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Research Article

Computational studies of antiviral properties of curry powder water extract against Norovirus infection

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ABSTRACT

Curry powder is a mixture of ground spices that are typically used to enhance flavor, aroma, color, and consistency in curries. The Sri Lankan roasted curry powder "*bedapu thuna paha*" consists of coriander seeds (*Coriandrum sativum* L.), cumin seeds (*Cuminum cyminum*), fennel seeds (*Foeniculum vulgare* Mill.), cinnamon sticks (*Cinnamomum zeylanicum*) and curry leaves (*Murraya koenigii*) as the main ingredients. *Norovirus* (NoV) is a single-stranded RNA virus belonging to the family *Caliciviridae*. The P domain capsid protein of this virus plays an important role in the host immune response and receptor recognition because when protruding domain 1 binds with receptor molecules, it is easy to penetrate the host cell. Therefore, twenty potential ligands contained in the curry powder decoction, which were identified through literature review, were docked to the active site in the P domain from norovirus strain saga4 in complex with HBGA, and Mahanimbine, Mahanine, and Fenchone ligands-binding energies were greater than -6.00 kcal/mol. According to these interactions between ligands and the protein, which were given binding energies greater than -6.00 kcal/mol, only the Mahanine ligand interacted with the highest number of amino acids in the binding pocket compared to the other two ligands (Asp374, His347, Gly346) and this ligand was subjected to Molecular Dynamics (MD) simulations. MD simulations were performed on the protein-ligand complex for 10 ns using the CHARMM36 force field. Rg, RMSD. The RMSF results indicated the stability of the protein-ligand complex throughout the simulation time and suggested that Mahanine phytochemical may be used as a potential anti-virus agent against *Norovirus.*

Keywords: Curry powder, mahanine, molecular docking, molecular dynamic, *Norovirus*

INTRODUCTION

Curry powder is a seasoning containing a variety of spices that is used to add flavor and aroma to food dishes, especially to curries. Different countries have different types of curry powders following different recipes. In Sri Lanka, curry powder is made using a variety of spices that are dried in the sun or roasted and then ground. The most popular roasted curry powder is made with coriander (*Coriandrum sativum*) seeds, cumin (*Cuminum cyminum*) seeds, fennel (*Foeniculum vulgare*) seeds, cinnamon (*Cinnamomum zeylanicum*) barks and curry leaves (*Murraya koenigii*) (Nishan and Subramanian, 2014).

Coriander is one of the main ingredients of Sri Lankan curry powder and is generally roasted and ground when the curry powder is cooked. When considering the culinary value of *Coriandrum sativum* is used to enhance the flavor of curries and is also used in condiments, desserts, liqueurs,

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perfumes, and candies (Takeda *et al*., 2008). In traditional and Ayurveda medicine. coriander is used to treat gastrointestinal ailments (anorexia, dyspepsia, flatulence, diarrhea, griping pain, vomiting, etc.), respiratory ailments, ailments related to the urinary system, local swelling and pain, headache, and burning sensations (Fayyad *et al.,* 2017).

Cumin seeds possess an aromatic and pungent flavor. Due to its flavor, aroma, and medicinal properties, it is used in cooking food, added to fragrances, and in different traditional medicines such as the Unani system of medicine, folk medicine, Ayurveda medicine, and Iranian traditional medicine for the treatment of toothache, diarrhea, common cold, fever, painful inflammatory conditions (Bhat *et al*., 2014).

Fennel (*Foeniculum vulgare*) is another spice that has a unique aroma and mostly cultivated in the Mediterranean and central Europe region. It is not only used for culinary purposes but is also used in industries such as cosmetics, pharmaceuticals, food, and medicinal purposes. It is used in many traditional medicine systems such as Ayurveda, Unani, and Siddha, in the Indian and Iranian traditional systems of alternative and balancing medicine to treat dyspeptic complaints, spasmodic, gastric-intestinal complaints, bloating and flatulence, diabetes, bronchitis, chronic cough, kidney stones, fever etc. (Badgujar *et al.*, 2014).

Cinnamon is a spice that is popular in many countries around the world and a constituent of Sri Lankan curry powder. Dried cinnamon bark powder from two most popular species of cinnamon are C*innamomum zeylanicum* which is known as Ceylon cinnamon, and *Cinnamon cassia* which is known as Chinese cinnamon are used to make curry powder (Hong *et al*., 2012). Apart from its use as a spice, cinnamon is also used in traditional and modern medicine (Prabhuji *et al.,* 2021; Yulion *et al.,* 2022).

In Sri Lanka, most curries are flavored with curry leaves (*Murraya koenghii*). Dried and ground curry leaves are used in preparing curry powder. It is also used in traditional systems of medicine to treat ailments such as diarrhea, stomach aches dysentery, insect bites, and to relieve the heat of the body. The oil is used externally to treat bruises. It has also been used as a blood purifier (Saini, 2015).

Noroviruses (NoVs) are the most important pathogens of epidemic nonbacterial gastroenteritis. Norovirus is a contagious, single-stranded RNA virus belonging to the family *Caliciviridae*. NoVs are a group of non-enveloped viruses encapsulated by an icosahedral protein capsid that primarily cause acute gastroenteritis (Singh *et al*., 2015). Human NoVs are also highly diverse and have multiple receptor binding patterns. NoVs recognize human histo-blood group antigens (HBGAs) types A, B, and H, secretor and Lewis antigens as receptors or attachment factors. The P domain plays an important role in host immune response and receptor recognition because when VP2 binds with receptor molecules it is important to penetrate the host cell during infection. Therefore, interaction between the P domain and receptors can be one of the most effective strategies in developing therapeutics against virus infection (Singh *et al*., 2015).

