuretic, and antinociceptive effects [33]. Diazepam is a benzodiazepine derivative with antianxiety, sedative, hypnotic, and anticonvulsant properties. Diazepam has been reported to be a potential inhibitor of gamma-aminobutyric acid [34]. Hexadecanoic acid, ethyl ester is a palmitic acid ester, and it has been reported as antioxidant, hemolytic,hypocholesterolemia, flavor, nematicide, anti-androgenic agents.

This present study aimed to understand the binding interactions between the nine compounds of ethanol extract and the NS2B-NS3 protease (PDB code 2FOM). Molecular docking was performed using glide XP module of Schrodinger 09. For each compound, the glide XP module of Schrodinger 09 program predicted several similar bound conformations that had energy differences. On examination and comparison of all the ligands, four ligands were obtained top- scored conformations, as it was apparent based on their low glide energy. In these conformations, interactions were between catalytic or P1 pocket residues of the active site and functional groups of the compounds. Significant interactions occurred between hydroxyl and nitro groups of the compounds and conserved residues that constituted the catalytic triad (His51, Asp75, and Ser135) and P1 pocket (TRP- 83, LEU-85, Gly151, and Gly153) of the protease. Compound 2 bound to the active site with docking score of −4.69 and a glide energy score of −40.22 Kcal/mol. The hydroxyl group on the left side of Compound 2 formed hydrogen bond interaction with the side chain of carboxyl group of Asp75 (2.7Å). An additional interaction occurred with P1 pocket residues of Gly151 and Gly153 (Fig. 1). The hydrogen bond interaction between the carboxyl group of extract compound 3 with P1 pocket residues of TRP-83, and LEU-85 (Fig. 2). Compound 3 bound with the glide energy of −43.16 kcal/mol and a docking score of −6.33. Favorable contact was predicted between nitro group of extracted compound 7 to form hydrogen bond interaction with the side chain of hydroxyl group Ser135 (2.9 Å) as shown in Fig. 3. The hydroxyl group of compound 8 has a hydrogen bond interaction 3 with carboxyl group of

Table 2: Glide XP docking results of ethanolic extract compounds docked with NS2B/NS3 protease

Compound name	Docking score	Glide energy (Kcal/mol)
Compound-1	−2.42	−29.30
Compound-2	−4.69	−40.22
Compound-3	−6.33	−43.16
Compound-4	−2.55	−32.88
Compound-5	−3.25	−30.20
Compound-6	−2.79	−31.04
Compound-7	−3.52	−33.88
Compound-8	−4.11	−32.84
Compound-9	−2.52	−29.71

Table 3: Induced fit docking results of ethanolic extract compounds docked with NS2B/NS3 protease

Compound name	Docking score	Glide energy (Kcal/mol)	Hydrogen bond interactions D-H...A	Distance (Å)
Compound -3	−6.33	−43.16	(TRP-83) N-H.O	3.1
			(TRP-83) N-H.O	3.0
			O-H.O (LEU-85)	2.7
Compound -2	−4.69	−40.22	O-H.O (ASP-75)	2.7
			N-H.O (GLY-151)	3.0
			(GLY-153) N-H.O	2.9
Compound -7	−3.52	−33.88	N-H.O (SER-135)	2.9
Compound -8	−4.11	−32.84	O-H.O (ASP-75)	2.8
			(HIS-51) N-H.O	3.2
			(GLY-153) N-H.O	3.1

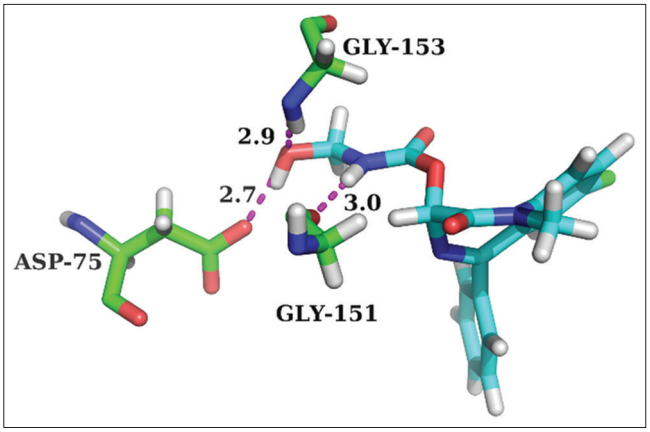


Fig. 1: Pymol view of the interaction between ethanol extract of compound 2 and NS2B/NS3 protease

