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RESEARCH PAPER

In-vitro and in-silico investigation of anti-dengue phytocompounds from *Bergenia ciliata* (Haw.) Sternb. via inhibition of host protein ER-α-glucosidase

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Abstract

Host-directed therapies target the host proteins/enzymes required for viral replication hence impeding antiviral activity irrespective of strains. The present study reports the efficacy of bioactive compounds from *Bergenia ciliata* (Haw.) Sternb. as host-directed antiviral agents against ER-α-glucosidase II, host protein involved in dengue viral pathogenesis. Bioactive compounds from *B. ciliata* rhizome extracts were identified and quantified using RP-HPLC (PDA) followed by inhibition of ER α-glucosidase II through *in-vitro* assay, *in-silico* molecular docking, molecular dynamics simulation and ADMET studies. Gallic acid and its 4-O-methyl glucoside derivative (bergenin) were identified/quantified in *B. ciliata* and exhibited mixed-type and uncompetitive-type of inhibition in *in-vitro* enzyme kinetic analysis with IC_{50} at 0.69 and 1.14 µg/mL. Both gallic acid and bergenin showed efficient binding *in-silico* against ER α-glucosidase II with docking scores − 7.3 and − 6.9 kcal/mol. The protein–ligand (binding complex) stability at various time intervals was validated by molecular dynamics and simulation studies, where both ligands formed stable complexes. The ADMET profiles predicted promising druglikeness of both the phytomolecules. Thus, the study concludes that *B. ciliata* and its phytocompounds, gallic acid and bergenin, are promising candidates as host-directed therapeutics and can be studied towards the development of a pan-strain antiviral agent against the dengue virus.

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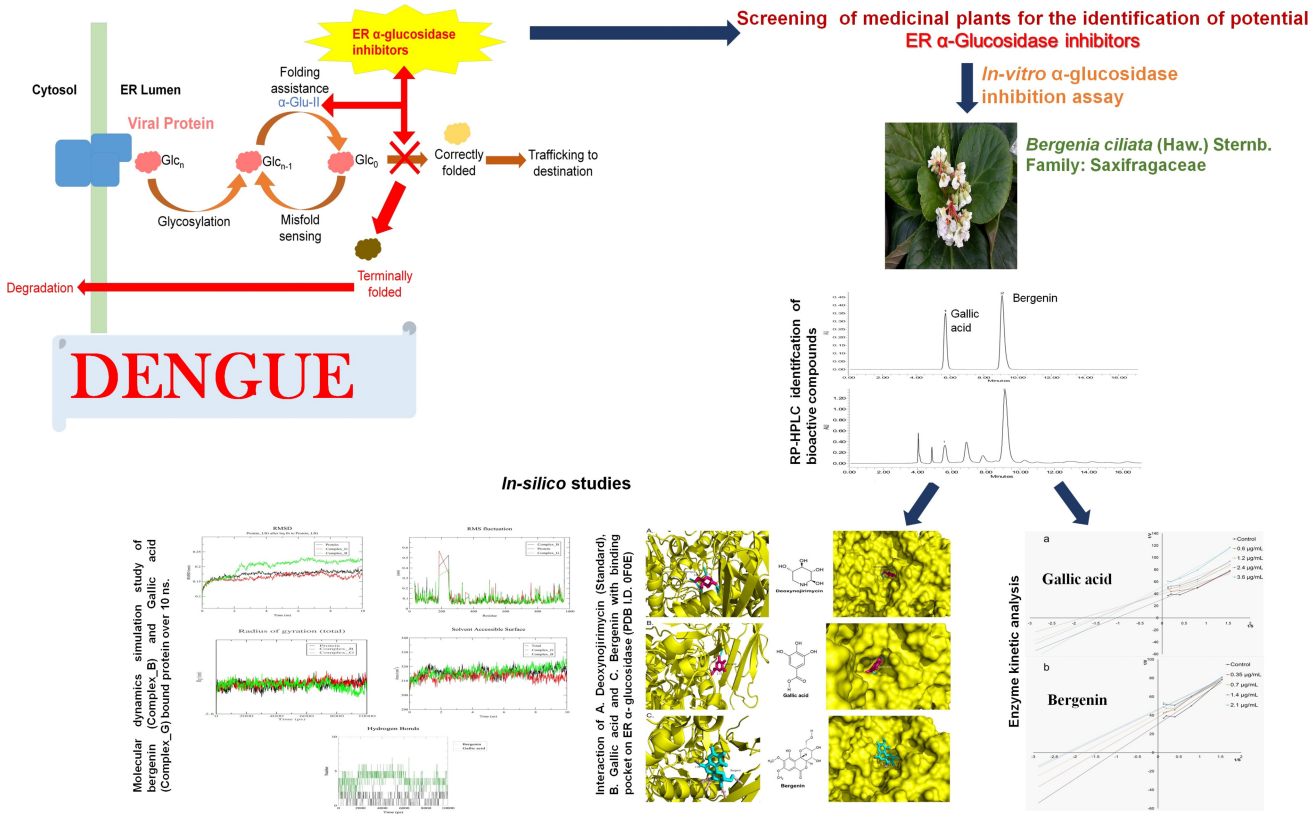
Graphical abstract



Keywords Host-directed therapies · *Bergenia ciliata* · Dengue virus · RP-HPLC · Molecular dynamics simulation

Introduction

Natural products of plant origin have played a major part in the development of novel drug candidates and are an important source of drug lead compounds (Cragg and Newman 2013). The antiviral efficacy of various medicinal plants against Dengue virus (DENV) has been widely reported and the majority of them are targeted towards the viral proteins involved in its replication cycle. However, in such cases where viral proteins are targetted, the emergence of drug-resistant viral strains is a major challenge (Purohit et al. 2022). It is essential to focus on alternative therapeutic targets with pan-strain action and the inhibition of host proteins can be a promising solution.

Host-directed therapies exploit the dependency of a virus on the host cellular machinery and the inhibition of such limiting factors may suspend the viral replication (Cakir et al. 2021). In this context, host protein i.e., ER-α-glucosidase (α-glucosidase found in the endoplasmic reticulum) assists in the post-translational modification of dengue viral proteins and hence, is a promising target for anti-dengue drug development (Courageot et al. 2000). Imino sugars such as deoxynojirimycin (DNJ) and castanospermine are well-studied α-glucosidases I and II inhibitors and selectively inhibit the virion assembly and secretion of many enveloped viruses, including DENV and are under clinical trial (Chang et al. 2011).