The P1 subdomain forms the leg of the P dimer connecting it to the S domain, while the P2 subdomain is located on the outermost surface and involved in the binding of norovirus to their host receptors – HBGAs. This has been confirmed by several crystallographic and mutagenesis analyses. Using multiple sequence alignments, homology modeling, and structural analysis of norovirus capsids, that identified a plausible receptor binding pocket in the P2 domain that may be responsible for binding to HBGA receptors. (Huang *et al*., 2004). The P domain dimers are stable over a broad range of pH (2 to 11) or under strongly denaturing conditions due to their inter-subunit disulfide bonds (Bereszczak *et al*., 2012). Since the P domain of norovirus can act as antigens in the human body, they have the potential to become a promising target for determining antiviral properties against noroviruses. Previous studies of the P domain have provided useful information about the virus-receptor interaction (Hegde *et al*., 2004).

The decoction extraction method is used to extract phytochemicals from each raw ingredient of curry powder. Decoction is the most preferred extraction process for extracting water-soluble, heat-stable constituents from fibrous plants (Hidayat and Wulandari, 2021).

Some groups of phytochemicals that may have significant impact on human health are carotenoids, phenolic compounds, phytosterols and phytosterols, tocotrienols, organosulfur compounds, and non-digestible carbohydrates (Otunola, 2022). Curry powder spice

ingredients were examined for their ability to provide health benefits against norovirus. (Anwar *et al*., 2009).

Most of the powerful computational methods used for prediction of drug designing, determination of action of mechanisms, activities, designing of materials, catalysts, and determination of desirable activities of proteins or peptides and their functions are the method of "Quantitative Structure-Activity Relationship" (QSAR). The basis of QSAR modeling is chemical descriptors which have made significant efforts and progress to develop a wide variety of chemical descriptors to describe different levels of chemical, physical, and structural characteristics of the target molecules or systems. Hence a predictive and interpretable QSAR model can help to further understand the mechanism of action of the explored molecules towards the target systems (Chen *et al*., 2015).

In this study, the potential phytochemicals contained in the water extracts of each ingredient in the curry powder were used to evaluate the antiviral activity against norovirus by *in silico* screening of phytochemicals by targeting norovirus P domain using molecular docking. Furthermore, to enhance the reliability of our findings, molecular dynamics simulations were conducted. This involved a detailed examination of the dynamic behavior and stability of the interactions identified during the molecular docking process. The combination of in silico screening, molecular docking, and molecular dynamics simulations would provide a robust and thorough evaluation of the antiviral potential of the phytochemicals found in curry powder against norovirus.

MATERIALS AND METHODS

Ligands preparation

At the beginning, 3D conformations of the ligands were searched from PubChem and downloaded in SDF format file types. These files were converted into the PDB format files and subjected to Avogadro software. The geometries were optimized by using Force Field MMFF94, algorithm steepest descent, 500 steps. The above-mentioned files were saved as PDB format files and subjected to AutoDock Tools 1.5.6 and converted to PDBQT file formats.

Protein preparation

The uniport id of the P domain from norovirus strain Saga4 (GII-4) in complex with HBGA type A was obtained from the literature survey. The bound P domain of GII-4 (4X07) with the removal of HBGA type A-trisaccharides was used as a model for the screening. The FASTA sequence of this protein was inserted into the BLAST server to find out suitable template. Then the same FASTA sequence of this protein was inserted to search suitable template from SWISS-MODEL server. The most appropriate template was identified based on the information obtained from these two servers and modeled via SWISS-MODELER. The energy optimization of the modeled protein and the refinement were done by Galaxy Refine webserver.

Model validation

The model protein was validated by using Verify 3D, ERRAT, and PROCHECK via the SAVES v6.0 server and ProSA server.

Binding site identification

A thorough literature survey was done to find the exact amino acids in the active site of the protein and protein structure was investigated to check whether each active site amino acid is in the correct position. The obtained amino acids in active sites can be further checked using the GASS-WEB server. The refined PDB file was uploaded to the server and the most probable active site was found. The list of amino acids is depicted below (Table 1).

Molecular docking

Water molecules were deleted which were adhered to the protein and polar hydrogen atoms were added. Finally, Kollman chargers were added to the modeled protein structure using AutoDock Tools 1.5.6. Then the above file was saved as a PDBQT format file. By detecting torsion root using AutoDock Tools 1.5.6, ligand files were

Table 1: List of amino acids associated with binding pocket

Protein	PDB ID	amino acids associated with binding pocket
P domain from norovirus strain saga4	4X07	Thr344, Arg 345, Ala 346, His347, Asp374,Ser442, Gly443

transformed to PDBQT format files. Both pdbqt files of ligands and protein were subjected to the AutoDock Tool 1.5.6. The grid parameter file was generated via Autogrid 4.2, while the Docking parameter file was generated by using AutoDock 4.2. The settings of the Lamarckian genetic algorithm (LGA) were adjusted as genetic algorithms (GA) runs: 100, size of the population: 300, and 25000000 of maximum evaluations. Other parameters were kept unchanged. For these docking studies, site-specific docking was performed and the macromolecule was kept rigid and the ligands kept flexible. Finally, the resultant files were generated as (dlg).

Analysis of docking results

Pymol software and ligplot plus online server were used to visualize the binding conformations between the protein and each ligand. A protein-ligand interaction profiler was used to validate the binding residues from the pymol.

