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A Mechanistic Study Combines *in Vitro* and *in Silico* Methods to Investigate the Antibacterial and Fungal Properties of *Centella Asiatica*

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KEYWORDS

Centella asiatica, Antimicrobial activity, Glucose assay, DNA gyrase B, *Aspergillus niger EstA.*

ABSTRACT:

Introduction: Ayurveda is an Indian traditional medicine based mainly on herbs. One such traditional plant is *Centella asiatica* (*C. asiatica*), which is used extensively in India and throughout Asia to treat a wide range of diseases. The plant has a broad range of therapeutic properties, such as neuroprotective, wound healing, anti-inflammatory, antibacterial, and anticancer actions.

Objectives: The main aim of the study is to understand the mechanism and action of *C. asiatica* biologically active compounds against bacteria and fungi.

Methods: Further antimicrobial properties were confirmed by glucose assays and bacterial membrane lysis. Moreover, we have tested the identified phytocompounds of *C. asiatica* (L) extract against bacterial and fungal targets using molecular docking studies.

Results: Results showed that the antimicrobial properties of the extract exhibited a substantial minimum inhibitory concentration (MIC) of about 90–100 µg/mL against *Staphylococcus and Bacillus sp.* Similarly, plant extract against *Aspergillus niger*, *Fusarium sp.*, and *Penicillium sp. showed a* MIC of about 90–100 µg/mL. Further glucose uptake analysis in the presence of plant extract showed reduced glucose uptake. This might be due to increased bacterial cell death or inhibition of glucose uptake and utilization. But our bacterial cell membrane lysis test reveals increased cell death, which is confirmed by the tryphan blue test. This might be due to increased cell membrane pores caused by phytocompounds present in plant extract.

Conclusions: Seven active bioactive compounds were selected for molecular docking based on LC-MS analysis, and the results showed ligands 2, 4, and 5 have shown strong binding affinity against target proteins such as DNA gyrase B and *Aspergillus niger EstA*. Hence, we conclude that bioactive compounds from *C. asiatica have a* synergistic effect on antimicrobial and antimicrobial activity. Our study provides a novel therapeutic strategy to treat bacterial and fungal infections.

1. Introduction

Medicinal plants are becoming recognized as a vital resource for the prevention and treatment of many diseases [1]. Every plant has a number of significant phytocompounds that can be utilized in the pharma industry and in the production of various medicines [2]. In the past few years, the focus on plant study has expanded all around the world. Plant extracts have antibacterial properties that can be obtained from a range of various components, such as phenolic compounds and flavonoids. *Centella asiatica* (*C. asiatica*) is a highly significant therapeutic plant utilized for various ailments in Asia and has recently become recognized in the West [3]. For thousands of years, *C. asiatica* has been used in Asian indigenous medicine for the treatment of a variety of varicose ulcers and skin diseases, including lupus, leprosy, psoriasis, and eczema [4]. Few studies have shown that antioxidant activity can decrease *in vivo* and *in vitro* oxidative stress in animal models [5]. The most important biologically active phytocompounds present in *C. asiatica* leaf extract are madecassic acid and Asiatic acid, madecassosides, and asiaticoside. All these compounds are triterpene saponosides, and they are reported to have a positive effect on brain aging [6]. The therapeutic effects of *C. asiatica* on skin diseases have been suggested to involve molecular processes such as MAPK, NF-κB, TGF-β/Smad, STAT, and Wnt/β-catenin signaling [7]. Many *in vitro* and *in vivo* studies have demonstrated the effectiveness of *C. asiatica* (L) extracts

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in treating a wide spectrum of diseases and disorders nowadays [7, 8]. *Kumari et al. (2016*) examined the potential of *C. asiatica* extracts as antioxidants and anti-hyperlipidemic agents [9]. Previous studies revealed that *C. asiatica* had the highest phytochemical concentrations and showed strong antioxidant and antihyperlipidemic properties [9, 10]. Studies also confirmed the antimicrobial activity of *C. asiatica* extracts, and they found strong antimicrobial activity against a number of bacterial and fungal species [11, 12].

In spite of its vast medicinal properties, no mechanical study was carried out using *C. asiatica* against bacterial and fungal strains [13]. Hence, this study investigates *the* potential and mechanism of action of *C. asiatica* (L) leaf methanolic extract against bacteria. two-gram positive bacteria *Staphylococcus and Bacillus sp.*, two-gram negative bacteria *Pseudomonas aeruginosa and E. coli*, and antifungal activity using *Aspergillus niger*, *Fusarium sp.*, and *Penicillium sp.* Further, we have studied the mechanism of action using a *g*lucose utilization assay and a membrane lysis test.

