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Antibacterial efficacy and antibiotic sensitivity augmentation potential of selected traditional medicinal plant extracts against *Vibrio cholerae* O1 E1 Tor Ogawa

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Abstract

Background The leaves of *Psidium guajava* (PGAE), fruit peel of *Punica granatum* (PGRPE), and the bark of *Careya arborea* (CAE) are widely used traditional medicinal plants for treating diarrheal diseases across India. Our previous studies showed that the hydroalcoholic extracts of these plant parts inhibit the cholera toxin activity. Hence, this study is framed to investigate these extracts for anti-*Vibrio cholerae* activity and their ability to augment antibiotic sensitivity through a combination of in vitro and computational methods. The hydroalcoholic extracts of PGAE, PGRPE, and CAE were tested for antibacterial activity against *Vibrio cholerae* by minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and virulence gene expression studies by reverse transcriptase-polymerase chain reaction (RT-PCR). Further, antibacterial activity in combination with selected antibiotics was performed by disk diffusion method. The possible mode of action of these extracts was predicted using docking and molecular dynamics studies on selected virulence factors of *Vibrio cholerae*.

Results All three plant extracts depicted antibacterial activity at different degrees. CAE showed MIC and MBC at 1.25 mg/mL and 10 mg/mL, PGAE at 1.25 mg/mL and 5 mg/mL; and PGRPE at 2.5 mg/mL and 10 mg/mL, respectively. RT-PCR confirmed these extracts significantly inhibited the expression of virulence genes like Flagellin A (FlaA), *Vibrio* polysaccharide synthesis transcription regulator (VpsT), and Lux Operon (LuxO). Further, the computational studies predicted that phytochemicals of these plants pose stable interaction with AphA, AphB, and ToxT playing the key role in inhibiting the expression of virulence factors.

Conclusion The results emphasize that these plant extracts potentially inhibit the growth of *Vibrio cholerae*, decrease the expression of virulence gene, and enhance the sensitivity of certain conventional antibiotics.

Keywords Antibiotic potentiation, Cholera toxin, Docking, Molecular dynamics, Traditional medicine, *Vibrio cholerae*

Background

Vibrio cholerae is a motile, gram-negative, non-spore producing, curved rod-shaped bacterium that includes both pathogenic and non-pathogenic strains. These strains are differentiated from one another by changes in the virulence gene content and polysaccharide surface [1]. *Vibrio cholerae* is responsible for cholera, a historically significant and severe acute diarrheal disease

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transmitted through water, posing a particularly high risk to children under five, where it stands as the second most common cause of diarrheal mortality and morbidity [2]. Globally, cholera remains a major concern, with yearly estimates indicating between 1.3 and 4.0 million cases, leading to 21,000 to 143,000 deaths [3]. Notably, cholera is classified among emerging and re-emerging infections, demonstrating the highest infection rates in children under the age of five [4].

The cholera toxin (CT) and the toxin co-regulated pilus (TCP) are the two most important virulence factors that are produced by *Vibrio cholerae*. TCP is involved in colonization of the *Vibrio cholerae* in the human intestine by mediating interactions between bacteria thus leading to the formation of microcolony [5]. An interaction between Cholera toxin (CT) and ganglioside GM1, which is found on the outer leaflet of the apical membranes of intestinal epithelial cells, enters the cells through an endocytic pathway and eventually causes persistent elevation in intracellular cyclic AMP levels. As a result, the cystic fibrosis transmembrane conductance regulator (CFTR) is activated, which results in a significant loss of ions and water from infected enterocytes, which in turn causes severe diarrhea characterized by "rice water" symptoms [6, 7]. The successful secretion of CT by *Vibrio cholerae* depends on the effective colonization of the small intestine by *Vibrio cholerae*. Microbial communities are spatially organized and immersed in a polymeric extracellular matrix in biofilms, a commonly reported growth form. Diverse environmental inputs are coordinated by the regulatory network that controls the decision to develop and disperse from biofilms. Numerous transcription factors, regulatory ribonucleic acids (RNAs), and secondary messengers, such as "bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP)," help to integrate these inputs.

The rise of multidrug-resistant *Vibrio cholerae* strains is rendering antibiotic treatments less effective. Recent studies have shown *Vibrio cholerae* developing resistance to antibiotics such as tetracycline, ampicillin, and ciprofloxacin, complicating treatment options [8]. Consequently, there's growing interest in traditional medicine, particularly plant extracts, as potential alternatives to conventional drugs with reduced side effects and environmental impact [9]. *Careya arborea*, *Punica granatum*, and *Psidium guajava* are some of the plants that have been used historically in India to treat diarrheal disorders. These plants have shown effectiveness against a wide variety of enteric infections and the toxins that they produce [8, 10, 11]. *C. arborea* Roxb, a medicinal plant from the family Lecythidaceae, is known as kumbhi in Ayurveda. The stem bark of *C. arborea*

has been traditionally used to treat conditions such as tumors, bronchitis, skin diseases, epileptic seizures, and as an astringent and antidote for snake venom. It is also employed in the treatment of abscesses, boils, and ulcers. The fruit is used in decoctions to aid digestion, while the leaves and flowers, when made into a paste, are applied to treat various skin conditions. Additionally, the plant is used as a remedy for diarrhea, dysentery with bloody stools, and ear pain. A paste made from the leaves and pulp can be applied as a poultice to speed up the healing of ulcers, while the roots are used for treating tuberculosis and skeletal fractures. Studies have shown that the methanol extract of *C. arborea* bark significantly reduces castor oil-induced diarrhea in mice [12, 13]. *P. granatum* L., from the family Punicaceae, has been used for centuries in various cultures to treat a broad spectrum of diseases. Known for its high nutritional value and numerous health benefits, the fruit is often consumed as part of the diet, particularly during recovery from diarrhea. It plays a significant role in traditional medicinal systems like Siddha, Yajurveda, and Unani, particularly in the treatment of gastrointestinal (GI) disorders. Nearly all parts of the plant are utilized in traditional medicine to address various health conditions. The bark and rind of the fruit are commonly used to treat dysentery, diarrhea, hemorrhoids, bronchitis, and to reduce the risk of cardiovascular disease, as well as serving as a natural anthelmintic. Methanol extracts from *P. granatum* seeds and dried peels have demonstrated antidiarrheal and wound-healing properties, respectively. Additionally, the rind extract has shown gastroprotective effects through its antioxidant properties. *P. granatum* is also a key ingredient in several Ayurvedic formulations for the treatment of diarrhea [14–16]. *P. guajava* Linn., commonly known as guava, belongs to the Myrtaceae family. This plant has been traditionally used for generations across various cultures to treat diarrhea and respiratory tract infections. It is also utilized for wound healing, hypertension, inflammation, and diabetes management. In studies on guinea pigs and rats, guava leaf extract was found to reduce coughing induced by capsaicin aerosol and demonstrated antibacterial properties against *Staphylococcus aureus* and *Streptococcus* bacteria. Additionally, decoctions made from the leaves and bark are employed to treat skin conditions, while young leaves are chewed to alleviate toothaches. Leaf tinctures are also used for managing convulsions in children. Furthermore, a crude leaf decoction has shown antibacterial effects against *Shigella flexneri* and *Vibrio cholerae*, and it inhibited the adherence of enteropathogenic and enteroinvasive *Escherichia coli* and *Shigella flexneri* to Hep-2 cells, as well as the binding of cholera toxin (CT) and heat-labile enterotoxin (LT) to the GM1 receptor.

This study aims to explore the antibacterial and antibiotic-enhancing properties of hydroalcoholic extracts from these plants, their impact on the expression of virulence genes, biofilm formation, and survival of *Vibrio cholerae*, alongside the identification of phytochemicals responsible for these effects through molecular docking and dynamics analysis.

Materials and methods

Plant collection, authentication, and extraction

The hydroalcoholic extract for the bark of *C. arborea* (CAE), leaves of *P. guajava* (PGAE), and fruit peel of *P. granatum* (PGRPE) was prepared. Fresh parts were collected from the Belagavi district in Karnataka, India, and were authenticated by a taxonomist. The authenticated voucher specimens were then deposited at the ICMR-National Institute of Traditional Medicine, Belagavi, with accession numbers 1590 (*C. arborea*), 1690 (*P. granatum*), and 1589 (*P. guajava*). The collected plant parts were dried and ground into coarse powder. The coarse powder was soaked in 70% ethanol (70:30 v/v) for 24 h, and extraction was carried out using cold maceration [11]. The filtrate was subjected to rota evaporation at 50 °C and then lyophilized. The extracts were stored at – 20 °C till further use.

Source of clinical isolates of *Vibrio cholerae*

The clinical isolates of *Vibrio cholerae* were obtained from several locations of Karnataka, India, during the outbreaks of cholera that occurred in 2010 and 2013. The present investigation made use of a single clinical isolate of *Vibrio cholerae* O1 E1 Tor Ogawa that was chosen from our previous research [17].

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the selected plant extracts were determined according to the methods by Kowalska et al. and Ohikhenia et al. [18, 19] with slight modifications. Initially, the plant extract stock was serially diluted to obtain concentrations ranging from 20 mg/mL to 0.156 mg/mL. A bacterial culture was streaked onto Muller Hinton Agar (MHA), and a single colony was inoculated into Muller Hinton Broth (MHB) and incubated for 16 h. After incubation, the bacterial suspension was standardized to a 0.5 McFarland turbidity and introduced into each tube at a 1:100 dilution, excluding the negative control. The tubes were incubated for 24 h and observed for signs of bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the plant extract that showed no visible bacterial growth after 24 h of incubation [20]. Additionally, the number of colony-forming units per mL (CFU/mL) was quantified by plating on Muller Hinton Agar (MHA) and incubating for 24 h.

