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Molecular docking analysis of bioactive compounds of *Senegalia rugata* against DENV-3 NS5 and DENV-1 NS2B/NS3 proteins of Dengue Virus

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Abstract
Dengue virus (DENV) is a major global health concern, infecting 100-400 million people annually, with around 96 million developing mild to severe clinical symptoms. In this study, phytochemicals from *Senegalia rugata* were screened to identify compounds with high binding affinity against the non-structural proteins NS5 and NS2B/NS3 of dengue virus. Methyl palmitate showed the highest binding affinity to NS5 (-6.2 kcal/mol), followed by geranylacetone (-5.6 kcal/mol), with 2D interactions revealing that both compounds interact with the target through van der Waals, hydrogen, and alkyl bonds. Methyl salicylate exhibited the highest binding affinity to NS2B/NS3 protease (-5.4 kcal/mol), forming hydrogen and van der Waals interactions. The binding of methyl palmitate to NS5 and methyl salicylate to NS2B/NS3 indicates the potential of these compounds as inhibitors of these targets. Further in vitro and *in vivo* validation is necessary, to exploit the potential of these compounds as a drug candidate for the treatment of dengue.

Keywords: *Senegalia rugata*, dengue virus, molecular docking, phytochemicals

1 Introduction
Dengue is a mosquito-borne disease transmitted by mosquitoes belonging to the *Aedes* genus, especially *Aedes aegypti* and *Aedes albopictus*. The number of people at the risk of contracting the disease ranges from 100 to 400 million annually, with around 96 million developing mild to severe clinical symptoms. The rapid increase in dengue cases, along with the lack of a specific drug, has contributed to its worldwide spread, affecting more than 100 countries and putting billions of lives at risk each year (World Health Organization, 2014) [32]. When the female *Aedes* mosquito feeds on an infected person, the virus replicates in the midgut of mosquitoes, eventually spreading to other tissues, including the salivary glands (Salazar *et al.*, 2007) [27]. There are predominantly four serotypes of dengue virus, namely DENV-1, DENV-2, DENV-3, and DENV-4, which stand as a major barrier in developing dengue vaccines. The dengue virus successfully multiplies within the host and produces proteins required for the formation of new viral particles. The DENV virus contains a single-stranded RNA inside a lipid bilayer, encircled by glycoproteins (Noble *et al.*, 2012) [18].
The key protein that plays a pivotal role in producing the new DENV viral particles includes the DENV NS2B-NS3 protease. The functional catalytic triad of the protein contains His-51, Asp-75, and Ser-135, found in the N-terminal region. The protease can be inhibited either by targeting its active site (Schüller *et al.*, 2011) [28] or by preventing the association of NS3pro with its cofactor NS2B (Phong *et al.*, 2011) [21]. Another 900 kDa non-structural protein, NS5, plays a crucial role in viral replication. This protein possesses an N-terminal methyltransferase activity and a C-terminal RNA-dependent RNA polymerase activity that aids in viral replication. As these regions are conserved among flaviviruses, NS5 has become a new target for drug development against DENV virus (Lim *et al.*, 2015) [14].
Since ancient times, plant extracts have been traditionally investigated for their therapeutic properties, including antiviral potential. As certain diseases like dengue and chikungunya still

lack an approved vaccine, ongoing research aims to identify molecules capable of inhibiting the replication or virulence of viruses. Numerous plant extracts have been evaluated for their antiviral activity against the dengue virus, with some exhibiting encouraging outcomes in suppressing the virus or easing disease manifestations. For instance, papaya leaf extract has proven to be an efficient natural product in increasing the platelet count in patients infected with dengue (Ahmad *et al.*, 2011) [4]. It was also found that the plant extracts of *Quercus lusitanica* showed efficient reduction in the replication of dengue virus (Rahman *et al*., 2006) [24].

The bioactive molecules responsible for antiviral properties include alkaloids, aurones, terpenoids, fatty acids and esters, phenolics, chalcones, flavonoids, lignans, isoflavonoids, coumarins, xanthones, quinones, and others (Powers & Setzer, 2016) [22]. Collectively, these compounds are referred to as phytochemicals. These compounds are generally produced by plants as secondary metabolites. Phenolics are one of the most diverse groups of phytochemicals, characterized by the presence of aromatic rings. They have been extracted from diverse plant sources belonging to Fabaceae (Sobeh *et al.*, 2016) [30], Zygophyllaceae (Lorenzo *et al.*, 2021) [16], Sapindaceae (Rakariyatham *et al.*, 2020) [25], Vitaceae (Acero *et al.*, 2025) [3], Crassulaceae (Singab *et al.*, 2011) [29], Punicaceae (He *et al.*, 2011) [10], and Rubiaceae (Jaiswal *et al.*, 2014) [11]. Previous studies reported the antiviral properties of phenolic compounds like rutin, with an IC_{50} value of 362.68 µg/mL. Viruses like DENV, Zika, etc., depend on the lipid metabolism of the host for successful replication. The non-structural proteins, including NS2B/NS3 and NS5, require lipid-rich membrane platforms for proteolytic processing and RNA synthesis. Some fatty acids have the potential to exert antiviral activity by disrupting the viral envelope. For instance, alpha-linolenic acid (ALA) has been shown to have promising antiviral activity against DENV-2 virus. Alpha-linolenic acid exerts its antiviral activity by disrupting the viral envelope and impairing early stages of DENV-2 infection (Feng *et al.*, 2023) [9]. Other phytochemicals like terpenoids, lignans, flavonoids, alkaloids, etc., have also been identified as efficient phytochemicals against various viruses (Jassim & Naji, 2003) [12].

In silico techniques, like molecular docking, play a remarkable role in predicting the interaction between plant-derived bioactive compounds and specific biological targets, such as proteins or enzymes. Plants produce a wide variety of compounds with unique chemical structures that are not commonly found in synthetic compound libraries. Docking allows researchers to explore the potential of these natural compounds in targeting diseases that are currently difficult to treat with existing drugs. By identifying novel interactions, docking can lead to the discovery of new classes of drugs with innovative mechanisms of action. By understanding these interactions, researchers can identify potential drug candidates more efficiently, saving both time and resources compared to traditional methods.

1.2 NS5 protein

The non-structural protein 5 (NS5) being the largest (900 amino acids) and most crucial protein that aids in viral replication. It consist of MTase and RdRp domains having inter-domain interface, that are stabilized by polar bonding between the residues present in both domains (Zhao *et al.*, 2015) [34]. The RdRp domain exhibits RNA dependent RNA polymerase activity responsible for the synthesis of viral RNA, and the MTase domain possess methyltransferase activity results in capping of viral RNA, resulting in recognition by the ribosomal machinery of the host (Bhatnagar *et al.*, 2021) [6].

1.2 NS2B/NS3 protein

The enzyme NS3 and its cofactor NS2B acts as a complex that plays a crucial role in replication of DENV-1 virus and in processing of viral polyprotein. The crystal structure of NS3 have been well elucidated (Luo *et al.*, 2008) [7]. NS3 is a protease with enzymatic function, having two major domains namely RNA helicase domain and protease domain. The catalytic domains found on the N-terminus of the protease and the substrate binding domain contains sequences similar to that of serine proteases. The aspartic acid residue found in the binding pocket of the substrate binding domain gives its binding specificity. NS2B is a 14 kDa protein containing 131 amino acids. It consist of hydrophobic (part of transmembrane domain) and hydrophilic domains, in which the hydrophilic domain plays an integral role in activation of NS3 protein. The beta-barrel present in the centre of the hydrophilic domain spirals the beta-barrel of NS3 protein imparting stability of the complex (Erbel *et al.*, 2006) [8].

2. Materials and methods

2.1 Bioactive compounds from *Senegalia rugata* and ligand preparation

In this study, the bioactive compounds identified by Balavignesh *et al.* (Balavignesh *et al.*, 2013), was used to dock with the non-structural proteins of dengue- DENV-3 NS5 and DENV-1 NS2B/NS3. The compounds include tetradecanoic acid, linolenic acid, methyl palmitate, methyl linoleate, cis-Linalool oxide, palmitic acid, geranylacetone, phenyl acetaldehyde, methyl salicylate, 5-Methyl-2-furfural, furfural, and trans-Linalool oxide. Tetradecanoic acid is a saturated fatty acid with proven antimicrobial properties (Abubakar & Majinda, 2016) [2]. The 3D structures of the ligands were downloaded from pubchem in (.sdf) format. The software openbabel, version 2.4.1, was used to convert (.sdf) format to (.mol) format (O'Boyle *et al.*, 2011) [19].