Moreover, molecular docking studies were also performed to find out active phytocompound inhibitory activity against bacterial and fungal target proteins DNA gyrase B and *Aspergillus niger EstA*.

2. Methods

2.1. Sample Preparation

C. asiatica leaves were thoroughly washed with tap water and then allowed to dry shade for a week, followed by grinding with an electric mixer grinder. About 3% of the yield was obtained using the Soxhlet method of extraction with 250 mL of methanol [14]. After the evaporation of solvent by using a rotary evaporator, the thick mixture of crude extract was kept at 4°C until further examination.

2.2. Antibacterial and Antifungal Activity

The minimum inhibitory concentrations (MIC) of the *C. asiatica* extract were tested against gram-negative and gram-positive bacterial and fungal strains under *in vitro* conditions [15].

Fungal strains *Aspergillus niger*, *Fusarium sp.*, and *Penicillium sp.*, gram-positive bacteria *Staphylococcus and Bacillus sp.*, and gram-negative bacteria *Pseudomonas aeruginosa and E. coli*, were used in the study [16]. The extract was dissolved in DMSO at concentrations that varied from 10 to 100 µg/mL. For bacterial growth, LB broth was taken, and then plates were prepared using Muller Hamilton Agar and Rose Bengal Agar for anti-bacterial and fungal activity, respectively. After streaking the respective bacteria and fungi on each plate, they were kept in an incubator for 72 h at 27°C for fungi and 24 h at 37°C for bacteria [17]. Every experiment was conducted in triplicate, and the MIC was determined as the lower concentration by macroscopic examination against a black background. Gram-positive bacteria were treated with penicillin sodium, gram-negative bacteria with streptomycin sulfate, and fungi with ketoconazole as positive control samples; DMSO was used as the blank control.

2.3. Glucose Assay

A working stock solution of glucose (1 mM) was prepared, and glucose working standards were prepared. 10 µL of glucose standards and the diluted bacteria samples (E. *coli*, *Pseudomonas sp., Bacillus sp., and S. aureus*) were added to each test tube [18]. 10µL of deionized water in a test tube was taken as a blank. 50µL of glucose assay solution was added to the standards and sample, mixed well, and incubated in the tubes at 37^0 C for 15 to 30 minutes in the dark [19]. The reaction was stopped by adding 50 µL of 3% acetic acid; the absorbance was measured at 492nm. A glucose standard graph was plotted to determine the glucose concentration in unknown samples.

2.4. Bacterial membrane lysis test

To check the bacterial membrane integrity, three test tubes were taken. In the first test tube, bacterial culture alone; in the second test tube, bacterial culture + plant extracts; in the third test tube, bacterial culture + glucose + plant extract [20]. Luria Bertani media is used as bacterial broth preparation, and the four bacterial strains used are *E. coli*, *Pseudomonas sp.*, *Bacillus sp.*, and *S. aureus.* All the test tubes were incubated overnight at 37 °C in an incubator. After incubation, from each test tube, 50 µl of sample and an equal amount of trypan blue (50 µl) were added and incubated for 5 minutes at RT. The suspension was taken with a hemocytometer and counted for the number of live and dead cells [21].

3. In silico method

3.1. Target preparation

The functional bacterial *Staphylococcus* DNA gyrase B and fungal *Aspergillus niger EstA* proteins were taken to study the anti-bacterial and anti-fungal activity *of C. asiatica* biologically active compounds at the molecular level [22]. The 3D (three-dimensional) structures of these proteins were retrieved using X-ray crystallography, which was acquired from the RCSB

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protein database: DNA gyrase B (PDB ID 4URM, resolution factor: 2.94 Å.) and *Aspergillus niger EstA* (PDB ID: IUKC, resolution factor: 1.60 Å). It serves as a useful resource to predict the pharmacophore analysis of structure-based proteins [23]. Using the CASTp calculation analysis, the computational model of active site amino acids was predicted. A graphical database called PDB Sum gives a quick overview of the information contained in each 3D structure that has been deposited in the Protein Data Bank and analyzes the active amino acids in each protein. A pro-check was done for residue-by-residue listing, standard deviations, secondary structure, and the Ramachandran plot [24].

