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In vitro evaluation of antibacterial and molecular docking studies of *Gardenia gummifera* fruit methanol extract

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Abstract
Gardenia gummifera Linn (Family: Rubiaceae) is used in traditional system of medication to treat numerous ailments such as flatulence for cleaning foul ulcers and wounds, anticonvulsants. To validate the traditional therapeutic claim this study has been undertaken to assess the antibacterial activity of the *Gardenia gummifera* fruit methanol extract (GFME). Antibacterial screening of the GFME was carried out against human pathogenic clinical isolates i.e. *Staphylococcus aureus, Streptococcus pneumoniae, Vibrio cholera, Pseudomonas aeruginosa, Salmonella typhi,* and *Klebsiella pneumonia* and further Molecular docking study of GCMS identified compounds was done by docking with bacterial enzyme DNA gyrase. The antibacterial screening of GFME against clinical pathogens showed significant bactericidal activity against the strains *Staphylococcus aureus* (16.67±0.33), *Streptococcus pneumonia* (12.00±0.58), *Vibrio cholera* (12.67±0.33), *Pseudomonas aeruginosa* (19.33±0.67), and *Salmonella typhi* (11.67±0.33) as compared to the standard drug ciprofloxacin. The molecular docking of quinic acid against the bacterial enzyme DNA gyrase exhibited a good binding affinity of -4.5 kcal/mol, 7 hydrogen bonds, and hydrophobic interaction with 7 amino acid residues so that quinic acid processes good inhibitor as compared to other 4 compounds. Thus the present study strengthened the traditional medicinal claim of the plant *Gardenia gummifera* Linn possessing the antibacterial drug.

Keywords: antibacterial, molecular docking, *Gardenia gummifera*, methanol, Linn, Family: Rubiaceae

Introduction
Infectious diseases represent a major problem to human health and represent one of the main causes of morbidity and mortality worldwide (World Health Organization, 2004) [1]. Bacterial infections are widespread and cause much discomfort and sickness. These bacterial pathogens continue to be a threat to human health and welfare as a result of new or resistant pathogens [2]. Antibiotics are one of our most important weapons in fighting microbial infections and have greatly benefited the health-related quality of human life since their introduction [3]. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions, but also due to the emergence of drug-resistant bacteria [4]. The development of new synthetic antimicrobial drugs was slow down due to the increase in the prevalence of multiple drug resistance. With the increasing incidence of chemotherapeutic failure and antibiotic resistance by several microbial agents, antimicrobial evaluation of medicinal plants has become the need of the hour. Plant-derived biomolecules have the added advantage of being less toxic in comparison to synthetic agents. The traditional medicinal practice is an important part of the primary health care system in most of the developed as well as developing countries [5]. Nearly, there are 1.5 million traditional medicine practitioners who are using medicinal plants in preventive, promotional, and curative applications. According to a report stated by the World Health Organization (WHO) that approximately 3.5 billion people in developing countries depend on the plants for their primary health care. Therapeutic plant lives have been used by many local healers and some urban people belonging to the interior parts of India still depends on the herbs for their medicinal practice [6].

The potential activity of medicinal plants against microbes depends on the nature of the plant drug either in crude form or as an isolated compound. It offers hope and fulfills its role in curing microbes related diseases. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The WHO estimates that the plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [7].

To expand the scope of antibacterial agents from natural resources, *Gardenia gummifera* Linn is a deciduous tree that belongs to the family Rubiaceae. It is commonly identified as a gummy gardenia. It is dispersed in the forests of south Indian region. This plant is well known for its application in traditional medicine. This plant is claimed to have numerous medicinal properties and treatment for many diseasese [8, 9, 10]. The preliminary phytochemical investigation of *Gardenia gummifera* Linn has shown that it is very rich in phytoconstituents. Based on the above information, the present study, the present study was designed to systematically screen the prophylactic effect of the fruit methanol extract, along with the antibacterial activity. The molecular docking of compounds extracted from fruit methanol extract against the target proteins was also performed to authenticate the therapeutic effects of *Gardenia gummifera* Linn.

