Uncovering of Anti-dengue Molecules from Plants Prescribed for Dengue: A Computational Investigation

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Abstract

Dengue fever is a tropical disease spread worldwide, transmitted by the mosquito *Aedes aegypti*. It affects 100 million people worldwide every year and half a million cases of dengue hemorrhagic fever are registered. At present, it poses sever health burden as combined infections of COVID-19. Currently, as a combined infection with COVID-19, it is becoming a serious health burden. To identify the active molecule, Maestro V12.7 was used with different tools including LigPrep, Grid Generation, SiteMap, Glide XP Docking, Pharmachophores and MM-GBSA. The UNRESS tool was also used to assess the protein stability with this dengue protein. The docking result showed that all examined phytocomponents except berberine and -(+)-ʟ-alliin had good docking scores of -8.577 (azadirachtin), -8.112 (curcumin), -7.348 (apigenin) and -6.028 (andrographolide). However, berberine and -(+)-ʟ-alliin possessed good hydrogen-bonding interactions with RdRp. In addition, molecular dynamic simulations demonstrate that the complex of azadirachtin and dengue protein has a solid understanding of the precise interactions. As per the research results, the present research suggests that this is the first statement of azadirachtin against NS5 RNA-dependent RNA polymerase domain (RdRp), despite extensive research on this molecule in previous investigations. Furthermore, we anticipate that molecules such as curcumin, apigenin, and andrographolide would show beneficial effects while in vitro and in vivo studies are conducted on virally related objects. Since we performed ADMET and pharmacokinetic properties in this research, we feel that the phytochemicals of the screened anti-dengue molecules may not need to be evaluated for toxicological effects.

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1 Introduction

Worldwide, dengue fever is considered to a most popular and severe flavivirus disease in the human races. It shows the sign ranging from moderate to severe. This virus is transmitted by the female *Aedes aegypti* mosquito infected with the dengue virus after biting humans. This virus belongs to the Flaviviridae family. In general, flaviviridae are trapped viruses with positive-strand RNA genomes that are classified into three genera: *hepacivirus*, *pestivirus*, and *flavivirus*. Among the genera, *Flaviviruses* are thought to be responsible for a number of clinically important arthropod-borne diseases, including dengue fever, yellow fever, Japanese encephalitis virus, tick-borne encephalitis virus, and West Nile virus, which mostly impact humans all over the world [1].

A person with dengue infection becomes known primarily after diagnosis based on symptoms, which include fever, headache, arthromyalgia, retro-orbital discomfort, and rash. Whereas, the severe dengue fever is known by plasma leakage, profuse bleeding, and severe organ failure, affecting about one in twenty people infected [2]. In the region of South East Asia, among mosquito-borne infections, severe dengue fever is the foremost cause of hospitalization and mortality [3]. It can be transmitted up to 2 days before dengue signs appear; while the virus can remain in the body for up to 2 days after it has been cured [4].

During viral replication, RNA-dependent RNA polymerase (RdRp) plays a key role in both positive and negative strand RNA synthesis [5]. Due to the lack of a similar protein in humans, it is an ideal target for the development of potential antiviral agents against the complication associated with the serotype dengue of NS5 [6]. Particularly, the RNA genomes direct the virus races. More specifically, survival of the virus in the host refers to the ability to adapt to rapid change through mutation; where, the RdRps helps to overcome the constant hurdles posed by the host's physiological functions [7]. Moreover, viral progeny undergo mutations due to pressure from the host's defence system and other environmental influences; while strand switching of RdRPs during copying allows for recombination, which can also rearrange genes or incorporate new genes from the host and other viruses [7].

Over the past two decades, the dengue infection rates have drastically surged by more than eightfold, from 505,430 cases in 2000 to over 2.4 million in 2010 and 5.2 million in 2019, according to the 2021 WHO report [8]. Furthermore, they reported that the fatality rate from these virus cases increased from 960 to 4032 between 2000 and 2015. In this decade, it is regarded to be an endemic disease due to its transmission in over 4 regions around the world, including Africa, America, the Eastern Mediterranean, South-East Asia and Western Pacific. More specifically, this virus is spread in over 70% of countries in Asia [4]. It had infected people from over 100 nations by 2020, according to the WHO report, including Bangladesh, Brazil, the Cook Islands, Ecuador, India, Indonesia, the Maldives, Mauritania, Colombia, Fiji, Kenya, Paraguay, Peru, Reunion Island and the list goes on [8].

In this scenario, dengue-endemic countries face a new battle from Covid-19 and its variants. If need to know more about the new health burden, as a dengue co-infection, the coronavirus and its variants are causing drastic health problems to people around the world. This co-infection not only causes health problems but also complicates the diagnosis of a person with the Covid-19 disease, thus delaying the diagnosing of corona infection and leading to further spread of this novel virus. Taking into account the current situation, cost and urgency of dengue antagonists, the computational biological tools were employed to identify possible candidates for anti-dengue treatment from recommended and other known antibiotic medicinal plants. Since it is the main source for viral replication, the present research selected this target to discover a drug to ward off viral replication.

2 Materials and Methods

2.1 Docking and Modeling Platform

Using a highly configurable Linux operating system with Schrödinger Maestro V.12.7 software including LigPrep, Grid Generation, SiteMap, Glide XP Docking, fingerprint analysis and MM-GBSA were applied to analyze the anti-dengue drug potential of the phytochemicals. Some research like pharmacophores and ADMET of docked phytochemicals were collected from established and reputable online databases after finding potential docking values.

2.2 Biological Data

The phytochemicals were selected from the referenced medicinal plants against dengue fever in the current context, on the advice of the Ministry of AYUSH. From them, nearly six phytochemicals were selected for docking with a dengue NS5 RNA-dependent RNA polymerase domain to discover active anti-dengue potential (Table 1). These were prepared for docking after being retrieved from a chemical database in molar format [9]. Then, the RNA-dependent RNA polymerase domain of the DENV3-NS5 crystallographic protein was obtained in the established database. The deposition and alphanumeric identification code of this protein is 2J7W [10].

2.3 Target Preparation

Before docking, the protein molecule was first subjected to an update of missing side chains and back chains using the Protein Preparation Wizard tool in Maestro V 12.7. In this tool, we have shifted two gears: preparation and refinement, which were used to identify and eliminate the water molecule. Protein molecules are often attached to and intertwined with the water molecules. In this phase, the protein molecule is not apt for docking, so the water molecules of the protein have been removed from it. Ultimately, the other two gears such as optimization and minimization were used during this protein production process. After protein preparation, the prepared target was subjected to site-map analysis to locate the active site for binding of

map tool. The grid-based ligand docking approach was used to study the interaction between phytochemicals and dengue protein. At this stage, a lattice box was formed to attach the ligands in the centroid of the selected dengue protein site. The grid box of 2J7W was built with X: 30.75, Y: 63.54 and Z: 31.14. Ultimately, according to our previous studies, the lattice-engineered dengue protein was docked with phytochemicals [13]

2.6 Ligand Preparation

Prior to docking, the collected phytochemicals such as azadirachtin, curcumin, apigenin, andrographolide, berberine and -(+)-L-alliin were loaded and processed by employing Ligprep module. With this module, the secondary plant

Table 1 Explored phytochemicals and their existence in plants

S. No.	Name of the phytcompounds	Possible medicinal plants to isolate the reported phytochemicals	Reported literature
1	*Azadirachtin*	Leaves, flowers and fruits of *Azadirachta indica* A. Juss. and *Melia azedarach* A. Juss	The availability of currently researched phytochem-icals is clearly disclosed according to previous reports
2	*Curcumin*	Rhizome of C*urcuma longa* L	
3	*Apigenin*	Leaves of *Allium fistulosum* L. *Caricca Papaya* L and flowers of *Gentiana veitchiorum* L. *Symphotrichum novea anglea* (L.) GL Nesom and *Matricaria recutita* L	
4	*Andrographolide*	Leaves of *Andrographis paniculata*	
5	Berberine	Roots, rhizome, and stem bark of *Berberis vulgaris* L. *Coptis chinensis* Franch. *Hydrastis Canadensis* L. R*hizoma coptidis, Xanthoriza simplicissima* L. *Phellodendron amurense* Rupr. and *Chelidonium majus* L	
6	(+)-L-Alliin	Bulb of *Allium sativum* L and *Allium ursinum* L	

the drug molecule according to Vijayakumar et al. [11].

