Indian Journal of Chemistry
Vol. 63, May 2024, pp. 437-443
DOI: 10.56042/ijc.v63i5.6713

In vitro and *in silico* studies of methanol extract of *Bridelia stipularis* on *S. aureus* and *E. coli*

Sini A[a], Bindu T K[a], Vinod P Raphael*[a] & Shaju K S[b]

[a] Department of Chemistry, Government Engineering College, Thrissur 680 009, India
(Affiliated to A P J Abdul Kalam Technological University, Thiruvananthapuram 695 016, Kerala, India)
[b] Department of Chemistry, Panampilly Memorial Government College, Chalakkudy 680 722, Kerala, India
E-mail: vinodpraphael@gectcr.ac.in

Received 13 November 2023; accepted(revised) 23 April 2024

Bacterial resistance to contemporary antibiotics is an emerging problem in allopathy. The pursuit of a novel therapeutic agent with superior activity to conventional antibacterial agents has piqued the interest of pharmaceutical scientists in recent decades. It has been established by several scientists that plant extracts are the source of many valuable phytochemicals that may have a variety of medicinal activities. Some of the compounds present in *Bridelia stipularis* (BS) are reported to have anti-cancer and anti-microbial activities. In the present work, we examined the phytochemicals present in the methanol extract of BS using GC-MS analysis. The extract was screened for anti-bacterial activity against *S. aureus* and *E. coli* in DMSO using the well-cut method. The extract's activity was comparable to that of standard antibiotics such as erythromycin, streptomycin, cefotaxime and cloxacillin. *In silico* investigations of the phytochemicals that have the highest proportion in the extract were conducted with the structural proteins of *S. aureus* and *E. coli*. Among the identified compounds, steroidal and sulphonyl hydrazone molecules showed high binding affinity on the enzymes of *E. coli* and *S. aureus*. Binding analysis of highly stable receptor-ligand complexes were discussed in the article.

Keywords: *Bridelia stipularis*, Transferase, Oxidoreductase, Phosphotransferase, Docking

Bacterial infections are the root cause of many diseases, and their severity may lead to fatalities for humans and animals. Infections are mainly controlled by synthetic pharmaceutical agents called antibiotics. Beta lactams, aminoglycosides, cephalosporins, and tetracyclines are the most popular antibiotics prescribed by medical practitioners nowadays. Repeated and unwise use of antibiotics and the unexpected mutations of bacterial strains may cause bacterial resistance, which in turn leads to the inefficacy of the existing antibacterial agents[1]. Research for novel therapeutic agents[2] that can overcome bacterial resistance is the need of the hour to fight against pathogens.

Staphylococcus aureus is a round, Gram-positive bacterium that is usually present in the upper respiratory tract and on the skin. Some opportunistic infections, such as sinusitis, respiratory infections, food poisoning, bone and joint infections, blood stream infections (bacteremia), *etc*., are caused by *S. aureus*. This pathogen is also responsible for orthopedic and cardiac implant infections. This pathogen greatly resists antibiotics[3,4] and causes the emergence of strains such as methicillin-resistant *S. aureus*. *Escherichia coli* is a Gram-negative bacterium that is frequently found in the intestines of humans and in the guts of some animals. Though *E. coli* bacteria keep our digestive tract healthy, some strains can cause food infections[5] that may lead to diarrhea. It is estimated that the root cause of 75–95% of urinary tract infections is *E. coli*. Some versions of *E. coli* cause infection by producing a toxic substance called shiga. This toxin damages the inner side of our intestines. One *E. coli* strain, O157:H7, causes vomiting and diarrhea, which may lead to kidney failure in children.

Apart from synthetic compounds, the antibacterial potency of natural compounds, especially plant extracts, has been reported by various researchers over the past 20 years[6,7]. Some phytochemicals, alone or when combined with other small molecules, can show antibacterial efficiency. *Bridelia ferruginea*[8], *Cordia wallichii*[9,10], *Elaeocarpus tectorius*[11] *etc*. are examples of medicinal plants that contain antibacterial compounds. Tanins, flavanols and compounds like methylpluviatilol[12] *etc*. exhibit antimicrobial activities.

