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Original Article

Antibacterial Property and Molecular Docking Studies of Leaf Calli Phytochemicals of *Bridelia scandens* Wild.

Ravikumar Shivakumar, Krishna Venkatarangaiah, Sudhesh Shastri, Ravishankara Burladinni Nagaraja, Ajith Sheshagiri

ABSTRACT

Background: *Bridelia scandens* Wild. (Euphorbiaceae) leaves are widely used to cure asthma, bronchitis pleurisy, exudation, sores in mouth and genital cancers. **Objective:** To evaluate antibacterial activity of the leaf calli methanol extract (LCME). **Materials and Methods:** Mass production of leaf calli was established on MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L 2, 4-D. Methanol extract of the dried calli was subjected to HR-LCMS analysis, antibacterial screening of the extract was carried out against human pathogenic clinical isolates. Molecular docking study of HR-LCMS identified compounds was performed by docking with bacterial enzyme DNA gyrase. **Results:** HR-LCMS analysis of LCME shows that the compounds azaperone bifonazole, fusidic acid, lasalocid and quinine as the major constituents. The antibacterial screening of LCME against clinical pathogens showed significant bactericidal activity against the strains *Staphylococcus aureus* (17.67±0.88 mm.d.), *Streptococcus pneumonia* (13.67±0.33), *Pseudomonas aeruginosa* (16.33±0.67), *Salmonella typhi* (17.67±0.33), and *Vibrio cholera* (15.33±0.33) as compared to the standard drug ciprofloxacin. The molecular docking of lasalocid against the bacterial enzyme DNA gyrase exhibited good binding affinity of -4.9 kcal/mol, good drug likeness (2.5589), 2 hydrogen bonds and hydrophobic interaction with 7 amino acid residues, so that lasalocid processes good inhibitor as compared to other 4 compounds. **Conclusion:** LCME of *Bridelia scandens* showed significant antibacterial activity against *Staphylococcus aureus and Salmonella typhi.* Lasalocid is the major phytocomponent of LCME which exhibited good inhibitory activity against bacterial enzyme DNA gyrase. This investigation supported traditional claim of LCME as potential antibacterial drug.
Key words: *Bridelia scandens,* Antibacterial, ADMET, DNA Gyrase, Molecular docking.

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INTRODUCTION

Since earliest times, medicinal plants have been known to exert healing properties against human infections as antimicrobial agents due to the presence of rich secondary metabolites. Unscientific collection and over exploitation of medicinal plants resulted in the dwindling of natural population and many of them are at the verge of threatening status. Induction of callus from the medicinal plant parts and *in vitro* production of secondary metabolites from the calli is a novel technique for sustainable conservation of medicinal plants. So that callus culture is the process that can reduce the time and season as it does not need to have the whole plant cultivation and sacrificing it for extraction.[1]

Bridelia scandens is a straggling climber belongs to the family Euphorbiaceae. It is distributed in the warm regions of Southeast Asia[2] and also in Western peninsular India especially in deciduous to semi-evergreen forests of Maharashtra, Kerala, Karnataka states (http://www.indiabiodiversity.org). In traditional medicine, decoction of the leaves has been used in the treatment of asthma, cough, fever, pleurisy, exudation and sores in mouth. The phytochemical examination of the *B. scandens* leaves showed the presence of flavonoids, carbohydrates, glycosides, phenolic compounds and tannins.[3] In the previous study an anticancerous compound anthrisine-deoxypodophyllotoxin was isolated from the leaves of *B. scandens*.[4]

Over the past decade, much attention has been placed on the study of phytochemicals for their antibacterial activity, especially against multidrug-resistant Gram-negative and Gram-positive bacteria.[5] The emergence of multidrug-resistance among bacteria has challenged the effectiveness of antibiotics in the advent of modern medicine and as such, antibiotic resistance has become one of the most serious health care problems in the world.[6] Considering the above, there is a need to develop new effective antibacterial agents that circumvent the emergence of resistance. Nevertheless, the discovery of new antibiotics is very expensive and time consuming, requiring about ten

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years to bring a new antibiotic to market. Therefore, the search for antibacterial substances derived from natural products, such as phytochemicals, has gained cumulative importance.[7]