Molecular dynamic simulation

The best negative binding energy was selected to perform molecular dynamics (MD) simulations to get further details about the protein-ligand complex. MD simulations were performed using the GROMACS (version 2021.4) software package, with CHARMM36 force field and default TIP3P water model. The protein-ligand complex was centered in a dodecahedron box with a minimum distance of 1.0 nm between the complex and any side of the box. The system was solvated with water and sodium and chloride ions were added to replace solvent molecules and to neutralize the systems at 0.15 M salt concentration. The LINCS bond length constraint algorithm was used to constrain bond lengths. Particle Mesh Ewald summation was used for electrostatic interactions and grid spacing of 0.12 nm combined with an interpolation order of 4 was used for long-range interactions. For van der Waals interactions, a cut-off of 1.4 nm was used. Energy minimization was performed using the steepest descent algorithm. The system was gradually heated from 50 K to 300 K throughout a 100 ps time. Finally, the MD production run was done in NPT ensembles at 300 K using a V^{-} V-rescale thermostat and at 1 bar using a Berendsen barostat. Results of the simulations were obtained after 10 ns production runs with 2 fs time steps. The trajectory obtained from the MD simulation was used to calculate the radius of gyration (R_g), root mean square deviation (RMSD), and root mean square fluctuation (RMSF).

RESULTS AND DISCUSSION

In *silico* analyses are rare related to the investigations of inhibition of Norovirus activities in humans from the natural products from different herbal extracts, as far as the authors are aware. According to referred literature (Obaid *et al.,* 2023), ZINC66112069 and ZINC69481850 showed higher binding affinities towards the NoV RdRp active site based on their binding energy (BE), physicochemical and drug-like characteristics, and molecular interactions. While the positive control had an interaction with RdRp with a BE of 9.0 kcal/mol, ZINC66112069, and ZINC69481850 had BEs of 9.7 and 9.4 kcal/mol, respectively. Additionally, the attached complexes displayed good stability throughout the 100 ns molecular dynamic simulation. It may be determined that ZINC66112069 and ZINC69481850 as potential inhibitors of the HNoV RdRp in future antiviral medication development investigations.

Moreover, in a 2021 study, discussed a class of peptidomimetic aldehydes that have strong antiviral activity against noroviruses both *in vitro* and in a small animal model (Van Dycke *et al.,* 2021). These results underline once more the HuNoV protease's appeal as a target for the creation of highly resistant norovirus antivirals. In a small animal model, a class of protease inhibitors was demonstrated to be effective against HuNoV GII.4 for the first time. Overall, these substances should be further researched to cure HuNoV-induced sickness (as well as to fight off infections from corona- and enteroviruses).

Another *in silico* study was conducted using the molecular docking technique, twenty (20) plant metabolites were evaluated against the norovirus VP1, VP2, P48, and P22 protein domains (Alam *et al.,* 2022). Asiatic acid, avicularin, guaiaverin, and curcumin had the highest binding affinities with all of the chosen proteins. The potential drug surface hotspots and binding sites for each viral protein with prospective medications were identified. The findings revealed that none of the compounds showed any unfavorable effects that might lessen their drug-like qualities. No measurable tumorigenic, mutagenic,

irritating, or reproductive effects of the compounds were found, according to the study of the toxicity pattern. However, curcumin exhibited the highest levels of cytotoxicity and immunotoxicity among the top four substitutes.

According to a study, for the development of anti-NV drugs, the enzyme NV 3 C L protease (3CLP) has been identified as a viable therapeutic target (He *et al.,* 2022). A virtual screening method was used based on the structure to test a library of 700 antiviral compounds against the residues in the active site of 3CLP. Three compounds were reported, Sorafenib, YM201636, and LDC4297 after sequential screening, detailed molecular docking and visualization, physicochemical and pharmacological property analysis, and molecular dynamics (MD) study, were found to have a higher binding energy (BE) value with 3CLP than the control (Dipeptidyl inhibitor 7). As compared to control, sorafenib, YM201636, and LDC4297 showed BEs of -11.67, -10.34, and -9.78 kcal/mol with 3CLP, respectively. The interactions were further optimized using MD simulations of the two best compounds and the control, and a 100 ns MD simulation showed that they create stable complexes with 3CLP.

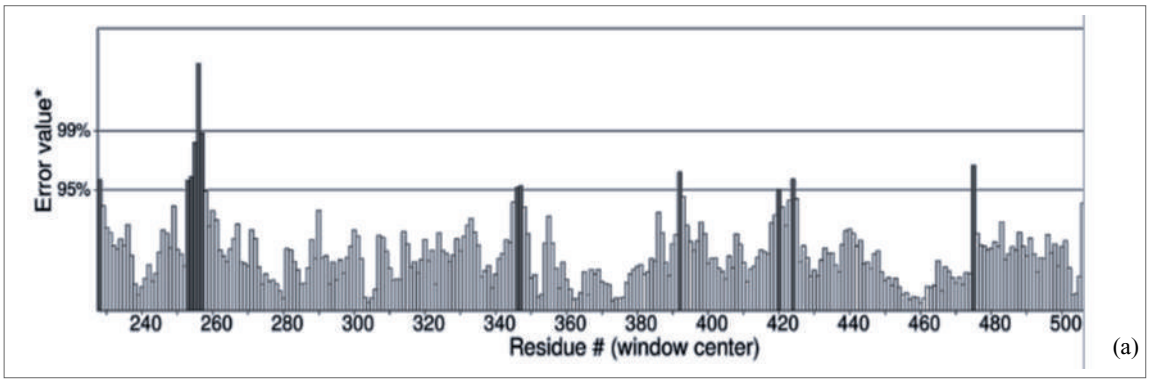
In this study, it was determined that if any natural ligand of curry powder ingredients that can block the HBGA type A binding site of P domain from norovirus strain Saga4 (GII-4) to prevent binding with HBGA type A antigens to initiation the infectivity. Based on literature reviews, 42 phytochemicals were identified which were able to extract into water. Those phytochemicals were again filtered into 20 ligands based on their percentage amount extracted into water from each five ingredients, and also with their drug-likeness properties. Phytochemicals that followed Lipinski's rule of five were chosen for the docking process (Table 2).