Minimum bactericidal concentration (MBC) is defined as the lowest concentration of the plant extract that causes 99% reduction of the bacterial population from the initial inoculum [21]. To determine the minimum bactericidal concentration (MBC), aliquots from concentrations above the MIC were plated onto Muller Hinton Agar (MHA) plates at various dilutions and incubated for 24 h at 37 °C [22]. The concentration at which there was a 99% decrease in colony-forming units per milliliter (CFU/mL) compared to the original culture was considered the minimum bactericidal concentration (MBC).

Augmentation of antibiotic susceptibility assay

The disk diffusion technique was used in order to assess the antibiotic-potentiating action of the plant extracts. This was done in conjunction with 10 routinely administered antibiotics against gastrointestinal diseases mentioned in Additional file 1: Table S1 [23]. In brief, both the minimum inhibitory concentration (MIC) and half the minimum inhibitory concentration (MIC/2) of each of the three plant extracts were prepared separately in Muller Hinton Broth (MHB) containing a 0.5 McFarland of *Vibrio cholerae* culture. 100 µL of these prepared culture with plant extract was spread uniformly on the Muller Hinton Agar (MHA) plate to form the bacterial lawn and untreated culture was kept as control. Afterward, sterile antibiotic disks of 6 mm in diameter were placed on the lawn, and plates were then incubated at 37 °C for 24 h. It was determined that the susceptibility was determined by utilizing the HIMEDIA susceptibility chart, and the diameter of the zones of inhibition was assessed [24, 25].

Real-time qPCR

Total RNA was extracted from overnight cultures of *Vibrio cholerae* in MHB containing sub-MIC concentration (MIC/2) of each plant extracts using the TRIzol reagent (Thermo Scientific, Cat No: 15596026). A UV/Vis nanospectrophotometer (NABI) was used in order to measure the amount of the RNA that was extracted. Using the PrimeScript RT Reagent Kit (DSS Takara Bio India, Cat No. 6110B), cDNA synthesis was carried out using 200 ng of RNA that had been isolated from each culture. Each sample was subjected to RT-qPCR using SYBR Green PCR Master Mix (DSS Takara Bio India; Cat No. RR820A), according to the instructions provided by the manufacturer. The relative expression levels of the *Vibrio cholerae* virulence genes, namely VpsA, FlaA, and LuxO, were measured and quantified (Additional file 1: Table S2 for primers). The PCR amplification was carried out with an initial denaturation step at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 35 s, using a Bio-Rad CFX Real-Time PCR system. Specific

transcript amplification was verified through dissociation curve analysis, with a gradual temperature increase from 60 °C to 95 °C at increments of 0.5 °C for 0.05 s. The relative mRNA expression levels of the VpsT, LuxO, and FlaA genes were analyzed using the $\Delta\Delta\text{CT}$ method [26, 27] and normalized against the expression levels of 16S rRNA.

Computational studies

Retrieval of phytocompounds

The reported phytocompounds from *Psidium gaujava* leaves, *Punica granatum* peel, and *Careya arborea* bark were obtained from IMPPAT medicinal plant database (<https://cb.imsc.res.in/imppat/>), and each phytocompound structural information was obtained from the PubChem chemical database (<https://pubchem.ncbi.nlm.nih.gov/>).

Molecular docking

The structural co-ordinates of the phytocompounds were retrieved in structural data file (*sdf*) format from PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). In order to obtain the lowest energy conformation, each compound energy was minimized using POAP_lig.bash script of POAP pipeline [28]. The compounds were saved in AutoDock vina (<https://vina.scripps.edu/>) accessible *pdbqt* format. On the other hand, the x-ray crystallographic structure of AphA (PDB ID: 1YG2), AphB (PDB ID: 3SZP), and ToxT (PDB ID: 4MLO) was obtained from the RCSB PDB (<https://www.rcsb.org/>) [29]. The structures were examined for missing amino acids and were filled using Swiss Model (<https://swissmodel.expasy.org/>) [30]. The active site information of the protein molecules was obtained from the P2Rank/PrankWeb (<https://prankweb.cz/>) [31]. The molecular docking of compounds with protein was performed using Auto Dock vina executed through the POAP pipeline. The system exhaustiveness was set to default, and nine conformations were generated. The compound having lowest RMSD score with lowest binding energy was generated for complex. The intermolecular interactions were visualized using DSV ver. 2019. The compound having lowest BE and interaction with active site was subjected to molecular dynamics (MD) studies.

Molecular dynamics

Molecular dynamics (MD) simulation has emerged as a crucial tool in computational biology, enabling the exploration within molecular complexes and intermolecular interactions under conditions that mimic biological environments. For validating the structural stabilities of the lead hit complexes, namely guavacoumaric acid with AphA, valoneic acid dilactone with AphB, and ellagic

acid with ToxT, an all-atom molecular dynamics simulation was performed using GROMACS version 2021 in an explicit solvent environment [32, 33]. The ligands and the entire complex topology were generated using the AmberTools xleap module (<https://ambermd.org/AmberTools.php>). The systems were solvated in a TIP3P water model in a rectangular box with boundary conditions set at a distance of 10.0 Å from the margins of the protein in all directions. The systems were neutralized by adding the required amount of counter ions. The lowest energy conformations were achieved using the steepest descent and conjugate gradient energy minimization methods. The systems were equilibrated using canonical (NVT) and isobaric (NPT) ensembles for 1 ns.

During NVT equilibration, a modified Berendsen thermostat was employed to keep the temperature constant at 300 K while maintaining the volume. For NPT equilibration, a Parrinello-Rahman barostat was used to maintain a constant pressure of 1 bar. Additionally, the particle mesh Ewald approximation with a 1-nm cut-off value was employed to calculate long-range electrostatic, van der Waals, and Coulomb interactions. Bond lengths were constrained using the LINear Constraint Solver (LINCS) approach. Finally, the system was simulated over a 100 ns production, with coordinates recorded every 2 fs. Trajectories were analyzed using built-in GROMACS tools. The stability and dynamics of ligand–protein interactions were assessed through molecular dynamics (MD) simulations, focusing on RMSD (root mean square deviation), RMSF (root mean square fluctuation), Rg (radius of gyration), SASA (solvent-accessible surface area), H-bond (hydrogen bond) formation, and principal component analysis (PCA).

The MM-PBSA approach, implemented with the "g_mmpbsa" tool [34] (Kumari and Kumar, 2014), assessed relative binding energies in MD simulations and thermodynamic calculations. Binding energies were computed by analyzing 50 representative snapshots from a stable trajectory spanning 50 to 100 ns. Entropy changes (ΔS) were determined using the Schlitter formula. The binding free energy (ΔG) was then calculated using $\Delta G = \Delta H - T\Delta S$, where ΔH represents enthalpy change, T is the temperature in Kelvin, and ΔS denotes entropy change. The resulting binding free energy from MM-PBSA was reported in kJ/mol. Additionally, the g_mmpbsa tool was used to analyze the contribution of individual residues to stable complex formation.

Statistical analysis

GraphPad Prism (version 8.0) was used for plotting graphs for *in-vitro* experimental data. The $\Delta\Delta\text{CT}$ method was utilized to examine the relative fold change of the mRNA expression. The xmgrace tool (version 5.1.25) was

employed to depict all the plots derived from the analysis of the MD trajectories. The binding free energy score and the docking score were expressed in units of kcal/mol.

Results

Determination of MIC and MBC

After 24 h of incubation of initial culture (5×10^7 CFU/mL), the bacterial density was estimated that 364×10^7 CFU/mL in positive control. The PGAE showed the MIC value at a concentration of 1.25 mg/mL and MBC at 5 mg/mL, followed CAE at MIC of 1.25 mg/mL and MBC at 10 mg/mL and PGRPE at MIC of 2.5 mg/mL and MBC at 10 mg/mL. The CFU/mL at each MIC and MBC concentrations along with dose at which 100% bactericidal activity is depicted in Table 1.

Augmentation of antibiotic susceptibility assay

Among the ten selected antibiotics, TE, AMP, and P were observed to be resistant to the selected isolate of *Vibrio cholerae*. Further, PGAE and PGRPE enhanced the antibiotic susceptibility of TE and AMP to an intermediate susceptibility level. The intermediate susceptibility of DOX and CIP in control was changed to sensitivity by all three extracts. The zone of inhibition of all the extracts along

with control is listed in Table 2. The percentage increase in the zone of inhibition (area in mm^2) of selected antibiotics by plant extracts is shown in Fig. 1.