In addition, *in silico* prediction of the ADMET properties plays an important role in antibiotic drug discovery process. Nowadays ADME (absorption, distribution, metabolism and elimination) is applied at an early phase of the drug development process, in order to remove molecules with poor ADME properties from the drug development pipeline and leads to significant savings in research and development costs. Lipinski "Rule of five" is widely used as a filter for drug-like properties.[8] Molecular docking is a frequently used method for evaluating the complex formation of small ligands with large biomolecules.[9] In view of the above, the present investigation was undertaken to isolate and characterize antibacterial compounds from the *in vitro* derived leaf calli and to authenticate the antibacterial property against human pathogenic clinical isolates.

MATERIALS AND METHODS

Plant material and callus culture

Mesristematic leaf explants of *B. scandens* was collected from Bhadra Wild Life Sanctuary of the Western Ghats (1 km from Kuvempu University) and was identified by Prof. V Krishna, professor and taxonomist, Dept. of Biotechnology, Kuvempu University.

The leaves were cleaned with deionized water, sterilized with 5% tween-20, 0.2% mercuric chloride, followed by distilled water wash and then 1cm aseptically inoculated on to MS semi solid media supplemented with hormonal concentration of 0.5 mg/L BAP and 0.5 mg/L 2,4-D for callus initiation. The calli was subcultured on the same media and mass propagated for 4-6weeks. The calli was harvested, dried in hot air oven at 40°C for 4 to 5 days.

Preparation of Extract

About 186 g of dried callus was subjected for cold extraction with methanol for about 48h. The extract was sieved (Whatman No.1 filter paper) and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and dried at desiccator.

Phytochemical screening

Determination of total phenol

Total phenol content in leaf callus methanol extract LCME was estimated by the Folin–Ciocalteu method.[10] 1 ml of LCME (50 μg) was mixed with Folin–Ciocalteu reagent (2 ml) (diluted 1:10, v/v) followed by the addition of 2 ml of sodium carbonate (7.5%, w/v) and mixed, allowed to reaction for 90 min at room temperature and absorbance was measured against the blank at 750 nm using spectrophotometer (Systronics, PC based double beam spectrophotometer 2202). Total phenol content of the extract was expressed in terms of equivalent to gallic acid (GAE, mg^{-1} of dry mass).

Determination of total flavonoid

Total flavonoid content of LCME was measured according to the modified method of Zhishen.[11] 5 ml of extract (200 μg) was mixed with 300 μl of 5% sodium nitrite and 300 μl of 10% aluminum chloride followed by the addition of 2 ml of 1 M sodium hydroxide after the incubation of reaction mixture at room temperature for 6 min. The volume in each test tube was made up to 10 ml by adding 2.4 ml of millipore water. Absorbance was measured at 510 nm against the blank. Total flavonoid content of the extract was expressed in terms of equivalent to catechin (mg^{-1} of dry mass).

Determination of alkaloid

Alkaloid determination using Harborne method:[12] 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered, and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

HR-LCMS analysis of LCME

The bioactive components of *B. scandens* leaf callus extract was analyzed by High Resolution Liquid Chromatograph Mass Spectrometer (HR-LCMS) G6550A system (Agilent technologies). The method used for Chromatography was 30 mins ± ESI 10032014_MSMS.m. The Gas temperature used for analysis was 250°C. The theoretical mass of protonated compound was used for identification. HR-LC-MS analysis of *B scandens* bark extracts was performed at Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Mumbai, India. The compounds were identified by comparison with their retention time (RT) and mass with stored metlin library available with IIT, Bombay.

Antibacterial activity

Microbial strains

The antibacterial activity of the LCME was individually tested against a set of five bacterial human pathogenic clinical isolates obtained from Shivamogga Institute of Medical Sciences, Shivamogga, Karnataka, namely: *Staphylococcus aureus, Streptococcus pneumoniae. Pseudomonas aeruginosa, Salmonella typhi*, and *Vibrio cholera*, Bacterial isolates were cultured overnight at 37°C in nutrient agar (NA) media.