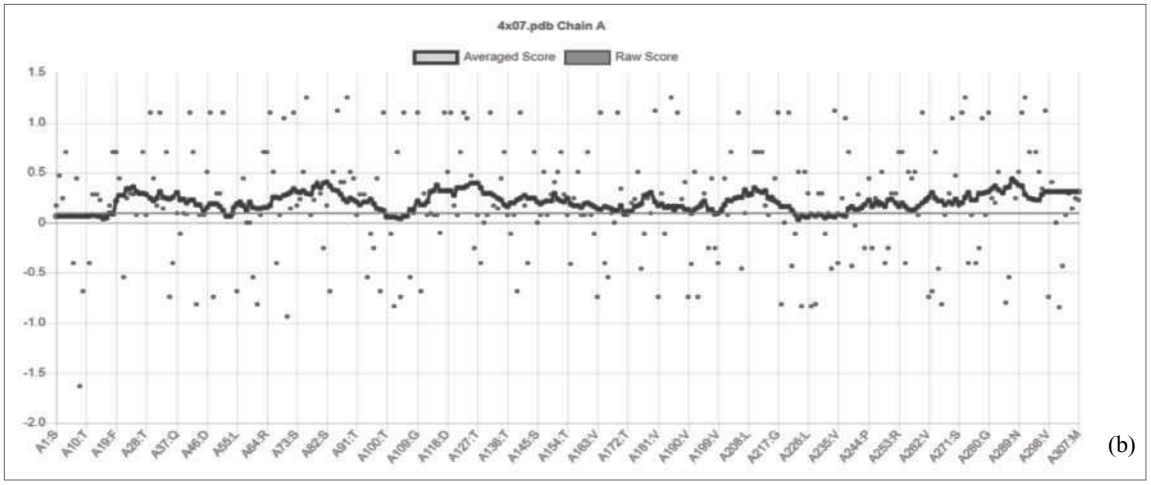
In protein preparation, after the energy optimization, the selected modeled protein PDB preceded to ERRAT, VERIFY 3-D, PROCHECK, and ProSA check web servers. The VERYFY 3-D score gives the matching of an atomic 3D model with its 1D peptide sequence by determining the structural class. The model's quality was determined by the score of the passed residues, which was larger than 0.2. According to the results, the modeled protein has 86.32% residues averaged 3D-1D score greater than 0.2. ERRAT server interprets the statistics of non-bound interactions between atom types. The acceptable value of ERRAT is greater than 60 and this modeled protein score was 95.13%. PROCHECK server was used to examine the placement of amino acid residues in the permitted and forbidden zones, and the protein structure's overall stereochemical quality. According to the results, the modeled protein had 87.70% residues in the favored region, indicating that this structure is reliable for docking studies. ProSAweb server is used to check the potential errors of the 3D structure of the protein. The Z score from this server gives the total quality of the model. Negative values for the z score represent the protein validity and the selected protein score was -4.67, which confirmed its validity (Figure 1).

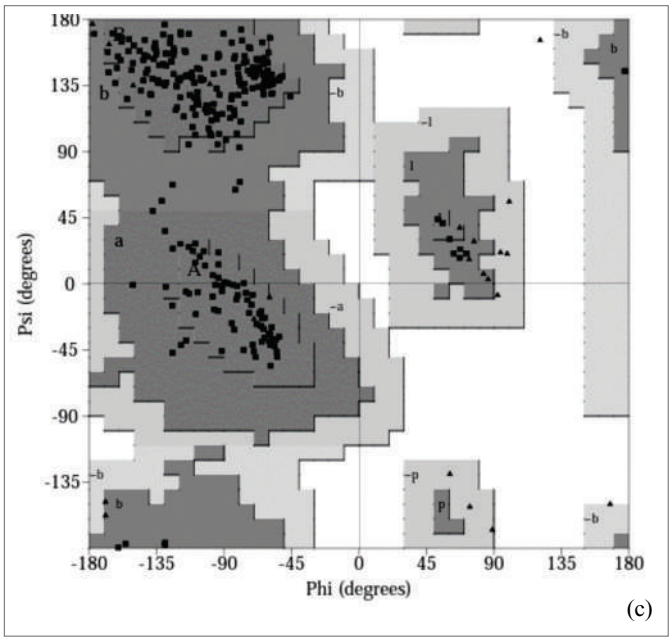
Table 2: Lipinski's rule of five test results for phytochemicals that gave higher docking results (Binding energy (kcal/mol) greater than -6.00)

Compounds (phytochemicals)	Molecular formula	Lipinski's rule of five
Mahanimbine	$C_{23}H_{25}NO$	Molecular weight (g/mol) (< 500) 331.45
		H-bond donors (< 5) 1
		H-bond acceptors (< 10) 1
		Rotatable bonds (< 10) 3
		Log $P_{o/w}$ (< 5) 5.62
Mahanine	$C_{23}H_{25}NO_2$	Molecular weight(g/mol) (< 500) 347.45
		H-bond donors (< 5) 2
		H-bond acceptors (< 10) 2
		Rotatable bonds (< 10) 3
		Log $P_{o/w}$ (< 5) 5.20
Fenchone	$C_{10}H_{16}O$	Molecular weight(g/mol) (< 500) 152.23
		H-bond donors (< 5) 0
		H-bond acceptors (< 10) 1
		Rotatable bonds (< 10) 0
		Log $P_{o/w}$ (< 5) 2.66