Bridelia stipularis (BS) is a woody evergreen climber found in the tropical and subtropical regions of many countries[13,14]. Many valuable medicinal compounds have been isolated from BS by various researchers.

Extracts from the various parts of the plant were studied for their antimicrobial, antioxidant[15,16], antidiabetic[17], nutritional, and other medicinal properties[18,19]. A systematic evaluation of the antibacterial properties of phytochemicals present in the BS extract, their pharmacology, and the mechanism of growth inhibition are not available in the literature.

The present investigation was conducted for analyzing the major components present in the methanol extract of BS leaves and to check its antibacterial potency[20]. We are also aiming to identify the major possible structural receptors of *S. aureus* and *E. coli* to interact with the active components present in the extract[21]. To analyze the interaction of receptor-ligand complexes and to check the binding efficacy of the complex is another objective of the present study.

Experimental Section

Extraction

BS plant leaves (Fig. 1) were collected from Thalikulam, Thrissur district, Kerala, India, in the month of March 2022.

The leaves were shade-dried for one week before being powdered. 200 g of powder was extracted with 750 mL methanol using a Soxhlet extractor. The volume of the solution was reduced and evaporated to dryness, and the yield was noted (31.6 g). In 2% DMSO, a solution of 1 mg/L of this powder was prepared. 50, 60, 70, and 80 microliters of this sample solution were used for antibacterial studies.

Fig. 1 — BS leaves

GC-MS analysis

Using a VF-5 Fused Silica Capillary Column (30 m x 0.25 mm x 0.25 micrometers), GC-MS analysis was performed on the methanol extract to identify the phytochemicals present in the methanol extract[16]. The program's temperature was 110°C for 2 minutes, then increased at 15 degrees per minute to 150 degrees, then increased at 10 degrees per minute to 250 degrees for 5 minutes. At a constant flow rate of 1 ml/min, He gas was used as a carrier gas. The total time required for this procedure was 20.67 minutes. The compounds were identified by comparison of their retention indices with those provided in the NIST Library.

Antibacterial studies

Bacterial cultures used included Gram-positive *Staphylococcus aureus* (MTCCNO 3103) and *Gram-negative Escherichia coli* (MTCCNO 68). The pure cultures were purchased from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Materials used for the preparation of nutrient broth and nutrient agar were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai. Nutrient broth consists of a peptic digest of animal tissue (5 g), sodium chloride (5 g), yeast extract (1.5 g) and 1000 mL of distilled water (*p*H – 7.2 + 0.2). Peptic digest of animal tissue (5 g), sodium chloride (5 g), yeast extract (1.5 g), agar (15 g), and distilled water (1000 mL ; *p*H 7.2 + 0.2) were used to prepare nutrient agar.

Well diffusion method (Collins & Lyner., 1987) with a slight modification was employed for carrying out antibacterial studies[12,13]. Nutrient broth was used to maintain the bacterial cultures. Using sterile swabs, a uniform distribution of the bacterial culture was made on the nutrient agar plates. Wells having 3 mm diameter were cut using a well borer on the hardened surface of agar. 2 cm of distance was kept between each well. A solution of 1 mg/L of the solid extract was prepared in a 2% DMSO solution and used as a stock solution. Plant extracts having different concentrations (50, 60, 70, and 80 µL) were applied using a micropipette into the wells made on the surface of the nutrient agar plates. All plates were incubated at 370°C for 24 h, and the zone of inhibition was measured.