Disk diffusion assay

Determination of antibacterial activity of LCME was evaluated by agar well diffusion method. The extract was dissolved in DMSO at different concentrations (500, 1000 and 1500 μg/m1 of DMSO μg). 100 μl of the suspension containing 10^8 colony forming units CFU/ml of bacteria were spread on NA media, respectively. Wells were made on agar plates using sterile cork borer, and 20 μl of LCME of each concentration were introduced into appropriately marked wells, ciprofloxacin (20 μg/ml) was taken as a positive control. Then culture plates incubated for 24 h at 37°C. Antibacterial activity was assessed by measuring the diameter of the growth inhibition zone in millimeters for the test organisms compared to the control. Activity index was calculated for comparison of the zone of inhibition of test material with standard antibiotic using the formula AI (Activity Index) = ZI of Test/ZI of Standard.[13]

Minimum Inhibitory Concentration (MIC)

The MIC of the LCME was evaluated by modified resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) microtitre plate assay.[14] 50 μl of test sample containing 250 μg of extract [5 mg/ml (w/v)] solutions in 10% dimethyl sulfoxide (DMSO, v/v) and 50 μg of standard antibiotic [1 mg/ml (w/v)] solutions in 10% DMSO. 50 μl of nutrient broth was added to all wells (microtitre plate). Two-fold serial dilutions were performed using a pipette such that each well had 50 μl of the test material in serially descending concentrations. 30 μl of 3.3 times stronger hi sensitivity broth and 10 μl of resazurin indicator solution (prepared by dissolving 27 mg resazurin in 4 ml of sterile distilled water) were added to each well. Finally, 10 μl of bacterial suspension was added to the appropriate wells to achieve a concentration of approx 5×10^6 CFU/ml. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software and Microsoft excel to determine the mean and standard error.

Molecular docking studies

Lipinski "Rule of five" is commonly used as a filter for drug-like properties.[8] The *in silico* pharmacokinetic properties and ADME (absorption, distribution, metabolism and elimination) and toxicity analysis were predicted using Data Warrior (http://www.openmolecules.org/data-warrior.html). Data Warrior tries to assess the toxicity risk by finding substructures within the chemical structure being indicative of a toxicity risk within one of said four major toxicity classes.

The chemical structure of HR-LCMS identified compounds namely, azaperone, bifonazole, fusidic acid, lasalocid and quinine and the standard drug ciprofloxcin were drawn using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper 2D orientation, and structure of each was checked for structural drawing error. Energy of each molecule was minimized using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina, in order to carry out the docking simulation. The protein data bank (PDB) coordinate file with the name '2XCT.pdb' was used as receptor molecule.[15] 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 Vina[16] was compiled and run in a Windows 8.0 professional operating system. LigPlot+[17] and PyMol educational version 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 colour, H-bonds with their respective distances are represented with cyan colour, and the interacting residues are represented in ball and stick model representation.

RESULTS

Callus culture

Leaf explants of *B scandens* proliferated into callus mass on MS media fortified with 0.5 mg/l BAP and 0.5 mg/l 2, 4-D. Callus induction was noticed from the cut end of the lamina and dorsal vein of the explants (Figure 1). Subculturing of the calli on to the same media induced luxuriant proliferation of the fleshy callus mass. After 6 weeks, the callus mass was harvested and fresh weight was found to be 3000 g the calli mass was dried in oven at 40°C for 4 days and dry weight was found to be 200 g of the dried calli mass was extracted with methanol used for phytochemical and antibacterial screening.

Phytochemical analysis

The preliminary phytochemical analysis of LCME showed positive result for qualitative tests for the phytochemicals alkaloids, flavonoids, and phenolics (Table 1). In quantitative analysis total polyphenolic content in LCME was expressed as equivalent to gallic acid (GAE) and is found to be 37.2 µg/mg of dry extract. The flavonoid concentration of LCME was expressed as equivalent to catechin and is found to be 56.9 µg/mg of dry extract. The alkaloid concentration of LCME was found to be 63.4 µg/mg of dry extract as depicted in the Table 1.