RT-qPCR

The expression level of all the three selected virulence genes was downregulated by all three extracts (Fig. 2). The PGAE was found reduce the expression of FlaA by 2^8 to 2^{10} folds, LuxO by $>2^{12}$, and VpsA 2^8 to 2^{10} folds, whereas PGRPE has decreased the expression level of FlaA by 2^3 to 2^8 folds, LuxO by 2^2 to 2^8 , and VpsA 2^5 to 2^9 folds, and CAE reduces the expression of FlaA by 2^5 to 2^8 folds, LuxO by 2^6 to 2^9 , and VpsA 2^5 to 2^9 folds with concentration ranging from 39 $\mu\text{g/mL}$ to 625 $\mu\text{g/mL}$ of overnight culture. CAE extract at 625 $\mu\text{g/mL}$ reduced FlaA, LuxO, and VpsT gene expression by approximately ninefold, with a 5- to tenfold reduction at 312 $\mu\text{g/mL}$. PGRPE extract consistently decreased gene expression by 5- to tenfold across all doses, suggesting its potential as a prophylactic against biofilm-related genes. PGAE extract showed the most significant reduction in gene expression, with a tenfold decrease in LuxO expression at 39 $\mu\text{g/mL}$ and ~ 12 -fold at 625 $\mu\text{g/mL}$ and also showed consistent decreases in FlaA and VpsT expression

Table 1 Plant extracts depicting MIC and MBC results along with the complete inhibition at their respective doses

Extract	MIC		MBC		Complete Inhibition	
	Dose (mg/mL)	Colonies (CFU/mL)	Dose (mg/mL)	Colonies (CFU/mL)	Dose (mg/mL)	Colonies (CFU/mL)
<i>Psidium guajava</i> (PGAE)	1.25	8.48×10^6	5	6.24×10^4	10	0
<i>Careya arborea</i> (CAE)	1.25	9.84×10^6	10	3.3×10^4	20	0
<i>Punica granatum</i> (PGRPE)	2.5	1.264×10^6	10	7.92×10^4	20	0

Table 2 Zone of inhibition (mm) of the plant extracts (himedia zone size interpretative chart (as per CLSI & EUCAST))

Antibiotics	Positive Control	PGRPE		PGAE		CAE	
		2.5 mg/mL	1.25 mg/mL	1.25 mg/mL	0.625 mg/mL	1.25 mg/mL	0.625 mg/mL
	Zone of Inhibition (mm)						
C	25	25	25	27	26	26	25
GEN	23	23	23	23	23	23	23
TE	9	14	13	15	14	16	12
AMP	13	14	13	15	13	13	13
DOX	13	15	14	18	13	17	16
K	15	15	15	16	15	15	15
AK	17	18	17	17	17	17	17
CIP	20	25	24	26	25	26	24
TOB	17	18	17	18	17	17	17
P	14	15	14	17	16	14	14

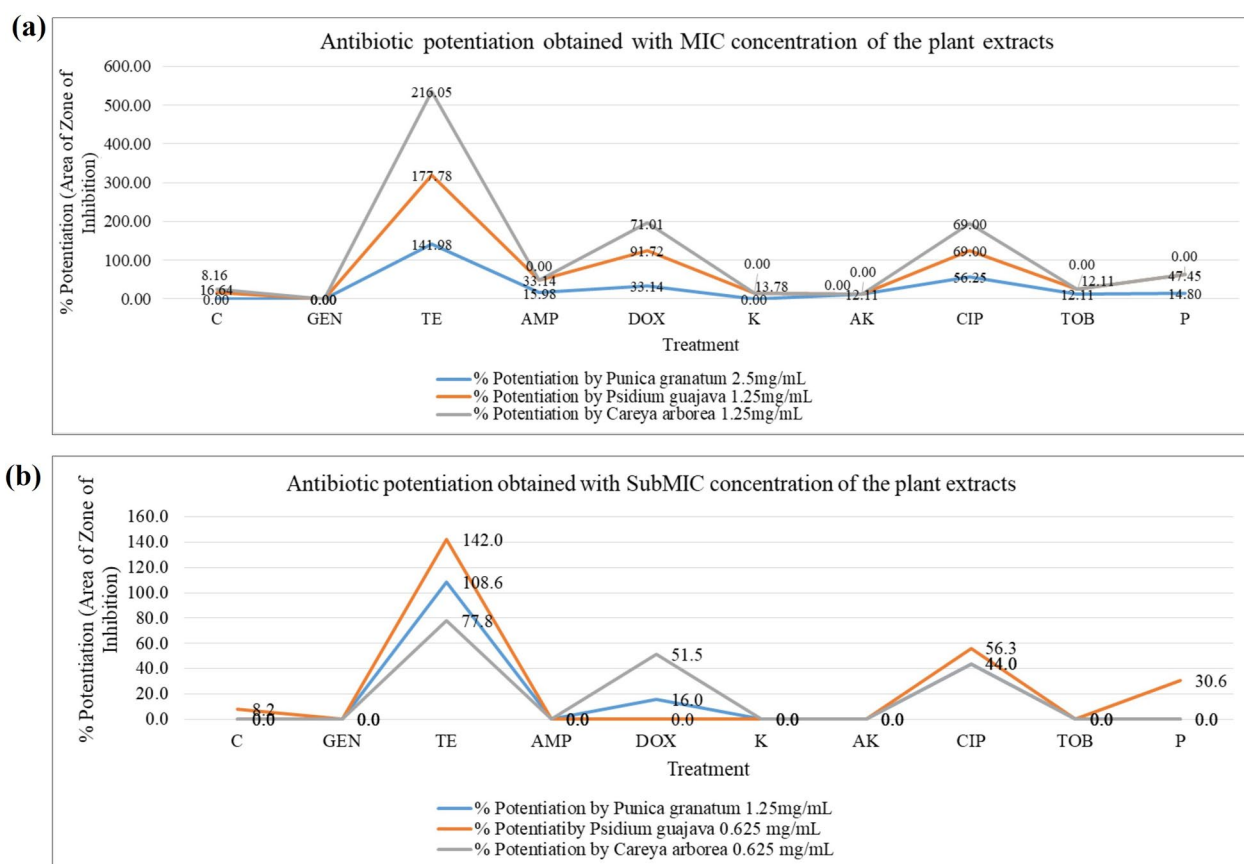


Fig. 1 Percentage of antibiotic potentiation of 10 different antibiotics with the three plant extracts (CAE, PGAE, PGRPE) **a** Antibiotic potentiation obtained with the MIC concentrations of the plant extracts. **b** Antibiotic potentiation was obtained with the sub-MIC concentrations of the plant extracts. The above graph indicates that sub-MIC concentrations of PGRPE at 1.25 mg/mL, PGAE at 0.625 mg/mL, and CAE at 0.625 mg/mL, as well as the extracts at 2.5 mg/mL (A), 1.25 mg/mL (B), and 1.25 mg/mL (C), can enhance the inhibitory effects of specified antibiotics. PGRPE notably enhances the effects of TE, DOX, and CIP at both MIC and sub-MIC levels. PGAE at sub-MIC shows significant enhancement of C, TE, and CIP, while PGRPE at MIC enhances TOB and P. PGAE at MIC enhances the activity of AMP, DOX, K, and TOB against *Vibrio cholerae*. CAE at sub-MIC enhances TE, DOX, and CIP, while CAE at MIC enhances chloramphenicol

upto ~tenfold. Overall, PGAE extract exhibited the greatest reduction in gene expression, followed by PGRPE and CAE extracts. In the in vitro qRT-PCR study, we found that, the PGAE extract to reduce the expression of FlaA by 2^8 to 2^{10} folds, LuxO by $>2^9$, and VpsA 2^8 to 2^{10} folds, while PGRPE decreases the expression level of FlaA by 2^3 to 2^8 folds, LuxO by 2^2 to 2^8 , and VpsA 2^5 to 2^9 folds.

Computational studies

Structural information

The active site residues of AphA (PDB ID: 1yg2) were Val6, Thr9, Val10, Glu24, Ser28, Ile29, Phe32, Trp82, Gln146, Leu149, Glu150, and Thr153. The active site residues of AphB (PDB ID: 3szp) were Lys23, Ser99, Asn100, Lys103, Arg104, Met125, Met126, Asn128, Gln129, Ser218, Ala219, Asn221, Arg224, and Met240. The active site residues of ToxT (PDB ID: 4mLo) were Gln8, Tyr20, Phe22, Leu25, Tyr26, Asn28, Asp29, Lys31, Phe33,

Leu61, Phe69, Leu71, Val81, Val83, Thr85, Ser223, Ile226, Ser227, Lys230, Met259, Val261, Tyr266, and Met269.

Docking studies

Among the 10 compounds targeting AphA, guavacoumaric acid scored the least BE (-9.4 kcal/mol) with AphA via forming 1 HBI with Gln146 and 3 NHBI with Val10, Leu149, Phe32, whereas the phyto compound jacoumaric acid scored the second lowest BE (-9.3 kcal/mol) via forming 3 HBI with Glu24, Ser28, Thr9 and 8 NHBI with Val6, Phe32, Arg156 (2), His89, Trp82, Phe83, Ile29. The phyto compounds punicalagin and castalagin did not show any interaction with active site residues and the remaining screened phyto compounds showed BE ranging from -9.4 to -8.2 and formed interaction with active site residues (Additional file 1: Table S3).

Among the 10 compounds targeting AphB, valoneic acid dilactone scored the least BE (-10.1 kcal/mol) with