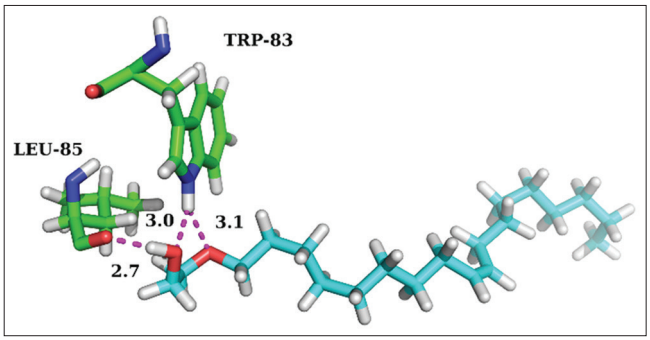


Fig. 2: Pymol view of the interaction between ethanol extract of compound 3 and NS2B/NS3 protease

the catalytic residue Asp75 (2.8 Å), and the carboxyl group of compound 8 occurred hydrogen bond interaction with imidazole ring of His51 (3.2 Å). An additional P1 pocket residue of hydroxyl group of Gly153 has a hydrogen bond with compound 8 (Fig. 4). Docking interaction between compound 8 and NS2B-NS3 protease has a dock score of −4.11and glide energy of −32.84.

CONCLUSION

A. paniculata, generally known as "king of bitters," could be potentially developed as an anti-DENV agent. In the present study, molecular docking was carried out with andrographolide derivatives against NS2B/NS3 protease. The compounds obtained from were docked by induced fit method against the catalytic triad (His51, Asp75, and Ser135) and P1 pocket (TRP-83, LEU-85, Gly151, and Gly153). Compound 2 (vor-diazepam 3-[[N-hydroxymethyl]aminocarbonyloxy]), compound 3 (ethanol, 2-(9, 12-octadecadienyloxy)- (Z,Z)-), compound 7 (dasycarpidan-1-methanol, acetate (ester)), and compound 8 gibberellic acid [Gibb-2-ene-1,10-dicarboxylicacid4a,7-dihydroxy-1-methylene-1,4a-lactone,10-methylester,1a,4aa,4ba,10a)] were found to be good with low energy value and showed crucial interactions with active site of NS2B/NS3 protease. Thus, *A. paniculata* is suggested to be a medicinally important plant to cure dengue fever. Therefore, these compounds positively inhibit the interaction of DEN2 virus with NS2B/ NSB protease preventing the dengue viral infection. This strategy reflects a logical progression for early-stage drug discovery that can be used to successfully identify new drug candidates.

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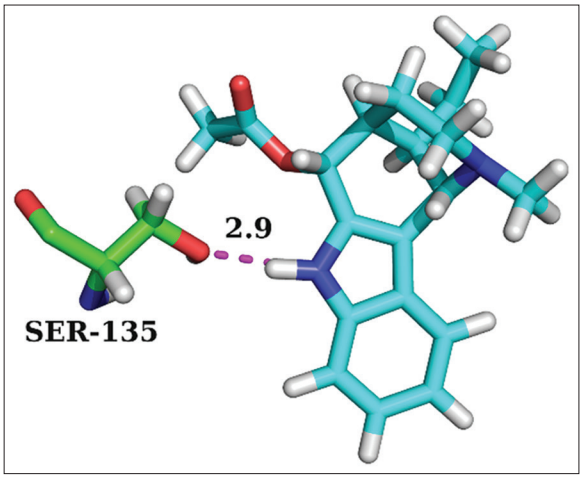

Fig. 3: Pymol view of the interaction between ethanol extract of compound 7 and NS2B/NS3 protease

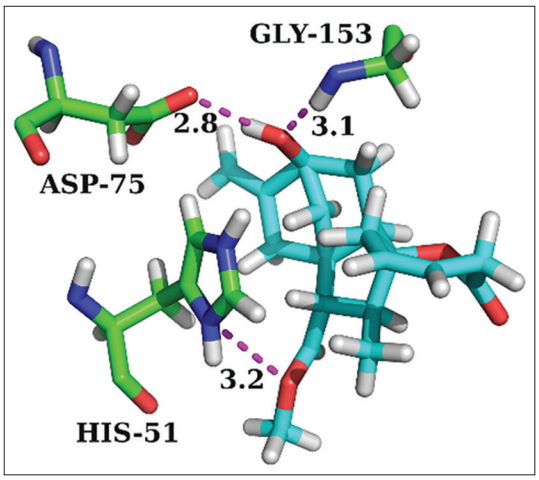

Fig. 4: Pymol view of the interaction between ethanol extract of compound 8 and NS2B/NS3 protease

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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