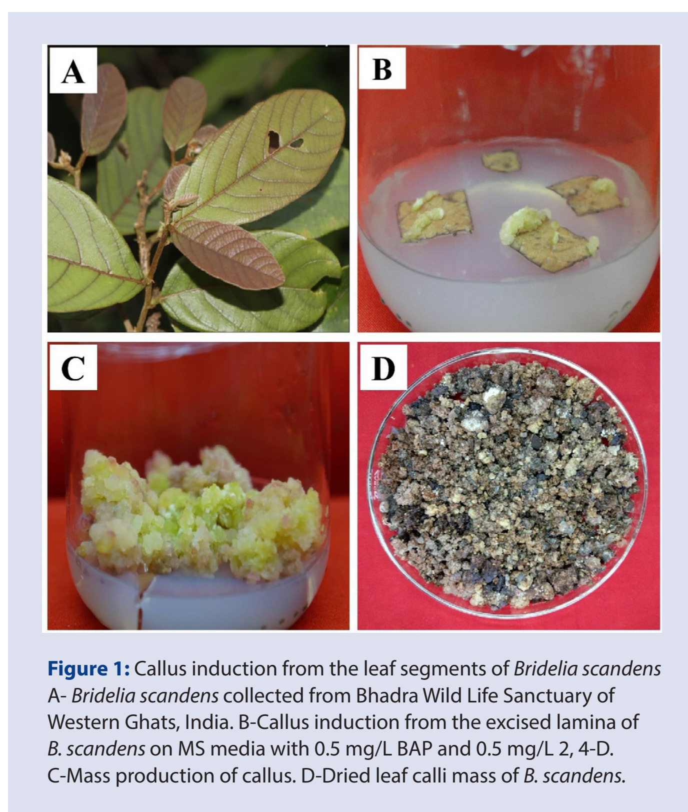

Figure 1: Callus induction from the leaf segments of *Bridelia scandens* A- *Bridelia scandens* collected from Bhadra Wild Life Sanctuary of Western Ghats, India. B-Callus induction from the excised lamina of *B. scandens* on MS media with 0.5 mg/L BAP and 0.5 mg/L 2, 4-D. C-Mass production of callus. D-Dried leaf calli mass of *B. scandens.*

Table 1: Qualitative and Quantitative analysis of *B. scandens* LCME.

	Qualitative test	Concentration µg/gm
Alkaloids	+	63.4 µg/gm
Flavonoids	+	56.9 µg/gm
Steroids	-	-
Terpenoids	-	-
Cardiac glycosides	-	-
Saponins	-	-
Tannins	-	-
Phenols	+	37.2 µg/gm

MCE – Methanol Callus extract, +: Present and -: Absent.

HR-LCMS analysis

HR-LCMS analysis of LCME resulted the presence of 100 phytoconstituents (Table 2) and the chromatogram of the phytoconstituents is shown in Figure 2. Among them the compounds azaperone, bifonazole, fusidic acid, lasalocid and quinine were known for antibacterial properties.

Antibacterial activity

The antibacterial activity of LCME was evaluated at the concentrations of 500, 1000 and 1500 µg/m1 of DMSO. 1500 µg/m1 concentration shows significant antibacterial property noticed against clinical pathogen strains *Staphylococcus aureus* (17.67±0.88 mm.d.), *Streptococcus pneumonia* (13.67±0.33), *Pseudomonas aeruginosa* (16.33±0.67), *Salmonella typhi* (17.67±0.33), and *Vibrio cholera* (15.33±0.33), as compared to the standard drug ciprofloxacin. The MIC assay was performed by modified resazurin assay the extract shows highest inhibitory activity against

Table 2: Phytoconstituents of leaf calli Methanol extract obtained from HR-LCMS.