2.4 Active Site Prediction

This plays a key role in this exploration of molecular docking as it revealed the correct binding pockets for ligand fixation in the target along with the active site volume and value. The sitemap tool in Maestro V12.7 was used for this analysis. Finally, the best ligand binding site for the lattice construction was selected according to the site values [12].

2.5 Grid Generation

It was carried out to fit the lattice for the binding of the ligand in the dengue protein after being evaluated by the site

metabolites were prepared for docking. To improve the topography of the selected phytochemicals, the OPLS force field was used in the ligand preparation. The compounds were even developed as 3D structures from 1D (Smiles) and 2D (SDF) presentations, while the tautomers and stereoisomers were also studied in the ligand to reduce their physical complexity. As the final product of ligand preparation, the appropriate molecular weight, functional groups, and chirality of ligands for docking to this target protein were obtained, which were available for each fully prepared input ligand [14].

2.7 Molecular Docking

Using the Grid-Glide docking module in Maestro V.12.7, the prepared ligands were docked with this viral protein in order to scrutinize the dengue-antagonistic effects of these phytochemicals. There we found two types of modes when docking: Standard Precision and Xtra Precision. In order to understand the precise interactions and other valuable docking metrics between ligand and protein, docking was performed in the Xtra-precision approach in the present study. Ultimately, the present investigation demonstrated workable potential outcomes between dengue and phytochemicals, including docking scores, hydrophobic interactions, hydrogen bonding interactions, pi–pi stacking, and salt bridge [15].

2.8 Pharmacokinetics Analysis

Using the two freely accessible online tools such as Swiss ADME and pkCSM were utilised to better assess the therapeutic potential of docked compounds. According to the five Lipinski rules, the likeness such as molecular weight (MW), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), rotatable bond (RB), and topological polar surface area (TPSA) were evaluated in the docked molecules by the Swiss ADME tool. Then, according to Mariya Jancy Rani et al. [4], the pkCSM server was used to know the drug parameters of docked molecules such as absorption, distribution, metabolism, excretion and toxicity (ADMET).

2.9 Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) Calculations

Following docking, it was employed to assess the drug robustness of the phytoconstituents using the Schrödinger Prime; specifically, it was used to assess the MM-GBSA energy levels of phytoconstituents with the dengue protein. This was done following the procedure of our previous publications as follows:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand}).$$

3 Results and Discussion

3.1 Medicinal Plants as Antiviral Agents

Since ancient times, over 80% of people worldwide have used plants as their primary health care to treat various diseases [16]. The plants have been used alone or mixed with other medicinal plants as a polyherbal formulation to treat diseases under various nature-based systems such as Siddha, Ayurveda, Unani, Folklore, etc. For instance, the Ministry of AYUSH recommended two polyherbal formulations as antiviral therapies to the Government of India to fight Dengue and Corona by enhancing the immune system. In accordance with this claim, the Siddha-based Chooranams like Nilavembu Kudineer Chooranam for dengue and Kabasura Kudineer Chooranam for corona have been prescribed for these viral diseases. After a thorough evaluation, they also reported that these chooranams act as immune stimulants and immune modulators to prevent dengue, chikungunya, and coronavirus replication and its associated consequences by boosting immunity and regulating the body's natural defense mechanisms. Moreover, several studies have recently demonstrated the effectiveness of Siddha-based herbal therapy, one of India's oldest traditional medicinal systems, in treating arbovirus and respiratory diseases.

Furthermore, this traditional naturopathy therapy, unlike modern drugs, has not shown any side effects after ingestion. Consistent with this statement, over the past two decades, due to population growth, shortage of medicines, treatment costs, side effects of modern drugs, etc., people in developing and developed countries have completely relied on plants to cope with diseases. Apart from that, the plants are considered a source of medicines worldwide due to their extensive biological potential. In order to know the exact therapeutic potential of dengue choornam and other antiviral herbal phytochemicals, the phytochemicals such as azadirachtin, curcumin, apigenin, andrographolide, berberine and -(+)-L-alliin were tested on RdRps of dengue whether these contributes in controlling of dengue fever or not. In a recent study, it was reported that numerous bioactive metabolites of Siddha drugs that possess antiviral properties against respiratory viruses have been isolated by many scientists to date [17]. From them, numerous polyphenolic compounds have been elucidated to explore their antiviral potential in various ways as in the present research. In general, the phytochemicals with phenols have a powerful antioxidant potential due to the existence of phenolic hydroxyl groups, which neutralizes various free radicals by releasing hydrogen atoms, resulting in fairly constant and less harmful molecules [4].

3.2 Computational Biology and Its Significance

Molecular docking methods have often been used to probe the feasible contacts of a target–ligand. It is currently being employed to explore the ligand–protein interactions at the atomic level, revealing the drug potential of small molecules at a target lattice site and important biological processes [11]. Assessing the ligand-binding pocket in the target is important to know the binding affinities between protein-ligands as it is the basis for the emergence of interactions

Fig. 1 Ligand binding pocket in the RdRp of dengue viral protein
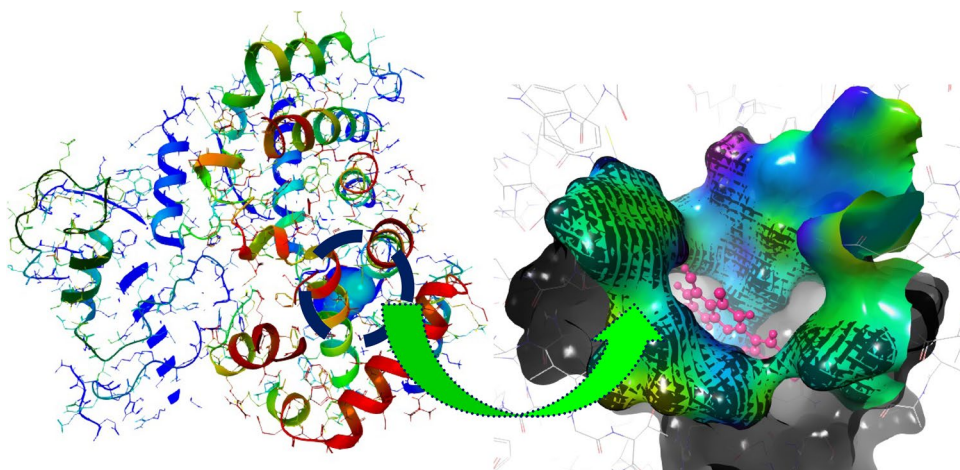
such as hydrogen bonding side chains, backbone, pi-pi stacking and salt bridge contacts. Electrostatic interactions between the ligand and target are also highlighted there. The stronger binding contacts and explicit protein–ligand distances are also promoted by this [12]. From a series of true and false results to finding the precise position of the ligand molecules, molecular docking algorithms are the possessed most significant role in computational biological research in terms of drug discovery. Similar to the present in-silico studies, we performed a series of molecular docking experiments on a variety of disease targets including diabetes, Alzheimer's, epilepsy, *Mycobacterium tuberculosis*, and the dengue mutant virus NS2B47-NS3. These disease targets were docked with a variety of phytopolyphenols to identify their drug potential.