2.2 Protein preparation

The structure of two proteins, NS5 and NS2B/NS3, were obtained from Protein Data Bank (PDB) with accession codes 5JJR (DENV-3) and 3L6P (DENV-1) respectively. These proteins were prepared for docking in BIOVIA Discovery Studio 2001, after removing water molecules, hydrogen atoms. Software algorithm is used to predict the active sites of the protein. The molecular docking analysis was performed using Autodock Vina 1.5.7 (Trott and Olson, 2010). The docked ligand-protein was visualized in BIOVIA Discovery Studio 2001.

3. Results: Molecular docking is used for analysing the binding interactions and binding affinities of bioactive compounds with target protein. In this study 12 bioactive compounds were docked with two DENV non-structural proteins - DENV-3 NS5 and DENV-1 NS2B/NS3.

3.1 Binding affinity of phytochemicals from *Senegalia rugata* on NS5 and NS2B/NS3 proteins

Docking of the 12 phytochemicals ***Se****negalia rugata* with

dengue NS5 and NS2/NS3 proteins revealed a range of binding affinities. The highest binding affinity was observed for methyl palmitate, which bound most effectively to NS5 (−6.2 kcal/mol) and moderately to NS2/NS3 protein (−5.4 kcal/mol) (Table 1.). Geranylacetone showed nearly similar affinities for NS5 and NS2B/NS3, with binding energies of −5.6 kcal/mol and −5.2 kcal/mol, respectively. Tetradecanoic acid showed moderate binding affinity toward NS5 (−5.3 kcal/mol) but exhibited weaker interactions with NS2B/NS3 (−4.1 kcal/mol). cis-Linalool oxide exhibited same binding affinity of -5.3 kcal/mol with both the proteins. Phenylacetaldehyde exhibited binding affinities of −5.3 kcal/mol and −4.7 kcal/mol toward NS5 and NS2B/NS3, respectively. 5-Methyl-2-furfural showed stronger binding affinity toward NS5 (−5.3 kcal/mol) but weaker interaction with NS2B/NS3 (−4.3 kcal/mol). Methyl salicylate exhibited higher affinity for NS2/NS3 (−5.6 kcal/mol) compared to NS5 (−4.7 kcal/mol). The trans-Linalool oxide displayed binding affinities of −5.5 kcal/mol for NS2/NS3 and −5.2 kcal/mol for NS5. Linolenic acid was bound nearly similar to both proteins, with affinities of −5.2 kcal/mol with NS2B/NS3 protein and -5.1 kcal/mol with NS5 protein. Methyl linoleate exhibited a binding affinity of −5.1 kcal/mol toward NS2B/NS3 and slightly lower affinity toward NS5 (−4.9 kcal/mol). Palmitic acid exhibited a binding affinity of -5.1 kcal/mol with NS5, whereas it had a binding affinity of -4.1 kcal/mol with NS2B/NS3 protein. The weakest binding was observed for furfural (−3.9 kcal/mol with NS2/NS3 and −4.1 kcal/mol with NS5). Overall, methyl palmitate (−6.2 kcal/mol) and methyl salicylate (−5.6 kcal/mol) displayed the strongest binding affinity towards NS5 and NS2B/NS3 proteins respectively (Table 1.)

Table 1. Docking of phytochemicals from *Senegalia rugata* with DENV-3 NS5 and DENV-1 NS2B/NS protein

Sl. No.	Ligands	Binding affinity (kcal/mol)	
		NS5	NS2B/NS3
1	Methyl palmitate	-6.2	-4.4
2	Geranylacetone	-5.6	-5.2
3	Tetradecanoic acid	-5.3	-4.1
4	cis-Linalool oxide	-5.3	-5.3
5	Phenyl acetaldehyde	-5.3	-4.7
6	5-Methyl-2-furfural	-5.3	-4.3
7	trans-Linalool oxide	-5.2	-5.5
8	Linolenic acid	-5.1	-5.2
9	Palmitic acid	-5.1	-4.1
10	Methyl linoleate	-4.9	-5.1
11	Methyl salicylate	-4.7	-5.6
12	Furfural	-4.1	-3.9

3.2 NS5 protein structure and interaction with ligands

The three-dimensional structure of the dengue virus (DENV)-3 NS5 protein (PDB ID: 5JJR) was modeled and visualized using BIOVIA Discovery Studio 2001. The structure displays two distinct functional domains: the N-terminal methyltransferase (MTase) domain and the C-terminal RNA-dependent RNA polymerase (RdRp) domain, connected via a flexible linker. The MTase domain is involved in viral RNA capping and methylation, while the RdRp domain adopts a characteristic right-hand conformation essential for RNA synthesis. The ribbon representation in colour, highlights the domain organization and spatial arrangement, providing insight into the multifunctional nature of NS5 (Fig. 1.).

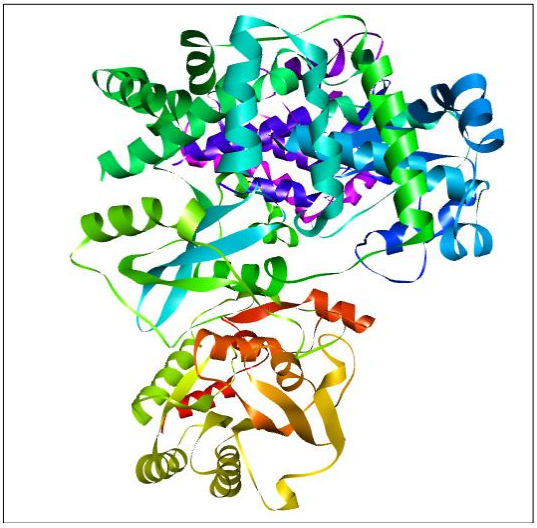

Fig 1: Ribbon diagram of dengue virus NS5 protein generated using BIOVIA Discovery Studio 2001

To illustrate the binding modes, 3D and 2D docking interaction diagrams were constructed for selected phytochemicals against NS5 protein, that exhibited higher binding affinities, among the ligands tested. The 3D models demonstrated stable orientation of the ligands (represented in light blue colour) within the active site pocket, while the 2D interaction maps detailed the key non-covalent interactions, including hydrogen bonding, van der Waals forces, alkyl, and π-π/π-alkyl contacts with crucial active-site residues.

3.3 Methyl palmitate

The 3D representation highlights the ligand, methyl palmitate, embedded within the protein's binding cavity, surrounded by hydrogen bond donor and acceptor regions, as well as hydrophobic patches (Fig. 2a). The 2D map reveals multiple stabilizing interactions, including carbon hydrogen bonds with ARG540 and GLY260, and van der Waals interactions with residues such as GLY93, ASP256, GLY258, ALA259, LEU257, GLU356, and LYS300, etc., supporting ligand stabilization within the active pocket (Fig. 2b). Additionally, hydrophobic interactions, including alkyl and π-alkyl contacts with TYR119, ARG361, PRO363, and ALA266, provide further nonpolar stabilization. The docking analysis yielded a binding affinity of −6.2 kcal/mol for methyl palmitate, indicating favourable and stable ligand-protein interactions.