Material and Methods
Plant material
The fresh fruit of *Gardenia gummifera* has been collected from the area of Sakarayapatna, Chikmagaluru District,

Karnataka, India. The plant has been identified and authenticated by inhouse taxonomist Dr. V. Krishna, Professor, Post Graduate Studies and Research in Biotechnology, Kuvempu University. Thereafter, the plant is maintained at Kuvempu University herbaria (KUBPHS80).

Preparation of extract

Plant material was washed with distilled water and dried at room temperature to constant weights. The dried fruit materials were crushed into powder form and subjected to successive solvent extraction from non-polar to polar solvents like petroleum ether, chloroform, and methanol respectively. The extraction has been carried out using soxhlet apparatus.

Bacterial cultures (collection and maintenance)

Pure cultures of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* used in this investigation were obtained from the Shimoga Institute of Medical Sciences (SIMS), Shimoga, Karnataka, India. From these strains, loop full bacteria were inoculated into the nutrient broth and incubated overnight at 37°C.

***In vitro* antibacterial activity**

This method was adopted to determine the antibacterial activity of plant extracts according to the standard protocol. 20 ml of Muller's Hilton agar media was poured into Petri plates and were allowed to solidify, followed by the addition of 100µl of 24 h fresh culture (105 cells/ml) of each test organism and were spread over the sterile agar plates. Subsequently, wells were created in plates using sterile cork borer (6 mm diameter). In each plate, four wells have been created, among which three wells were induced with 500µg, 1000µg, and 1500 µg/ml of dissolved extract in 10% DMSO, and the remaining one well with positive control ciprofloxacin. These plates were incubated in the upright position at 37ºC for 24 h. The incubated plates were observed for the formation of a clear zone of inhibition around the well which indicates the induction of antibacterial activity [11].

Minimal inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) of the GFME was evaluated by modified resazurin microtitre plate assay. 50 µl of nutrient broth was added to all the wells. 50 µl of a test sample containing 400 µg of extract sample dissolved in 10% dimethyl sulfoxide was added to the first six wells of the first row of titrator plate and then continued with the serial dilution down the column to get concentrations200,100,50,25,12.5,6.25,3.125µg/50µl.Then, wells of first row were inoculated by 10µl (5×106 cfu/ml) of different microbial strains followed by the addition of 10µl (0.015 %) resazurin dye to all the wells as an indicator. Seventh well of first row was used as reference positive control (load ciprofloxacin instead of extract), eighth well as negative control (without extract) and another one column as normal control (only media and dye without strains. Thereafter, 30µl of iso-sensitized broth to all the wells and covered with clean wrap to avoid the dehydration of bacteria, individual concentration was maintained in triplicates. Plates were incubated at 37˚c for 24 h, and color change was observed. The growth was indicated by color changes from blue to pink and MIC was confirmed by visual observation where no change of color was observed at a minimum concentration of plant extract [12].

Molecular docking studies

For molecular docking studies, the compounds were selected based on the previously published reports [13]. The chemical structure of the compounds namely, -2-Furancarboxaldehyde, 5-(hydroxymethyl), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 1,3,5-Triazine-2,4,6-triamine, Hydrazinecarboxamide, 2-(2 methylcyclohexylidene) and Quinic acid and the standard drug ciprofloxacin was drawn using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper two-dimension (2D) orientation, and the structure of each was checked for structural drawing error. The energy of each molecule was minimized using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina to carry out the docking simulation. The protein data bank match file with the name "2XCT.pdb" was used as a receptor molecule, and all the water molecules were removed from the receptor. The graphical user interface program MGL tool was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The grid box volume was set to 8, 14, and 14 Å for x, y, and z dimensions, respectively, and the grid center was set to 3.194, 43.143, and 69.977 for x, y, and z center, respectively, which covered all the ten amino acid residues in the considered active pocket. The docking algorithm provided with AutoDock Vina was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of ten conformers were considered for each ligand. Molecular docking was performed in Corei5 Intel processor CPU with 6 GB DDR3 RAM. AutoDock Vina16 was compiled and run in a Windows 8.0 professional operating system. LigPlot+17 and PyMol educational versions were used to deduce the 2D and 3D pictorial representation of the interaction between the ligands and the receptor. The ligands are represented in green color, H-bonds with their respective distances are represented with cyan color, and the interacting residues are represented in ball and stick model representation [14].