3.2. Ligand and Pharmacophore analysis:

The identified compounds such as Androst-11-en-17-one, 3-formyloxy-, (3alpha, 5alpha), Methandriol dipropionate, 3beta-Hydroxy-21-oxoolean-12-en-28-oic acid methyl ester, 3beta-Hydroxy-21-oxoolean-12-en-28-oic acid methyl ester, Lanosterol, trimethylsilyl ether, 3beta-Hydroxy-21-oxoolean-12-en-28-oic acid methyl ester, Estra-1,3,5(10)-trien-17-one, 3,12-bis[(trimethylsilyl)oxy]-, (12beta),2-Hexenoic acid, 6-cyclohexyl and Cholesta-5,17(20)-dien-3-ol, acetate, (3beta,17E) were taken for in silico studies based on our LC-MS analysis. Ligand molecules were retrieved from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) [25]. By using the professional molinspiration (http://www.molinspiration.com/cgi-bin/properties) and Hyperchem 7.5 the drug-likeness properties along with the pharmacophore and biological activity against different enzymes has been calculated [26].

4. Results and Discussion

In this study, we determined *in vitro* antibacterial activity using two gram-positive bacteria (*Staphylococcus and Bacillus sp.) and* two gram-negative bacteria (*Pseudomonas aeruginosa and E. coli)* by the cup-plate agar diffusion method [27]. All the bacteria we isolated in Luis Hamilton agar media from the master plate and 400 µl of each bacteria were cultured in the agar plate [28]. **Table 1** displays the inhibition zone diameters for the tested gram-positive bacteria with positive controls penicillin sodium, and gram-negative bacteria with streptomycin sulfate DMSO served as controls. We noticed significant zone clearance started at a concentration of 90 µg/mL, and it increased with the concentration of extract **(Figure 1)**. Further, *in vitro* antifungal activity against *Aspergillus niger*, *Fusarium sp., and Penicillium sp*. by the cup-plate agar diffusion method was used [29]. **Table. 2** displays the inhibition zone diameters for the tested fungi in the presence of plant extract. Ketoconazole was used as a positive control, and DMSO served as a negative control. Our results showed that significant zone clearance started at an 80 µg/mL concentration and increased with the concentration of the extract **(Figure 2)**. However, it is more effective against *Aspergillus* strains than *Fusarium* and *Penicillium* fungal strains. Secondary metabolites are the active ingredients in numerous drugs that are identified in plants [30]. The primary triterpene derivatives of *C. asiatica*, such as asiaticoside, madecassic acid, asiatic acid, madecassoside, and brahmic acid, have been widely linked to the plant's biological effects [31, 32]. *C. asiatica* is an exciting source of triterpenoids, volatile oils, and phenolic compounds, particularly flavonoids. Based on our results, we conclude that triterpenoids and other phenolic compounds might be responsible for these antibacterial and antifungal activities [33].

To find out the underlying molecular mechanism behind *C. asiatica* anti-bacterial activity, we have done a glucose uptake assay. For this assay, four different bacteria were taken to compare glucose uptake in different conditions, as shown in **Table 3**. The first tube served as a negative control to get reference glucose, as the tube was not treated with any type of bacterial strain [34]. The second tube served as a positive control, as the tube contained bacteria but not plant extract. To determine the glucose uptake in the third tube, bacteria were grown with plant extract and a substantial amount of glucose [35]. After culturing with plant extract, the glucose uptake decreased due to cell death, and the data is presented in **Table 4**. Glucose is the primary source for the survival of bacteria. When the availability of glucose is diminished or uptake is inhibited under any conditions, the bacteria die. Our results clearly indicate that the phytocompounds inhibited glucose uptake by bacteria and led to cell death. Bacterial cell wall or membrane integrity is crucial for bacteria's survival under any circumstances. Gram-positive and Gram-negative bacteria are protected by an external cell wall composed of varying layers of peptidoglycan [36]. Damage to the bacterial cell wall compromises its integrity and creates an imbalance of electrolytes that triggers cell death [37]. The cell wall of most bacteria is made of a polymer called peptidoglycan, which is made of amino sugars, namely N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) [38].

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Table: 1. Antibacterial activity of *C. asiatica* against gram positive and gram negative bacteria.