Phenolic compounds and their derivatives such as gallic acid, epigallocatechin gallate, bergenin, curcumin, resveratrol, etc. have been reported to possess antiviral activity against several viruses including hepatitis B virus, influenza, chikungunya, HIV and Zika viruses (Loaiza-Cano et al. 2020). They inhibit virus multiplication at various stages of the viral life cycle, e.g., attachment, penetration, replication, assembly and maturation and release of virus particle. Phenolic compounds also influence the viral life cycle by affecting the host cell's biochemical processes that viruses use for their own benefit (Kowalczyk et al. 2021).

Bergenia ciliata (Haw.) Sternb. (Saxifragaceae), commonly known as *Pashanbhed*, is a source of wide range of bioactive phenolic compounds such as bergenin, gallic acid, arbutin, protocatechuic acid, ferulic acid, etc. (Pandey et al. 2017). The rhizome is widely used in various traditional systems of medicine for the treatment of fever, cough, cold and

viral diseases, renal calculi, etc. (Rajbhandari et al. 2009; Sinha et al. 2010; Shankar and Rawat 2013; Ahmad et al. 2018). There are several reports on the antiviral activity of *B. ciliata* against varoius viruses viz. influenza virus A and herpes virus HSV-1 (Rajbhandari et al. 2009), but it has not been explored against dengue virus so far.

Hence, the present study explores the efficacy of bioactive compounds from the rhizome of *B. ciliata* as host-directed antiviral agents through *in-vitro* α-glucosidase inhibition assay, *in-silico* molecular docking, ADMET profiling and molecular dynamics simulation. The study attempts to validate the traditional claims of *B. ciliata* as antiviral agent and provides insights in host directed therapeutic action of its bioactive metabolites. The study provides leads towards potential anti dengue compounds having pan-strain efficacy in the form of host-directed therapeutics.

Materials and methods

Chemicals and reagents

α-Glucosidase from *Saccharomyces cerevisiae* (100 U/mg), p-nitrophenyl-α-D-glucopyranoside (> 99%) and deoxynojirimycin (≥ 95%) were procured from Sigma Aldrich (USA). Gallic acid (purity ≥ 98%) and bergenin (purity ≥ 98%) were also procured from Sigma Aldrich (USA). All the other chemicals and reagents were of analytical grade and purchased from Thermo Fisher Scientific Pvt. Ltd. (USA).

Plant material

Fresh rhizome of *B. ciliata* was collected from natural habitats of Bhowali, Uttarakhand and passport data sheet was prepared (Table S1). The sample was authenticated by Dr. Sharad Srivastava (Chief Scientist & Head, Pharmacognosy Division, CSIR-NBRI, Lucknow, Uttar Pradesh, India), and raw drugs were submitted to the Institutional Raw Drug Repository. The collected rhizome was washed, chopped, shade dried, followed by oven drying at 30 °C and then pulverized to coarse powder (40 mesh) for further studies.

Extraction protocol

5 g powder of sample was subjected to cold extraction in methanol, followed by continuous shaking for 6 h and allowed to stand at room temperature (25 °C ± 3) for 18 h. The sample was then filtered through whatmann filter paper (No. 42) and the residue was re-suspended in fresh solvent for further extraction. The process was repeated thrice and the pooled filtrates were concentrated in rotatory evaporator (Buchi, Switzerland), concentrated extracts was lyophilized and stored at 4 °C till future use (Ayurvedic Pharmacopoeia of India 2004).

RP-HPLC–PDA characterization

The stock solution of standards and *B. ciliata* extract was freshly prepared (1 and 10 mg mL-1) in HPLC-grade methanol and diluted to 0.1 and 5 mg mL^{-1} as a working solution. Working dilutions and mobile phase were filtered through a 0.22 µm Millipore membrane filter (Pall, USA), prior to HPLC analysis.

The quantification of secondary metabolites was done on a high-pressure liquid chromatography (HPLC) system by Waters (USA), consisting a pump for delivering the mobile phase (Waters-1525), autosampler (Waters-2707), column heater (Waters-1500) and photodiode array detector (Waters-2998). All these units were operated and monitored on Empower 3 Quick start software. The separation of metabolites was achieved on C_{18} RP-HPLC column (4.6 × 250 mm internal diameter, 5 µm particle size) thermostated at 35 °C. The mobile phase consisted of 0.01 M sodium acetate buffer in HPLC grade water (pH 3 by adding glacial acetic acid) (A) and acetonitrile (B). The mobile phase was eluted in isocratic manner (65:35 v/v; A:B), with a flow rate of 0.7 mL min-1. The run time was 17 min and detection was done at 268 nm. The amount of standards in plant extract was calculated with the help of linear regression curve plotted between concentration and peak area of standards. The developed method was validated following ICH Q2 (R2) (International Conference on Harmonization) guidelines (Anonymous 2022).

In-vitro inhibition assay

In-vitro α-Glucosidase inhibition assay was performed as per previously reported method (Dong et al. 2012) with some modifications. α-Glucosidase from *Saccharomyces cerevisiae* was used for screening due to unavailability of commercial human ER α-Glucosidase. 50 µL of α-Glucosidase solution (0.2U/mL) prepared in 0.1 M Phosphate buffer was added to 60 µL of test compound (0.2–1 µg/mL) and incubated at 37 °C for 10 min. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (p-NPG) was added to each well and incubated at 37 °C for another 20 min. To stop the reaction, 160 µL of 0.2 M Na_2CO_3 was added into each well and absorbance was measured at 405 nm using Cytation 5 cell imaging multimode plate reader (Agilent, USA). To avoid sample interference, sample control was applied for each concentration. Percentage inhibition was observed and IC_{50} values were calculated. Deoxynojirimycin (DNJ), a well-known α-glucosidase inhibitor was used as standard.