Compound Label	RT	Mass	Formula	DB Diff (ppm)	Hits
Cpd 1: Trolamine	2.27	149.1039	$C_6 H_{15} N O_3$	8.74	1
Cpd 2: Diaminopimelic acid	4.767	190.0929	$C_7 H_{14} N_2 O_4$	12.97	15
Cpd 3: Trolamine	4.82	149.104	$C_6 H_{15} N O_3$	7.85	1
Cpd 4: METARAMINOL	4.82	167.0937	$C_9 H_{13} N O_2$	5.43	14
Cpd 5: Trp Tyr	7.109	367.1537	$C_{20} H_{21} N_3 O_4$	-1.3	15
Cpd 6: Trp Tyr	7.268	367.1537	$C_{20} H_{21} N_3 O_4$	-1.42	15
Cpd 7: 1,2-Benzenediol, 4- [[4-(4-fluorophenyl)-3-piperidinyl] methoxy]-, (3S- trans)	7.269	317.1424	$C_{18} H_{20} F N O_3$	0.9	15
Cpd 8: Ser Leu Met	7.269	349.1683	$C_{14} H_{27} N_3 O_5 S$	-3.25	15
Cpd 9: Asn Cys Ser	7.271	322.0974	$C_{10} H_{18} N_4 O_6 S$	-8.31	9
Cpd 10: 17-phenyl-trinor- PGF2alpha	7.59	388.2207	$C_{23} H_{32} O_5$	10.89	1
Cpd 11: 18-acetoxy- PGF2alpha-11-acetate	7.854	454.2561	$C_{24} H_{38} O_8$	1.3	7
Cpd 12: His Ile Trp	7.854	454.227	$C_{23} H_{30} N_6 O_4$	12.92	15
Cpd 13: N-(4- benzenesulfonamide) arachidonoyl amine	8.081	458.2605	$C_{26} H_{38} N_2 O_3 S$	-0.31	4
Cpd 14: 26,26,26,27,27,27- hexafluoro-1alpha,25- dihydroxyvitamin D3 /26,26,26,27,27,27-hexafluoro1alpha,25-	8.278	524.2677	$C_{27} H_{38} F_6 O_3$	9.26	3
Cpd 15: Tolbutamide	8.605	270.1058	$C_{12} H_{18} N_2 O_3 S$	-7.26	10
Cpd 16: Hydroxylysine	10.089	162.1008	$C_6 H_{14} N_2 O_3$	-2.28	5
Cpd 17: sebacic acid	10.089	202.1161	$C_{10} H_{18} O_4$	21.62	4
Cpd 18: Zolazepam	10.277	286.1242	$C_{15} H_{15} F N_4 O$	-4.4	15
Cpd 19: Val Asn Gly	10.445	288.143	$C_{11} H_{20} N_4 O_5$	1.4	12
Cpd 20: 8- cyclopentyltheophyllin	10.448	248.125	$C_{12} H_{16} N_4 O_2$	9.3	9
Cpd 21: Tolbutamide	10.451	270.1037	$C_{12} H_{18} N_2 O_3 S$	0.41	11
Cpd 22: Met Asn Gly	12.312	320.1119	$C_{11} H_{20} N_4 O_5 S$	11.16	14
Cpd 23: 6-Ketoestriol	12.312	302.1524	$C_{18} H_{22} O_4$	-2.04	15
Cpd 24: Laminaribiose	12.313	342.1198	$C_{12} H_{22} O_{11}$	-10.54	15
Cpd 25: 12.8	12.8	331.1328		1.23	15
Cpd 26: Hydroxythiopental	13.006	258.1066	$C_{11} H_{18} N_2 O_3 S$	-10.79	5
Cpd 27: Asn Asn Gly	13.077	303.116	$C_{10} H_{17} N_5 O_6$	6.35	6
Cpd 28: Methylergonovine	13.094	339.1978	$C_{20} H_{25} N_3 O_2$	-9.14	15
Cpd 29: Esmolol	13.364	295.1777	$C_{16} H_{25} N O_4$	2.17	15
Cpd 30: 13.607	13.607	186.1163			
Cpd 31: Methylergonovine	13.703	339.1976	$C_{20} H_{25} N_3 O_2$	-8.5	15
Cpd 32: Asp Val Glu	13.703	361.1529	$C_{14} H_{23} N_3 O_8$	-12.05	15
Cpd 33: EVOXINE	13.802	347.1403	$C_{18} H_{21} N O_6$	-9.96	15
Cpd 34: Leu Ser Glu	13.804	347.17	$C_{14} H_{25} N_3 O_7$	-2.08	15
Cpd 35: azaperone	13.988	327.1735	$C_{19} H_{22} F N_3 O$	3.57	15
Cpd 36: Glu Ala Ile	14.786	331.1721	$C_{14} H_{25} N_3 O_6$	6.71	15
Cpd 37: Cuscohygrine	14.884	224.1873	$C_{13} H_{24} N_2 O$	6.82	2
Cpd 38: Anandamide 0- phosphate	14.986	427.2497	$C_{22} H_{38} N O_5 P$	-2.12	15
Cpd 39: (E)-2- Methylglutaconic acid	15.639	144.0434	$C_6 H_8 O_4$	-7.86	8
Cpd 40: Arg Arg Glu	16.304	459.2509	$C_{17} H_{33} N_9 O_6$	9.77	13
Cpd 41: 2-[3-Carboxy-3- (methylammonio)propyl]-L- histidine	16.537	270.1321	$C_{11} H_{18} N_4 O_4$	2.44	4
Cpd 42: DEOXYGEDUNOL ACETATE	16.893	510.2715	$C_{30} H_{38} O_7$	-19	2
Cpd 43: 16.893	16.893	537.3174			
Cpd 44: GPCho(9:0/9:0)	16.894	538.357	$C_{26} H_{53} N O_8 P$	-11.35	11