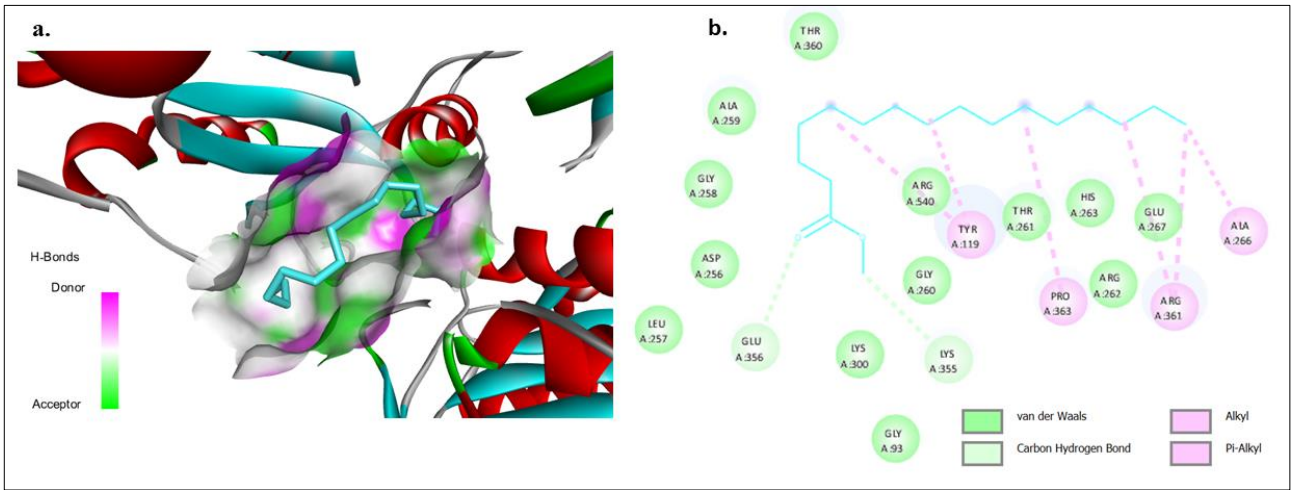

Fig 2: Interactions of methyl palmitate with the NS5 protein: (a) 3D interaction and (b) 2D interaction.

3.4 Geranylacetone: The 3D visualization shows geranylacetone fitted well within the binding pocket, forming hydrogen bonds and hydrophobic interactions, with donor and acceptor sites highlighted in magenta and green (Fig. 3a). The 2D image revealed a strong hydrogen bond interaction with ARG540, which likely stabilizes the ligand within the active site. Van der Waals interactions were observed with residues such as GLY93, ASP256, GLY258, ALA259, GLY260, THR360, etc., contributing to structural complementarity (Fig. 3b). Additionally, alkyl and π-alkyl interactions (hydrophobic) with LYS355, GLU356, LEU257, and TYR119, were observed, suggesting additional stabilization via non-polar interactions. Geranylacetone exhibited a docking score of -5.6 kcal/mol for NS5 protein.

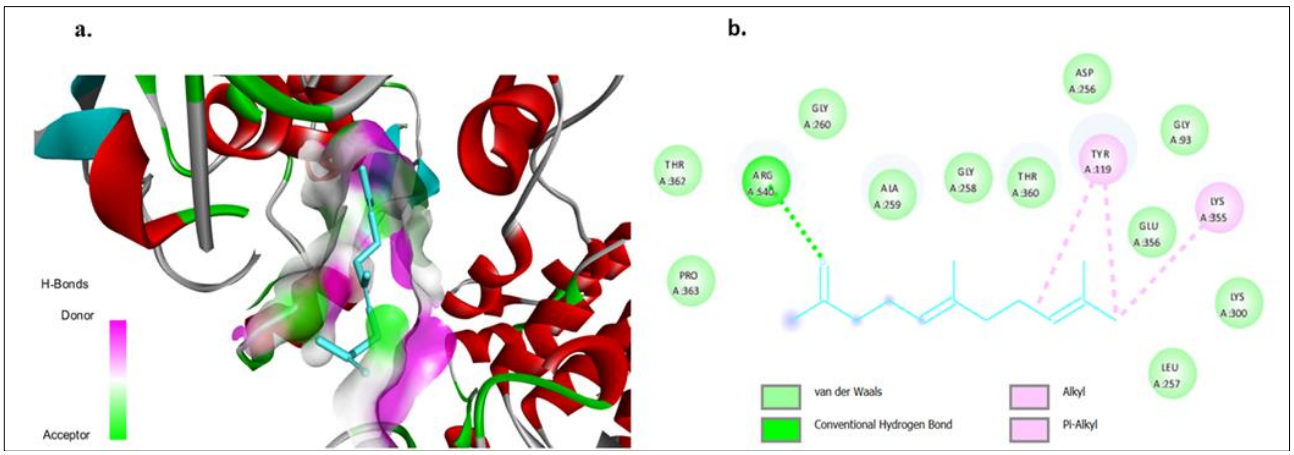

Fig 3: Interactions of geranylacetone with the NS5 protein: (a) 3D interaction and (b) 2D interaction.

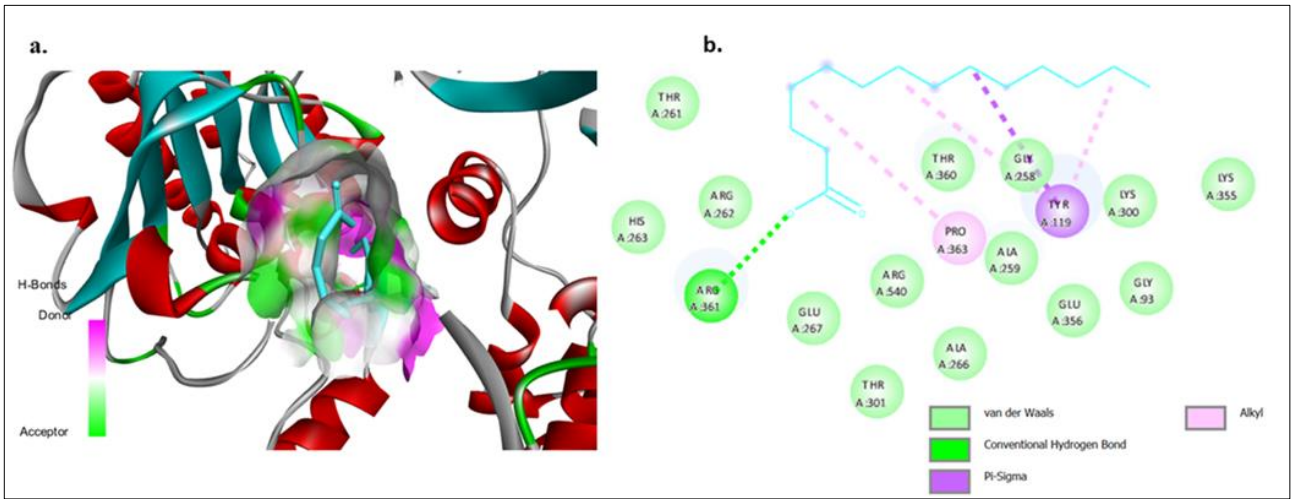

Fig 4: Interactions of tetradecanoic acid with the NS5 protein: (a) 3D interaction and (b) 2D interaction.

3.5 Tetradecanoic acid

The 3D structure shows that, tetradecanoic acid was embedded within the active site, stabilized by hydrogen bonding, hydrophobic interactions, and aromatic contacts (Fig. 4a). The 2D interaction map revealed a conventional hydrogen bond with ARG361, providing a key polar interaction for anchoring the ligand (Fig. 4b). Weak van der Waals interactions are contributed by GLY93, ALA259, GLY258, GLU356, GLU267, ALA266, THR301, THR360, ARG262, and HIS263, supporting structural complementarity. Hydrophobic stabilization is further enhanced through alkyl and π-alkyl interactions with PRO363 and TYR119 respectively. The docking analysis revealed a binding affinity of −5.3 kcal/mol for tetradecanoic acid, confirming a moderately stable ligand-protein interaction.

3.6 Cis-Linalool oxide: The interaction analysis of cis-Linalool oxide with the NS5 protein is represented as both 3D (Fig. 5a) and 2D (Fig. 5b) images. The 3D docking image shows the ligand fitting within the active site of the protein. It is found to be stabilized by hydrogen bond and hydrophobic interactions, with donor and acceptor regions highlighted in magenta and green, respectively. The 2D interaction map reveals key contacts, including hydrogen bonding with ASP533, van der Waals interactions with residues such as ALA531, ASP532, LYS698, LYS689, SER697, TRP700, ASP690, ARG688, and an alkyl interaction with ILE691. Additionally, an unfavourable donor-donor interaction was observed with ASP533, suggesting possible steric or electronic repulsion at this site. The docking score was −5.3 kcal/mol, indicating moderate binding affinity of cis-Linalool oxide with the NS5 protein.