Kinetics of α-glucosidase inhibition

To study the inhibitory mechanism of the bioactive phytocompounds on the enzyme α-Glucosidase, kinetic studies were conducted using Lineweaver–Burk plot. The experiments were carried out with different concentrations of the substrate p-NPG (1.25–7.5 mM) and inhibitor concentrations ranging from half to three times the IC50 value. While keeping the enzyme concentration constant, enzyme kinetics was measured at different concentrations of inhibitors corresponding to the varying substrate concentrations (Dong et al. 2021). Double reciprocal plot of initial velocity against the substrate concentration was plotted to form the Lineweaver–burk plot. Resulting plot was analysed to determine the type of inhibition.

In-silico molecular docking

Bioactive compounds identified in *B. ciliata* extract (from RP-HPLC analysis) were further studied *in-silico* for binding against ER-α-glucosidase. The 3D structure of the phytocompounds was downloaded in SDF (structure data file) format from pubchem database and were converted to PDB (protein data bank) format using OpenBabel tool (OPENBABEL—Chemical file format converter, cheminfo.org). For protein structure of ER α-glucosidase, several files were found in the Protein Data Bank of which two structures were found most relevant. One was from *Chaetomium thermophilum* and the other a murine protein. Out of these, the murine α-glucosidase II was showing more sequence similarity to the human ER protein i.e., 90%, thus it was selected and downloaded from RCSB-PDB database (https://www.rcsb.org/) using the PDB ID 5F0E and was optimized along with the ligands using Autodock Tools 1.5.7 (Sanner 1999; Morris et al. 2009).

For docking, both the protein and ligands were converted to.pdbqt format in Autodock Tools 1.5.7. Docking performed using Autodock vina program 1.5.7 (http://vina.scripps.edu/) using grid dimensions of 40*40*40 $Å^3$ and their binding energies (dG) were generated. The generated protein–ligand interactions were analyzed and illustrated using LIGPLOT$^+$ v1.4.5 (Wallace et al. 1995; Laskowski and Swindells 2011) to generate 2D plots of the interactions. The generated plots showed hydrophobic interactions and hydrogen bonds between the ligand and binding site of protein. For 3D visualisation, the output file was opened in PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.sx) and interactions between the ligand and protein were visualised in their respective binding pockets. DNJ was also docked as standard.

Molecular dynamics simulation

The molecular docking outputs were subjected to molecular dynamics (MD) simulation studies to evaluate the effects of ligand binding on the protein and stability of the ligand bound complex. The simulations were carried out for free protein and ligand-bound protein complexes using GROMACS (Version: 2020.1) (Abraham et al. 2015) with Charmm27 force field. SwissParam server was used for generating the ligand topologies and parameter files (Zoete et al. 2011). The protein and the complexes were solvated using single-point charge (SPC) water model extended about 10 nm in a cubic periodic box. Sodium and chloride ions were added for the electro neutralization of the systems. Energy minimization of 50,000 steps was done using steepest decent method to resolve the bad contacts and clashes in the protein. Following the energy minimization, all the systems underwent two steps of equilibration, first 100 ps of canonical (NVT) equilibration followed by 100 ps of isobaric-isothermic (NPT) equilibration. The temperature and pressure of the system were maintained at 300 °C and 1 Bar by using modified Berendsen thermostat and Berendsen barostat (Berendsen et al. 1984). MD simulations were performed for 10 ns saving the co-ordinates every 10 ps for all the systems. The resulting trajectories were visualized using PyMol software (L. 2015) and graphical representations were made using Grace Software (Version 5.1.22).

ADMET studies of bioactive phytocompounds

The bioactive phytocompounds were analysed for their drug likeness studies, bioavailability and ADME (absorption, distribution, metabolism and excretion) using SwissADME (Daina et al. 2017). Toxicity of the compounds were tested using ProTox-II (https://tox-new.charite.de/protox_II/) (Banerjee et al. 2018).

Statistical analysis

Each observation was taken in triplicate and the data were subjected to one-way analysis of variance (ANOVA) to test the level of significance (XLSTAT, 2010; Microsoft Corporation, Redmond, WA, USA).

Results

RP-HPLC characterization of bioactive phytocompounds in *B. ciliata* extract

Bioactive phytocompounds are responsible for therapeutic effectiveness of herbal extracts (Swargiary et al. 2022). RP-HPLC was used for the identification and quantification of

bioactive compounds present in *B. ciliata* rhizome extract. The analysis resulted in identification of two compounds namely gallic acid and a 4-O-methyl glucoside derivative of gallic acid (also known as bergenin) in the plant extract. Chromatogram of standard mixture and sample (Fig. 1) showed the presence of gallic acid and bergenin at retention time (R_t) 5.73 ± 0.07 and 9.09 ± 0.05 min. and coincides with peak of sample. Linear regression curve plotted between concentration and peak area (Table 1) was used to calculate the amount of gallic acid and bergenin in the plant sample.