Code	Concentration of extract in µg/ml	**Zone of inhibition (mm)**			
		Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacillus sp.*	*E.coli*
1	10	6.11 ± 0.028	6.13 ± 0.033	6.17 ± 0.039	6.15 ± 0.032
2	20	6.05 ± 0.035	6.17 ± 0.039	6.13 ± 0.032	6.13 ± 0.036
3	30	6.15 ± 0.026	6.12 ± 0.031	6.13 ± 0.033	6.19 ± 0.038
4	40	6.21 ± 0.032	6.14 ± 0.037	6.11 ± 0.031	6.23 ± 0.033
5	50	6.46 ± 0.022	6.19 ± 0.042	6.14 ± 0.033	6.44 ± 0.032
6	60	6.53 ± 0.039	6.22 ± 0.033	6.17 ± 0.032	6.29 ± 0.037
7	70	6.74 ±0.022	7.51 ± 0.76	6.14 ± 0.033	7.54 ± 0.78
8	80	6.91 ± 0.044	8.83 ± 0.95	6.17 ± 0.031	8.71 ± 0.93
9	90	11.6 ± 1.53	9.22 ± 1.34	6.29 ± 0.041	10.11 ± 1.12
10	100	13.7 ± 1.87	11.4 ± 1.67	7.13 ± 0.84	11.72 ± 1.73
11	Positive Control	16.36 ± 2.34	15.5 ± 2.13	18.7 ± 3.12	15.23 ± 2.03
12	Negative Control	6.12 ± 0.035	6.23 ± 0.032	6.16 ± 0.031	6.21 ± 0.31

Values are mean ± SD of three independent experiments. Pencillin and Streptomycin were used as positive controls at 20 µg/mL, DMSO is used as negative control.

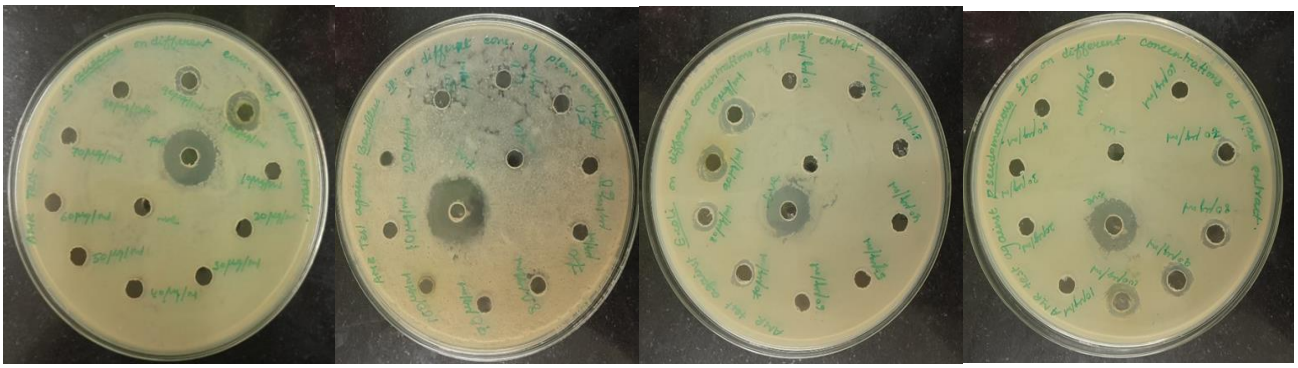

Figure 1. Anti-bacterial activity of *C.asiatica* extract. Experiment performed in triplicate as described in the methodology.

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Table: 2. Antifungal activity of *C. asiatica* against gram positive and gram negative bacteria.

Code	Concentration of extract (µg/ml)	Zone of inhibition (mm)		
		Aspergillus niger	*Fusarium sp.*	*Penicillium sp.*
1	10	6.11 ± 0.036	6. 21± 0.031	6.17 ± 0.033
2	20	6.16 ± 0.032	6.14 ± 0.037	6.13 ± 0.031
3	30	6.12 ± 0.031	6.16 ± 0.032	6.16 ± 0.025
4	40	6.14 ± 0.038	6.18 ± 0.035	6.21 ± 0.033
5	50	6.16 ± 0.033	6.19 ± 0.032	6.16 ± 0.027
6	60	6.15 ± 0.031	6.21 ± 0.036	6.23 ± 0.032
7	70	6.18 ± 0.032	6.23 ± 0.039	6.19 ± 0.029
8	80	7.34 ± 0.039	6.28 ± 0.034	6.17 ± 0.022
9	90	8.82 ± 1.24	6.31 ± 0.032	6.51 ± 0.034
10	100	9.36 ± 1.76	6.29 ± 0.032	7.34 ± 0.95
11	Positive Control	14.42 ± 2.54	16.21± 3.45	9.72 ± 1.32
12	Negative Control	6.11 ± 0.032	6±0.032	6.12 ± 0.032

Values are mean ± SD of three independent experiments. Ketoconazole was used as positive controls at 20 µg/mL. DMSO is used as negative control.