3.3 Antiviral Potential of Explored Phytochemical Contains Herbs

Firstly, azadirachtin is a unique and important phytochemical from the genus *Azadirachta* that we can find in the leaves, flowers and fruits of *Azadirachta melia* and *A. indica*. Various parts of these plants have insecticidal properties; hence the farmers of South India use it as an insecticidal agent on the agricultural lands after grinding it as a fine powder with sheep or cow dung. From the point of view of traditional medicinal use, leaves of this plant with the leaves of *Andrographis paniculata* and *Caricca papaya* are used in the treatment of dengue fever as a decoction after boiling the dried powder in water. They also use the fresh leaves of these plants for dengue after boiling them in water. Also, the leaves of *Azadirachta indica* are soaked in water mixed with turmeric powder by local people for 8 h to treat chickenpox. This formulation is used topically as a bathing agent for 3 days after 1 week post-infection with chickenpox. Prior to this treatment, the cluster of the leaves with rachis is kept for those who are infected by this virus. These treatment

measures are carried out by them without scientific reason. However, this treatment helps them avoid further spread of this viral infection. Prior to researching the phytochemicals from dengue-prescribed plants, the antiviral potential of these plants is collected from the South Indian peoples in accordance with our previous ethnobotanical investigation.

3.4 Active Site

In order to dock the phytochemicals with this dengue protein, an active binding pocket was investigated in the dengue protein for ligand docking, which was thoroughly explored in both its outer and inner regions. In this ligand binding site analysis, the two most active binding sites were discovered in the present study, from which only one site was selected for further exploration based on its site score and volume. From the site values, the site 1 was found to be an appropriate ligand binding site (Fig. 1). As a result, the sitemap was ultimately found to be better for lattice generation and a suitable site for ligand docking. The sitemap outcomes show that the residues such as GLN350, GLN351, THR345, PHE354, MET589, ILE592, TRP302, VAL358, TRP477, GLY599, SER600, ARG481, GLY601, GLN602, LYS575, VAL576, VAL450, VAL577, LYS578, VAL579 and GLN580 have been located in and around the selected lattice site. Moreover, it can be seen from Table 2 that Site 1 consisted of a maximum number of residues due to its larger area volume.

3.5 Molecular Docking

In this computer-aided drug design exploration, the selected phytochemicals were docked with a dengue target in order to identify whether they have potential as dengue antagonists. Following the docking results, the current study examined three docking outcomes including docking score, glide energy, and ligand-target interactions to see if these
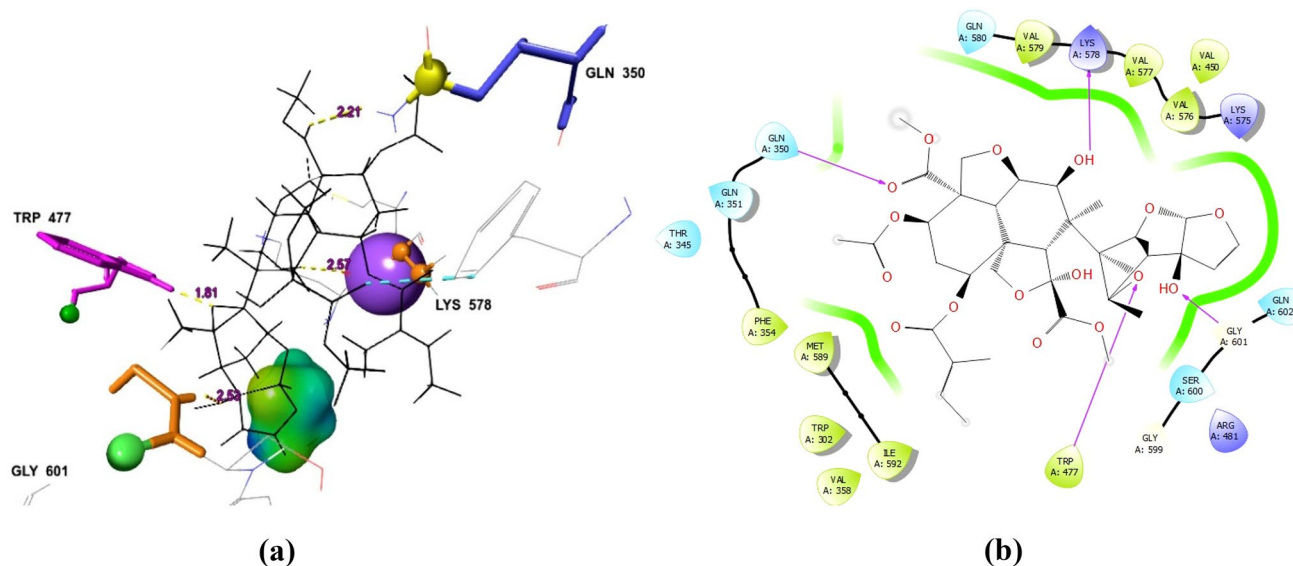
Fig. 2 a Azadirachtin: interactions and the values of the hydrogen bonding distances, **b** 2D template plot shows the variety of contacts formed between the hydroxy and methoxy groups of azadirachtin with the RdRp of the DENV protein

Table 2 Active binding pocket of 2J7W

S. No.	Sites	Score	Volume	Ligand binding pocket residues within 3 Å	Chain
1	Site 1	1.647	524.42	GLN350, GLN351, THR345, PHE354, MET589, ILE592, TRP302, VAL358, TRP477, GLY599, SER600, ARG481, GLY601, GLN602, LYS575, VAL576, VAL450, VAL577, LYS578, VAL579, GLN580	A

phytochemicals have anti-dengue potential. According to the outcomes examined, the present study observed that azadirachtin had the highest docking scores and energy values. Apart from that, among the phytoconstituents tested, it has also been known to have good hydrogen bonding with dengue protease amino acid residues as well. Likewise, curcumin and apigenin were found to have better docking values and hydrogen bonding. Such compounds were noticed

to have better effects against dengue proteins, similar to azadirachtin. While other phytochemicals such as andrographolide, berberine and—(+)-L-alliin showed modest docking scores, they have good hydrogen bonding contacts with this dengue protein (Tables 3 and 4). Examining the docked complex, the present study was assessed the H- bond interactions around 3 Å from the ligand pose. From the two-dimensional interaction diagram, we found that the oxygen, hydroxyl, and ammonia groups of these phytocompounds showed positive binding affinities with the residues of target. The protein–ligand H-bonding interactions clearly show the sort of integration involved among them.

3.5.1 Azadirachtin

It has a superior docking score of − 8.577 and glide energy values of − 52.684 (Table 3). This docked complex has been studied in depth to understand the interaction between *Azadirachtin* and dengue amino acids. Approximately four lines of contact between these molecules were observed during the interpretation of the docked complex, as explain below. Accordingly, the residues including LYS578, GLY601, TRP477 and GLN350 were involved

Table 3 Docking scores, glide energy and GG/MMGBSA values of phytoconstituents with the RdRp of dengue virus

S. No.	Name of the Phytocon- stituents	Docking Score	Glide Energy	GG/MMGBSA
1	Azadirachtin	− 8.577	− 52.684	− 70.869
2	Curcumin	− 8.112	− 39.927	− 65.461
3	Apigenin	− 7.348	− 34.507	− 59.863
4	Andro- grapholide	− 6.028	− 22.634	− 41.643
5	Berberine	− 5.114	− 35.792	− 37.410
6	- (+)-L-Alliin	− 4.097	− 22.836	− 33.511