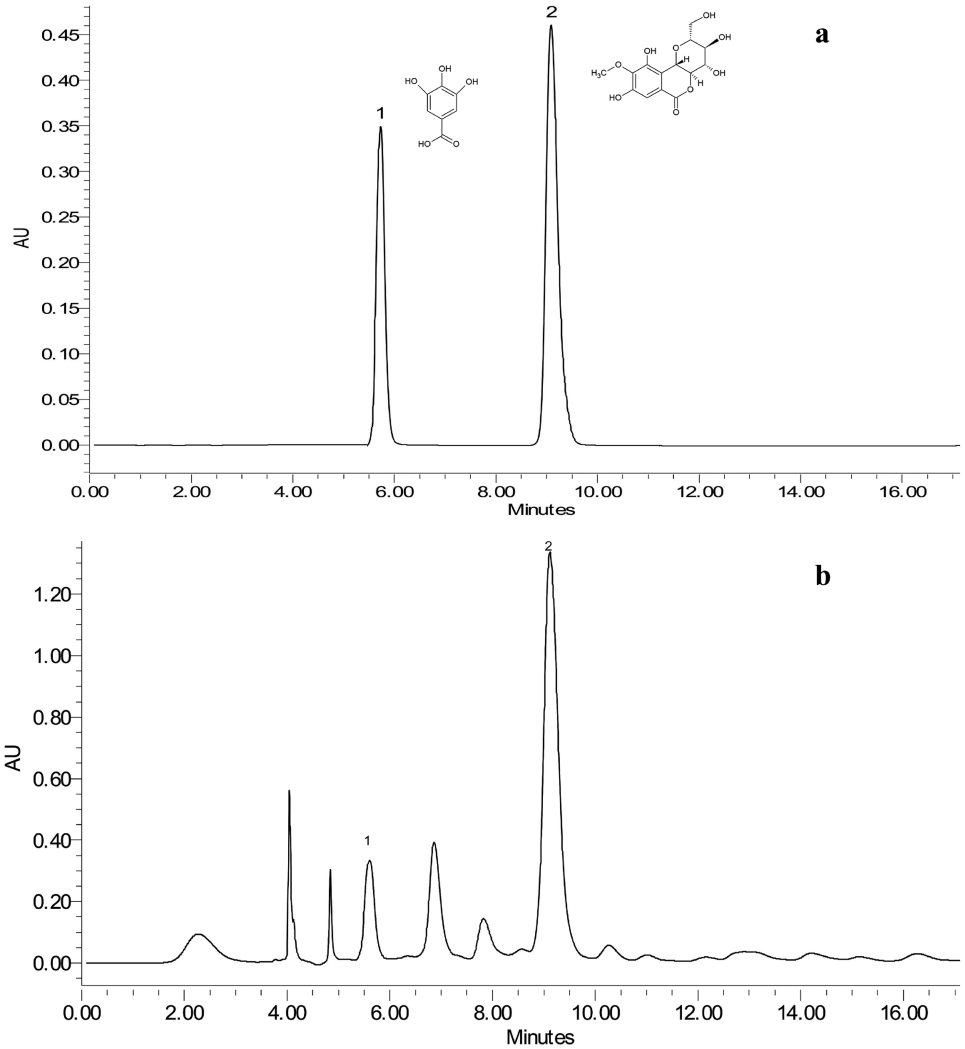


Fig. 1 RP-HPLC chromatogram of standards and plant extract **a** HPLC chromatogram of gallic acid (1) and Bergenin (2) **b** Identification of standards in the plant extract

Table 1 Statistical parameters for the calibration of gallic acid and bergenin for the developed RP-HPLC method

Parameters	Gallic acid*	Bergenin*
Retention time (min.)	5.73 ± 0.07	9.09 ± 0.05
Linearity range (ng/run)	100–500	100–500
Calibration equation	Y = 2439.9 × − 66,354	Y = 2124.9 × − 109,110
Correlation coefficient (r^2)	0.997	0.987
Limit of detection (ng[@])	33.51	72.62
Limit of detection (ng)	101.56	220.07
Precision study		
Intra-day (Repeatability)[a] (%RSD[#])	1.36	1.49
Inter-day (Reproducibility)[b] (%RSD)	1.44	1.68

*n = 6, [@]ng = nanogram, [a]Mean %RSD within the day (Intra-day), [b]Mean %RSD per day over 3 days (Inter-day), [#]*RSD* Relative standard deviation

The amount of gallic acid and its derivative in the sample was found to be 72.80 and 114.32 µg mg^{-1} of plant extract. The developed RP-HPLC method was validated and the results are described in the supplementary material.

In-vitro α-glucosidase inhibition assay

Inhibitory assay of identified bioactive metabolites, bergenin and gallic acid against α-glucosidase was performed where both the molecules showed significant activity having IC_{50} at 0.69 µg/mL and 1.14 µg/mL and were at par with positive control, deoxynojirimycin (IC_{50} 0.87 µg/mL). Lineweaver–Burk plot of α-glucosidase inhibition at variable concentration of bergenin and gallic acid vs. different concentration of p-NPG (substrate) was plotted to identify the type of inhibition. The linear regression and extrapolation of the data represent a number of lines intersecting the horizontal and vertical axis. In case of gallic acid, on increasing the concentration, the vertical axis intercept ($1/V_{max}$) decreased while the horizontal axis intercept (1/Km) increased, indicating mixed-type of inhibition (Fig. 2). Whereas, in case of bergenin, both the vertical axis intercept ($1/V_{max}$) and the horizontal axis intercept (1/Km) increased with increasing bergenin concentration. This indicates a typical case of uncompetitive inhibition where the inhibitor binds to the substrate-enzyme complex and forms an inactive substrate-enzyme-inhibitor complex (Strelow et al. 2004).

In-silico molecular docking analysis

Bioactive phytocompounds characterized in *B. ciliata* were studied *in-silico* against murine ER α-Glucosidase II to analyse their binding responsible for the inhibitory potential of the plant. Both the molecules exhibited better binding score