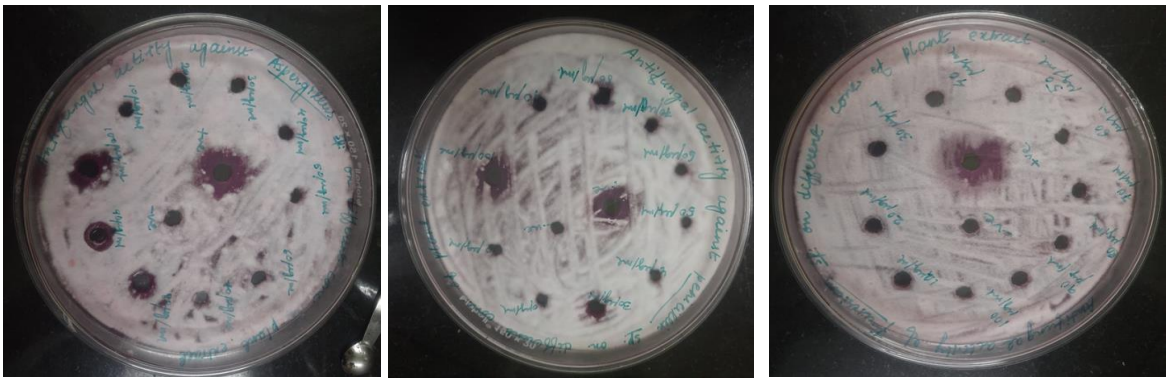

Figure .2. Anti-fungal activity of *C.asiatica* extract. Experiment performed in triplicate as described in the methodology.

Table. 3. Effect of *C.asiatica* on glucose uptake in different bacterial strains.

Bacterial strains	Glucose + No bacteria (Glucose concentration (µM)	Glucose + bacteria (Glucose concentration (µM)	Glucose + bacteria + plant extract (Glucose concentration (µM)
Staphylococcus aureus	120 ± 0.82	70 ± 4.82	108 ± 7.33
Pseudomonas aeruginosa	120 ± 0.55	84 ± 6.16	111 ± 9.58
Bacillus sp.	120 ± 0.33	78 ± 4.21	103 ± 4.71
E.coli	120 ± 0.45	61 ± 5.44	97 ± 8.49

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Values are mean ± SD of three independent experiments.

Table. 4. Effect of *C.asiatica* on bacterial cell wall lysis.

Sl.No	Name of bacteria	Experimental groups	No of dead cells	No of live cells	% of live cells	% of dead cells
1	*E. coli*	Control	12 ± 1.2	82 ± 6.2	87.2 ± 5.7	12.7 ± 1.6
		Glucose + Extract	66 ± 3.4	32 ± 2.6	32.2± 4.1	67± 4.9
		Extract	69 ± 3.5	29 ± 2.2	29.1 ± 1.8	70.5 ± 6.1
2	*Pseudomonas sp.*	Control	21 ± 1.7	87 ± 5.7	80.2 ± 6.2	19.9 ± 4.2
		Glucose + Extract	72 ± 4.4	33 ± 2.2	31.3 ± 2.5	68.5 ± 3.4
		Extract	75 ± 4.2	25 ± 1.1	25.2 ± 1.5	75 ± 3.9
3	*Bacillus sp.*	Control	24 ± 1.6	89 ± 5.5	78.1 ± 4.2	21.5 ± 1.7
		Glucose + Extract	75 ± 5.2	12 ± 0.96	13.2 ± 1.1	86.1 ± 6.1
		Extract	79 ± 8.1	10 ± 0.6	11.2 ± 1.3	88.4 ± 7.1
4	*S. aureus.*	Control	10 ± 0.7	84 ± 6.3	89 ± 7.2	10.6 ± 0.8
		Glucose + Extract	62 ± 4.1	36 ± 2.2	36.7 ± 2.6	63.2 ± 4.4
		Extract	68 ± 4.2	34 ± 2.2	33.3 ± 2.2	66.6 ± 5.2

Values are mean ± SD of three independent experiments.