Table 4 Interactions between dengue residues and the phytocomponents

S. No.	Name of the Phyto-constituents	Contacts between phytochemical and dengue protein (3 Å)			
		Hydrogen bond	Pi–Pi Stacking	Salt Bridge contacts	Aromatic H-bond
1	Azadirachtin	LYS578, GLY601, TRP477 and GLN350	–	–	VAL579
2	Curcumin	GLY601 and PHE354	–	–	LYS355
3	Apigenin	VAL450, THR571 and GLN597	TRP302	–	–
4	Andrographolide	ARG598, SER600, LYS578 and GLN350	–	–	–
5	- (+)-L-Alliin	ARG598 and SER600 (Covalent)	–	ASP538	–
6	Berberine	GLN602 and GLN350	–	–	–

in the interactions with the Azadirachtin (Fig. 2a and Table 4). The hydrogen bond contact distances between these molecules were measured to be 2.57 in LYS578, 1.81 in TRP477, 2.53 in GLY601, and 2.21 in GLN350 (Fig. 2b and Table 4). Besides the contacts of hydrogen bonds, an aromatic contact has also formed between them. In Fig. 2a, the aromatic bond contact is shown in blue color. Besides the aromatic H-bond contact, other contacts, pi–pi stacking, salt bridge, backbone H-bond and H-bon side chain can be found precisely in the interaction map. It also shows whether the residues of the dengue protein interacted with which group of phytochemicals. Accordingly, residues LYS 578 and GLY 601 are connected as backbone hydrogen bonds to the hydroxyl (OH) group of azadirachtin. Conversely, the other two contacts were such as TRP477 and GLN350 are connected as backbone hydrogen bonds to the oxygen (O) group of azadirachtin. Figure 2b displays this very clearly.

Currently, it has been shown to be a viable antiviral drug candidate for the RdPd of dengue. Previously, since 1990 to till date, numerous studies have stated that azadirachtin is reported to be the primary dynamic

insecticidal molecule as it has potential anti-feedant, growth retardant, sterile and anti-insect larvicidal properties [18, 19]. Besides, the seed kernel of *A. indica* has ovicidal and oviposition deterrent effects against the dengue vectors *A. aegypti* and *A. Albopictus* [20–22]. Based on reports from previous exploration, we believe that activities may have been uncovered due to the presence of this molecule that could have unveiled these potentials either alone or synergistically with other phytochemicals of this plant. This molecule was chosen for its broad antiviral activity against a range of viral infections.

3.5.2 Curcumin

It has second docking scores in this research with high glide energy values against this dengue protease (Table 3). Based on the results, the docked complex was then thoroughly investigated to understand the binding affinities. From the investigation, it was found that about three interactions between these molecules were observed in Fig. 3a. After a thorough investigation, LYS 355 was found to have aromatic interactions with curcumin. In addition, the other
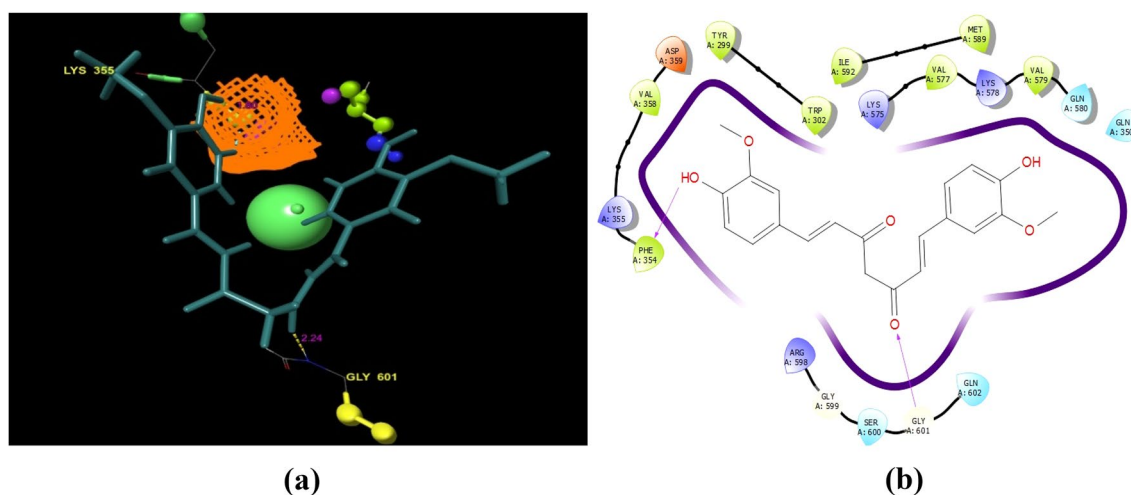

(a)   **(b)**

Fig. 3 **a** Curcumin: interactions and the values of the hydrogen bonding distances, **b** 2D template plot shows the variety of contacts formed between the hydroxy and methoxy groups of curcumin with the RdRp of the DENV protein

two residues such as PHE 354 and GLY 601 have interacted with curcumin as hydrogen bonds (Fig. 3a and Table 3). The hydrogen bond distances of these residues were measured to be 1.80 and 2.24 as shown in Fig. 3a. This interaction diagram showed only the hydrogen bonding backbone contacts; here, residue PHE 354 was linked with a hydroxyl group of the ligand (Fig. 3b). Conversely, residue GLY 601 was connected with an oxygen group of this molecule. Figure 3b shows this very precisely.

It is a unique molecule in the C*urcuma longa*. It is commonly known around the world as turmeric, which also holds numerous patents for its therapeutic potential. In addition, it is widely used as a dye, antibiotic, and cooking agent by people all over the world. Consistent with the therapeutic potential statement, a recent review reported that a variety of viral research, including in silico, in vitro, and in vivo studies, reported that curcumin had a broad spectrum of antiviral potential against has different viruses [23]. For instance, our previous computational research has shown that it has antiviral properties against NS2B47-NS3 [4]. Based on their results, they have reported that it effectively suppressed viral replication while administered at different doses in the *in-vitro* and *in-vivo* trials.

3.5.3 Apigenin

It has good docking and glides energy similar to azadirachtin against this dengue protease (Table 2). Almost five interaction lines are observed in this docked complex, which can be seen in Fig. 4a. Accordingly, residues including VAL

350, THR 571, TRP302 and GLN 597 contributed in contacts with apigenin (Fig. 4a). Among the contacts, a contact line was found as π-π stacking line. The distances of the hydrogen bond contacts were measured to be 1.99 in VAL 350, 2.37 in THR 571, and 1.90 in GLN 597 (Fig. 4a and Table 4). Furthermore, it was found that all connections of hydrogen bonds between dengue and apigenin originate from the hydroxyl group of apigenin. Figure 4b reveals this very clearly.

It is found in the leaves of the *Carica papaya* [24]. Consistent with this claim, Nugroho et al. [25] also reported in 2017 that this molecule is present in the leaves of this plant. In 2016, Kasture et al. [26] reported that the extract of *Carica papaya*, due to its platelet rejuvenating property, was clinically tested by the Indian Medicinal Plant Research against Dengue Infections. Furthermore, a recent study by Sivaraman and Pradeep [27] reported that apigenin has suitable drug potential for RdPd of dengue. There, it has the highest docking values of − 8.28 kcal/mol in the docked ligands. It also had good hydrophobic contact with this protein residue such as LEU511, SER710, ARG729, ARG737, MET761, MET765, TYR766, THR794, SER796, ALA799 and TRP803. However, they did not show the hydrogen bonding contacts as accurately as the current research.