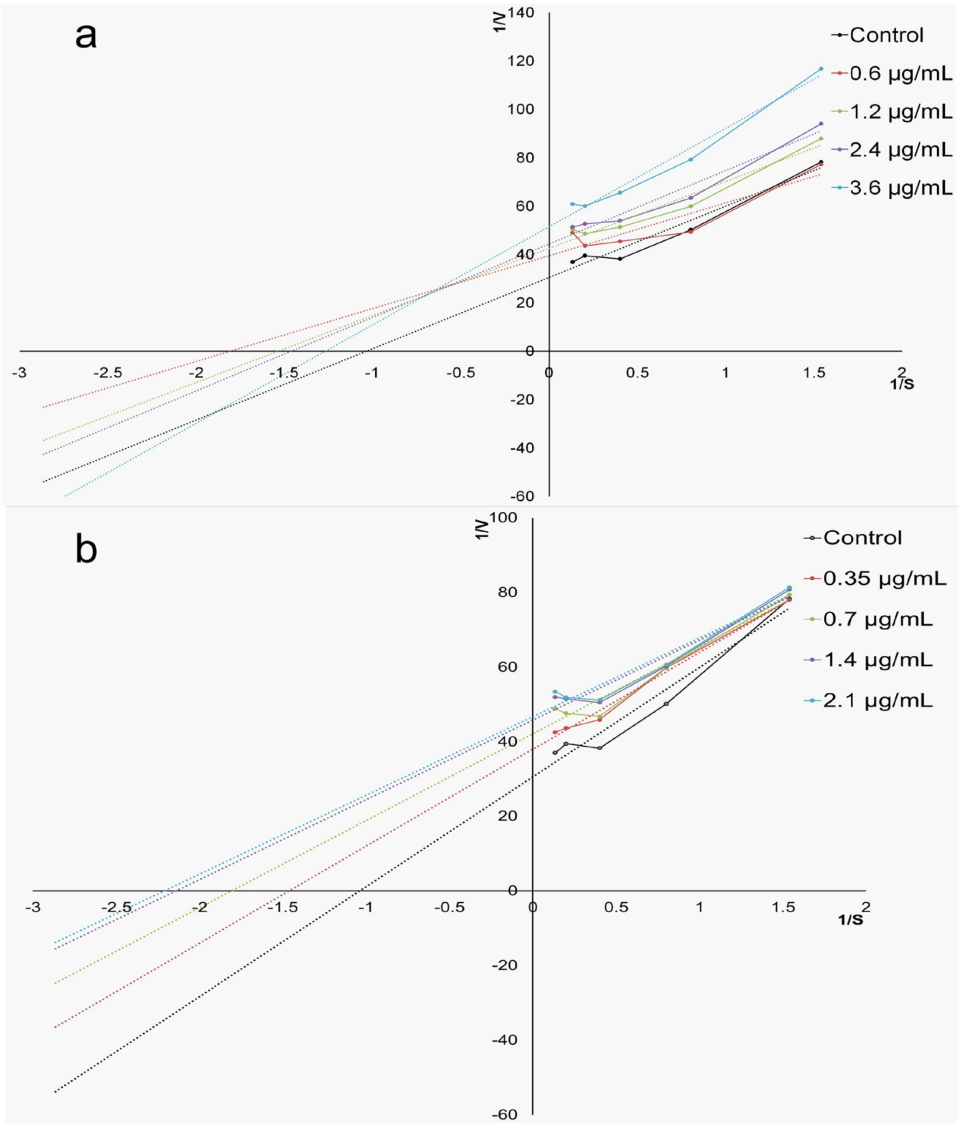


Fig. 2 Enzyme kinetic analysis. Lineweaver–Burk plot analysis of inhibition kinetics of α-glucosidase at variable concentration of **a** gallic acid and **b** bergenin

than the standard, DNJ (− 5.0 kcal/mol). Maximum binding affinity was observed in bergenin (− 6.9 kcal/mol) followed by gallic acid (− 5.6 kcal/mol). The molecules were found to have similar site of binding overlapping with the binding site of the standard DNJ (Fig. 3a). The analysis of interactions of ligands with the protein was visualized as 2D plots generated by Ligplot + . The standard, DNJ forms an interaction of − 5.0 kcal/mol owing to its 5 hydrogen bonding interactions with the protein at Glutamine$_{739,}$ Aspartate$_{772}$, Glutamate$_{865}$ and Argenine$_{749}$. There are also 4 hydrophobic interactions of the molecule with the protein (Fig. 3b). Among phytocompounds, bergenin exhibited highest docking score to the protein with 5 hydrogen bonds, one each with Glutamate$_{865}$, Glutamine$_{863}$, Argenine$_{749}$ and 2 with Glutamine$_{749}$ and 6 hydrophobic interactions between the ligand and protein (Fig. 3d). Gallic acid also exhibited great