In this study, molecular docking can be defined as a molecular modeling technique that can be used to predict the interactions between a large molecule such as a protein (enzyme), nucleic acid, and a small molecule (ligand). Knowledge of the preferred orientation may be used to predict the strength of association or binding affinity between protein and the ligand. Normally docking gives binding energy. The energy needed to separate the protein-







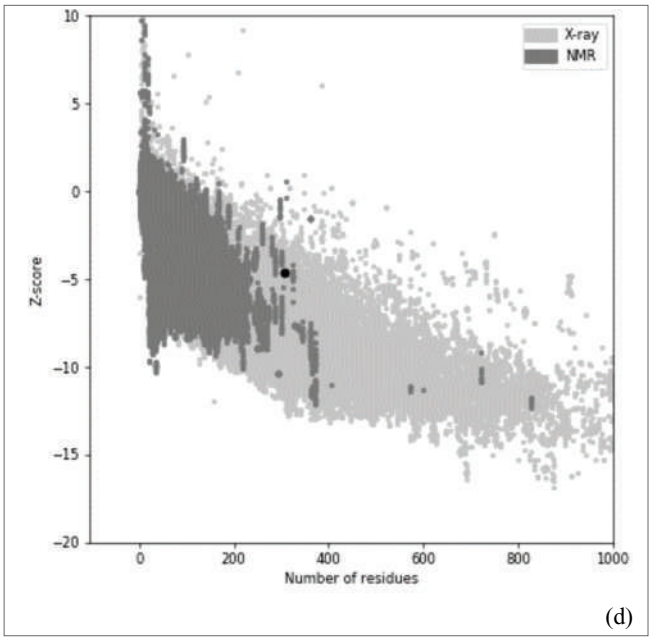


Figure 1: The ERRAT plot (a) indicates its accuracy. Red, yellow, and white bars represent errors, warnings, and acceptable scores. VERIFY3-D plot (b) shows that 86.32% of its residues have a score ≥0.2. Ramachandran plot (c) indicates that 87.70% of residues are in the favored region. ProSA Z-score diagram (d) shows that the model is within the range of scores recorded for native proteins of equal size

Table 3: Docking results of crystal structure of P domain from norovirus strain GII-4 with curry powder ligands.

Ingredients of curry powder	Phytochemicals	Binding Energy (kcal/mol)
Fennel	Anethole	-4.83
	Fenchone	-6.31
	Limonene	-5.41
	Quercetin	-4.99
Curry leaves	Mahanimbine	-7.07
	Mahanine	-6.57
	Murrayanol	-5.96
Coriander	Alpha_pinene	-5.97
	Alpha-terpinene	-5 .52
	Geraniol	-5.39
	Limonene	-5.33
Cinnamon	Alpha-phelendrene	-5.52
	alpha-terpinene	-5.52
	beta-phellendrene	-5.57
	Limonene	-5.41
Cumin	2-allylphenol	-5.01
	alpha_pinene	-5.97
	Cuminaldehyde	-5.55
	p-menthatriene	-5.29
	Terpinolene	-5.47

ligand complex is defined as $\Delta G_{binding}$. In another way, it is the energy difference between bound form and unbound ligand and protein separately.

Twenty ligands were docked to the site mentioned in (Table 3), and out of those 3 ligands' binding energies were greater than -6.00 kcal/mol. The above docking results were obtained for the site-specific docking of the P domain from norovirus strain saga4 in complex with HBGA type A and a grid box was drawn to that site. The highest binding energy (BE) (-7.07 kcal/mol) was seen between the receptor molecule and Mahanimbine (a derivative of curry leaves). The phytochemicals of curry leaves had much more negative binding energies than other derivatives of curry powder. The order of increasing binding affinity of proteins to ligands is mahanimbine > mahanine > fenchone > alpha-pinene murrayanol > beta-phellendrene (Figure 2). In addition, the curry leaves' phytochemicals, in curry powder ingredients, had higher negative binding energies than other ingredients. Moreover, fennel, cinnamon, and coriander have some potential to inhibit the protein-HBGA binding.

In addition, it should be noted that binding energy alone cannot predict the effect on the target protein function due to ligand-protein interaction. To clarify the relationships between the ligands which showed the greatest binding