Results

Antibacterial activity

The antibacterial capacity of GFME was screened according to their zone of inhibition against selected clinical pathogens and the results (zone of inhibition) were compared with the standard ciprofloxacin. The results showed that the extract acted against all the microorganisms studied in the present investigation. 1500 µg/ml concentration shows significant antibacterial property noticed against clinical pathogen strains of *Staphylococcus aureus* (16.67±0.33), *Streptococcus pneumonia* (12.00±0.58), *Vibrio cholera* (12.67±0.33), *Pseudomonas aeruginosa* (19.33±0.67) and *Salmonella typhi* (11.67±0.33) as compared to the standard drug ciprofloxacin. The MIC assay was performed by modified resazurin assay the extract shows the highest inhibitory activity against *P.aeruginosa* with a significant MIC value of 20.83 µg. The inhibition of bacterial strains is summarized in Table 1.

Table 1: Antibacterial Activity by Agar Well Plate Method

SL. no	Inhibition zone diameter (mm) and MIC			
	Microorganism	ZI of GFME (1500µg/well)	Activity index	MIC (µg)
1	*S.aureus*	16.67±0.33	0.658	33.33
2	*S.pneumoniae*	12.00±0.58	0.580	133.33
3	*V.cholerae*	12.67±0.33	0.624	133.33
4	*P.aeruginosa*	19.33±0.67	0.620	20.83
5	*S.typhi*	11.67±0.33	0.479	133.33

Values are mean±standard error (n=3) of three different samples, analyzed individually in triplicate, AI (Activity Index) = ZI of Test/ZI of Standard. Readings are presented in Mean ± S.D. ZI of ciprofloxacin for SA, SP, VC, PA and ST is 25.33±0.67, 20.67±0.33, 20.30±0.00, 26.33±0.33 and 24.33±0.33 respectively.

In silico studies

In association with *in vitro* antimicrobial activity, it is useful to carry out *in silico* studies to predict the orientation and binding affinity at the active site of the receptor. The molecular docking of previously reported GCMS identified ligand molecules -2-Furancarboxaldehyde, 5-(hydroxymethyl), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 1,3,5-Triazine-2,4,6-triamine, Hydrazinecarboxamide, 2-(2-methylcyclohexylidene) and Quinic acid with bacterial enzyme DNA gyrase is shown in Figure 1.