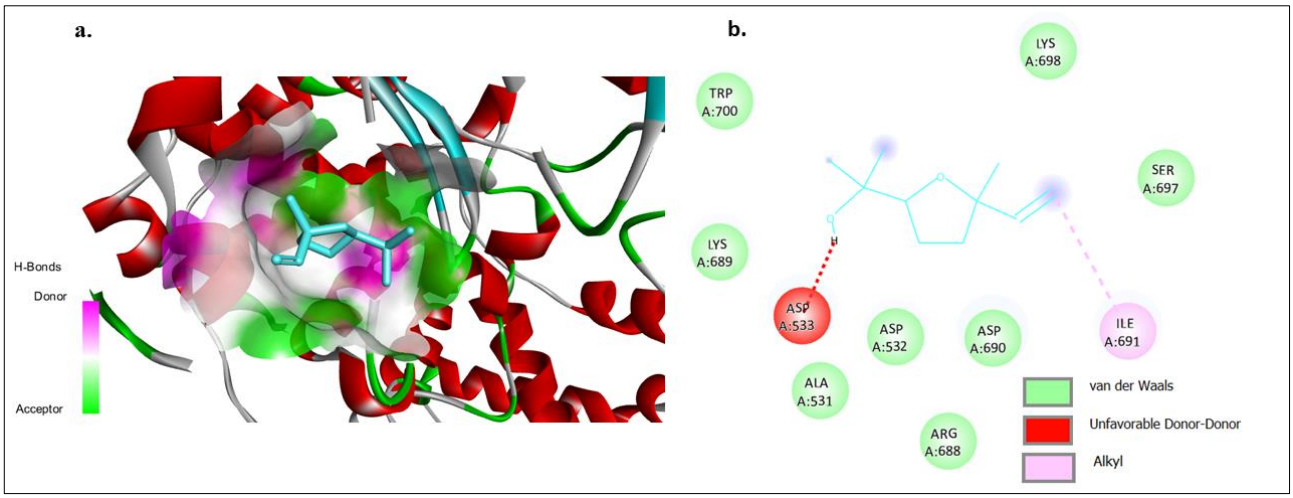

Fig 5: Interactions of cis-Linalool oxide with the NS5 protein: (a) 3D interaction and (b) 2D interaction.

3.7 Phenylacetaldehyde

The binding interaction of phenylacetaldehyde with NS5 protein is illustrated in the 3D pocket view (Fig 6a) and the 2D interaction diagram (Fig. 6b). The 3D visualization shows the ligand accommodated within the binding site, surrounded by hydrogen bond donor and acceptor regions, with additional hydrophobic patches stabilizing the complex. The 2D interaction map showed hydrogen bond interactions with LYS355 and carbon hydrogen bond with GLY93, both of which enhance ligand anchoring within the active pocket. Moreover, strong π-π stacking interaction with TYR119 and a π-sigma interaction with THR360 further stabilize the aromatic region of the ligand. Van der Waals contacts with residues such as GLY258, ALA259, LEU257, ASP359, GLU356, and LYS300 provide complementary nonpolar support. Phenylacetaldehyde had a binding affinity of -5.3 kcal/mol with NS5 protein.

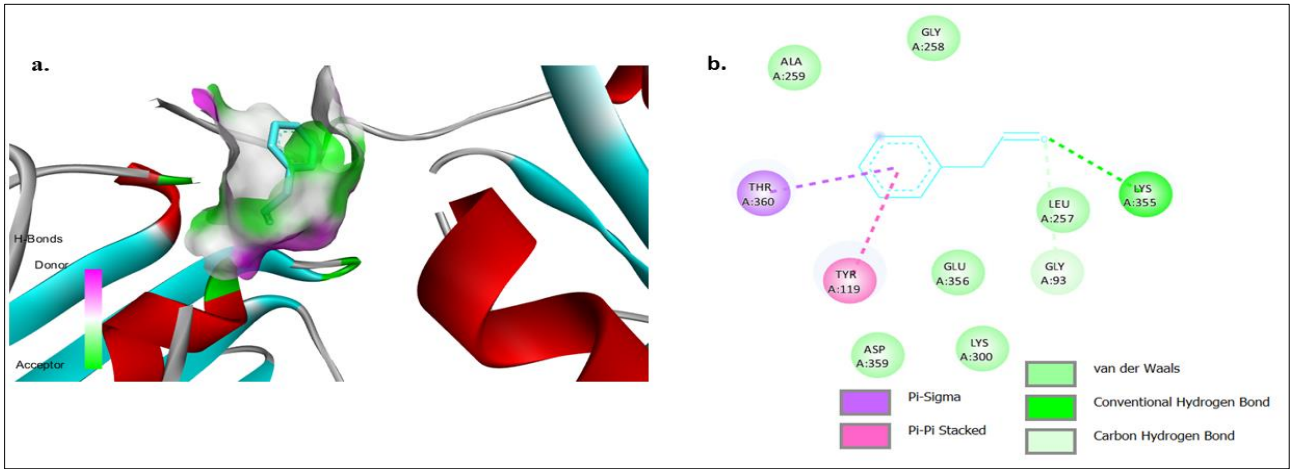

Fig 6: Interactions of phenylacetaldehyde with the NS5 protein: (a) 3D interaction and (b) 2D interaction.

3.8 Methyl 2-furfural

The 3D view shows 5-Methyl 2-furfural positioned within the binding pocket, forming hydrogen bonds and hydrophobic contacts with surrounding residues, where donor and acceptor regions are highlighted (Fig. 7a). The 2D interaction data shows a hydrogen bond interaction with LYS355 and a carbon hydrogen bond with GLU356, suggesting strong polar stabilization (Fig. 7b). Hydrophobic interactions are observed, including π-alkyl interaction with TYR119 and further van der Waals contacts with residues such as ALA259, ASP256, LEU257, GLU356 and THR360, which support ligand accommodation. 5-Methyl 2-furfural also exhibited a binding affinity of -5.3 kcal/mol with NS5 protein.

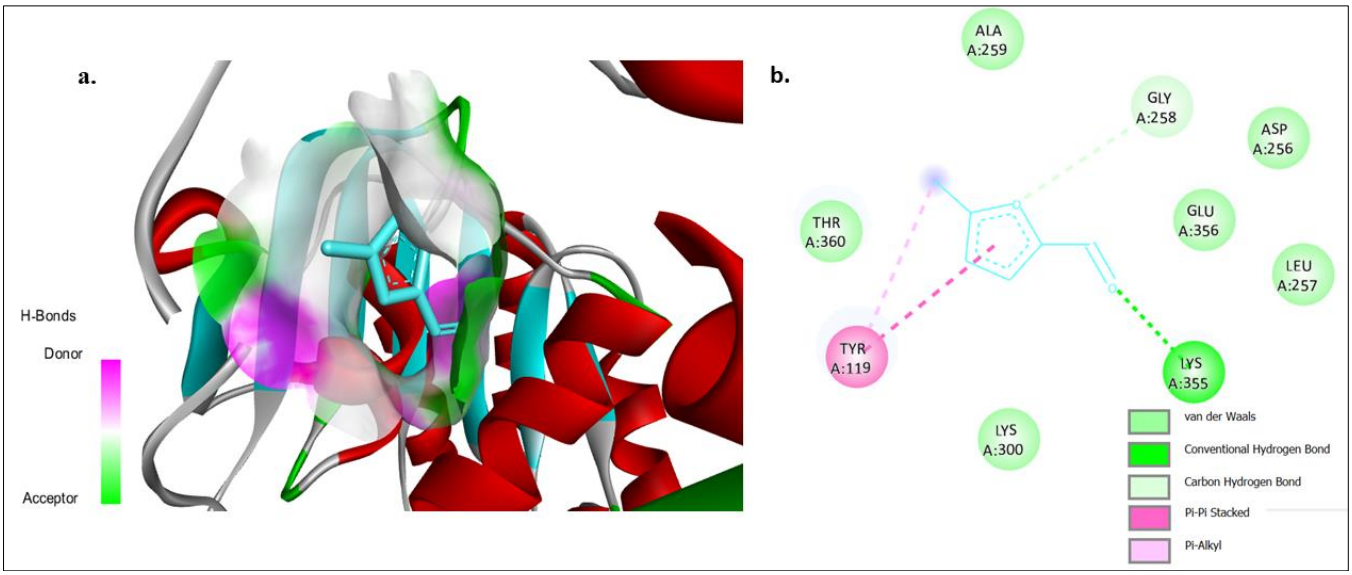

Fig 7: Interactions of 5- Methyl 2-furfural with the NS5 protein: (a) 3D interaction and (b) 2D interaction.