In silico methods

The 3D molecular structures of the compounds present in the BS leaf extract were downloaded from the Pubmed server as sdf files and converted into

Table 1 — Structural proteins present in *S. aureus* and *E. Coli*

PDB code	Function	Resolution	Sequence Length
	S. aureus		
4BXI	Accessory Gene Regulator-ATP binding domain. Used for Quorum sensing	2.2 Å	153
6UEX	LcpA- phosphotransferase enzyme; catalyzing the glycan attachment to surface proteins	1.9 Å	273
2ZCO	Dehydrosqualene synthase-involves in the synthesis of staphyloxanthin pigment which has an antioxidant property to survive bacterium in the host cell	1.58 Å	293
1N67	Ligand-binding segment of the Staphylococcus aureus MSCRAMM, clumping factor A- helps in the attachment of bacteria to host cell	1.90 Å	359
1T2P	Sortase A- It is peptidase involves in the catalysis of cell wall sorting reaction	2.0 Å	146
3U2D	DNA gyrase-catalyzes the super coiling of DNA and control the topological transitions of DNA	1.85 Å	198
	E. coli		
1JG0	Transferase- involves in the synthesis of thymidylate; a precursor of DNA biosynthesis	2.00 Å	264
6F86	Gyrase-involves in topological transitions of DNA	1.90 Å	206
1FJ4	beta-ketoacyl-acyl carrier protein synthases-Regulate fatty acid synthesis and acts as targets for thiolactomycin (TLM) and cerulenin	2.35 Å	406
2Q85	Oxidoreductase-Involves in the synthesis of peptidoglycan precursor	2.51 Å	342
5BNR	Transferase- involves in the branched chain fatty acid synthesis	1.98 Å	317

mol2 format using Openbabel software. Structures six proteins of *S. aureus* and five proteins of *E. coli* which have significant catalytic role in the metabolic activities of pathogens was downloaded from the Protein Data Bank and prepared for molecular docking studies using Chimera freeware. All the protein structures were characterized by X-ray diffraction techniques. Descriptions of proteins, their function, and structural features are listed in Table 1. Structures of the prepared proteins and the natural products were uploaded to the online web server Swissdock to get the possible interactions and the binding energy values. The most feasible protein-ligand complex was taken from the docking results and analysed using Biovia Discovery Studio software.

Results and Discussion

GC-MS analysis

Fig. 2 represents the gas chromatogram of the methanol extract of BS leaves. The components present in great proportion were identified with the help of the mass spectrum and NIST library and provided in Table 2. The structures of major components present in the methanol extract of BS were screened for their drug-likeness using SwissAdme webserver and Lipinski rule. Out of the thirteen compounds listed in the table, eight were selected (designated from M1 to M8) for *in silico* studies on the various structural proteins of *E. coli* and *S. aureus*. Fig. 3 represents the molecular structures of the compounds selected for docking studies.

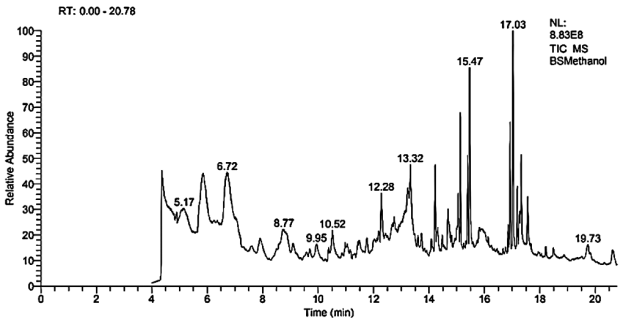

Fig. 2 — Gas chromatogram of methanol extract of BS leaves

Antibacterial assay

Antibacterial activities of methanol extract of BS leaves are provided in Table 3. Values provided in the table represent the zone of inhibition (diameter in cm) of the growth of bacterial strains. The extract showed appreciable activity against the growth of *S. aureus* and *E. coli*. As the concentration of extract increased, antibacterial response increased. At all concentrations *S. aureus* inhibited than *E. coli* by the BS leaf extract. At a concentration of 80 µl, the zone of inhibition of the extract on *S. aureus* and *E. coli* were 17 cm and 16 cm respectively. Antibacterial activity of standard antibiotics such as erythromycin, streptomycin,