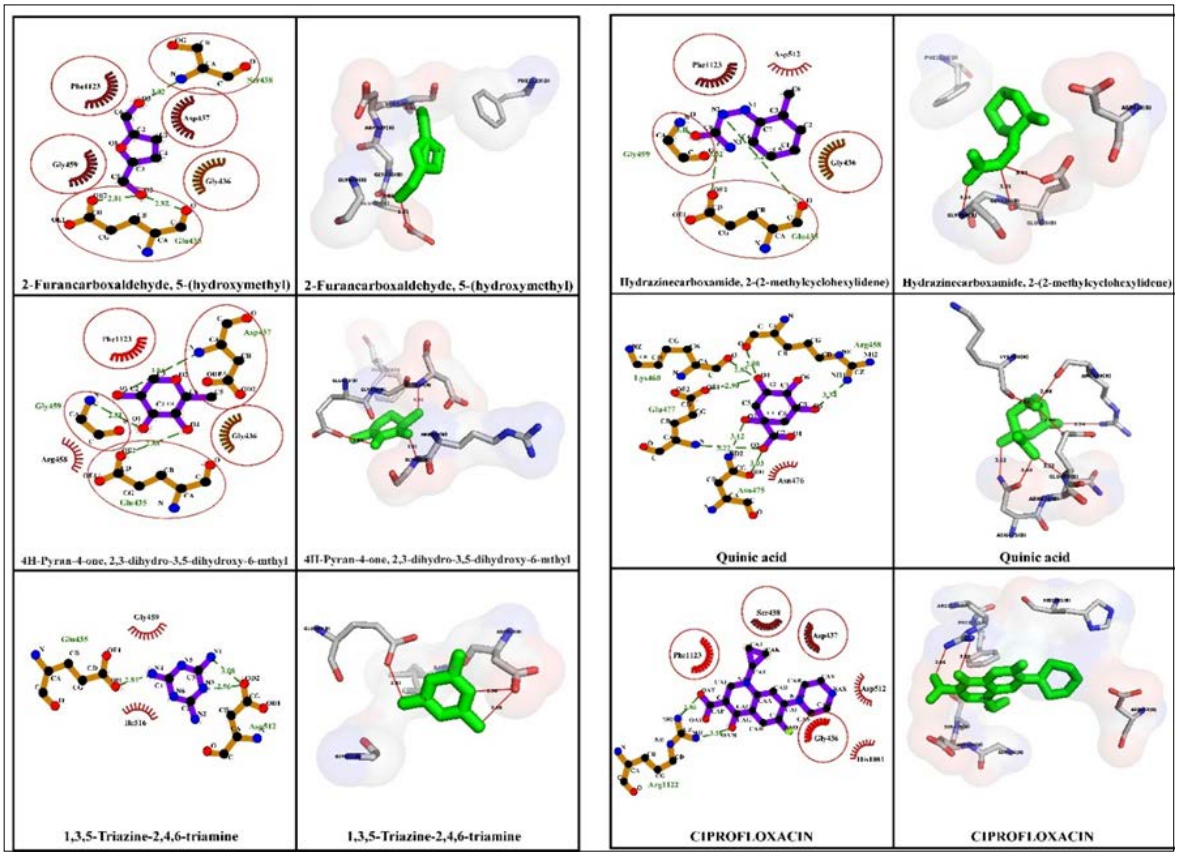

Fig 1: 2D and 3D protein-ligand interaction DNA gyrase with the ligands 2-Furancarboxaldehyde,5-(hydroxymethyl), 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-mthyl, 1,3,5-Triazine-2,4,6-triamine, Hydrazinecarboxamide, 2-(2-methylcyclohexylidene) Quinic acid and ciprofloxacin

The compound quinic acid showed higher docking efficiency with DNA gyrase. It forms 7 hydrogen bonds with amino acids Lys460, Glu477, Arg458, Asn475, Arg475, Glu477, and Arg458 in the active site of the target protein with bond lengths of 2.82, 2.90, 2.98, 3.03, 3.12, 3.22, and 3.34 Å respectively with a less binding affinity of -4.5 and hence considered as the best dock conformation (Table 2). The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl forms three hydrogen bonding with Gly459, Glu435 and Asp437 with bond length 2.81, 2.88 and 3.01 Å respectively and followed by both 2-Furancarboxaldehyde, 5-(hydroxymethyl) and 1,3,5-Triazine-2,4,6-triamine forms three hydrogen bonds with -3.7 binding affinity and Hydrazinecarboxamide, 2-(2-methylcyclohexylidene) forms two hydrogen bonds with a less binding affinity of -4.4 as depicted in table 2. However, all these docked molecules exhibited good hydrophobic interaction.