Furthermore, Hakobyan et al. [28] conducted the research on replication of African swine fever (ASFV) in Vero cells by in vitro exploration to find drug efficacy against it. They treated this cell line with various phytochemicals including apigenin, catechin, genistein, luteolin and quercetin, in a dose-dependent manner. Based on their findings, they concluded that apigenin has anti-ASFV properties and
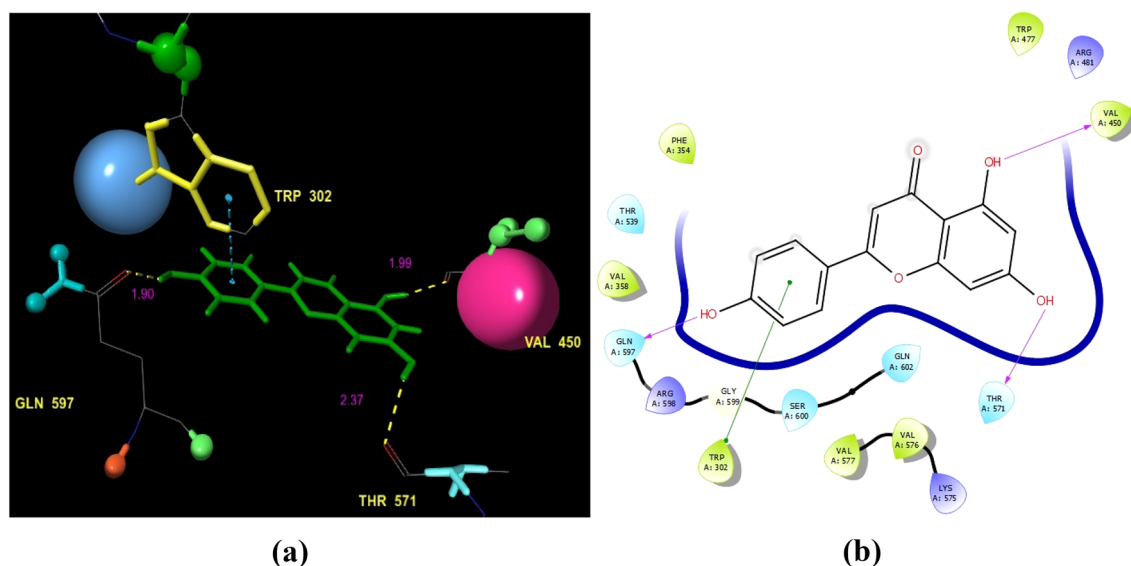

(a)   **(b)**

Fig. 4 **a** Apigenin: interactions and the values of the hydrogen bonding distances, **b** 2D template plot shows the variety of contacts formed between the hydroxy and methoxy groups of apigenin with the RdRp of the DENV protein

recommended further research into this molecule to develop a new drug for ASFV. It had also been used in the treatment of antiviral and immunomodulatory effects on macrophages infected with dengue virus serotypes 2 and 3 to determine whether or not it improved antibodies [29]. Ultimately, they reported that apigenin alone showed a significant antiviral potency in dengue serotypes 3; whereas interleukin 10 was also down-regulated in the dengue infected BHK-21 cell lines [29].

3.5.4 Andrographolide

It has a fourth noble docking score and good glide energy values, which is pretty similar to the phytochemicals discussed above in terms of docking metrics (Table 3). This docked complex was then carefully probed in order to better understand the binding affinities of these compounds according to the docking outcome. From the investigation, it was found that about four contacts between these molecules were observed in three-dimensional view as detailed below. According to the above examination, residues such as LYS 578, GLN 350, SER 600, and ARG 198 were examined to determine whether they were strongly bound to andrographolide in terms of binding affinities (Fig. 5a and Table 4). The hydrogen bond distances of these residues were measured to be 1.99, 1.77, 1.87, and 2.19 (Fig. 5a). Assessment of the interaction plot showed that residues LYS 578, SER 600, and ARG 198 were revealed to have hydrogen-bonding backbone interactions with the hydroxyl of this phytochemical (Fig. 5b). Another residue, GLN 350, was linked to an oxygen group of

andrographolide as a backbone contact. Figure 5b shows this very precisely.

Andrographolide is a unique chemical found in *A. Paniculata*. it was found to have antiviral potential for hepatitis C virus and chikungunya virus (CHIKV) at a 50% effective concentration (EC50) of 77 μM with no cytotoxicity [30, 31]. According to Iwu et al. [32], it has emerged as a plausible, multifaceted antiviral with potential value as it possesses potential mechanisms of action to inhibit Ebola virus disease, dengue fever, and coronaviruses. Although there is such a plausible drug potential against the viruses, it has a place in this research as a third antiviral drug in terms of molecular docking and hydrogen bonding.

3.5.5 Berberine

It has the position of fifth docking scores and good glide energy scores (Table. 3). This docked complex was then carefully probed in order to better understand the binding affinities of these compounds according to the docking outcome. From the investigation, it was found that about four contacts between these molecules were observed in Fig. 6a. According to the above examination, residues such as GLN602 and GLN350 were examined to determine whether they were strongly bound to berberine in terms of binding affinities (Fig. 6a and Table. 4). The hydrogen bond distances of these residues were measured to be 2.28 and 2.30 (Fig. 6a). Assessment of the interaction plot showed that residues GLN602 and GLN350 were revealed to have hydrogen-bonding backbone interactions with the hydroxyl of this phytochemical (Fig. 6b). Figure 7b shows this very precisely.
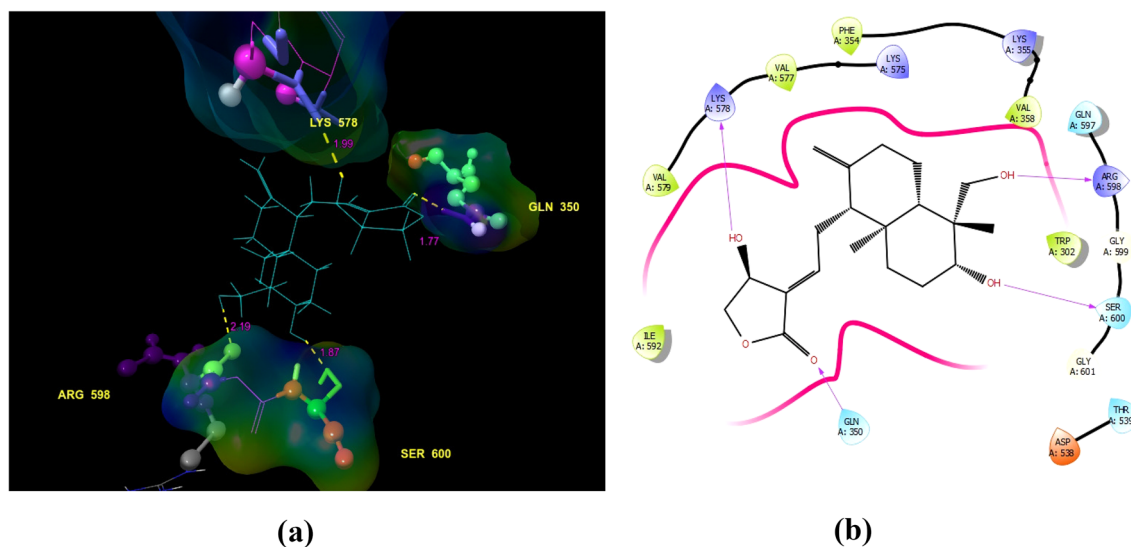