Table. 5. The molecular docking binding affinity of DNA gyrase B against phytochemicals

Compound name	Docking score (kcal/mol	Interacting residue	Type of bond	Bond distance (A^o)
Ligand_1	-11.0	Ile 427 Ile 432 Ala 517 Pro519	PI-Sigma Pi-Alkyl Pi-Alkyl Pi-Alkyl	3.99 4.68 5.25 4.53
Ligand_2	-9.8	Ala517 Glu520 Ser521 Thr510 Pro519	H-bond H-bond H-bond Pi-Alkyl Pi-Sigma	2.20 3.35 2.66 3.25 4.63
Ligand_3	-9.7	Gln509 Ile432 Ala517 Ile427 Pro519	H-bond Pi-Alkyl Pi-Alkyl Pi-Alkyl Pi-Alkyl	3.54 4.11 4.95 4.11 4.55

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Ligand_4	-9.3	Asp397 Arg423 Thr401 Arg505 Ser522 Ala517	H-bond Pi-Alkyl Pi-Alkyl Pi-Sigma Pi-Alkyl Pi-Alkyl	2.15 3.31 3.65 3.59 3.09 3.59
Ligand_5	-8.2	Thr510 Glu520 Ile432 Ala517 Ile427	H-bond H-bond Pi-Alkyl Pi-Alkyl Pi-Alkyl	3.52 2.04 4.18 4.16 4.92
Ligand_6	-8.3	Lue301 Ile427 Pro519 Ile32 Ala517	Pi-Sigma Pi-Sigma Pi-Alkyl Pi-Alkyl Pi-Alkyl	5.43 4.69 4.66 4.69 5.37
Ligand_7	-7.3	Ala517 Ala427	Pi-Alkyl Pi-Alkyl	3.90 4.39
Delafloxacin	-10.7	Ala517 Gln509 Ile427 Ile432 Pro519	H-bond H-bond Pi-Sigma Pi-Alkyl Pi-Alkyl	3.55 3.41 4.18 4.57 4.60

Table. 6. The molecular docking binding affinity of *Aspergillus niger EstA* against phytochemicals.

Compound name	Docking score (kcal/mol	Interacting residue	Type of bond	Bond distance (A°)
Ligand_1	-7.7	Glu91 Asn54 Ala 517 Ile120	H-bond H-bond Pi-Alkyl Pi-Alkyl	2.02 3.39 2.24 3.29
Ligand_2	-8.1	Ser521 Glu520 Asn511 Ile 432 Ile 472	H-bond H-bond H-bond Pi-Alkyl Pi-Alkyl	2.41 2.01 1.99 2.45 3.25
Ligand_3	-8.2	Gln509 Ile427 Pro519 Ile432	H-bond H-bond Pi-Alkyl Pi-Alkyl	3.54 4.11 4.55 4.11
Ligand_4	-9.2	Arg505 Ser522 Ala517 Thr401	H-bond H-bond Pi-Alkyl Pi-Sigma	2.15 3.09 3.59 3.31

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Ligand_5	-8.2	Thr510 Glu520 Ile432 Ala517	H-bond H-bond Pi-Alkyl Pi-Alkyl	3.52 2.04 4.18 4.16
Ligand_6	-8.9	Pro519 Ile32 Ala517 Lue301 Ile427	Pi-Sigma Pi-Sigma Pi-Alkyl Pi-Alkyl Pi-Alkyl	4.66 4.69 5.37 5.43 4.69
Bifonazole	-8.3	Arg517 Asn425 Ile427 Ile432	H-bond H-bond Pi-Sigma Pi-Sigma	2.54 3.12 3.30 4.27

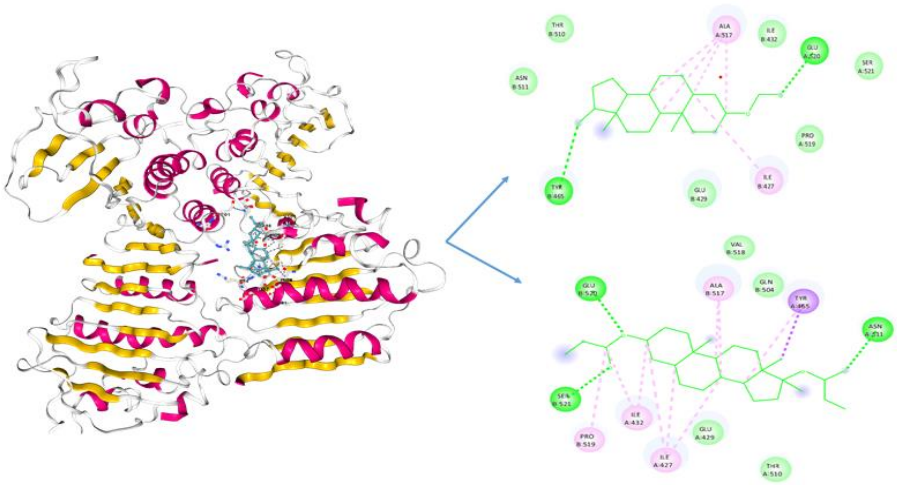

Figure .3. The Binding affinity of DNA gyrase B against *C. asiatica* phytochemicals