Table 2: Molecular docking values of GFME compounds obtained from GCMS analysis

Ligand	Affinity (kcal/mol)	H-Bonds	H-Bond Length (Å)	H-Bond With	Hydrophobic Interactions
2-Furancarboxaldehyde,5-(hydroxymethyl)	-3.7	3	2.81	2XCT:Glu485::1:O2	Gly436, Asp437, Gly459, Phe1123
			2.82	2XCT:Glu485::1:O2	
			3.02	2XCT:Ser438::1:O3	
4H-Pyran-4-one, 2,3-dihydro-3,5-	-3.9	3	2.81	2XCT:Gly459::2:O1	Gly436, Arg458, Phe1123

dihydroxy-6-mthyl			2.88	2XCT:Glu435::2:O4	
			3.01	2XCT:Asp437::2:O3	
1,3,5-Triazine-2,4,6-triamine	-3.7	3	2.81	2XCT:Glu435::3:N4	Gly436, Asp512, Phe1123
			2.96	2XCT:Asp512::3:N3	
			3.08	2XCT:Asp512::3:N1	
Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)	-4.4	2	3.02	2XCT:Glu435::4:N2	Glu435, Gly436, Arg458, Gly1082, Arg1122, Phe1123
			3.21	2XCT:Glu435::4:N2	
Quinic acid	-4.5	7	2.82	2XCT:Lys460::5:O4	Asn476
			2.90	2XCT:Glu477::5:O4	
			2.98	2XCT:Arg458::5:O4	
			3.03	2XCT:Asn475::5:O2	
			3.12	2XCT:Arg475::5:O3	
			3.22	2XCT:Glu477::5:O2	
			3.34	2XCT:Arg458::5:O5	
Ciprofloxacin	-6.0	2	2.86	2XCT:Arg1122::CIP:OAT	Gly436,Asp437, Ser438,Asp512, His1081, Phe1123
			3.30	2XCT:Arg1122::CIP:OAM	

Discussion

Medicinal plants are a vital source of potentially beneficial structures for the progress of novel chemotherapeutic agents [15]. During the last several years, natural products with antimicrobial effects were explored to remove the usage of synthetic antibiotics which cause the resistance of the microorganisms and can show side effects to human health [16]. Various studies have been conducted with the extracts of numerous plants measuring antimicrobial activity as well as for the detection of novel antimicrobial phytocompounds [17]. Phytochemicals derived from the plant products assist to develop less toxic and more effective medicines in controlling the development of microorganisms and curative of numerous illnesses [18]. The traditional claim indicated that the *G. gummifera* plant parts are used to treat wounds, roundworms, cough. Scientific inquiries for natural plants have been started in various countries because they assisted in human health maintenance. Nowadays, herbal drugs are becoming more popular because using medicinal plants derived medications are comparatively safer than synthetic substitutes. The exploration for antimicrobials from natural sources has received much care these days with regard to certain ailments such as respiratory diseases, fever, and skin diseases.

In the present study, *G, gummifera* fruit methanol extract showed significant inhibitory result on both gram positive *Staphylococcus aureus*, *Streptococcus pneumoniae. Pseudomonas aeruginosa* and gram negative *Salmonella typhi*, *Vibrio cholera*, strains that cause pneumonia, osteomyelitis, endocarditis, phlebitis, mastitis, and meningitis in humans. The fruit methanol extract displayed the highest activity against *P.aeruginosa* 19.33 mm and for *S.aureus* of 16.67 mm ZI. The antibacterial property of GFME is due to the cumulative effect of the compounds -2-Furancarboxaldehyde, 5-(hydroxymethyl), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 1,3,5-Triazine-2,4,6-triamine, Hydrazinecarboxamide, 2-(2-methylcyclohexylidene) and Quinic acid, and it was supported by molecular docking studies.

DNA gyrase is an essential bacterial enzyme that catalyzes the introduction of negative (−) supercoils into chromosomal and plasmid DNA. Gyrase was discovered soon after it was clear that *in vitro* recombination of bacteriophage λ required a negatively supercoiled DNA substrate. DNA gyrase cleaves and religate DNA to regulate DNA topology and are a major class of antibacterial and anticancer drug targets [19, 20]. The *in silico* docking of quinic acid with the DNA gyrase showed greater binding affinity as well as hydrogen bonding and good hydrophobic interaction with the receptor. Among the 5 ligands, quinic acid showed the highest binding affinity and hydrophobic interaction with the amino acids of the active pocket. The 5 ligand molecules showed antibacterial activity by hindering the function of DNA gyrase.