3.9 NS2B/NS3 structure and interaction with ligands

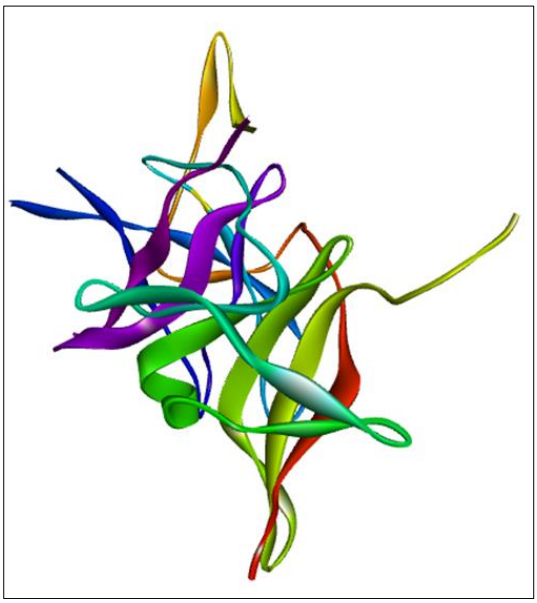

Fig 8: Ribbon diagram of NS2B/NS3 protease generated by BIOVIA Discovery Studio 2001.

The three-dimensional structure of DENV-1, NS2B/NS3 protease (RCSB PDB: 36LP) was generated and visualized using BIOVIA Discovery Studio 2001 (Fig. 7.). The structure was represented as a 'ribbon model' that showed a compact folding pattern. The 3D structure revealed β-sheets of the protein interlinked with short connecting loops and helices, which contribute to the structural stability of the protease. NS2B was found closely associated with NS3 protease forming the structural basis of catalytic pocket. The active site was found accessible in the 3D structure which plays a crucial role in substrate binding and enzymatic activity. This structure was used as the template for subsequent molecular docking analysis with 12 phytochemical compounds.

Among the 12 phytochemicals, six compounds with highest binding affinities are represented in 3D and 2D form using BIOVIA Discovery Studio 2001. To visualize the molecular interactions, 3D and 2D docking interaction diagrams were generated for these phytochemicals against NS2B/NS3 protease. The 3D representations confirm their stable orientation within the binding pocket, in which the ligands are represented in light blue colour. The 2D interaction map highlight the specific non-covalent contacts, including hydrogen bonding, van der Waals forces, alkyl, and π-π/π-alkyl interactions with critical residues in the active sites.

3.10 Methyl Salicylate

The docking analysis of methyl salicylate with DENV-1 NS2B/NS3 protein revealed a well-defined binding interaction within the active site pocket, as illustrated in both the 3D (Fig. 8a) and 2D (Fig. 8b) interaction profiles. The 3D visualization shows that the ligand is stably accommodated within the catalytic pocket, surrounded by donor and acceptor residues contributing to hydrogen bonding interactions. The 2D interaction diagram further highlights key contacts, including conventional hydrogen bonds with Gly203 and Asn202, as well as π-π T-shaped interactions with Tyr200, which are critical for stabilizing the ligand-protein complex. Additional van der Waals interactions with surrounding residues such as Phe180, Ser213, Val212, etc., further enhance the binding affinity. Notably, the docking score revealed a binding affinity of −5.6 kcal/mol, supporting the stable interaction of methyl salicylate with NS2B/NS3 protein.

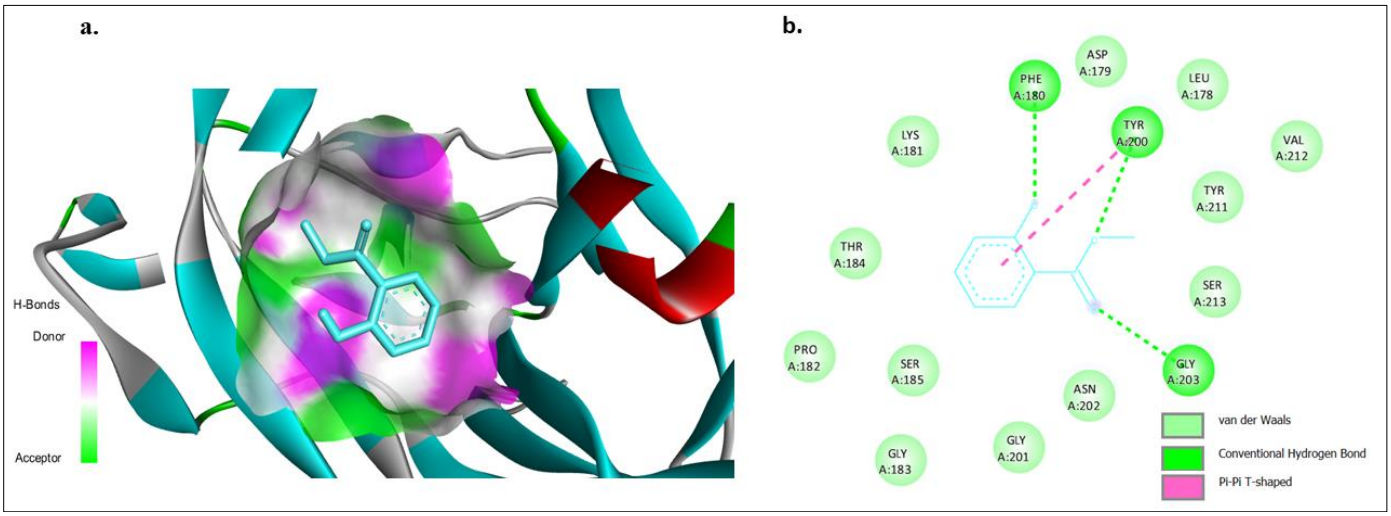

Fig 9: Interactions of methyl salicylate with the NS2B/NS3 protein: (a) 3D interaction and (b) 2D interaction.

3.11 Trans-Linalool oxide

The 3D model shows an efficient adherance of trans-Linalool oxide within a hydrophobic pocket of the protein, suggesting a strong and stable interaction (Fig. 9a). The overall conformation indicates that trans-Linalool oxide is well-accommodated within the active site. The 2D interaction map highlights two key conventional hydrogen bonds: one formed between the ligand's oxygen and the side chain of Tyr200 and another with the backbone of Phe180 (Fig. 9b). Additionally, the analysis shows a π-alkyl interaction with Tyr211, and several van der Waals interactions with residues including Lys181, Pro182, Asp179, Gly201, Ser185, Asn202, Gly203, Val205, and Ser213. trans-Linalool oxide had a binding affinity of -5.5 kcal/mol with NS2B/NS3 protein.