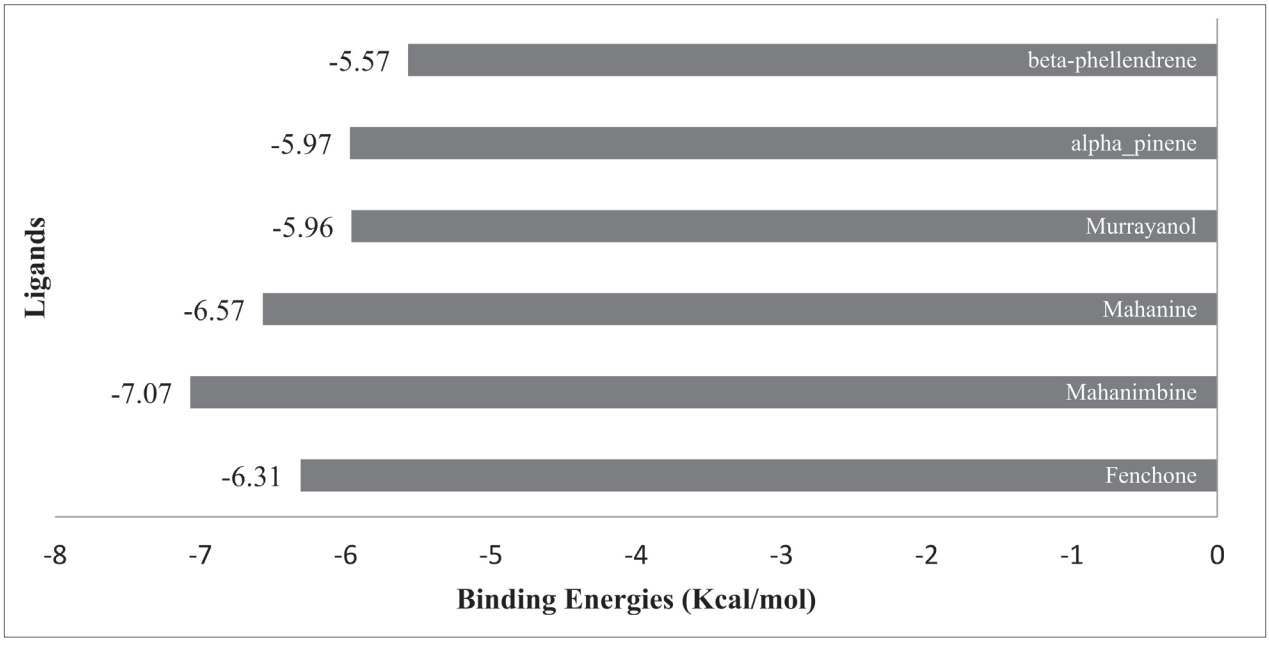


Figure 2: The order of increasing binding affinities of ligands with the HBGA type A receptor

Table 4: Amino acids responsible for ligand-protein interactions (* - represents amino acids included in the binding pocket)

Ingredients of curry powder	Phytochemicals	Binding energies (kcal/mol)	Amino acids responsible for ligand-protein interactions
Fennel	Fenchone	-6.31	Pro439, Gln336, Lys382, Thr335, Try308, Asn380, Gly440*,Met447, Thr337
Curry leaves	Mahanimbine	-7.07	Pro439, Lys382, Thr437, Try308, Lys339, Asn380*, Thr337,Thr335, Gln336
	Mahanine	-6.57	Asp374*, His347*, Gly346*, Ile389, Ser442, Lys348, Asp370,Thr350, Thr369, Thr352, Gln331, Ser368, Arg345
	Murrayanol	-5.96	His347, Gly346, Gln331, Cys441, Ser442, Lys348, Ile389,Tyr352, Ser368, Thr350, Asp370*
Coriander	Alpha-pinene	-5.97	Asn380, Gln336, Thr337, Thr335, Trp308, Lys382, Gly440,Met447, Pro439, Met438, Thr437
Cinnamon	Beta-phellendrene	-5.57	Gly440, Gln336, Thr335, Asn380, Thr381, Lys382, Trp308,Met438, Thr437, Pro439, Thr337

energies with the amino acids included the protein, each complex was subjected to the protein-ligand interaction profiler and the results are presented in Table 4. LigPlot and pymol software were used to visualize the 2D and 3D interactions, and their results are listed Table 5.

According to the interactions between ligands and the protein, which were given binding energies greater than -6.00 kcal/mol, only the Mahanine ligand interacted with the highest number of amino acids in the binding pocket compared to the other two ligands (Asp374, His347, Gly346) and this 4X07-MAH complex was subjected to Molecular dynamics (MD) simulations.