(a)   **(b)**

Fig. 5 **a** Andrographolide: interactions and the values of the hydrogen bonding distances, **b** 2D template plot shows the variety of contacts formed between the hydroxy and methoxy groups of andrographolide with the RdRp of the DENV protein
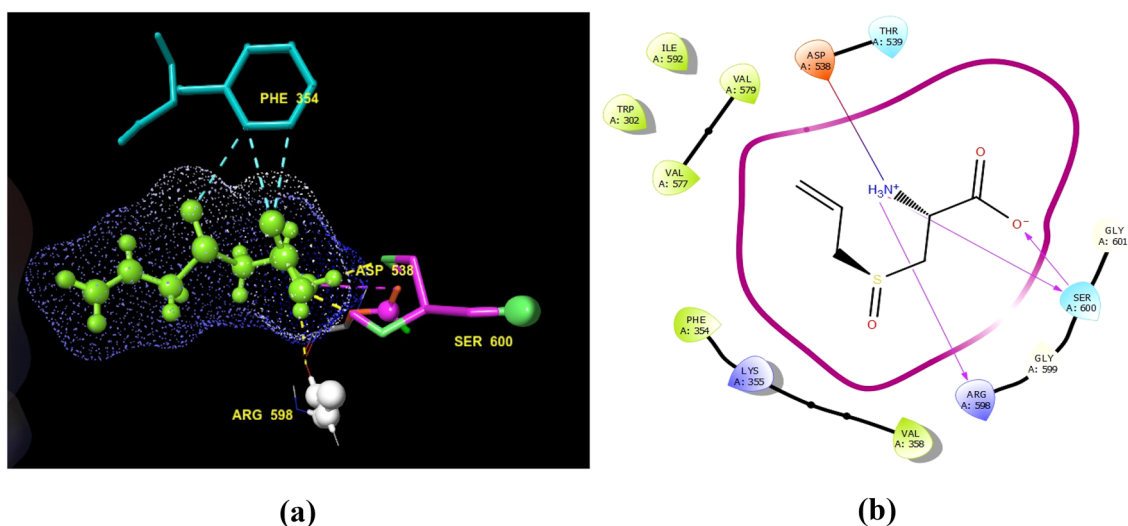
Fig. 6 **a** (+)-L-Alliin: interactions and the values of the hydrogen bonding distances, **b** 2D template plot shows the variety of contacts formed between the hydroxy and methoxy groups of—(+)-L-alliin with the RdRp of the DENV protein
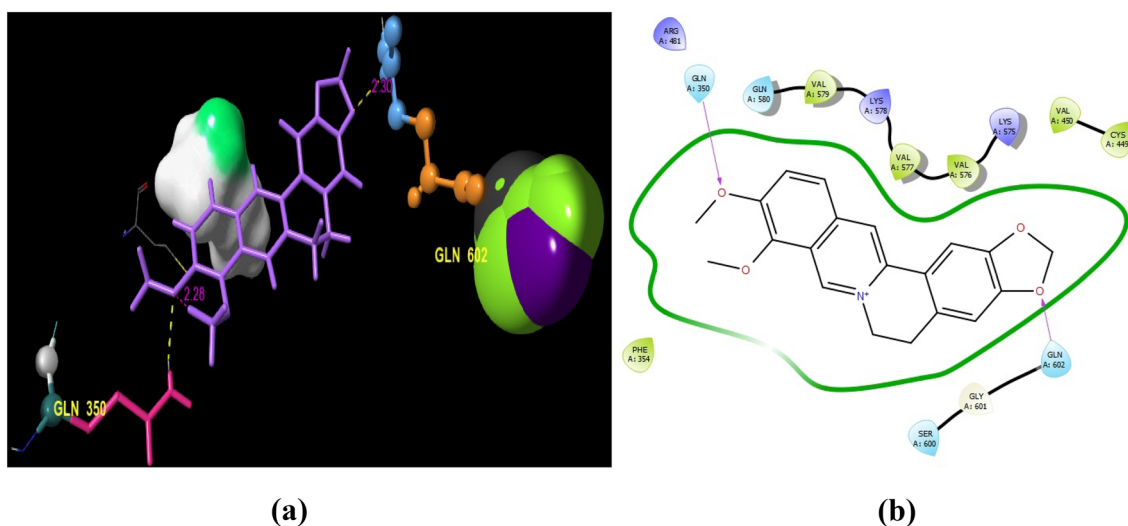

Fig. 7 **a** Berberine: interactions and the values of the hydrogen bonding distances, **b** 2D template plot shows the variety of contacts formed between the hydroxy and methoxy groups of berberine with the RdRp of the DENV protein

It is a natural, low-toxicity isoquinoline alkaloid. A recent review documented that it can be found in the plants of *Berberis vulgaris, Coptis chinensis, Hydrastis canadensis, Coptidis rhizoma, Xanthoriza simplicissima, Phellodendron amurense* and *Chelidonium majus* [33]. Over the past two decades, viruses belonging to the Flaviridae family have been causing serious health complications in humans. A study in 2019 has reported that berberine inhibits the replication of the hepatitis C virus by targeting the E2 glycoprotein of this virus [34]. After concluding the results with molecular docking, they suggested that it could be a potential drug candidate to suppress entry into prophylaxis and HCV complications. It also shows antiviral activity against

the health complications caused by the *Aedes aegypti* and *Aedes albopictus* mosquitoes [34]. There are two viral infections such as dengue (hemorrhagic fever (DHF) and dengue shock syndrome) and zika (microcephaly, spasticity, craniofacial disproportion, irritability, seizures, and other brain dysfunctions) that are commonly caused by these vectors [35, 36].

Similar to the present research, a recent study by Srivastava et al. [37] analyzed this molecule computationally with NS5 methyltransferase from DENV and the NS3 protein from ZIKV. Based on their results, they reported that Berberine could be a potential drug candidate for the complications related to DENV's NS5 methyltransferase and

ZIKV's NS3 protein. Although it has a potential outcome, they suggested it needs to be investigated through invitro and invivo. Despite having such plausible drug potential against dengue and Zika viruses, it holds a place after three phyto-ingredients as an antiviral drug in this research in terms of molecular docking and hydrogen bonding.

3.5.6 (+)-L-Alliin

This molecule has a fifth notable docking value and good glide energy values (Table 3). It was then carefully examined to understand the interaction with this dengue protein. Of the interactions studied, approximately four interactions were observed in Fig. 7a. Accordingly, residues including SER 600, ARG 598 and ASP 538 were found to be involved in the interactions with (+)-L-Alliin (Fig. 7a and Table 4). Among the residues interactions, SER 600 was covalently connected with this molecule. The hydrogen bonding distances were shown as 1.77 and 1.98. In contrast, ASP 538 was shown to form a salt bridge contact with (+)-L-Allin (Table 4). Apart from the hydrogen bonding and salt bridge contacts, the interpretation of the docked complex showed that residues PHE 354 had three aromatic compounds with this ligand molecule. The interaction plot revealed that SER 600 was covalently bound, with one end connected to ammoniumyl of (+)-L-Allin and the other to an oxygen group. ARG 598 is shown to interact with the ammoniumyl group of (+)-L-Allin. ASP 538 is shown as salt bridge contact with ammoniumyl group. Figure 7b shows this very precisely.

It is a nature of organosulfur found in *Allium sativum* and *Allium ursinum* [38]. Hall et al. [38] previously reported that the biosynthetic pathway to—(+)-L-alliin is not yet clear.

They also reported that when human cells were infected with the dengue virus, garlic's organosulfur compounds could minimize inflammation and oxidative stress. Hall et al. [38] suggested that based on their findings, organosulfur will be an alternative drug candidate to treat infected individuals and/or prevent the progression of a serious disease. Despite the fact that it showed better biologic drug potential against dengue inflammation and oxidative stress, it has not shown better result in the present investigation to suggest it as a potential drug candidate for dengue.

3.5.7 Residue Frequencies

The interaction fingerprint plot shows the hydrogen-bonding interactions between the phytocomponents and RdRp of the dengue protein, with the approximately 24 hydrogen-bonding contacts found among them. Among the contacts, residue GLN350 was frequently found as backbone contacts of hydrogen bonds with three phytochemicals. In addition, the highest contact frequency with residues was found for azadirachtin and andrographolide (Fig. 8).