Thus, the current study specifies that *G. gummifera* fruit methanol extract contains important phytochemicals. That may be responsible for the inhibition of the growth of pathogens. The present results are promising for the isolation and characterization of biologically active constituents as anti-microbial agents.

Conclusion

The findings of this study showed that fruit methanolic extract of *Gardenia gummifera* Linn displayed possible antibacterial action against the gram positive and negative strains. The plant extracts against resistant bacteria lead to new choices for the treatment of infectious diseases.

References

1. Bloom DE, Cadarette D. Infectious disease threats in the twenty-first century: strengthening the global response. Frontiers in immunology,2019:28:10:549.
2. Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. Journal of Orthopaedic Research,2018:36(1):22-32.
3. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. Journal of advanced pharmaceutical technology & research,2011:2(2):104.
4. Gunjan M, Naing TW, Saini RS, Ahmad A, Naidu JR, Kumar I. Marketing trends & future prospects of herbal medicine in the treatment of various disease. World Journal of Pharmaceutical Research,2015:28:4(9):132-55.
5. Hassan A, Ullah H. Antibacterial and Antifungal Activities of the Medicinal Plant Veronica biloba. Journal of Chemistry,2019:7:2019.
6. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention.
7. Hassan A, Ullah H. Antibacterial and Antifungal Activities of the Medicinal Plant Veronica biloba. Journal of Chemistry,2019:7:2019.
8. Nagar HK, Ranawat MS. Preliminary phytochemical screening, in vitro antioxidant activity, topical and oral

formulation of extract of Woodfordia fructicosa and Gardenia gummifera. Int J Pharmacol Phytochem Ethnomed,2017:8:16–26.
9. Kekuda PT, Raghavendra HL, Shilpa M, Pushpavathi D, Petkar T, Siddiqha A. Antimicrobial, antiradical and insecticidal activity of Gardenia Gummifera L. F. (Rubiaceae). Int J Pharm Pharm Sci,2017:9:265–72.
10. Reddy GC, Rangaswami S, Sunder R. Triterpenoids of the stem bark of Gardenia gummifera. Planta Med,1977:32:206–11.
11. Alam MT, Karim MM, Khan SN. Antibacterial activity of different organic extracts of Achyranthes aspera and Cassia alata. Journal of scientific research,2009:23:1(2):393-8.
12. Valgas C, Souza SM, Smânia EF, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. Brazilian journal of microbiology,2007:38(2):369-80.
13. Vinaykumar NM, Mahmood R, Krishna V, Ravishankara B, Shastri SL. Antioxidant and in vivo hepatoprotective effects of Gardenia gummifera Lf fruit methanol extract. Clinical Phytoscience,2020:6(1):1-4.
14. Shivakumar R, Venkatarangaiah K, Shastri S, Nagaraja RB, Sheshagiri A. Antibacterial Property and Molecular Docking Studies of Leaf Calli Phytochemicals of Bridelia scandens Wild. Pharmacognosy Journal, 2018, 10(6).
15. Greenwell M, Rahman PK. Medicinal plants: their use in anticancer treatment. International journal of pharmaceutical sciences and research,2015:1:6(10):4103.
16. Cheesman MJ, Ilanko A, Blonk B, Cock IE. Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?. Pharmacognosy reviews,2017:11(22):57.
17. De Zoysa MH, Rathnayake H, Hewawasam RP, Wijayaratne WM. Determination of in vitro antimicrobial activity of five Sri Lankan medicinal plants against selected human pathogenic bacteria. International Journal of Microbiology,2019:6:2019.
18. Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian journal of pharmaceutical sciences,2012:74(5):443.
19. Crisona NJ, Strick TR, Bensimon D, Croquette V, Cozzarelli NR. Preferential relaxation of positively supercoiled DNA by E. coli topoisomerase IV in single-molecule and ensemble measurements. Genes & development,2000:15:14(22):2881-92.
20. Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. Applied microbiology and biotechnology,2011:1:92(3):479-97.