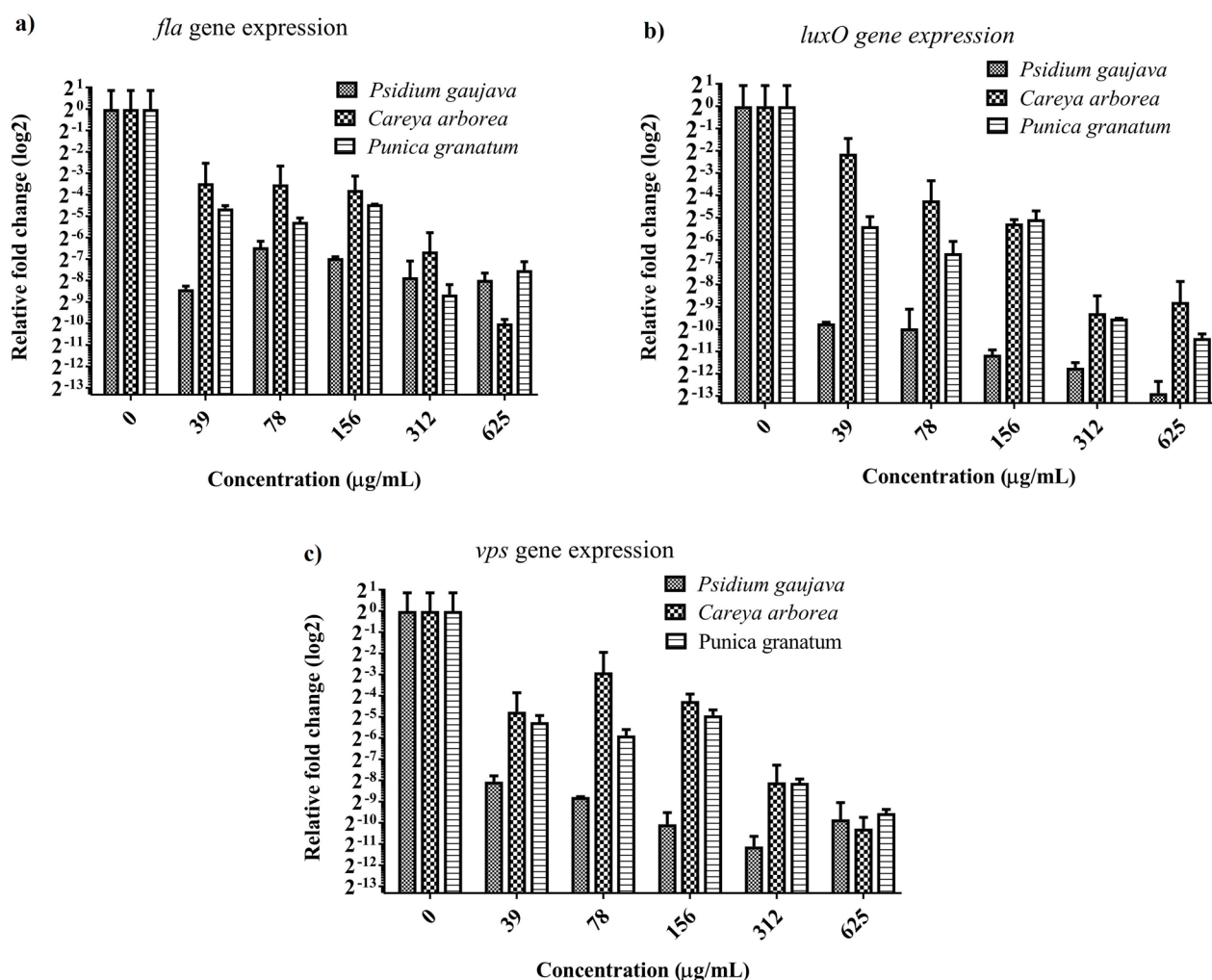


Fig. 2 Effect of the selected plant extracts PGAE, CAE, and PGRPE on the expression levels of three different genes a) *FlaA*, b) *LuxO*, and c) *Vps* associated with the virulence factors of *Vibrio cholera*

3zsp via forming 3 HBI with Ser99, Asn128, Gln129 and 7 NHBI Ala219 (2), Ser99, Ser127, Ser218, Ala219, Met126, whereas the phytochemical rutin scored the second least BE (− 9.9 kcal/mol) via forming 9 HBI with Asn221, Asn221 (2), Asn128, Ser218, Ser99, Ser218 (2), Asp131 and 4 NHBI with Met126, Lys23, Ser99, Ala219. The phytochemical granatin A did not form interaction with active site, whereas the remaining phytochemicals showed interactions with the active site as shown in the (Additional file 1: Table S4).

Among the 10 compounds targeting ToxT, ellagic acid scored the least BE (− 9.5 kcal/mol) via forming 11 NHBI with Val81, Val83(4), Phe33, Tyr12(3), Phe22(3), whereas luteolin was the second lead hit as it scored lowest BE of − 8.7 kcal/mol via forming 9 NHBI with Leu25, Ile226, Lys31, Phe22(2), Tyr12(2), Val83(2). Jacoumaric acid, granatin A, guavacoumaric acid, and ilelatifol D did not show any interaction with the active site, whereas

the remaining phytochemicals showed interactions with the active site as shown in the (Additional file 1: Table S5).

Stability of guavacoumaric acid -AphA

In the guavacoumaric acid -AphA_1yg2 complex, stable dynamics were observed throughout a 100 ns simulation following a 20 ns equilibration period. Initially, backbone and complex RMSD values increased steadily from 2.0 Å to 4 Å and approximately 3 Å to 5 Å, respectively, over the first 20 ns. By 20 ns, both the backbone and complex RMSD stabilized with reduced fluctuations (Fig. 3A). Residues Leu57 to Lys67 exhibited fluctuations around 4.0 Å, while residues Gln85 to Asp94 showed higher fluctuations of about 6.0 Å. C-terminal residues also fluctuated up to 5 Å. In contrast, residues Gln146, Val10, Leu149, and Phe32, involved in stable non-bonded interactions with guavacoumaric acid during docking, did not

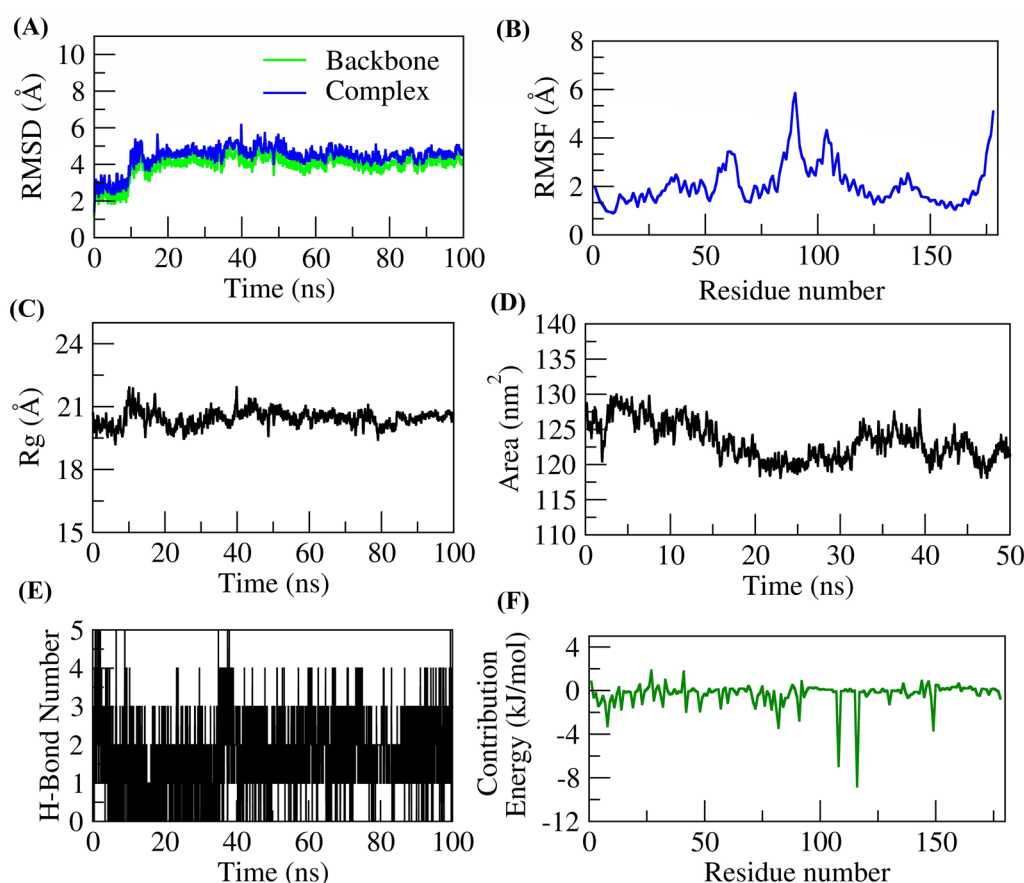


Fig. 3 Parameters describing guavacoumaric acid -AphA complex structural stabilities during 100 ns MD simulation. **(A)** RMSD of backbone and complex atoms, **(B)** RMSF of C-alpha atoms, **(C)** protein atoms Rg, **(D)** protein atoms SASA, **(E)** number of H-bond interactions, and **(F)** contribution energy plot highlighting the importance of the binding pocket residues in stable complex formation with guavacoumaric acid

exhibit significant fluctuation. The complex adopted a compact globular shape, as indicated by a decrease in Rg from 20.0 Å to 19.0 Å initially, stabilizing around 20.0 Å (Fig. 3C). The initial and final surface areas occupied by the Guavacoumaric acid -AphA complex were 125 nm² and 120 nm², respectively, with the complex typically occupying 123 nm² (Fig. 3D). Throughout the simulation, the complex formed 5 hydrogen bonds, with three persisting consistently (Fig. 3E). Guavacoumaric acid scored a relative BE of -185.794 ± 20.667 kJ/mol with AphA. Per residue energy decomposition revealed that three residues in the binding pocket (Ala116, Ala108, Leu149) significantly contributed to the stable complex formation, each contributing between -3.6 kJ/mol and -8.8 kJ/mol (Fig. 3F).