Table 2 — Phytochemicals identified in the methanolic extract of BS using GC-MS

No.	Name of the compound	RT	Area%	M. wt
1	phytol	17.03	6.78	296
2	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (M1)	15.47	3.22	276
3	2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone (M2)	14.32	1.33	204
4	Ethyl iso-allocholate (M3)	14.09	0.7	436
5	5-Thio-D-glucose	13.32	3.68	196
6	4,4,5,8-Tetramethylchroman-2-ol (M4)	12.28	2.35	206
7	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl- (M5)	11.43	0.55	206
8	4-Phenyl-3-penten-2-one p-toluenesulfonylhydrazone (M6)	10.52	1.99	328
9	2,4,6-Octatrienoic acid	9.95	0.92	138
10	1,2,3-Benzenetriol	8.77	2.09	126
11	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (M7)	5.85	8.0	144
12	L-Glucose	5.17	1.12	180
13	N-cyclohexyl-3,4-methylenedioxyamphetamine (M8)	5.13	1.8	261

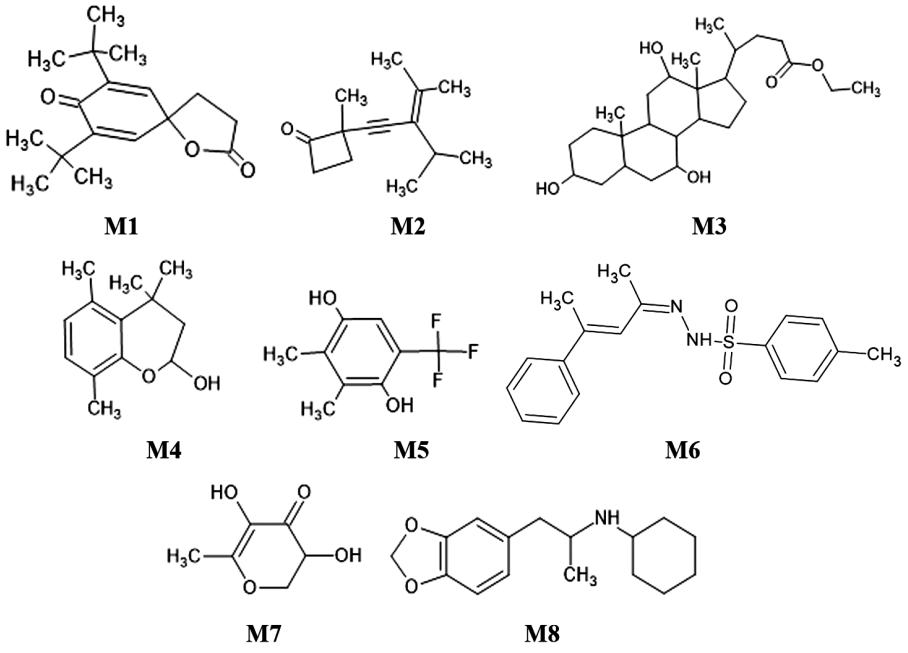

Fig. 3 — Molecular Structures of compounds present in the BS extract taken for *in silico* studies

cefotaxime and cloxacillin in DMSO are also reported in Table 3. Methanol extract showed comparable antibacterial potency with that of erythromycin and streptomycin. It is worthwhile to note that the BS extract performed well than cefotaxime and cloxacillin to inhibit the growth of *S. aureus*. Also, BS extract showed higher activity for *E. coli* than the standard antibiotic cloxacillin. Fig. 4 represents the zone of inhibition of methanol extract against *S. aureus* and *E. coli*. Extract showed slightly low inhibition zones for *E. coli* than the *S. aureus* inhibition.