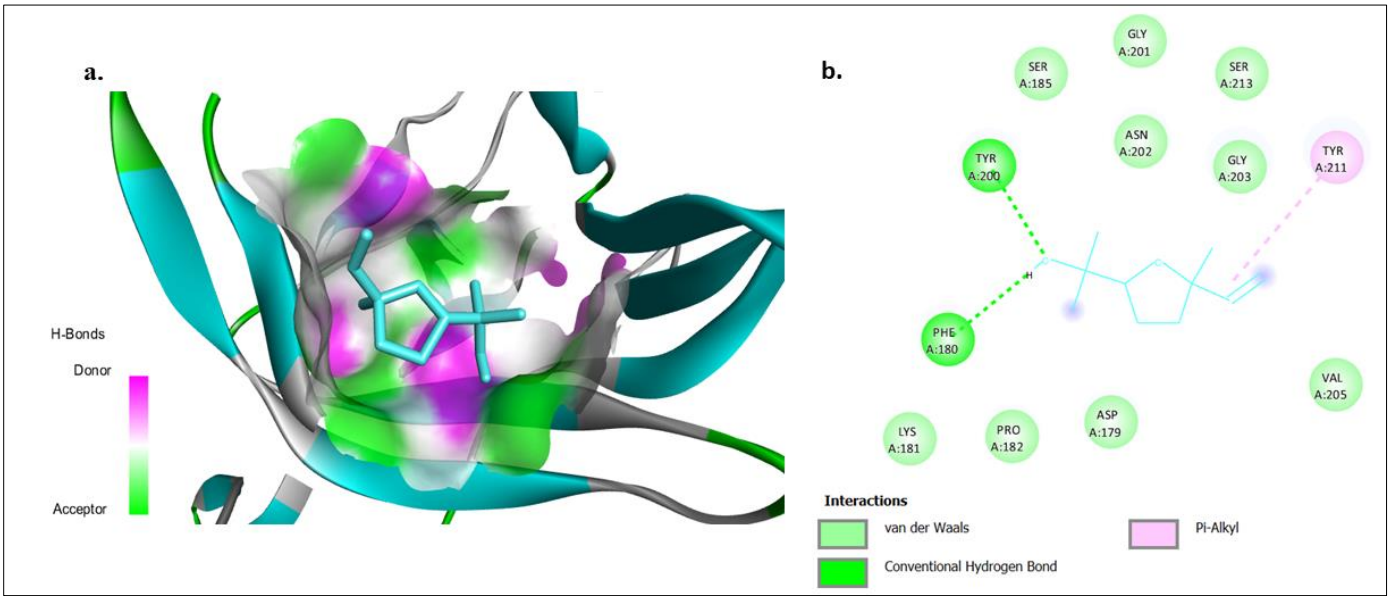

Fig 10: Interactions of trans-Linalool oxide with the NS2B/NS3 protein: (a) 3D interaction and (b) 2D interaction.

3.12. cis-Linalool oxide

The docking study of cis-Linalool with the NS2B/NS3 protease revealed stable binding interactions within the active site. The 3D docking visualization revealed that cis-Linalool was well accommodated in the binding cavity, of the protein (Fig. 10a). In the 2D interaction plot, cis-Linalool showed a hydrogen bond interaction with Leu178, while surrounding residues such as ASP179, TYR200, PHE180, PRO182, GLY203, SER185, VAL212, SER213, TYR211, THR184, ASN202 and LYS181 contributed to van der Waals interactions, enhancing the ligand's stability within the catalytic pocket (Fig. 10b). The ligand had a binding affinity of -5.3 kcal/mol with NS2B/NS3 protein.

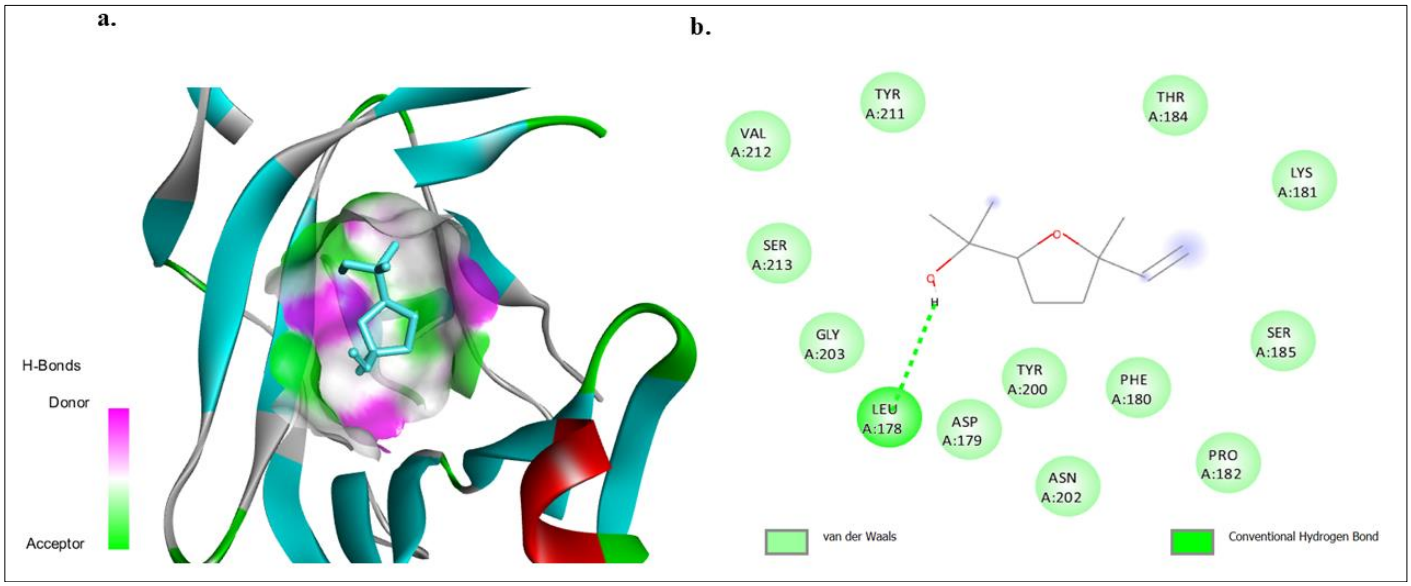

Fig 11: Interactions of trans-Linalool oxide with the NS2B/NS3 protein: (a) 3D interaction and (b) 2D interaction.

3.13 Geranylacetone

The interaction analysis depicted in the figure reveals that geranylacetone forms multiple non-covalent interactions within the binding pocket of the protein. The 3D docking model provides a clear spatial representation of geranylacetone nestled within the protein's active site (Fig. 11a). The image visually confirms the ligand's deep insertion into the binding pocket, where its hydroxyl group is positioned to form a hydrogen bond with the protein. Specifically, three conventional hydrogen bonds are observed with residues THR184, SER185, and GLY183, suggesting strong and specific anchoring in 2D interactions (Fig. 11b). Additionally, van der Waals interactions with LYS181, PRO182, PHE180, TYR200, GLY201, ASP179, SER213, and GLY203 residues, alkyl interactions with VAL205 and π-alkyl interactions with TYR211 were also observed. Geranylacetone showed a binding affinity of -5.2 kcal/mol with NS2B/NS3 protein.

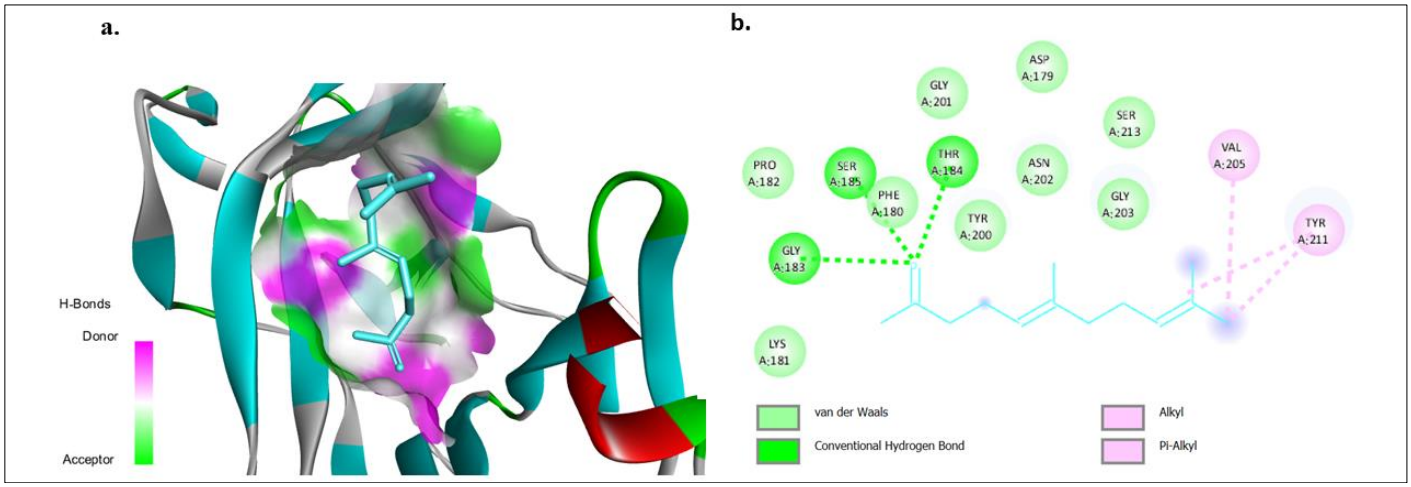

Fig 12: Interactions of geranylacetone with the NS2B/NS3 protein: (a) 3D interaction and (b) 2D interaction.