Stability of AphB-valoneic acid dilactone complex

The AphB-valoneic acid dilactone complex exhibited stable dynamics throughout a 100 ns simulation following a 20-ns equilibration period. Initially, both backbone and complex RMSD values increased steadily from 3.0 Å to

8 Å and approximately 4 Å to 9 Å, respectively, over the first 20 ns. By 20 ns, these values stabilized with reduced fluctuations (Fig. 4A). Chain B, particularly the amino acid region from Met1 to Ser90, showed greater fluctuations. However, residues such as Ser99, Asn128, Gln129, Ala219, Ser127, Ser218, and Met126, which interacted with AphB during docking studies, remained stable due to their involvement in non-bonded interactions. The complex adopted a compact globular shape, evidenced by a gradual decrease in Rg from 32 Å to 29 Å initially, stabilizing at 31.0 Å (Fig. 4C). The initial and final surface areas occupied by the AphB-valoneic acid dilactone docked complex were 300 nm² and 295 nm², respectively, with the complex typically occupying 297 nm² (Fig. 4D). Throughout the simulation, the complex formed 6 hydrogen bonds, with three persisting consistently (Fig. 4E). Valoneic acid dilactone scored a relative binding energy (BE) of -257.590 ± 19.069 kJ/mol with AphB. Per residue energy decomposition revealed that 12 residues in the binding pocket (Asp132, Asp131, Asp119, Asp263, Leu290, Asp67, Asp256, Glu115, Glu205, and Met126)

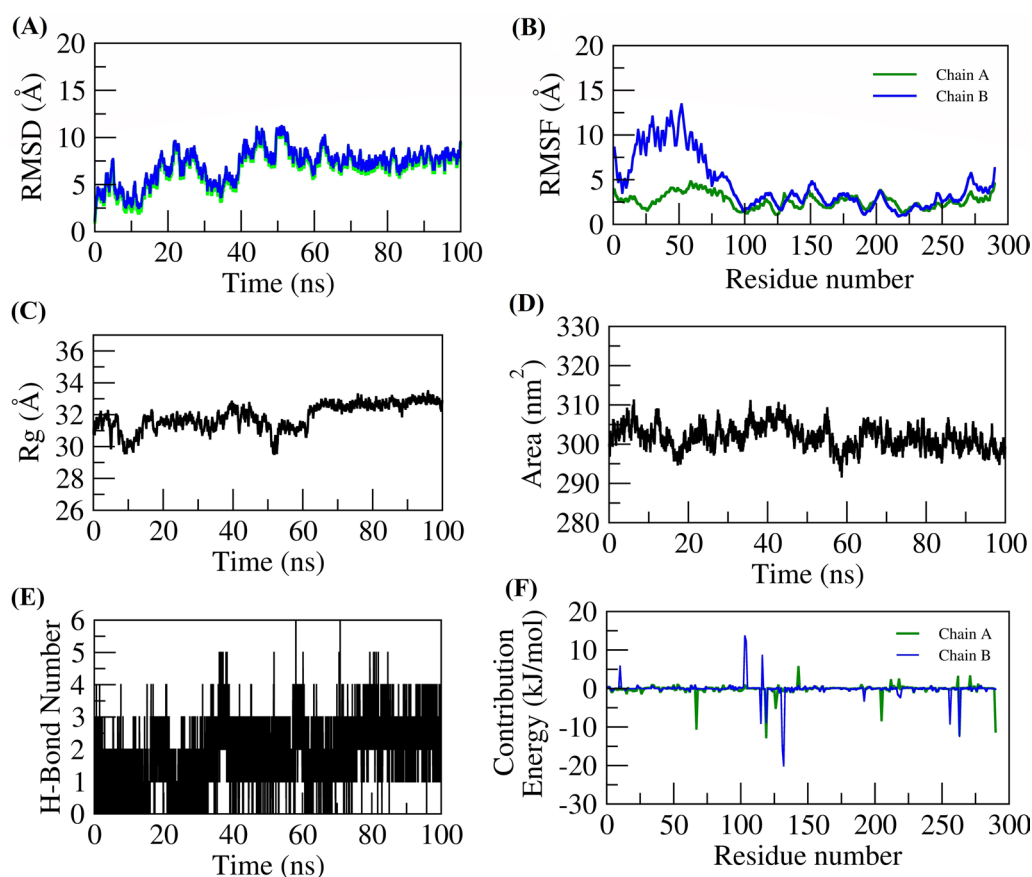


Fig. 4 Parameters describing AphB–valoneic acid dilactone complex structural stabilities during 100 ns MD simulation. **(A)** RMSD of backbone and complex atoms, **(B)** RMSF of c-alpha atoms, **(C)** protein atoms Rg, **(D)** protein atoms SASA, **(E)** number of H-bond interactions, and **(F)** contribution energy plot highlighting the importance of the binding pocket residues in stable complex formation with valoneic acid dilactone

significantly contributed to the stable complex formation, each contributing between -5.0 kJ/mol and -20.0 kJ/mol (Fig. 4F). Conversely, residues Arg262, Arg143, Lys271, and Arg10 exhibited positive energies, indicating their opposing effect on the interaction.

Stability of ToxT–ellagic acid complex

The ToxT–ellagic acid complex maintained stable dynamics throughout a 100 ns simulation following a 20-ns equilibration phase. Initially, backbone and complex RMSD values increased steadily from 1.0 Å to 2.5 Å and approximately 1.5 Å to 2.8 Å, respectively, over the first 20 ns. By 20 ns, these values stabilized with reduced fluctuations (Fig. 5A). Residues Gly133 to Ser140, which form loops connecting to helices, exhibited greater fluctuations around 4.0 Å. However, residues such as Val81, Val83, Phe33, Tyr12, and Phe22, which interacted with ToxT during docking, remained stable due to their involvement in non-bonded interactions. The complex adopted a compact globular shape, supported by a gradual decrease in Rg from 19.5 Å to

19.3 Å, stabilizing around 19.3 Å during the simulation (Fig. 5C). The initial and final surface areas occupied by the ToxT–ellagic acid docked complex were 145 nm² and 150 nm², respectively, with the complex typically occupying 148 nm² (Fig. 5D). Throughout the simulation, the complex formed 5 hydrogen bonds, with two persisting consistently (Fig. 5E). Ellagic acid exhibited a relative BE of -90.663 ± 10.767 kJ/mol with ToxT. Per residue energy decomposition highlighted that 9 residues within the binding pocket (Asn22, Asn20, Ser21, Leu71, Tyr12, Val81, Met259, Met14, and Asn260) significantly contributed to the stable complex formation, each contributing between -2.1 kJ/mol and -10.9 kJ/mol (Fig. 5F).

Principal Component Analysis of the complexes

Principal component analysis (PCA) was performed to investigate the essential dynamics and collective motions of the protein-ligand complexes during MD simulations. The projections of the trajectories along the first two principal components (PC1 and PC2) revealed distinct patterns of motion. For

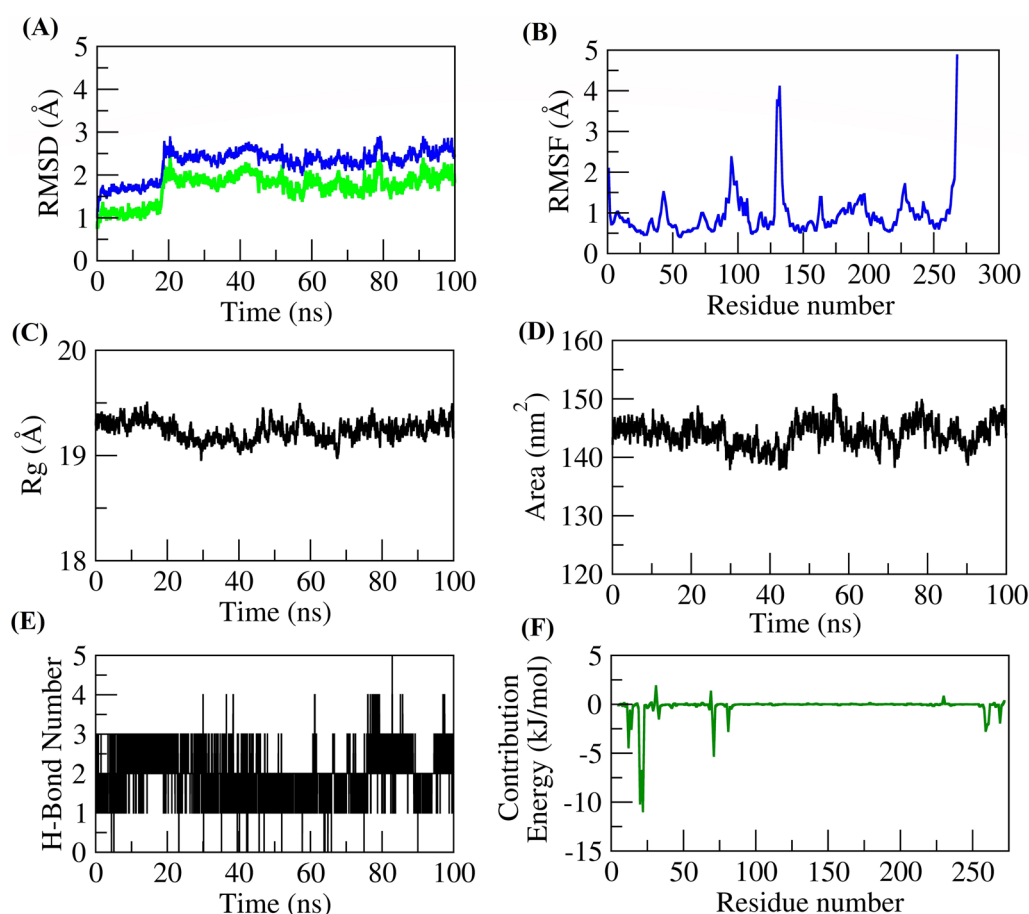


Fig. 5 Parameters describing ToxT–ellagic acid complex structural stabilities during 100 ns MD simulation. **(A)** RMSD of backbone and complex atoms, **(B)** RMSF of c-alpha atoms, **(C)** protein atoms Rg, **(D)** protein atoms SASA, **(E)** number of H-bond interactions, and **(F)** contribution energy plot highlighting the importance of the binding pocket residues in stable complex formation with ellagic acid

Guavacoumaric acid with AphA, two well-defined clusters were observed, indicating the presence of two stable conformational states. The eigenvalue plot showed a sharp decay after the initial components, suggesting that most of the variance is captured by the first few eigenvectors. Whereas, Valoneic acid dilactone with AphB exhibited a broader distribution along PC1 and PC2, signifying greater conformational flexibility. The corresponding eigenvalue plot displayed higher initial values, reflecting significant collective motions in the system. Ellagic acid with ToxT demonstrated a tightly clustered distribution in the projection, indicating restricted conformational variability and enhanced stability of the complex. The eigenvalue plot for this complex showed limited variance, with most motions confined to the first few principal components. These results highlight the varying degrees of flexibility and stability in the protein-ligand complexes, with ToxT–ellagic acid displaying the highest stability and Valoneic acid dilactone–AphB showing greater flexibility (Fig. 6).