Computational studies

All major compounds identified in the methanol extract were screened for their drug-likeness using the SwissAdme. Eight compounds which fairly obey Lipinski and ADME properties were subjected to docking studies on various structural receptors of *S. aureus* and *E. coli*. The binding energies (kcal/mol) of receptor-ligand complexes of *S. aureus* and *E. coli* are given in Tables 4 and 5, respectively. High values of binding affinities (≥8.93 kcal/mol for *S. aureus* and ≥8.37 kcal/mol for *E. coli*) are given as bold numbers in the tables.

Table 3 — Antibacterial activity of BS extract and standard antibiotics on *S. aureus* and *E. coli*

Species	Methanol Extract				Erythromycin				Streptomycin				Cefotaxime				Cloxacillin			
Conc.(µL)	50	60	70	80	50	60	70	80	50	60	70	80	50	60	70	80	50	60	70	80
S. aureus	13	14	15	17	12	13	14	14	14	17	19	20	4	7	8	9	8	8	9	10
E. coli	10	12	13	16	13	15	15	15	8	10	12	14	12	16	17	18	2	4	6	6

Table 4 — Binding Energy (kcal/mol) of phytochemicals in BS extract with various targets of *S. aureus*

PDB code	M1	M2	M3	M4	M5	M6	M7	M8
4BXI	–7.26	–7.06	–7.34	–6.78	–7.20	–7.46	–5.99	–7.21
6UEX	–7.99	–7.98	**–9.77**	7.14	–8.28	–8.39	6.15	–8.21
2ZCO	–7.16	–7.26	–8.54	–6.78	–7.78	–8.93	–6.29	–7.87
1N67	–7.41	–6.86	**–9.0**	–6.92	–7.34	–8.03	–6.17	–7.18
1T2P	–6.80	–6.83	–7.87	–6.65	–7.02	–9.10	–5.96	–7.01
3U2D	–6.86	–7.02	–8.00	–6.95	–7.25	–8.05	–6.89	–7.4

Table 5 — Binding Energy (kcal/mol) of phytochemicals in BS extract with various targets of *E. coli*

PDB Code	M1	M 2	M3	M4	M5	M6	M7	M8
1JG0	–7.17	–7.11	–8.50	–6.77	–7.20	–8.29	–5.93	–7.20
6F86	–6.67	–7.09	–7.57	–6.59	–7.03	–7.47	–6.76	–6.87
1FJ4	–6.69	–7.32	–8.37	–6.62	–8.08	–8.07	–6.16	–8.08
2Q85	–7.33	–7.31	–8.33	–7.30	–7.81	–9.17	–6.26	–8.10
5BNR	–6.76	–7.10	–8.03	–6.73	–7.86	–8.47	–6.40	–8.00

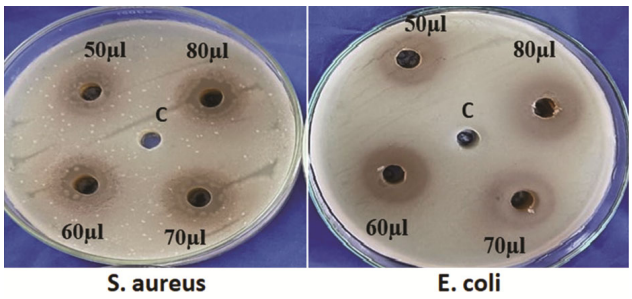

Fig. 4 — Antibacterial activity of *S. aureus* and *E. coli* by BS extract

According to the binding energy data, molecule Ethyl iso-allocholate (M3) and sulfonyl hydrazone derivative M6 displayed more binding affinity towards the structural proteins of *S. aureus* and *E. coli* than all other compounds. Out of six structural proteins of *S. aureus*, 6UEX-M3 and 1N67-M3 complexes were highly stable. The sulfonylhydrazone was also active against various enzymes of S. *aureus*. The peptidase (PDB:1T2P) and dehydrosqualene synthase (PDB:2ZCO) showed a very good binding energy with (–9.1 and –8.93 kcal/mol) M6 than all other structural receptors of *S. aureus.*