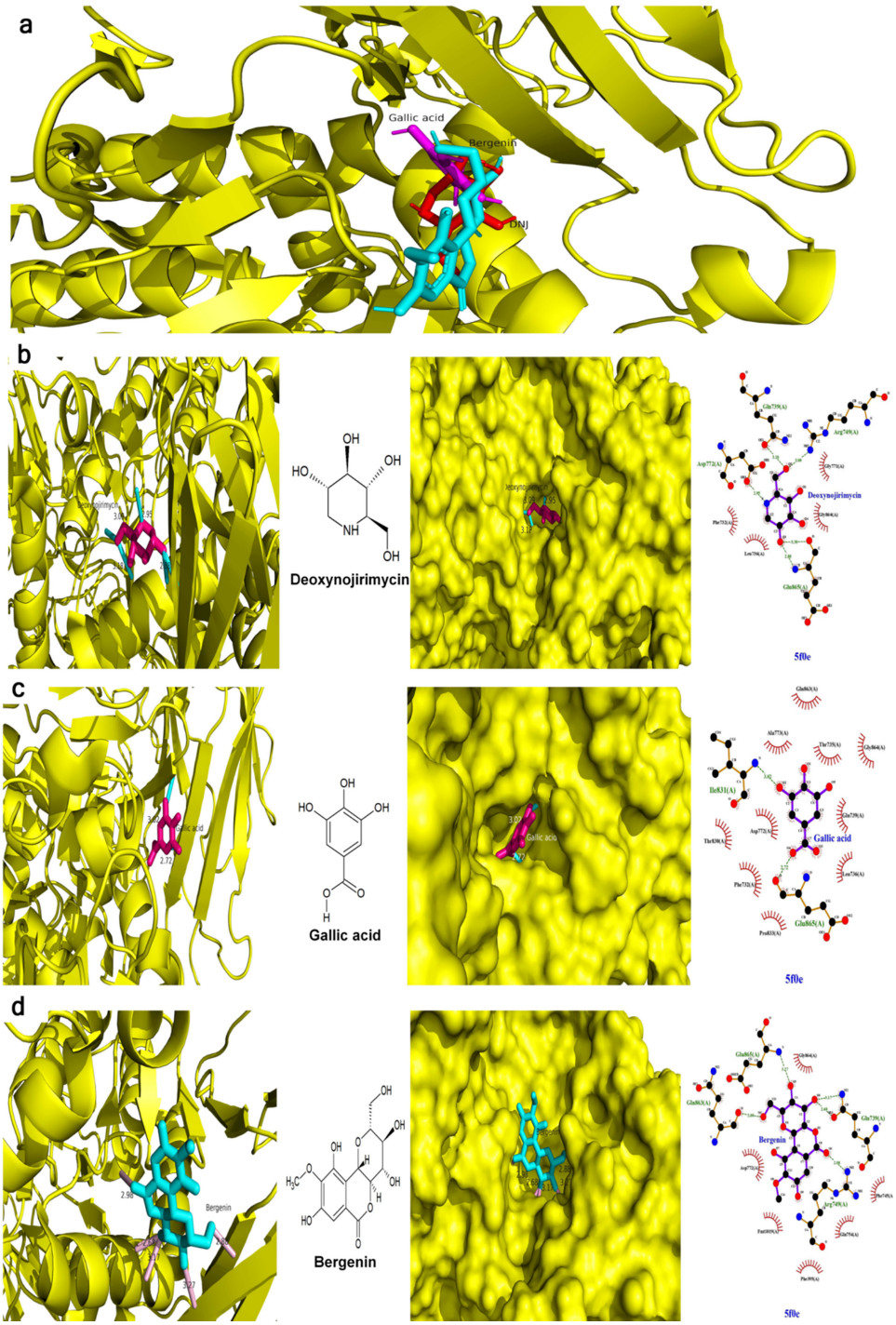


Fig. 3 3D and 2-D diagrammatic representation of interactions of ligands with the binding pocket of ER α- glucosidase (PDB I.D. 0F0E) **a** Overlapped binding of ligands. 3D and 2-D diagrammatic representation of interactions of **b** Deoxynojirimycin (DNJ; Standard), **c** Gallic acid and **d** Bergenin with the binding pocket

binding affinity owing to its smaller size and presence of 4 polar hydrogens in its structure. The ligand formed 2 hydrogen bonding interactions with Isoleucine$_{831}$ and Glutamate$_{865}$ along with 10 hydrophobic interactions with other residues in the binding pocket (Fig. 3c).

Molecular dynamics simulation

To understand the dynamics of bergenin and gallic acid binding on the ER- α-glucosidase II protein, simulations were carried out for free-protein and protein–ligand complex. The protein stability and the protein–ligand complex stability was monitored using the time evolution of the root mean square deviation (RMSD) and are represented in Fig. 4a. The RMSD of the un-bound protein as well as the complexes stayed > 0.25 nm throughout the simulation which depicts the stability of the systems (Fig. 4a). In bergenin bound complex, the RSMD of remained below 0.17 nm similar to unbound protein till 2 ns, after which a sharp rise in complex RMSD was observed and reached 0.21 nm at 2.45 ns. The plots of complex and unbound protein diverged with 0.03 nm difference in their final RMSDs. This indicates minute positional deviation in the ligand–protein complex affecting structural stability. In gallic acid bound complex,

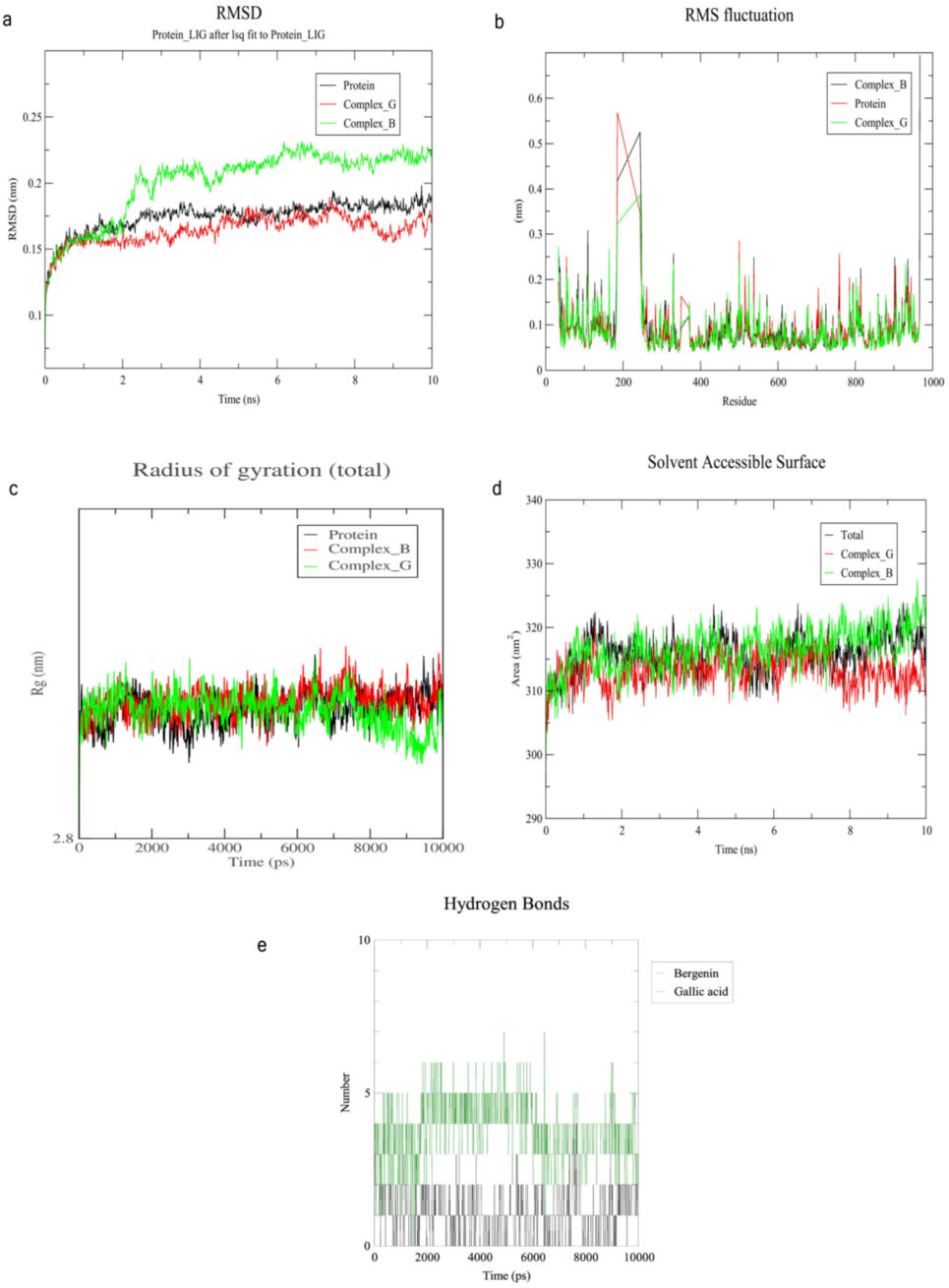


Fig. 4 MD Simulation trajectory plot of unbound ER α-glucosidase (PDB I.D. 0F0E) and ligand (standard inhibitor / lead phytocompound) bound complex over 10 ns. **a** RMSD, **b** RMSF, **c** Radius of Gyration (Rg), **d** SASA, **e** number of hydrogen bond interactions (Complex_B: Bergenin bound complex, Complex_G: Gallic acid bound complex, Protein: Unbound Protein). The plots are generated using Grace Software (Version 5.1.22 1. Available at https://plasma-gate.weizmann.ac.il/Grace/)

the RMSD values remained similar in the beginning of simulation, however, the RMSD of complex drifted below from unbound protein until 5.5 ns when it again neared the protein. This rise in RMSD was transient and after 6.3 ns, the RMSD value lowered, stayed 0.18 nm and below the unbound protein at the end of simulation. The decrease in RMSD of the complex indicates the increased rigidity and stability of protein upon ligand binding.