3.6 MM-GBSA

This examination revealed that the free energy values were − 60.869 for azadirachtin, − 61.461 for curcumin, − 59.863 for apigenin, − 41.643 for andrographolide, − 37.410 for berberine, and − 33.511 for -(+)-L-alliin (Table 3). These were estimated to have probable binding energy values with dengue virus RpRd.
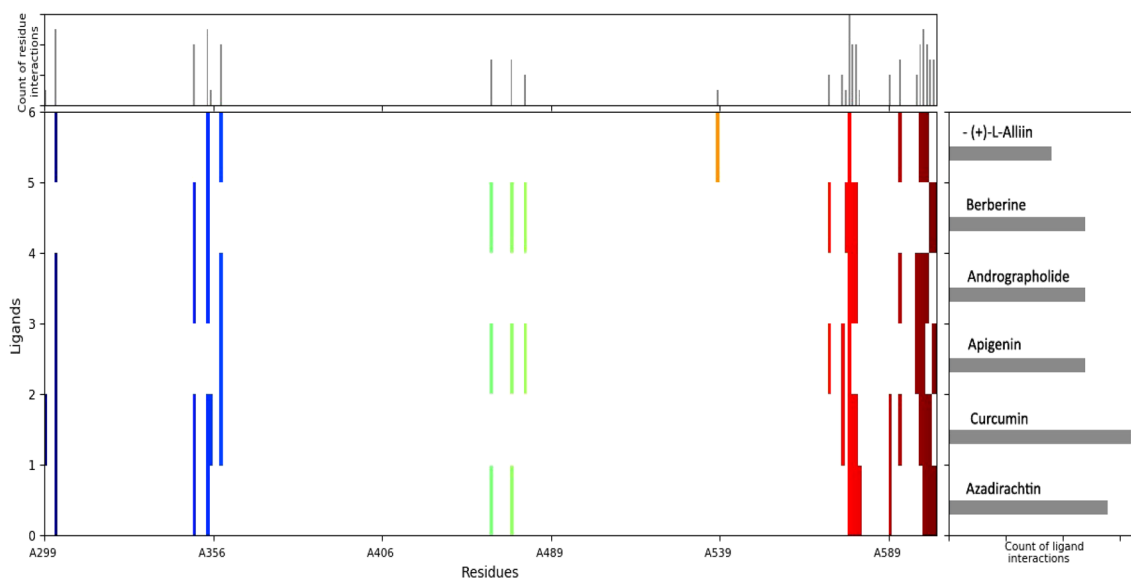

Fig. 8 The fingerprint plot reveals the residues frequency in interactions among phytoconstituents and RdRp of Dengue virus

Table 5 Drug likeness parameters of explored phytochemicals in this research

S. No.	Name of the Polyphenols	MW (g/mol)	HBA	HBD	RB	Log P	TPSA (Å^2)
1	Azadirachtin	720.71	16	3	10	− 0.47	215.34
2	Curcumin	368.38	6	2	8	1.47	93.06
3	Apigenin	270.24	5	3	1	0.52	90.9
4.	Andrographolide	350.45	5	3	3	1.98	86.99
5.	Berberine	336.36	4	0	2	2.19	40.8
6.	- (+)-L-Alliin	177.22	4	2	5	− 2.88	99.6

MW molecular weight, *HBD* hydrogen bond donors, *HBA* hydrogen bond acceptor, *RB* rotatable bond, *TPSA* topological polar surface area

3.7 Drug Likeness

The drug-likeness of probable dengue antagonist phytochemicals was explored as described in method 2.1. The drug likeness parameters such as molecular weight (MW), hydrogen bond donors (HBD), hydrogen bond acceptor (HBA), rotatable bond (RB), and topological polar surface area (TPSA) were shown in Table 5.

3.8 Pharmacokinetics Properties of Docked Molecules

This pharmacokinetic study plays a vital role in assessing the value of drug molecules in terms of absorption, distribution, metabolism, excretion and toxicity (ADMET) (Table 5 and Fig. 9). This exploration was revealed that the tested molecules have the active potential for development as a drug to

this dengue protein-related function. Through this research we have clearly understood that despite its deviation from Lipinski's rule, azadirachtin possesses remarkable pharmacokinetic properties. It was also reported as a non-toxic substance as having no risk to human health in a fact sheet report by CERTIS, USA, LLC [39]. This statement assisted in the understanding of the toxicity of azadirachtin. Ultimately, we believe these are the most relevant and important findings for drug toxicity assessment.

As shown in Table 6, the pharmacokinetic studies were aided in determining the status of screened anti-dengue phytochemicals for drug development. The results, as well as the 95% drug-likeness, are shown in Table 4 according to the pharmacokinetic properties. Almost more than 20 drugs have been withdrawn from the market in the past two decades due to their significant side effects after treatment [40]. This could be due to a lack of adequate research

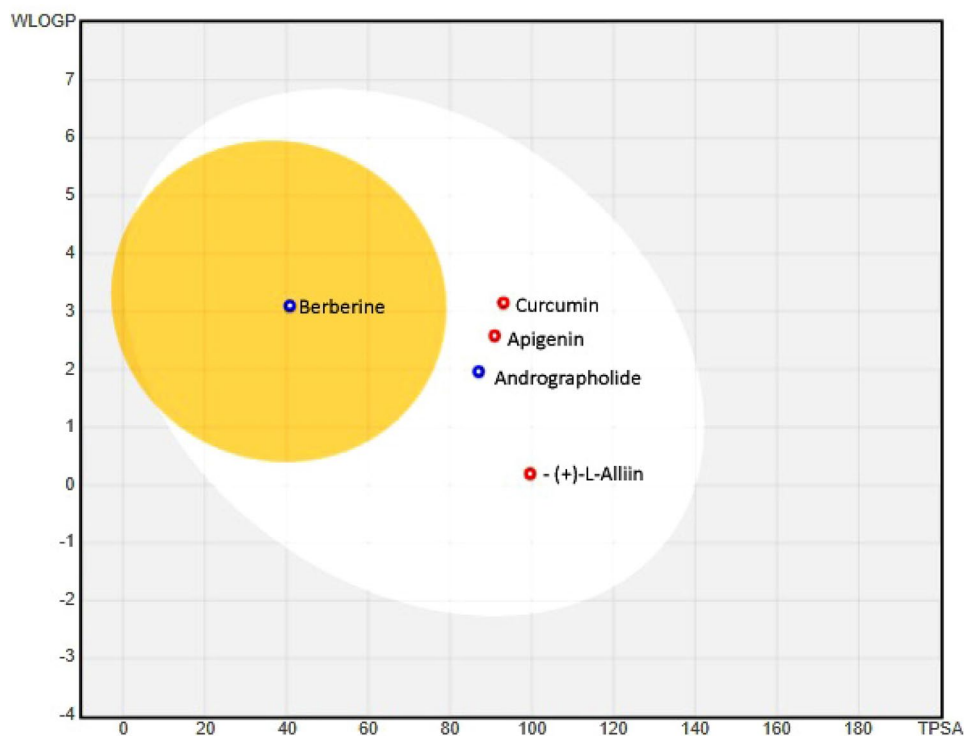**Fig. 9** The boiled egg plot shows the ADME parameters of the phytoconstituents explored in this study