In general, the inhibitory potency of the molecules present in the methanol extract against the structural receptors of *E. coli* was slightly less than that of *S. aureus*. Antibacterial assay using the extract also showed the same trend in the *in vitro* studies. The steroidal ester molecule (M3) showed good binding affinity towards transferase (1JG0; –8.5 kcal/mol) and protein synthase (1FJ4; –8.37 kcal/mol) which involve in the biosynthesis of DNA and protein synthesis respectively. The sulfonyl hydrazone displayed great affinity for the oxidoreductase (2Q85) and the transferase (5BNR) involved in the synthesis of fatty acids. These receptor-ligand complexes possess binding energy values –9.17 and –8.47 kcal/mol, respectively.

Analysis of selected protein-ligand complexes: *S. aureus*

The steroid molecule M3 displayed fair binding energies on all other protein receptors taken for the *in-silico* studies (Table 4). The compound M3 in complexation with the phosphotransferase enzyme of *S. aureus* (6UEX) has got the greatest binding affinity (–9.77 kcal/mol) than all other complexes even in the absence of conventional hydrogen bonds. The complex was stabilized by four alkyl interactions (Val254, Leu258, Met235, Leu238; 4.8-5.49 Å) and one pi-alkyl bond (Phe171; 5.21 Å).

A binding energy –9.0 kcal/mol was observed for 1N67-M3 complex. Amino acid residues Arg395 interacted with two conventional hydrogen bonds with M3 (2.81 and 2.03 Å). One more hydrogen bond was established between val288 and M3 at 3.04 Å. Hydrophobic alkyl interactions were noticed between

Phe455, Ile488, Tyr399, Pro341 and the steroid molecule M3 was also very effective in attaching the host binding region of the structural protein 1N67 with M3 (4.27-5.33 Å). Moderate affinity was noticed for the M3 molecule to other structures.

Structural proteins dehydrosqualene synthase (2ZCO) and peptidase (1T2P) of *S. aureus* showed great affinity to sulfonylhydrazone molecule present in the extract. 2ZCO-M6 and 1T2P-M6 complexes displayed –8.93 and –9.1 kcal/mol binding energies, respectively. M6 made two conventional hydrogen bonds with Arg45 (3.06 Å), Tyr41 (2.05 Å) and seven hydrophobic interactions with Phe22, Phe26, Lue141, Leu160, Ala134, Leu164 and Val133 (4.5-5.17 Å). One pi-cation interaction was also noted between Arg45 (3.1 Å) and the sulfonylhydrazone which also contributed to the stability of the complex. 1T2P-M6 complex was mainly stabilized by two conventional (Val72; 2.03, 2.7 Å) and two non-conventional hydrogen bonds (Lys134; 2.87 Å). Also, four hydrophobic interactions were observed between the amino acid residues Pro66, Ile123, Lys198, Lys196 and the sulphonyhydrazone. The 3D and 2D interaction plots of 6UEX-M3 and IT2P-M6 receptor-ligand complexes are depicted in Fig. 5.

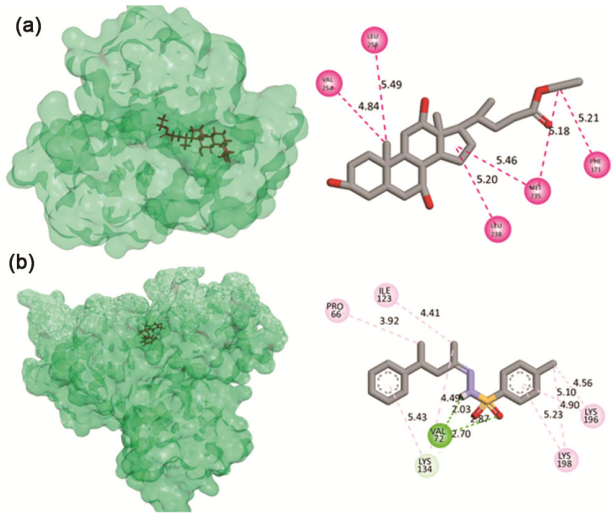

Fig. 5 — 3D and 2D binding interactions of molecules with the structural proteins of *S. aureus* (a) 6UEX-M3(b) 1T2P-M6