A root mean square fluctuation (RMSF) was computed to analyze the impact of binding of lead phytochemicals on the flexibility of the protein. The simulation results show comparable RMSF values for both the complexes and the unbound protein with gallic acid bound protein showing slightly reduced RMSF values than the unbound protein (Fig. 4b).

Radius of gyration (Rg) and Solvent accessible surface area (SASA) analysis for bergenin bound complex, gallic acid bound complex and unbound protein are shown in Fig. 4c and d. Result showed that bergenin bound complex showed Rg pattern similar to the free protein contrary to gallic acid that reduced the Rg values in comparison to the free protein. Analysis of the solvent accessible surface area during the MD simulation trajectory revealed bergenin binding caused a slight increase in the SASA value in comparison to the free protein whereas the gallic acid bound complex exhibited reduction in the SASA values. Hydrogen bond interaction between the protein and the lead compounds was assessed (Fig. 4e) and bergenin formed a maximum 4 hydrogen bonds and gallic acid formed a maximum of 6 hydrogen bonds with the protein. Post simulation, the maximum number of hydrogen bonds decreased in bergenin bound complex (as compared to docking) while increased in gallic acid bound complex.

ADMET studies

The drug likeness of gallic acid and bergenin were predicted using swissADME platform on the basis of Lipinski's rule of 5, according to which for a druglike molecule should have molecular weight less than 500 Dalton, hydrogen bond donors not more than 5, 10 or fewer hydrogen bond acceptors, less than 5 consensus logP (indicator of lipophilicity). Both gallic acid and bergenin have been predicted to show no violation of Lipinski's rule of five. The ADME studies of gallic acd and bergenin involving several pharmacokinetic parameters as shown in Table S2. The toxicity of bergenin and gallic acid were predicted to be of Class 6 and Class 4 with predicted LD_{50} of 10,000 mg/kg and 2000 mg/kg. Both the molecules were inactive against more than 95% of the targets as shown in Fig. 5.

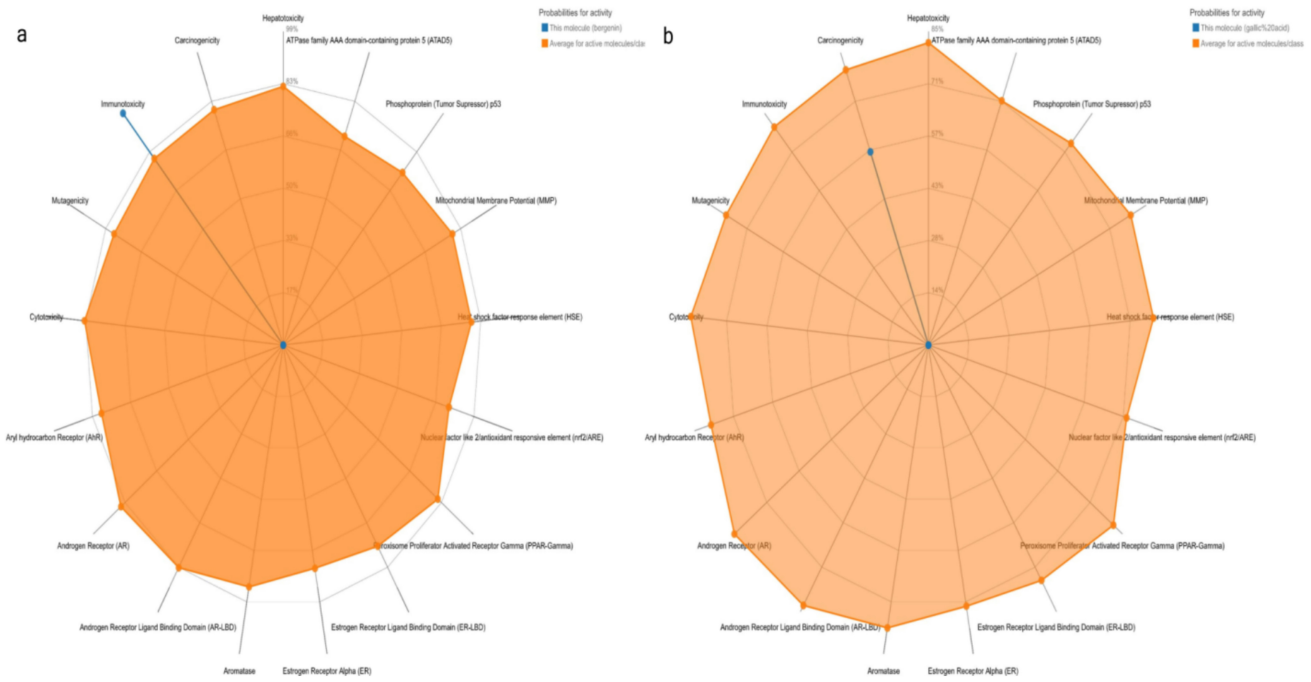


Fig. 5 Toxicity radar chart of **a** bergenin and **b** gallic acid against different toxicological targets predicted using Protox-II (https://tox-new.charite.de/protox_II/)

Discussion

Host proteins play an essential role in viral replication inside the host cell and are a key target for antiviral activity. Host-directed therapeutics selectively target the proteins/enzymes of the host that are required for viral replication. Further, the therapeutic efficacy of the drug is maintained even with the development of new resistant viral strains and this will lead to broad-spectrum therapeutics (Cakir et al. 2021). N-linked oligosaccharide processing within the host endoplasmic reticulum plays a crucial role in the folding and release of viral envelop glycoprotein (Sayce et al. 2016). ER α- glucosidase is an enzyme found in the endoplasmic reticulum of host cells and is involved in the initial steps of dengue virus assembly (Chambers et al. 1990). Translation of dengue viral proteins such as prM, E and NS1 utilizes this enzyme and its inhibition has been reported to reduce dengue viral load through protein misfolding (Courageot et al. 2000). Several medicinal plants and phytocompounds have been found useful in dengue conditions due to their ability to inhibit ER α- glucosidase enzyme (Sayce et al. 2016; Ariavianti et al. 2020).