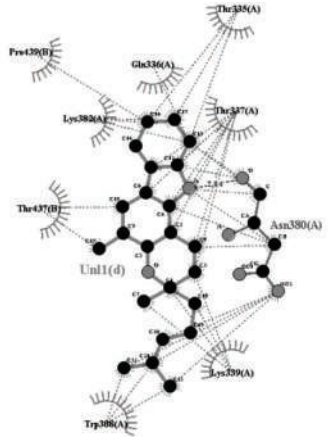
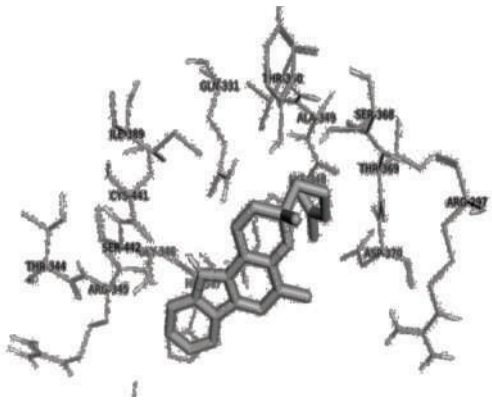
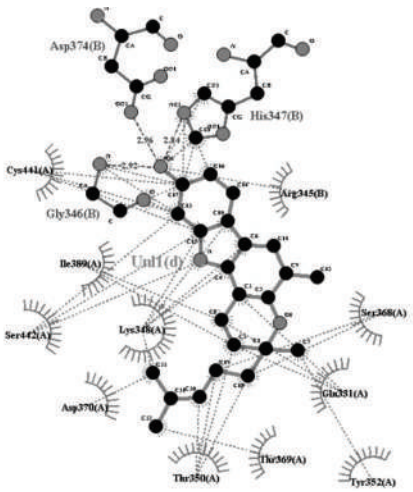
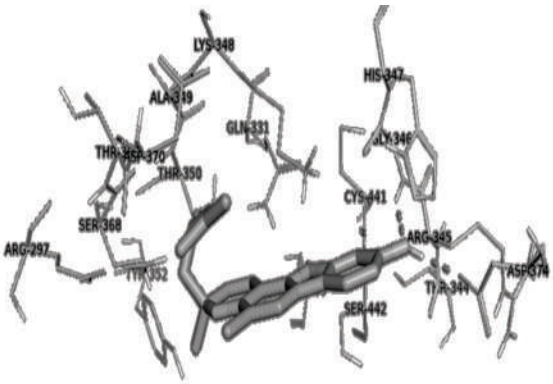
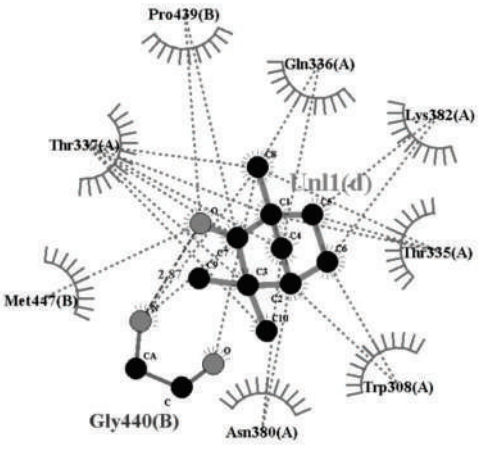
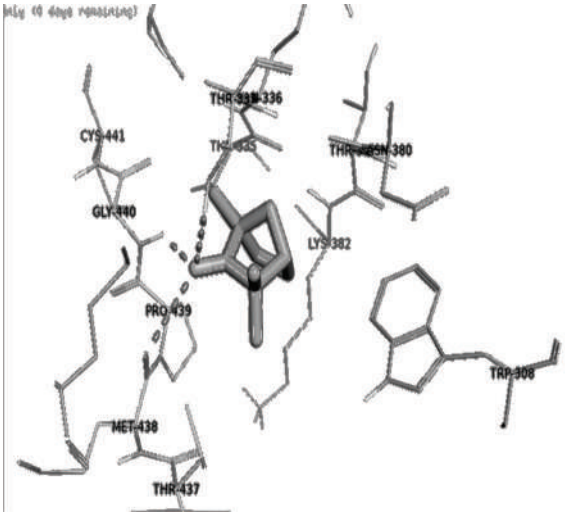
Molecular Dynamics (MD) simulation is defined as an operation of giving the movements to a particular protein or protein-ligand complex internally, which is produced by increasing the temperature of the system and cooling them rapidly in a very short time scale (Poulikakos *et al*., 1957). As a result, defective bonds between amino acid residues and peptides or steric interactions are removed or modified (Trespalacios and Pla, 2007). Finally, the most stable and energy-minimized conformations of the protein or protein-ligand complex are generate. In this study, GROMACS was used due to its efficiency compared to other MD software. In addition, it is very user-friendly and allows trajectory data to be stored compactly. GROMACS also provides Xmgrace which is a basic trajectory data viewer. In this study qtgrace software was used. The root mean square deviation (RMSD), and radius of gyration (Rg) of the protein ligand complexes were obtained to analyze conformational variations and stability. The flexibility of protein residues was obtained from root mean square fluctuation (RMSF) (Hong *et al*., 2012).

RMSD can be simply defined as a measure of the deviation from the overlap of the configurations from the trajectory file with reference structures which is the initial configuration of the simulation. This calculation is used both as an indicator of the accuracy of the relevant protein-ligand complex and to ensure that the particular systems are in equilibrium and undergo no conformational changes. In an ideal situation, the RMSD value should be equal to zero but due to statistical uncertainties, deviation can be observed. Hence, the smaller the deviation, the two compared structures may be more spatially equivalent to each other. The 4X07-MAH complex RMSD graphical result (Figure 3a) leveling off around ~2.5 nm indicated its stability throughout the simulation time.

The mass-weighted root mean square distance of a set of atoms from their common center of mass is known as the Rg. The Rg was plotted to determine the structural flexibility of the complex over simulation time. The radius of gyration analysis gives an idea of the protein's total dimensions which is the complex may fold or unfold after the MD simulation. According to the 4X07-MAH plot analysis (Figure 3b) higher numbers of fluctuation between ~2.00 nm and 2.75 nm indicate 4X07-MAH complex stabilization during the simulation time of 10 ns.

The RMSF method is used to investigate local changes in protein chain residues in a protein-ligand complex and to examine the effect of these moving residues to interact with ligands. The variations in the constituent residues were observed for the 4X07-MAH complex, and the flexibility of each residue in the 4X07-MAH complex was measured and plotted after the simulation period. However, a higher