3.14 Linolenic acid

The 3D interaction diagram (Fig. 12a) shows linolenic acid fitting into the binding pocket, forming hydrogen bond interactions with nearby residues and occupying a hydrophobic cavity. The 2D interaction reveals that the ligand primarily binds through extensive hydrophobic interactions, including multiple alkyl and π-alkyl contacts with residues such as VAL173, VAL204, VAL212, ALA175, ALA214, ALA216, and PHE166 (Fig. 12b). Further, van der waals interaction was observed with residues including GLY203, SER213, LYS123, ASP125, GLY201 and THR168. The compound had a binding affinity of -5.2 kcal/mol with the target protein.

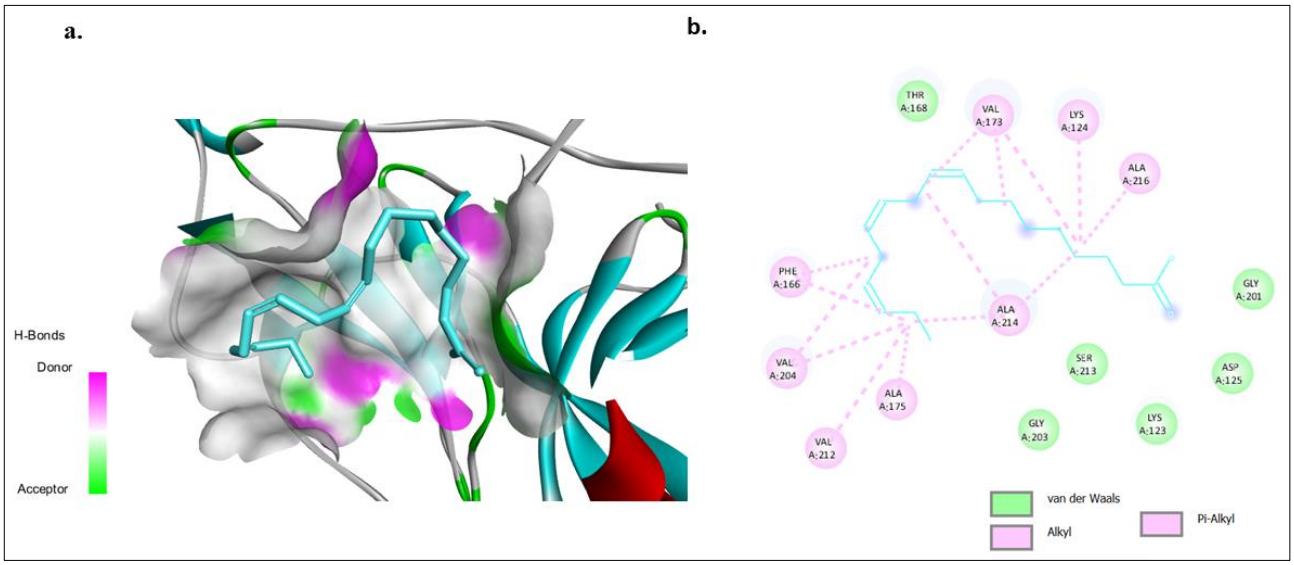

Fig 13: Interactions of linolenic acid with the NS2B/NS3 protein: (a) 3D interaction and (b) 2D interaction.

3.15 Methyl linoleate

The 3D structure shows methyl linoleate embedded within a hydrophobic cavity of the protease, stabilized primarily by van der Waals forces and hydrophobic contacts (Fig. 13a). Distinct donor (magenta) and acceptor (green) regions are visible around the ligand, indicating the presence of weak polar interactions in addition to dominant hydrophobic effects. The 2D interaction map confirms this, showing multiple alkyl and π-alkyl interactions with key non-polar residues such as VAL173, ALA214, ALA216, and LYS124 (Fig. 13b). A few residues, including GLY203, SER213, and ASP125, etc., engage in van der Waals interactions, suggesting a close molecular fit. Methyl linoleate exhibited a binding score of -5.1 kcal/mol with the target protein.

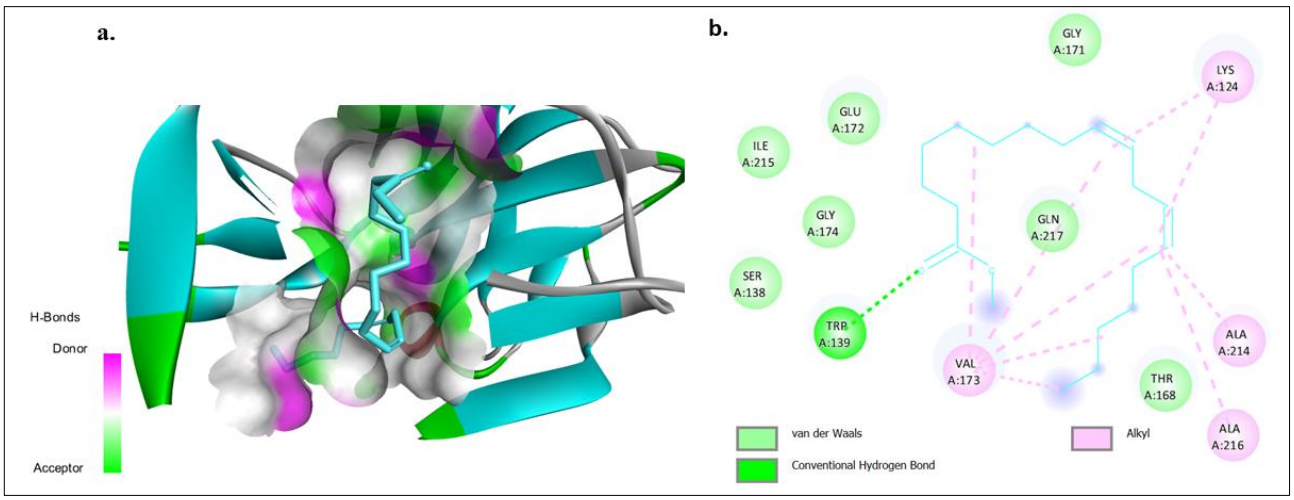

Fig 14: Interactions of methyl linoleate with the NS2B/NS3 protein: (a) 3D interaction and (b) 2D interaction.

4. Discussion

The present study employed in silico molecular docking studies to investigate the binding affinities and interaction profiles of twelve phytochemical ligands from *Senegalia rugata* against two essential dengue viral proteins, NS2B/NS3 protease and NS5. Both proteins are non-structural proteins that play crucial roles in DENV viral replication. NS5 contributes to RNA replication and capping (Wahaab *et al.*, 2021) [31] while NS2B/NS3 protease mediates polyprotein cleavage (Yangsheng *et al.*, 2007) [33]. Therefore, targeting these proteins provides a dual strategy to hinder viral propagation.

Overall, the docking analysis revealed differential binding affinities of ligands against the two proteins. Methyl palmitate exhibited the highest binding affinity to NS5 (-6.2 kcal/mol), primarily mediated by extensive van der Waals and alkyl interactions with hydrophobic residues in the NS5 binding pocket (ALA259, LEU257, TYR119, ARG540). Minimal hydrogen bonding was observed, consistent with the compound's lipophilic character. These interactions explain the stronger binding to NS5 compared to NS2B-NS3, whose active site is shallower and more polar, limiting accommodation of long-chain hydrophobic ligands.