Discussion

Cholera is an acute diarrheal disease responsible for approximately 2,420 deaths annually. The available vaccines offer only short-term protection, and the chemotherapy used for treatment has contributed to the emergence of multi-drug resistant bacterial strains. This is particularly challenging with the O1E1 Tor Ogawa strain of *Vibrio cholerae*, which has developed resistance mechanisms against conventional drugs. In our previous studies, the three plant extracts PGRPE, PGAE, and CAE have been shown to effectively neutralize cholera toxin activities [11, 35]. Continuing to this, our current study investigated the ability of these extracts for their antibacterial properties, their potential to enhance antibiotic susceptibility, and their impact on the expression levels of virulence regulatory genes in *Vibrio cholerae*. Bioinformatics tools were used to dock the plant compounds, and the in silico results revealed that these herbal compounds effectively interact with the virulent genes of the cholera-causing *Vibrio cholerae*. The extracts were tested through

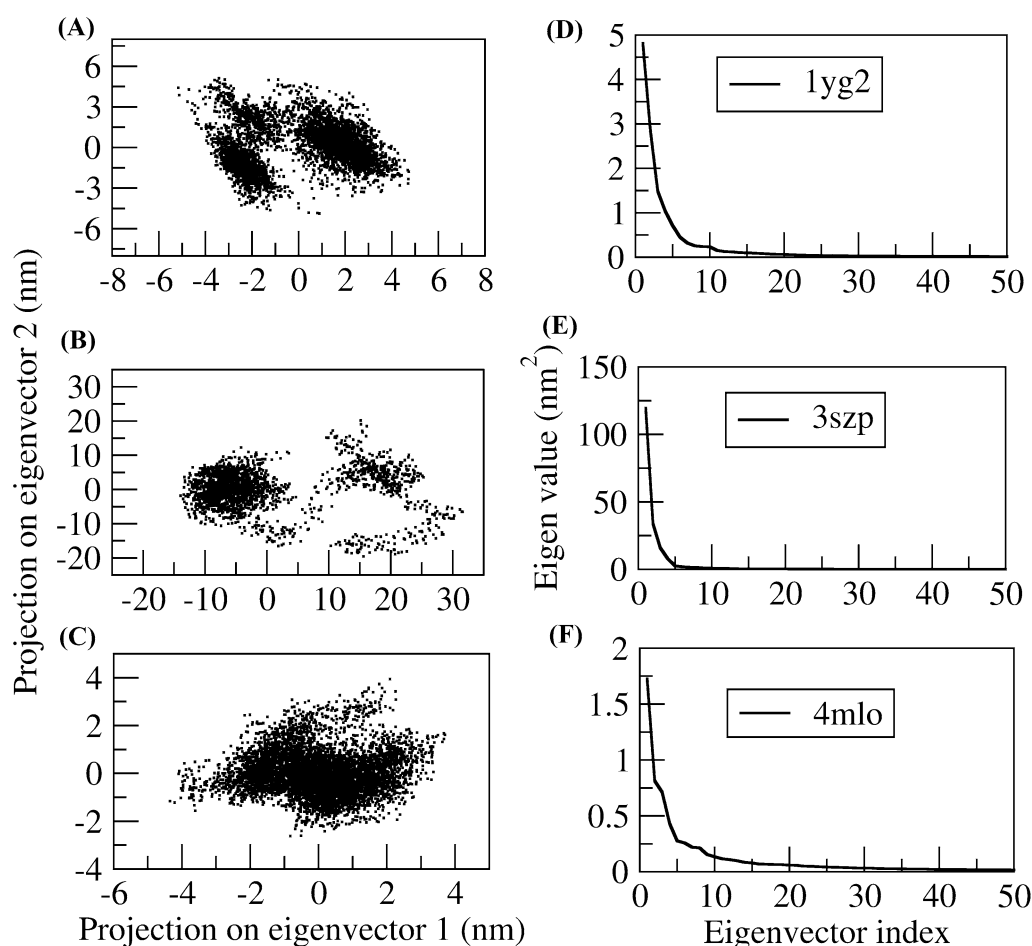


Fig. 6 Principal component analysis of protein–ligand complexes: the collective motion of **(A)** guavacoumaric acid with AphA, **(B)** valoneic acid dilactone with AphB, and **(C)** ToxT with ellagic acid using projections of MD trajectories on two eigenvectors corresponding to the first two principal components. The first 50 eigenvectors were plotted versus eigenvalue for **(D)** guavacoumaric acid with AphA, **(E)** valoneic acid dilactone with AphB, and **(F)** ToxT with ellagic acid

in vitro microbial assays. The findings from this study could lead to the identification of natural compounds from the extracts that target antimicrobial resistance in the O1E1 Tor Ogawa strain, providing a promising approach to treating cholera. Additionally, the study also supports the efficacy of traditional medicinal plants in combating diarrheal infections.

The MIC values recorded were ranging from 1.25 to 2.5 mg/mL suggesting that these extracts could be considered as potent antibacterial agents. PGAE and CAE showed strong antibacterial activity when compared to PGRPE. The traditional use of *Punica granatum* peel, *Psidium guajava* leaves, and *Careya arborea* bark in treating various gastrointestinal ailments is well documented. The leaves of PGAE have been used in traditional medicine for treating gastroenteritis, diarrhea, and dysentery [36], while PGAE leaf extracts have attracted considerable interest due to their diverse properties,

encompassing anticancer, antidiabetic, antidiarrheal, antimicrobial, antimutagenic, and antioxidant effects [37]. The PGRPE, rich in bioactive compounds like tannins, flavonoids, polyphenols, and anthocyanins, has demonstrated significant antibacterial and antioxidant effects. These properties, validated *in-vitro*, suggest its potential as a source of broad-spectrum antimicrobial agents effective against various pathogens like *S. aureus*, *P. aeruginosa*, and *E. Coli* [38, 39]. The CAE bark is used for its anti-diarrheal, antioxidant, and hepatoprotective effects, and also as a remedy for dysentery with bloody stools and ear pain [40], and it is reported to mitigate the castor oil-induced diarrhea in mice [10]. Further, it is reported against various conditions like tumors, bronchitis, epileptic fits, and skin diseases [41].

The escalating prevalence of pathogenic bacterial strains resistant to a broad spectrum of conventional antibiotics represents a critical global health issue.

Moreover, the lack of success in developing novel antibiotics over the past two decades has further intensified this concern. Consequently, research is increasingly focused on enhancing the efficacy of existing antibiotics through various strategies. In this context, our study specifically investigated the synergistic effects of combining commonly used antibiotics with certain extracts to enhance their antimicrobial activity [42].

Our study elucidates the significant role of plant extracts in augmenting the susceptibility of several widely utilized antibiotics, specifically with TE, DOX, and CIP. It was found to substantially enhance the inhibitory effects with TE by CAE (216.05% and 77.78%), PGAE (177.78% and 141.98%) and PGRPE (141.98% and 108.64%), DOX by CAE (71.0% and 51.48%), PGAE (91.7% and 0%), and PGRPE (33.13% and 15.98%), CIP by CAE (69% and 44%), PGAE (69% and 56.25%) and PGRPE (56.25% and 44%), at its MIC and sub-MIC levels, respectively. Additionally, PGAE at MIC levels exhibited potentiation of AMP, K, and TOB by 33.14%, 13.78%, and 12.11%, respectively.

The pathophysiological processes like secretion of toxins, survival of bacteria in different physical conditions, quorum sensing, and biofilm formation by *Vibrio cholerae* are a complicated process involving several genes and regulatory mechanisms [43]. The synergistic actions of FlaA, VpsT, and LuxO in *Vibrio cholerae* culminate in enhanced virulence by governing pivotal processes including biofilm formation, quorum sensing, and motility. These processes are indispensable for effective colonization, survival, and pathogenesis within the host milieu. Deciphering the intricate molecular mechanisms regulated by these genes is imperative for devising precise interventions aimed at combating cholera infections. *Vibrio cholerae* VpsT transcriptional regulator is essential for controlling the development of biofilms. It does this by turning on the genes that produce exopolysaccharide (EPS), an essential part of the biofilm matrix. By acting as a barrier, this biofilm matrix increases *Vibrio cholerae*'s resistance to adverse conditions and encourages its survival in aquatic settings. Increased ability to survive under these circumstances promotes colonization and persistence of *Vibrio cholerae* within host habitats, increasing its pathogenic potential. In *Vibrio cholerae*, LuxO functions as a key regulator of quorum sensing (QS), coordinating gene expression in response to variations in cell population density. LuxO is phosphorylated in low-cell density environments, which in turn stimulates the expression of short regulatory RNAs (sRNAs) such as quorum regulatory RNAs (Qrr). The expression of genes encoding virulence factors, such as cholera toxin (CTX) and toxin co-regulated pilus (TCP), is subsequently post-transcriptionally suppressed by these sRNAs. Thus, LuxO helps *Vibrio cholerae* adapt to host

colonization by indirectly modulating virulence by modifying the expression of important virulence factors in response to changes in cell density. The structural protein FlaA makes up the flagellum, the whip-like projection of *Vibrio cholerae* bacterium that is responsible for their motility. *Vibrio cholerae* uses its flagellar motility to travel toward nutrient-rich settings and to colonize host epithelial surfaces. Apart from their motility, flagella are involved in the adherence and colonization of host cells, which further adds to the pathogenicity of *Vibrio cholerae*. In the early stages of infection, *Vibrio cholerae* needs FlaA-mediated motility and adhesion to establish infection in the host intestine and start the pathogenic process that results in cholera sickness [44, 45]. The impact of these extracts on the expression levels of key regulatory genes, namely VpsT, LuxO, and FlaA, necessary for the virulence of *Vibrio cholerae*, was analyzed using RT-PCR. Furthermore, to elucidate the probable mechanisms of action, major bioactives from these plant extracts targeting key regulatory proteins such as AphA, AphB, and ToxT of *Vibrio cholerae* were studied using computational methods. The results showed that CAE bark extract was effective against FlaA, LuxO, and VpsT at a concentration of 625 µg/mL, causing a nearly nine-fold reduction in expression. At a lower concentration of 312 µg/mL, the extract induced a reduction in the range of fivefold to tenfold.