Analysis of selected protein-ligand complexes: *E. coli*

The steroid molecule M3 and the sulphonylhydrazone derivative present in the extract bound strongly with the structural receptors of *E. coli* (Fig. 6). The transferase which involves in the synthesis of thymidylate (1JG0) showed a binding energy –8.5 kcal/mol with M3 molecule (Table 5). This complex was stabilized by one conventional hydrogen bond with the residue Asn177 (2.66 Å) and two carbon-hydrogen bonds with Tyr209 (3.07 Å) and Ala263 (2.8 Å), respectively. In addition to these, four hydrophobic alkyl interactions were observed in this complex (Cys146, Ile79, Trp80, Phe176) in the range 3.75-5.2 Å. 1JG0 also displayed –8.29 kcal/mol binding energy with the sulphonylhydrazone (M6).

The protein synthase 1FJ4 showed good affinity towards the molecule M3 (–8.37 kcal/mol). The complex 1FJ4-M3 was stabilized only by hydrophobic interactions. The amino acid residues Pro303, Ala206, Val304, Ala271, Phe392 and Pro272 made alkyl interactions with the steroid molecule within the range 2.4-4.9 Å.

The sulphonylhydrazone also exhibited good binding scores with the oxidoreductase (2Q85) and

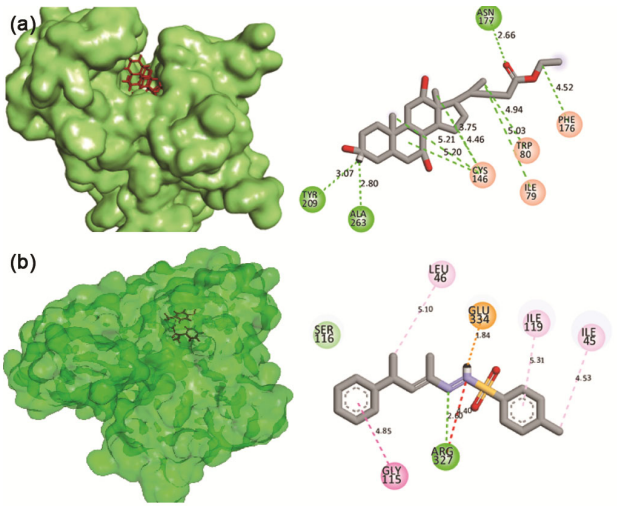

Fig. 6 — 3D and 2D binding interactions of molecules with the structural proteins of *E. coli* (a) 1JG0-M3 (b) 2Q85-M6

transferase (5BNR) enzymes. The phytochemical M6 bound well with the Arg327 residue of oxidoreductase 2Q85 (–9.17 kcal/mol) using one conventional hydrogen bond (2.6 Å). Four hydrophobic bonds were also seen in the receptor ligand complex between the alkyl/aromatic moieties and the amino acid residues Leu46, Ile119 and Ile45 in the range 4.5-5.31 Å. A one stacked pi-amide interaction with Gly115 (4.85 Å) was also noticed in the complex. Basic azo group accepted one proton from the residue Glu334 and formed a salt bridge at a distance of 1.84 Å was also favorable to enhance the stability of the 2Q85-M6 complex.