Geranylacetone is an acyclic monoterpene ketone, that consist of a long isoprenoid chain and a carbonyl group. The interaction of geranylacetone with the dengue virus NS5 protein, showing a binding score of −5.6 kcal/mol, indicating a favourable binding affinity. The 3D interaction shows that the ligand is positioned within the binding pocket of NS5, capable of forming Hydrogen bond and van der Waals interactions. Likewise, hydrogen bonding with Ser138 and Gly185 residues serves as the strongest interaction that secure

the ligand into the pocket of active site of NS2B/NS3 protein. The long isoprenoid chain of geranylacetone was found engaged in van der Waals interactions and hydrophobic stabilization through alkyl and π-alkyl interactions of NS2B/NS3 protein. These binding patterns might suggest that geranyl acetone effectively occupies the substrate-recognition cleft, potentially blocking substrate accessibility to the catalytic triad (His51, Asp75, Ser135). Previous report suggests other acyclic monoterpenes acting as NS2B/NS3 protease inhibitors (Purohit *et al.*, 2022) [23].

Methyl salicylate demonstrated a notable binding affinity of -5.6 kcal/mol towards the NS2B/NS3 protease of dengue virus, indicating its potential as a moderate inhibitor. The 3D and 2D interaction profiles revealed that its ester and hydroxyl groups contributed to hydrogen bonding while the aromatic ring engaged in π-π and hydrophobic contacts within the catalytic pocket. Key interactions were observed with amino acid residues TYR200, GLY203, PHE180, and TYR200, where conventional hydrogen bonds contributed to the overall stability of the ligand-protein complex. Previous reports also suggest the antiviral activities of salicylic salts, as it inhibited DENV-2 replication *in vitro* (Ching-Len *et al.*, 2001) [7]. These interactions suggest that methyl salicylate might interfere with the catalytic function of the NS2B/NS3 protease, thereby impeding viral replication.

Terpenoids and fatty acid derivatives such as cis-Linalool oxide (-5.3), geranylacetone (-5.2), linolenic acid (-5.2) methyl linoleate (-5.1), methyl salicylate (-5.6), and trans-Linalool oxide (-5.5), exhibited comparatively stronger binding affinities against NS2B/NS3 protease. In an *in vitro* study, it was shown that linalool oxide has efficiently reduced the NS2B/NS3 protease activity of dengue virus by 18.42% at a concentration of 2mM (Abdullah *et al.*, 2023) [1]. In the 2D interaction of cis-Linalool oxide with NS2B/NS3 protease, hydrogen bond interaction with Leu178 residue was observed. The hydrogen bond interaction represents efficient binding affinity with the binding pocket of the protease. For instance, glycyrrhizic acid conjugates was found to interact with NS2B-NS3 protease through hydrogen bonds (Asp75, Tyr150, and Gly153 residues). It has proved later to have inhibitory activity against NS2B/NS3 protease with EC_{50} value of 0.034 μM (Lin *et al.*, 2024) [15]. Also, van der Waals interaction between residues serves as a supplementary stabilizing force for ligand-protein interaction. cis-Linalool oxide showed similar binding affinities towards both NS2B/NS3 and NS5 proteases. It also exhibited stable binding with the NS5 protein, forming van der Waals interactions with residues such as Ala531, Asp532, and Trp700, along with hydrophobic contacts with Ile691. Although an unfavourable donor-donor interaction was observed with Asp533, the overall interaction profile suggests that this monoterpene oxide could contribute to the inhibition of NS5 activity, consistent with previous reports highlighting the antiviral potential of oxygenated monoterpenes (Ching-Len *et al.*, 2001) [7].

Tetradecanoic acid exhibited a higher binding affinity for the NS5 protein (−5.3 kcal/mol) compared to the NS2B/NS3 protease (−4.1 kcal/mol). The binding is stabilized through van der Waals contacts and hydrogen bonding, particularly with residues such as ARG361, while additional non-covalent interactions like Pi-Sigma and alkyl contacts contribute to the overall complex stability. This might be due to the fact that the molecule may interfere with its RNA-dependent RNA polymerase function rather than the proteolytic activity mediated by NS2B/NS3. These findings align with previous reports demonstrating that long-chain fatty acids often show higher affinity for polymerase active sites due to hydrophobic and hydrogen-bonding complementarity (Pant & Jena, 2022) [20].

Phenyl acetaldehyde (−5.3 kcal/mol) and 5-Methyl-2-furfural (−5.3 kcal/mol) showed stronger binding affinities toward the NS5 protein, while exhibiting comparatively lower binding scores with the NS2B/NS3 protease (-5.3 kcal/mol with NS5 and -4.7 kcal/mol with NS2B/NS3). The lower binding affinity towards NS2B/NS3 might be due to the shallow binding pocket of the protease, surrounded by negatively charged residues, that makes interaction of slightly polar molecule like phenylacetaldehyde difficult (Pant & Jena, 2022) [20]. On the other hand, NS5 protein's binding site is more accommodating to such compounds, allowing for effective interaction and inhibition.

The antiviral activities of fatty acids and their derivatives have been widely explored, due to their amphipathic nature, which allows them to interact with both hydrophobic enzyme pockets and polar catalytic residues. In this study, linolenic acid and its methyl ester derivative methyl linoleate exhibited moderate binding affinities toward the dengue virus NS2B/NS3 protease. The 3D conformation indicated deep embedding of the hydrophobic aliphatic chain within the catalytic cleft, while the terminal polar group aligned toward hydrogen bond donor/acceptor regions. Previous studies have highlighted the antiviral potential of fatty acids against flaviviruses and other enveloped viruses. Linolenic acid has been reported to inhibit viral replication through both membrane disruption and direct enzyme interaction, with evidence suggesting its ability to interfere with protease-mediated cleavage events (Feng *et al.*, 2023) [9]. Similarly, extracts of *Ocimum basilicum* containing methyl linoleate, has also shown to have antiviral property against DENV (Joshi *et al.*, 2023) [13]. Computational screenings of fatty acid derivatives against flaviviral enzymes also support their ability to dock stably within catalytic pockets (Rasool *et al.*, 2019) [26].

5. Conclusions

The present study provides a comprehensive in silico evaluation of selected phytochemicals from *Senegalia rugata* against the NS2B/NS3 and NS5 proteins, which are critical for the replication and proteolytic processing of the dengue virus. The phytochemicals obtained from the GC-MS analysis of *Senegalia rugata* demonstrated strong binding affinities and favourable interaction profiles, highlighting their promise as potential antiviral agents targeting dengue virus proteins. Among the 12 docked ligands, methyl palmitate showed the highest binding affinity of -6.2 kcal/mol, followed by geranylacetone (-5.6 kcal/mol). cis-Linalool oxide, 5-Methyl 2-furfural, phenyl acetaldehyde, and tetradecanoic acid each exhibited binding affinities of -5.3 kcal/mol, indicating comparable interaction strengths with NS5 protein. Among the docked ligands, methyl salicylate showed the highest binding affinity of -5.6 kcal/mol, followed by trans-Linalool oxide (-5.5 kcal/mol). cis-Linalool oxide (-5.3 kcal/mol), geranylacetone (-5.2 kcal/mol), linolenic acid (-5.2 kcal/mol), and methyl linoleate (-5.1 kcal/mol) also demonstrated strong interactions with the DENV-1 NS2B/NS3 protease, suggesting their potential as promising inhibitory compounds.

Among the tested compounds, ligands like methyl palmitate,

geranylacetone, cis-Linalool oxide, tetradecanoic acid, phenyl acetaldehyde, and 5-Methyl-2-furfural exhibited comparatively high binding affinities and strong hydrogen bond interactions with NS5 protein. Additionally, ligands including methyl salicylate, trans-Linalool oxide, cis-Linalool oxide, geranylacetone, and methyl linoleate exhibited high binding scores and formed stable hydrogen bonds, hydrophobic interactions, and π-π stacking interactions with essential amino acid residues within the active sites of the NS2B/NS3 protein. These findings suggest that these phytochemicals can effectively occupy critical regions within the protein structures, potentially hindering the enzymatic activity required for viral replication. The docking results also revealed the structural features necessary for effective binding, such as hydroxyl groups, aromatic rings, and polar moieties, which facilitate hydrogen bonding and electrostatic interactions with the target proteins. These structural insights can guide future chemical modifications or optimization of lead compounds for enhanced efficacy and bioavailability.

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