Among the three plant extracts studied, PGAE showed the most significant reduction in the expression of genes involved in biofilm formation. LuxO, FlaA, and VpsT gene expressions were reduced up to 2¹⁰-fold on dose-dependent manner. These findings suggest that PGAE leaf extract demonstrated the most dramatic decrease in gene expression, followed by CAE bark extract and PGRPE peel extract. These results highlight the ability of these plant extracts as potent agents for inhibiting biofilm formation, quorum sensing secretion of toxins and motility of bacteria.

Currently on going researchers emphasize the potential of natural compounds to inhibit pathogenic factors and enhance treatment strategies which are majorly validating through the synergy of experimental and computational studies, for instance, cordycepin's, forodesine, and riboprime success in inhibiting viral replication [46, 47], 1-(5-bromopyrazin-2-yl)-1-[3-(trifluoromethyl)benzyl]urea [48] and galloyl hydrazide [49] for cancer treatment, heterocyclic derivatives of citric acid [50] and sperm-shaped amphiphilic heterocyclic compounds derived from natural palmitic acid [51] as upcoming novel pharmacologically active lead molecules, and these studies inspire similar investigations into plant-based compounds for targeting other infectious agents, and this study also further focused

on the computational study to understand the predicted mode of action of these extracts; the reported phytocompounds from these extracts were virtually screened against the major regulator of virulence factors such as AphA, AphB, and ToxT protein targets.

Guavacoumaric acid was identified as a lead hit molecule against AphA (BE, -9.4 kcal/mol) followed by jacoumaric acid. Likewise, valoneic acid dilactone was against AphB (BE, -10.1 kcal/mol) followed by rutin, whereas ellagic acid was found to be the lead hit against ToxT. The results revealed that the compounds from *P. guajava* and *P. granatum* as a valuable lead against *V. cholera* virulence factors and inhibit the regulation of quorum sensing via forming interactions with active site residues of AphA, AphB, and ToxT [44]. Guavacoumaric acid, valoneic acid dilactone, and ellagic acid are the polyphenols, and their ability to form multiple hydrogen bonds, as well as their interaction with aromatic residues through π - π stacking, highlights the importance of these functional groups in stabilizing protein-ligand complexes. Additionally, the presence of hydroxyl groups in these compounds promotes the formation of extensive hydrogen bonding networks, a common feature observed in many bioactive phytochemicals with therapeutic potential. Previous literature on phenolic and coumaric acid derivatives emphasizes their role as potent inhibitors of enzymes, owing to their ability to interact with active site residues via hydrogen bonds and hydrophobic interactions. This aligns with the findings in the present study, where these compounds demonstrated high binding affinities and stable MD profiles during 100 ns; as a result, it was found that the residues Ala116, Ala108, and Leu149 of AphA were the residues forming stable interactions with guavacoumaric acid with binding free energy of -185.794 kJ/mol. Likewise, valoneic acid dilactone was the lead hit compound against AphB and it formed stable contacts with Asp132, Asp131, Asp119, Asp263, Asp263, Leu290, Asp67, Asp256, Glu115, Asp119, Glu205, Met126 throughout 100 ns MD simulation and scored the relative binding energy of -257.590 kJ/mol, whereas ellagic acid was the lead hit compound against ToxT and it formed stable contacts with Asn22, Asn20, Ser21, Leu71, Tyr12, Val81, Met259, Met14, and Asn260 residues throughout the simulation period and scored the relative binding energy of -90.663 kJ/mol. The identified lead molecules also formed a stable RMSD for backbone and complex atoms throughout 100 ns MD simulation [52] indicated these identified phytocompounds may serve as key molecules in regulating the virulence factors of *Vibrio cholerae*.

Conclusion

This study showed that PGAE, CAE, and PGRPE exhibit significant inhibitory effects on *Vibrio cholerae* growth. These extracts, notably PGAE extract, enhance the efficacy of certain commonly using antibiotics. Among the extracts, CAE exhibited the strongest antibacterial effect, followed by PGAE and PGRPE. Further, the combination of gene expression studies and computational analysis showed that these plant extracts not only exhibit antibacterial properties but also significantly suppress the expression of key virulence genes FlaA, LuxO, and Vps required for biofilm formation and virulence. The computational predictions suggest a stable interaction of plant-derived phytocompounds with crucial regulators of virulence, highlighting a promising avenue for natural adjunct therapies in cholera management. Despite these promising results, further research utilizing *in-vivo* models and clinical studies are necessary to fully ascertain their therapeutic value and potential role in managing cholera outbreaks thereby contributing to a holistic approach in combating antibiotic resistance and enhancing cholera treatment strategies.

Abbreviations

AMP	Ampicillin
AK	Amikacin
C	Chloramphenicol
CAE	<i>Careya arborea</i> extract
CFU	Colony-forming unit
CT	Cholera toxin
CIP	Ciprofloxacin
DOX	Doxycycline
flaA	Flagellin A
GEN	Gentamycin
K	Kanamycin
luxO	Lux operon
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MD	Molecular dynamics
P	Penicillin G
PGPRE	<i>Punica granatum</i> fruit peel extract
PGAE	<i>Psidium guajava</i> extract
RT	Reverse transcriptase
TE	Tetracycline
TOB	Tobramycin
vpsT	Vibrio polysaccharide synthesis transcription regulator

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-024-00750-x>.

Additional file 1.