5BNR-M6 complex displayed two conventional hydrogen bond interactions with the residues Arg249 (2.54 Å) and Asn247 (2.73 Å). The tranferase enzyme also interacted with the sulphonyl hydrazone using one pi-sigma bond (Gly209; 2.66 Å), one pi-sulphur bond (Cys112; 4.79 Å), five alkyl linkages (Val122, Met207, Ile156, Ala246, Lue189; 3.9-5.17A) and one pi-pi T shaped bond (HSD244; 5.21A) to make the complex a stable one. The binding energy of this molecule on 5BNR was –8.47 kcal/mol.

Conclusions

In this work, we extracted the phytochemicals present in *Bridelia stipularis* using methanol and characterized using GC-MS analysis. *In vitro* antibacterial potency of the BS extract against the pathogens *E. coli* and *S. aureus* was investigated. The extract showed comparable antibacterial activities to that of standard antibiotics erythromycin, streptomycin, cefotaxime and cloxacillin. To find out the effective components responsible for the antibacterial action, *in silico* studies were performed using the selected phytochemicals present in the extract on the structural receptors of *E. coli* and *S. aureus*. Molecular docking studies revealed that the steroid molecule and the sulphonyl hydrazone molecule present in the BS extract were highly active against the structural proteins of the pathogens.

References

1 Kokoska L, Kloucek P, Leuner O & Novy P, *Curr Med Chem*, 26 (2019) 5501.

2 Agbo M O, Uzor P F, Akazie-Nneji U N, Eze-Odurukwe C U, Ogbatue U B & Mbaoji E C, *Dhaka Uni J Pharm Sci*, 14 (2015) 35.

3 Velasco J, Contreras E, Buitrago D & VelazcoCiencia E, 13 (2005) 411-415. (Reference not found kindly provide a valid DOI number or journal name).

4 Ghosh A, Ricke S C, Almeida G & Gibson K E, *Curr Microbiol*, 72 (2016) 426.

5 Reyes-Munguía A, Carrillo-Inungaray M L, Carranza-Álvarez C, Pimentel-González, D & Alvarado-Sánchez B, *Front Life Sci*, 9 (2016) 90.

6 Olarewaju OO, Fajinmi O O, Naidoo K K, Arthur G D & Coopoosamy R M, *J Med Plants Eco Dev*, 6 (2022) 15.

7 Dutta S & Chakraborty S, *Int J Pharm Life Sci*, 11 (2020) 2860.

8 Tadros SH, Sleem AA, *Bull Fac Pharm (Cairo University)*, 42 (2004) 225.

9 He X, Wang X, Fang J, Chang Y, Ning N, Guo H, Huang L & Huang X, *J Ethnopharm*, 203 (2017) 260.

10 Dias D A, Urban S & Roessner U, *Metabolites*, 2 (2012) 303.

11 Manoharan K & Chitra P, *Asian J Biomed Pharm Sci*, 5 (2022) 597.

12 Kotigadde S, Jose S, Zachariah A, Premanathan P & Bhat K G, *J Comm Dis*, 37 (2005) 135.

13 Hasan S, *Int J Sci Env Tech*, 3 (2014) 123.

14 Kosalec I, Kremer D, Locatelli M, Epifano F, Genovese S & Carlucci G, *Food Chem*, 136 (2013) 335.

15 Anjum A, Sultan M Z, Sikder M A A, Hasan C M, Al-Mansoor M A & Rashid M A, *Dhaka Uni J Pharm Sci*, 15 (2016) 221.

16 Khan I, Jan S A, Shinwari Z K, Ali M, Khan Y & Kumar T, *Med Online J Bio Med*, 1 (2017) 9.

17 Dhale D A & Bhoi S, *Curr Agri Res J*, 1 (2013) 51.

18 Ngueyem T A, Brusotti G, Caccialanza G & Finzi P V, *J Ethnopharm*, 124 (2009) 339.

19 Thomas S, Patil D A, Patil A G, Naresh F, *J Herb Med Toxi*, 2 (2009) 51.

20 Chaturvedula V S P & Prakash I, *J Chem Pharm Res*, 5 (2013) 261.

21 Morsy N, *Main Group Chem*, 13 (2014) 7.