In the present study, *B. ciliata* was selected for characterization and *in-silico* studies after a preliminary *in-vitro* screening of 65 plant extracts in which *B. ciliata* showed maximum activity (Supplementary material). The bioactive compounds from *B. ciliata*, gallic acid and bergenin exhibited great binding affinity against the target protein, ER α-glucosidase, better than the standard i.e., DNJ. Kinetic analysis of *in-vitro* α-glucosidase inhibition revealed gallic acid and bergenin to have mixed type and uncompetitive type of inhibition against the enzyme. Inhibition kinetics of α-glucosidase enzyme has been well studied and several phenolics have been reported to exhibit mixed type of inhibition (Proença et al. 2017; Singh et al. 2016). Uncompetitive inhibitors are also attractive drug candidates as an increase in substrate concentration leads to a further increase in compound potency (since the compound binds to enzyme–substrate complex, rather than free enzyme) (Heise et al. 2012). The no nucleoside reverse transcriptase inhibitors (NNRTIs) used in the treatment of AIDS, provide interesting examples of clinically relevant uncompetitive inhibitors (Coster and Kumar 2012).

Chemically, both gallic acid and bergenin are derivatives of trihydroxybenzoic acid (having 6 carbon phenyl ring) while DNJ is a derivative of trihydroxypiperidine (having 6 carbon aliphatic ring in which one of the carbon is replaced with nitrogen) (Fig. 3). This structural similarity of the phytomolecules with DNJ might be responsible for their equivalent inhibitory potential. However, the aromatic nature of gallic acid and bergenin is a significant feature that distinguishes them from DNJ, which is not aromatic. Difference in the shape of these molecules influence their ability to interact with biological targets and plays a crucial role in interactions with enzymes/receptor which may lead to distinct inhibitory mechanisms. The correlation of molecular shape and the potential of bioactive molecules in drug development has been extensively studied considering their lipophilicity, solubility, torsion, bond interactions, etc. (Aldeghi et al. 2014).

Regarding the general chemical aspects of antiviral activity, phytocompounds with phenyl ring(s), carboxyl residues, glycosides, hydroxyl, and methoxy groups appear to manifest antiviral efficacy (Kamboj et al. 2012). Gallic acid (having three hydroxyl groups and a carboxyl group in its structure) along with its derivatives have been previously reported to be a potent antiviral compound with good bioavailability and are proved to interfere with virus replication and/or viral essential protein synthesis (Kamboj et al. 2012). Bergenin is an O-methyl glycoside derivative of gallic acid and glycosides are well-known inhibitors of α-glucosidase enzyme (Khan et al. 2019) and also has five hydroxyl and one methoxy group in its structure which may assist in its antiviral activity.

Further, validation of the results through MD simulation revealed that both the compounds formed stable complexes, gallic acid bound complex showed greater stability than bergenin bound complex in all the assessed parameters. RMSD is a parameter that calculates the distance between protein atoms. The comparative evaluation of the average distance between the atoms in the ligand–bound and unbound protein allows us to analyze the relative stability and conformation of the protein (Gupta et al. 2021). The present study indicated that gallic acid stabilized the protein upon binding, however, the binding of bergenin leads to minute deviations in the protein structure (Dinesh et al. 2017). Root mean square fluctuations (RMSF) were used to analyze the impact of the binding of lead phytochemicals on the residues of the protein and the results indicate that the binding of ligands does not disrupt or restrict the motion of residues significantly [20]. The altercations in the folding of the regular secondary structure of protein before and after ligand binding were analyzed through radius of gyration (Rg) (Kushwaha et al. 2021). Similarly, the Rg value in bergenin-bound complex shows no significant alteration in protein folding, however, the binding of gallic acid reduced the Rg values indicating compact and stabilized folding in the complex. Ligand binds to the protein through solvent-substitution at the active site, so, SASA provides important information about potential ligand binding (Kushwaha et al. 2021). SASA increase in the case of bergenin-bound complex indicates comparatively less stability than gallic acid bound complex where SASA value decreased on ligand binding. Hydrogen bond interactions play a significant role

in the protein–ligand complex stabilization (Kushwaha et al. 2021). In the present study, the increased hydrogen bond-forming potential of gallic acid indicates the potential stability of the complex during simulation.

For a compound to be considered as a drug candidate, it must possess optimal pharmacokinetics and safety characteristics. The ADMET analysis predicted both bergenin and gallic acid to have good solubility (with optimal lipophilicity) as an indication to have good oral bioavailability. Both the compounds complied with the Lipinski's rule of five and possessed druglikeness property. To determine the toxicity of chemicals, they are labelled as per globally harmonized system of toxicity classification (Miyagawa 2010), where category IV may be harmful if ingested and Category VI chemicals are non-toxic and hence, both bergenin and gallic acid are predicted to be safe for prescribed drug use.

Several α-glucosidase II inhibitors such as deoxynojirimycin (DNJ), castanospermine (CST), celgosivir and their derivatives have been evaluated for their anti-dengue potential and are under clinical trial (Low et al. 2014; Caputo et al. 2016). Structural similarity of Gallic acid and bergenin with DNJ as well as the traditional use of rhizome of *B. ciliata* against fever and viral diseases, support the study and hence, can be explored against dengue virus in the form of host directed therapeutics.

Conclusion

In the present study, the rhizome of *B. ciliata* and its bioactive compounds, gallic acid and its O-methyl glycoside derivative bergenin, reflect potential responses in both *in-vitro* and *in-silico* models as host-directed antiviral agents against dengue. The study validates the traditional claims of *B. ciliata* as antiviral agent and concludes that an extensive *in-vivo* safety and efficacy study may facilitate the development of a pan-strain targeting anti-dengue agent in form of host-directed therapeutics.

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Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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