Table 6 ADMET profiling parameters of phytochemicals explored in this research

Pharmacokinetic Properties	Azadirachtin	Apigenin	Curcumin	Andrographolide	Berberine	- (+)-L-Alliin
Absorption						
Water solubility	− 3.038	− 4.073	− 4.01	− 3.419	− 2.967	− 2.888
Caco2 permeability	− 0.316	0.033	− 0.093	1.195	1.738	0.78
Intestinal absorption (human)	100	82.441	82.19	93.613	98.815	77.35
Skin Permeability	− 2.735	− 2.768	− 2.764	− 3.751	− 2.599	− 2.736
P-glycoprotein substrate	No	Yes	Yes	Yes	Yes	No
P-glycoprotein I inhibitor	No	Yes	Yes	Yes	No	No
P-glycoprotein II inhibitor	No	Yes	Yes	No	Yes	No
Distribution						
VDss (human)	− 1.733	− 0.258	− 0.215	− 0.237	0.695	− 0.545
Fraction unbound (human)	0.189	0	0	0.216	0.396	0.459
Blood–Brain barrier permeability	− 2.097	− 0.51	− 0.562	− 0.638	0.553	− 0.296
Central Nervous System permeability	-2.146	− 3.044	− 2.99	− 2.522	− 1.618	− 3.474
Metabolism						
Cytochrome P2 D6 substrate	No	No	No	No	No	No
Cytochrome P3 A4 substrate	No	Yes	Yes	Yes	Yes	No
Cytochrome P1 A2 inhibitor	Yes	No	Yes	No	Yes	No
Cytochrome P2 C19 inhibitor	No	Yes	Yes	No	No	No
Cytochrome P2 C9 inhibitor	No	Yes	Yes	No	No	No
Cytochrome P2 D6 inhibitor	No	No	No	No	Yes	No
Cytochrome P3 A4 inhibitor	No	Yes	Yes	No	No	No
Excretion						
Total Clearance	1.851	0.014	− 0.002	1.179	1.282	
Renal OCT2 substrate	No	No	No	No	Yes	
Toxicity						
AMES toxicity	Yes	No	No	No	No	No
Max. tolerated dose (human)	0.744	0.048	0.081	− 0.64	0.023	1.149
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	Yes	No
Oral Rat Acute Toxicity (LD50)	2.482	1.915	1.833	2.589	3.007	2.01
Oral Rat Chronic Toxicity (LOAEL)	3.193	1.586	2.228	0.599	2.051	1.908
Hepatotoxicity	No	No	No	No	Yes	No
Skin Sensitisation	No	No	No	No	No	No
Minnow toxicity	0.285	− 0.634	− 0.081	0.553	− 0.294	2.332

on the drug-likeness and the ADMET factors of potential molecules.

3.9 MD Simulation

Molecular dynamics (MD) simulations were performed on the dengue protein-docked complex of azadirachtin and RdRp, which was selected as a result of molecular docking based on docking scores and protein–ligand interaction. The result of the MD simulation showed that the residual flexibility, a radius of gyration based on C-coordinates, potential energy and temperature stability is as described in Fig. 10a–d.

4 Conclusion

Dengue fever is recognized as the deadliest pandemic disease in the world, becomes spreading rapidly. Although this virus infection is the deadliest for humans, as a co-infection with Corona disease, it is currently becoming an additional health burden for humans. Therefore, there is currently a need for novel drug candidates from natural sources that act either as agonists for the human body or as antagonists for the viruses. According to the latest Dengue Treatment report and document, the unique phytochemicals were selected from plants that have been prescribed for dengue. These were chosen because of the adverse effects of synthetic drugs and lack of specific drug for dengue. Eventually, the present
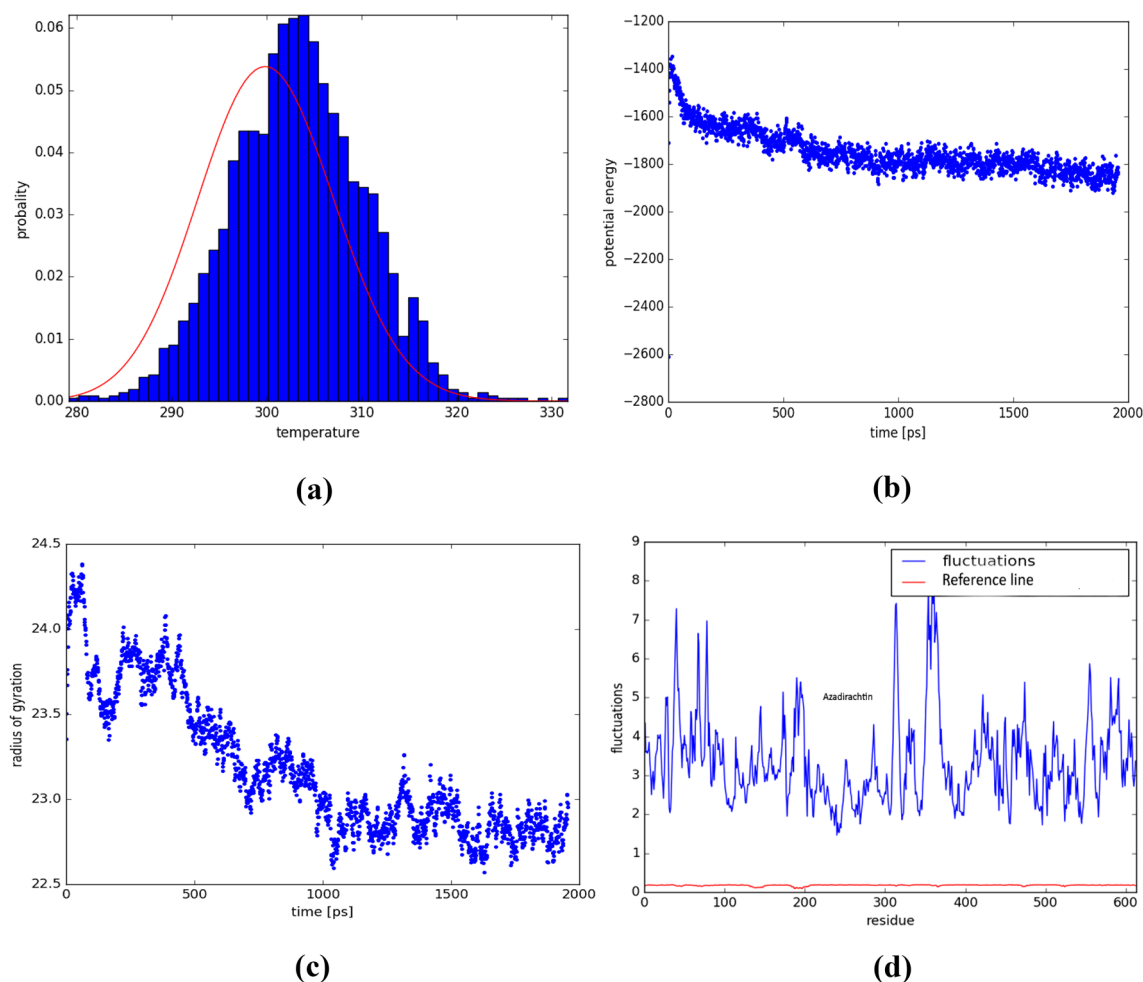
Fig. 10 Molecular dynamic simulation of the docked complex of azadirachtin and RdRp of dengue virus: **a** Temperature histogram, **b** Potential energy, **c** Radius of gyration of RMSD-Cα coordinates, **d** Fluctuations (Blue line denotes rosmarinic acid and red line denotes reference line)

research revealed that all of the docked phytochemicals have good docking values and glide energy values with notable hydrogen bonding with this dengue target. Although phytochemicals such as curcumin, apigenin, andrographolide, berberine and -(+)-L-alliin have been reported to have potent antiviral potential. The current results, compared to previous in-silico studies, propose that azadirachtin is identified as a potential drug candidate for dengue protein-related activities and complications. Interestingly, molecular dynamic simulations reveal that the combination of azadirachtin and dengue target has an excellent understanding of the specific interactions and has also supported stable acquisition at a binding site. In conclusion of the present findings, we believe that not only the azadirachtin but also other molecules such as curcumin, apigenin, andrographolide and berberine would reveal significant therapeutic potential against dengue virus replication in both in-vitro and in-vivo experiments. Moreover, we anticipate that this research will be a useful resource

for the discovery of drugs to inhibit further replication of the viruses in the infected individual.

Data Availability The datasets used for analysis during these studies were included in this published study.

Declarations

Conflict of interest The author(s) have disclosed no potential conflicts of interest in researching, authoring and/or publishing this article.

Ethical Approval This research does not require ethical approval.

Consent for Publication On behalf of the authors, I hereby assign the right of this entire article content to this journal.

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