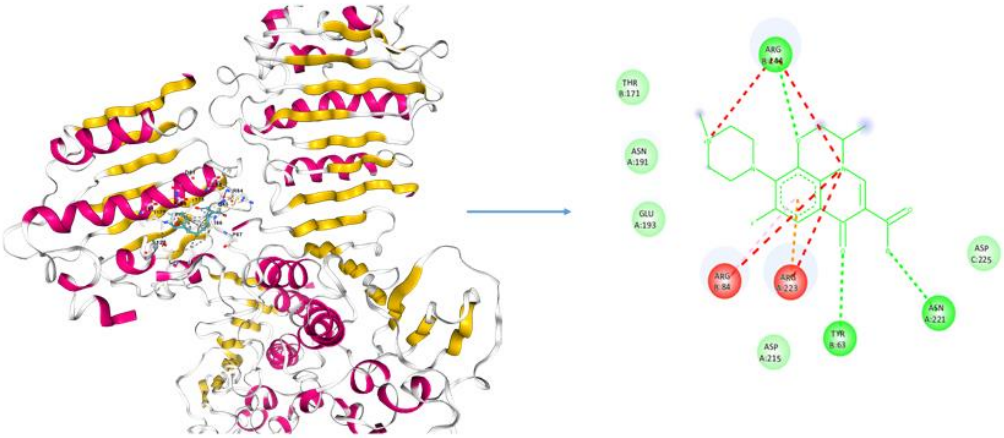

Figure .4. The Binding affinity of DNA gyrase B against reference drug Delafloxacin

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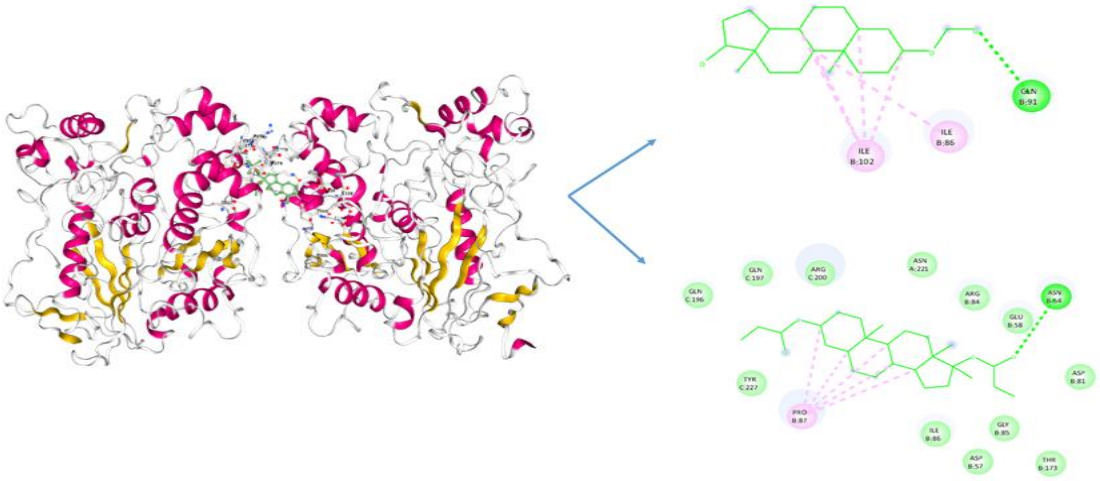

Figure.5. The Binding affinity of *Aspergillus niger EstA* against *C. asiatica* phytochemicals