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Author contributions

Pruthviraj Chavan and Kamakshi Naik involved in investigation, writing—original draft. Soumika Ghosal took part in investigation, writing—review & editing. Rajitha Charla, Rajalakshmi Patil, Jyoti N Guruputranavar involved in investigation, formal analysis. Vishal S. Patil took part in investigation, formal analysis, computational studies, writing—original draft, revision, and writing—review & editing. Darasaguppe R. Harish involved in conceptualization, supervision, project administration, methodology, writing—review & editing. Subarna Roy took part in formal analysis, co-supervision, resources, writing—review & editing.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and in the supplementary files provided.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by Institutional Biosafety Committee (IBSC), RCGM-DBT, Govt. of India, for the conduct of research on *Vibrio cholera*, highly virulent enteric bacteria isolated from clinical samples and their toxins. Letter No: BT/IBKP/475/2021 Dated 03/11/2021; IBKP UAC ID: ICMRD6071217.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Faruque SM, Albert MJ, Mekalanos JJ (1998) Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae*. *Microbiol Mol Biol Rev* 62(4):1301–1314
2. Cholera Annual Report (2019) World Health Organization. *Wkly Epidemiol Rec* 95(38):441–448
3. Ng QX, De Deyn MLZQ, Loke W, Yeo WS (2020) Yemen's cholera epidemic is a one health issue. *J Prev Med Public Health* 53(4):289
4. Bryce J, Boschi-Pinto C, Shibuya K, Black RE (2005) WHO estimates of the causes of death in children. *Lancet* 365(9465):1147–1152
5. Kirn TJ, Bose N, Taylor RK (2003) Secretion of a soluble colonization factor by the TCP type 4 pilus biogenesis pathway in *Vibrio cholerae*. *Mol Microbiol* 49(1):81–92
6. Gill DM, Meren R (1978) ADP-ribosylation of membrane proteins catalyzed by cholera toxin: basis of the activation of adenylate cyclase. *Proc Natl Acad Sci* 75(7):3050–3054
7. Cassel D, Pfeuffer T (1978) Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. *Proc Natl Acad Sci* 75(6):2669–2673
8. Rahim N, Gomes DJ, Watanabe H, Rahman SR, Chomvarin C, Endtz HP, Alam M (2010) Antibacterial activity of *Psidium guajava* leaf and bark against multidrug-resistant *Vibrio cholerae*: implication for cholera control. *Jpn J Infect Dis* 63(4):271–274
9. Kharisma A, Tjahjandingsih W, Sigit S (2020) Determination of minimum inhibitory and minimum bactericidal concentration of ketapang (*Terminalia catappa*) leaves extract against *Vibrio harveyi*. *IOP Conf Ser Earth Environ Sci* 441(1):012012
10. Rahman MT, Khan OF, Saha S, Alimuzzaman M (2003) Antidiarrhoeal activity of the bark extract of *Careya arborea* Roxb. *Fitoterapia* 74(1–2):116–118
11. Charla R, Patil PP, Bhatkande AA, Khode NR, Balaganur V, Hegde HV, Roy S (2022) In vitro and in vivo inhibitory activities of selected traditional medicinal plants against toxin-induced cyto-and entero-toxicities in cholera. *Toxins* 14(10):649
12. Mishra A, Seth A, Maurya SK (2016) Therapeutic significance and pharmacological activities of antidiarrheal medicinal plants mention in Ayurveda: a review. *J Intercult Ethnopharmacol* 5(3):290
13. Satish KB, Vrushabendra SB, Kamal KG, Mohan BG (2010) Review on *Careya arborea* Roxb. *Int J Res Ayurveda Pharm* 1(2):306–315
14. Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP, Pal M (1999) Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. *J Ethnopharmacol* 68(1–3):205–208
15. Qnaies EY, Elokda AS, Abu Ghalyun YY, Abdulla FA (2007) Antidiarrheal activity of the aqueous extract of *Punica granatum* (pomegranate) peels. *Pharm Biol* 45(9):715–720
16. Shamkuwar PB, Pawar DP (2012) Evaluation of antidiarrhoeal potential of *Punica granatum* L. (Punicaceae) in Ayurvedic formulation. *J Chem Pharm Res* 4:1489–1492
17. Bhattacharya D, Dey S, Roy S, Parande MV, Telsang M, Parande AV, Mantur BG (2015) Outbreak of cholera by multidrug resistant *Vibrio cholerae* O1 in a backward taluka of Bagalkot, North Karnataka. *Jpn J Infect Dis* 2:14
18. Kowalska-Krochmal B, Dudek-Wicher R (2021) The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance. *Pathogens* 10(2):165
19. Ohikhen A, Wintola OA (2017) Afolayan AJ (2017) Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. *Sci World J* 1:9658598
20. Acharya S, Patra A, Bag PK (2009) Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multi-drug resistant *Vibrio cholerae*. *Trop J Pharm Res* 8(3):63. <https://doi.org/10.4314/tjpr.v8i3.44538>
21. Pankuch GA, Jacobs MR, Appelbaum PC (1996) MIC and time-kill study of antipneumococcal activities of RPR 106972 (a new oral streptogramin), RP 59500 (quinupristin-dalfopristin), pyostacine (RP 7293), penicillin G, cefotaxime, erythromycin, and clarithromycin against 10 penicillin-susceptible and -resistant pneumococci. *Antimicrob Agents Chemother* 40(9):2071–2074
22. Kamruzzaman M, Bari SN, Faruque SM (2013) In vitro and in vivo bactericidal activity of *Vitex negundo* leaf extract against diverse multidrug resistant enteric bacterial pathogens. *Asian Pac J Trop Med* 6(5):352–359
23. Beesoo R, Bhagooli R, Neerghen-Bhujun VS, Li WW, Kagansky A, Bahorun T (2017) Antibacterial and antibiotic potentiating activities of tropical marine sponge extracts. *Comp Biochem Physiol C Toxicol Pharmacol* 196:81–90
24. Cheesman MJ, Alcorn S, Verma V, Cock IE (2021) An assessment of the growth inhibition profiles of *Hamamelis virginiana* L. extracts against *Streptococcus* and *Staphylococcus* spp. *J Tradit Complement Med* 11(5):457–465
25. Joe MM, Jayachitra J, Vijayapriya M (2009) Antimicrobial activity of some common spices against certain human pathogens. *J Med Plants Res* 3(11):1134–1136
26. Patil VS, Harish DR, Charla R, Vetrivel U, Jalalpure SS, Bhandare VV, Deshpande SH, Hegde HV, Roy S (2023) Structural insights into modeling of hepatitis B virus reverse transcriptase and identification of its inhibitors from potential medicinal plants of Western Ghats: an in silico and in vitro study. *J Biomol Struct Dyn* 2:1–9. <https://doi.org/10.1080/07391102.2023.2264400>
27. DasNandy A, Patil VS, Hegde HV, Harish DR, Roy S (2022) Elucidating type 2 diabetes mellitus risk factor by promoting lipid metabolism with gymnemagenin: an in vitro and in silico approach. *Front Pharmacol* 13:1074342
28. Samdani A, Vetrivel U (2018) POAP: a GNU parallel based multithreaded pipeline of open babel and AutoDock suite for boosted high throughput virtual screening. *Comput Biol Chem* 74:39–48
29. Rose Y, Duarte JM, Lowe R, Segura J, Bi C, Bhikadiya C, Chen L, Rose AS, Bittrich S, Burley SK, Westbrook JD (2021) RCSB Protein Data Bank: architectural advances towards integrated searching and efficient access

- to macromolecular structure data from the PDB archive. *J Mol Biol* 433(11):166704
30. Schwede T, Kopp J, Guex N, Peitsch MC (2003) SWISS-MODEL: an automated protein homology-modeling server. *Nucleic Acids Res* 31(13):3381–3385
 31. Jendele L, Krivak R, Skoda P, Novotny M, Hoksza D (2019) PrankWeb: a web server for ligand binding site prediction and visualization. *Nucleic Acids Res* 47(1):63
 32. Bekker H, Berendsen HJ, Dijkstra EJ, Achterop S, Vondrumen RV, Vander-spoel D, Sijbers A, Keegstra H, Renardus MK (1993) Gromacs—a parallel computer for molecular-dynamics simulations. In: 4th International Conference on Computational Physics (PC 92), World Scientific Publishing, pp 252–256
 33. Patil VS, Deshpande SH, Harish DR, Khanal P, Abduljalil JM, Roy S (2024) Computational investigation of remdesivir, favipiravir, ribavirin, and their phosphate derivatives against Nipah virus RNA-dependent RNA polymerase. *Mol Simul* 50(15):1170–1182
 34. Kumari R, Kumar R, Lynn A (2014) g_mmpbsa: a GROMACS tool for high-throughput MM-PBSA calculations. *J Chem Inf Model* 54(7):1951–1962
 35. Charla R, Patil PP, Patil VS, Bhandare VV, Karoshi V, Balaganur V, Joshi RK, Harish DR, Roy S (2023) Anti-Cholera toxin activity of selected polyphenols from *Careya arborea*, *Punica granatum*, and *Psidium guajava*. *Front Cell Infect Microbiol* 13:1106293
 36. Gutiérrez RMP, Mitchell S, Solis RV (2008) *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol* 117(1):1–27
 37. Biswas B, Rogers K, McLaughlin F, Daniels D (2013) Yadav A (2013) Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *Int J Microbiol* 1:746165
 38. Soliman FM, Fathy MM, Salama MM, Saber FR (2016) Comparative study of the volatile oil content and antimicrobial activity of *Psidium guajava* L. and *Psidium cattleianum* Sabine leaves. *Bull Fac Pharm Cairo Univ* 54(2):219–225
 39. Swain SS, Padhy RN (2015) In vitro antibacterial efficacy of plants used by an Indian aboriginal tribe against pathogenic bacteria isolated from clinical samples. *J Taibah Univ Med Sci* 10(4):379–390
 40. Ambardar N, Aeri V (2013) A better understanding of traditional uses of *Careya arborea* Roxb.: phytochemical and pharmacological review. *Cell Med* 3(4):28–1
 41. Navya AS, Anitha S (2019) Review on pharmacognostic and pharmacological activities of *Careya arborea* plant. *J Pharmacogn Phytochem* 8(3):4165–4169
 42. Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health* 109(7):309–318
 43. Tai JSB, Ferrell MJ, Yan J, Waters CM (2023) New insights into *Vibrio cholerae* biofilms from molecular biophysics to microbial ecology. In: *Vibrio spp. infections*. Cham: Springer International Publishing, pp 17–39
 44. Haycocks JR, Warren GZ, Walker LM, Chlebek JL, Dalia TN, Dalia AB, Grainger DC (2019) The quorum sensing transcription factor AphA directly regulates natural competence in *Vibrio cholerae*. *PLoS Genet* 15(10):63
 45. Ramamurthy T, Nandy RK, Mukhopadhyay AK, Dutta S, Mutreja A, Okamoto K, Ghosh A (2020) Virulence regulation and innate host response in the pathogenicity of *Vibrio cholerae*. *Front Cell Infect Microbiol* 10:572096
 46. Rabie AM (2022) Potent inhibitory activities of the adenosine analogue cordycepin on SARS-CoV-2 replication. *ACS Omega* 7(3):2960–2969. <https://doi.org/10.1021/acsomega.1c05998>
 47. Rabie AM, Abdalla M (2022) Forodesine and riboprine exhibit strong anti-SARS-CoV-2 repurposing potential: in silico and in vitro studies. *ACS Bio Med Chem Au* 2(6):565–585. <https://doi.org/10.1021/acsbiochemchem.2c00039>
 48. Mohammed YHI, Shamkh IM, Alharthi NS, Shanawaz MA, Alzahrani HA, Jabbar B, Beigh S, Alghamdi S, Alsakhen N, Khidir EB, Alhuthali HM, Karamalla THE, Rabie AM (2023) Discovery of 1-(5-bromopyrazin-2-yl)-1-[3-(trifluoromethyl) benzyl] urea as a promising anticancer drug via synthesis, characterization, biological screening, and computational studies. *Sci Rep* 13(1):22824. <https://doi.org/10.1038/s41598-023-44662-x>
 49. Nashaan FA, Al-Rawi MS, Alhammer AH, Rabie AM, Tomma JH (2022) Synthesis, characterization, and cytotoxic activity of some imides from galloyl hydrazide. *ECC* 4(10):966–75
 50. Rabie AM (2021) Four three-winged nitrogenous heterocyclic derivatives of citric acid scaffold: the first synthesis and characterization of these newly discovered fan-like compounds. *Russ J Org Chem* 57(3):417–421. <https://doi.org/10.1134/S1070428021030131>
 51. Rabie AM (2022) Design, synthesis, and characterization of novel series of pharmacologically-important sperm-shaped amphiphilic heterocyclic compounds derived from natural palmitic acid. *Natr Resour Human Health* 2(2):287–292
 52. Xu X, Stern AM, Liu Z, Kan B, Zhu J (2010) Virulence regulator AphB enhances toxR transcription in *Vibrio cholerae*. *BMC Microbiol* 10:1–8

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