Table 5: Ligand-protein interactions in 2D and 3D visualization

Ligands*	2D visualization	3D visualization
Mahanambine		
Mahanine		
Fenchone		

* Ligands that showed significant binding energies greater than -6.00 kcal/mol

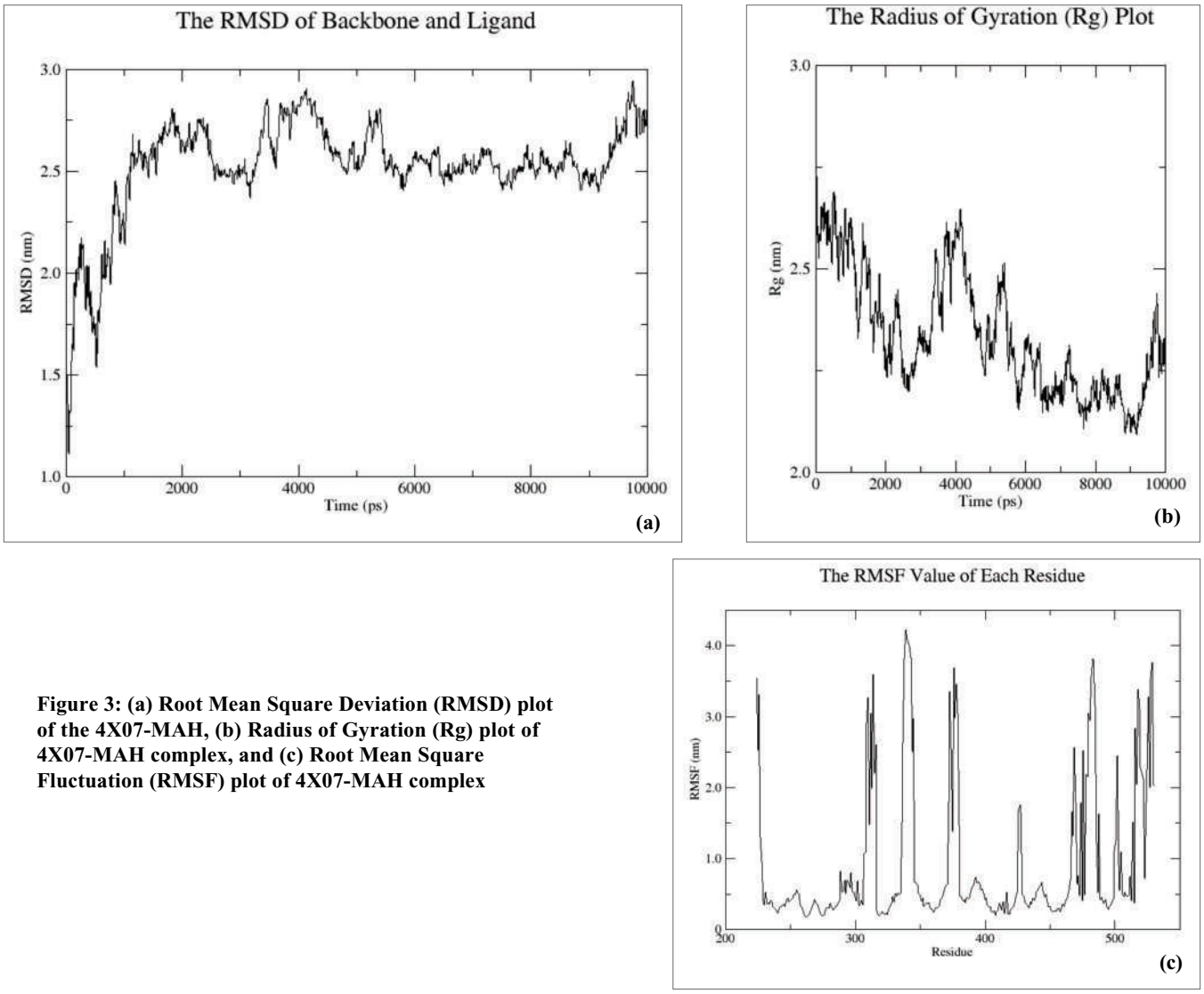


Figure 3: (a) Root Mean Square Deviation (RMSD) plot of the 4X07-MAH, (b) Radius of Gyration (Rg) plot of 4X07-MAH complex, and (c) Root Mean Square Fluctuation (RMSF) plot of 4X07-MAH complex

RMSF value might indicate the whole structure fluctuates or a large portion of protein fluctuates during the simulation. Fluctuations below ~1.0 nm explain the smooth interaction between 4X07 and MAH during the 10 ns of simulation time (Figure 3c). However, a higher intense fluctuation between ~2.5 nm and ~3.5 nm residues may not be involved in the ligand (MAH) interaction, because the involvement of these protein chain residues accounts for the local changes of the protein (4X07) structure.

According to the MD simulations that were carried out for the 4X07-MAH complex, in all the graphs of Rg and RMSD, a higher number of significant fluctuations (flattening) can be seen, and due to that 4X07-MAH may maintain a stable complex throughout the 10 ns simulation time. By analyzing the RMSF graph of this 4X07-MAH complex, more information was found on interactions within the complex. For the most significant intense peaks on RMSF graphs, information was found on what residues fluctuate more and thereby what residues are more flexible. These results further support the results found through docking studies revealing that more flexible residues contribute significantly to the bond formations such as H bonding and hydrophobic interactions (Trespalacios and Pla, 2007). Therefore, the results obtained through docking and MD studies can be concluded that Mahanine has a major impact on the P domain capsid protein binding mechanism to the receptor molecules in our body and alleviates the potential of having this deadly disease.

CONCLUSION

Forty two phytochemicals were identified which were able to extract to curry powder water extract. Those phytochemicals were again filtered into 20 ligands based on their percentage amount extracted into water from each five ingredients. Among the twenty ligands, only three ligands had negative binding energies greater than -6.00 kcal/mol. Among those ligands, the Mahanine (MAH) ligand perfectly fits into the binding pocket of norovirus P domain protein via forming hydrogen bonds and hydrophobic interactions. Since the binding energy was considerably negative for this phytochemical with norovirus P domain protein (4X07), it reveals the formation of a very stable protein-ligand complex. These results are further clarified by MD simulations. Rg, RMSD, and RMSF results indicated the stability of the protein-ligand complex throughout the simulation time. The results derived from both Docking and Molecular Dynamics studies lead that Mahanine significantly influences the binding mechanism between the P domain capsid protein and receptor molecules within human body. Hence, Mahanine has the potential to mitigate the risk of virus infection.

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Conflict of interest

Authors have no conflict of interest to declare.

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