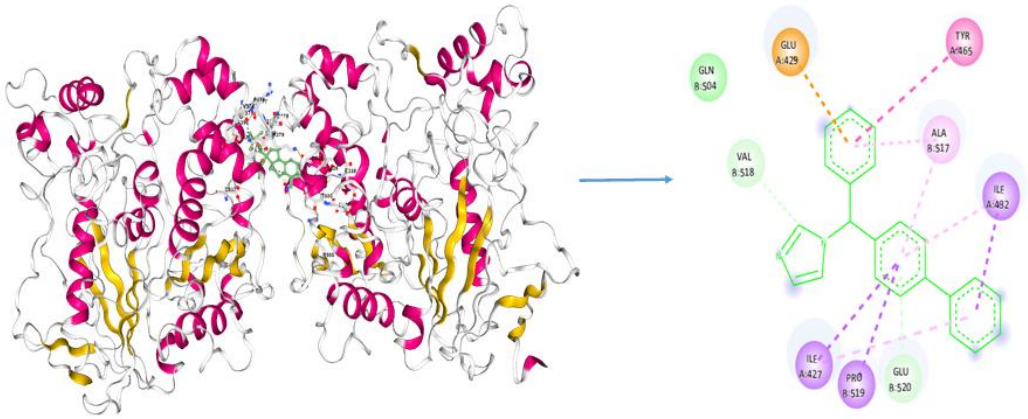

Figure.6. The Binding affinity of *Aspergillus niger EstA* against reference drug Bifonazole

Dead cells take up the tryphan blue stain, which was observed under a microscope. In the present study, we have tested the ability of *C. asiatica* biologically active phytocompounds on bacterial cell wall lysis and the results presented in **Table 4.** Our results clearly indicate that the percentage of dead cells increased when the bacterial cells were incubated with plant extract. The phytocompounds present in plant extract may have any of the following mechanisms to kill the bacteria: i. the compounds kill bacteria through binding of the beta-lactam ring to DD-transpeptidase, inhibiting its cross-linking activity and preventing new cell wall formation; ii. cleaving the β-(1,4) linkages between the NAM and NAG saccharides.

Docking studies reveal the molecular interactions. In the present study, a docking study was performed between identified active compounds from *C. asiatica* and target proteins DNA gyrase B and *Aspergillus niger EstA* using the autodock vina 4.2 to find out the binding affinity of the protein-ligand interaction to check the antimicrobial activity [39]. Grid space was defined in the Auto grid by selecting important residues with grid box sizes of x = 40, y = 40, and z = 40 and grid spacing of 0.642, which provides search space. The grid center was chosen with dimensions of x = 14.709, y = 51.140, and z = 10.562. It utilized exhaustiveness score 10 and used it to compute grid parameters that help comprehend the grid energy with stabilized energy distribution, as mentioned in **Tables 5 & 6**.

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Molecular docking studies have been conducted to find out the antimicrobial activity of *C. asiatica*-extracted phytochemicals identified from LC-MS analysis against selected target proteins such as DNA gyrase B and *Aspergillus niger EstA*. The target protein DNA gyrase B interacts with phytocompounds from *C. asiatica* extract and shows a binding affinity of -11.0 kcal/mol with 2 to 3 hydrogen bonds. The conformation of amino acids is Tyr 465, Glu520, Asn511, and Ser521, with binding distances of 2.20 A^0, 3.35 A^0, 2.66 A^0 & 3.52 A^0. The interactions were compared with reference drugs such as Delafloxacin with target and showed binding affinity of -10.7 kcal/mol with 2 hydrogen bonds. The conformation of amino acids is Arg517 & Asn425 with binding distances of 2.54 A^0 & 3.12 A^0 **(Figure 3 &4.)**. *Aspergillus niger EstA* protein interacts with phytochemicals and showed binding affinity -9.2 kcal/mol with 2 hydrogen bonds. The conformation of amino acids is Gln91 & Asn54 with binding distances of 2.02 A^0 & 3.39 A^0. The interactions were compared with reference drugs such as Bifonazole with target and showed binding affinity -8.3 kcal/mol with 2 hydrogen bonds. The conformations of amino acids are Arg517 & Asn425 with binding distances of 2.54 A^0 & 3.12 A^0 **(Figure 5 and 6)**.

5. Conclusion

In this study, we found that bioactive compounds from *C. asiatica* exhibited promising effectiveness against bacterial and fungal microbes. The glucose assay demonstrated decreased glucose uptake due to cell death. Furthermore, membrane lysis assays indicated enhanced cell death in extract-treated bacterial cells. Our molecular docking studies also indicated that Ligands 2, 4, and 5 have the highest inhibitory activity against DNA gyrase B and *Aspergillus niger EstA*. As a result, we conclude that bioactive chemicals derived from *C.asiatica* have synergistic effects and can be employed therapeutically to treat bacterial and fungal infections.

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Authors' contributions

SBR performed experiments. VDR and RN designed the work. SBR KG VDR and RN wrote the paper. All authors read and approved the final manuscript.

Conflict of interests

None

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