Continued...

Table 2: Cont'd.

Cpd 45: GPGro(18:1(9E)/0:0) [U]	16.894	510.3029	$C_{24}H_{47}O_9P$	-13.98	3
Cpd 46: Enkephaline, (D- Ala)2-Leu	17.972	569.2743	$C_{29}H_{39}N_5O_7$	18.73	5
Cpd 47: Maltotriitol	17.973	506.1786	$C_{18}H_{34}O_{16}$	11.97	2
Cpd 48: 13-Hydroxypergolide glucuronide	17.978	506.2118	$C_{25}H_{34}N_2O_7S$	-6.15	3
Cpd 49: 3- Hydroxydodecanedioic acid	18.248	246.1438	$C_{12}H_{22}O_5$	11.8	3
Cpd 50: Isosorbide-2- glucuronide	18.561	322.0913	$C_{12}H_{18}O_{10}$	-4.2	9
Cpd 51: Doxapram	18.782	378.2307	$C_{24}H_{30}N_2O_2$	0.07	15
Cpd 52: BIFONAZOLE	19.072	310.1477	$C_{22}H_{18}N_2$	-2.29	4
Cpd 53: 4-Amino-6,7- dimethoxy-2-(1- piperazinyl) quinazoline	19.346	289.1545	$C_{14}H_{19}N_5O_2$	-2.28	7
Cpd 54: Met Tyr	19.347	312.1146	$C_{14}H_{20}N_2O_4S$	-0.76	5
Cpd 55: 3,5,3',5'-Tetra-tert- butyldiphenoquinone	19.752	408.3045	$C_{28}H_{40}O_2$	-4.14	3
Cpd 56: 3beta,6alpha,7alpha- Trihydroxy-5beta-cholan-24-oic Acid	19.758	408.2823	$C_{24}H_{40}O_5$	12.96	15
Cpd 57: 19.860	19.86	792.5443			
Cpd 58: GPIns(16:0/16:0)	19.861	810.5154	$C_{41}H_{79}O_{13}P$	12.92	1
Cpd 59: GPA (18:0/22:6(4Z,7Z,10Z,13	19.973	748.4841	$C_{43}H_{73}O_8P$	26.94	1
Cpd 60: 19.975	19.975	770.4228			
Cpd 61: Di- demethylcitalopram	20.067	296.1309	$C_{18}H_{17}FN_2O$	5.22	3
Cpd 62: GPGro(16:0/16:0) [U]	20.081	722.5023	$C_{38}H_{75}O_{10}P$	10.3	3
Cpd 63: 20.082	20.082	704.4151			
Cpd 64: GPEtn(18:0/18:1(11Z))	20.085	717.5187	$C_{39}H_{76}NO_8P$	16.89	13
Cpd 65: 20.087	20.087	704.4538			
Cpd 66: 20.203	20.203	682.4211			
Cpd 67: 20.206	20.206	677.453			
Cpd 68: 20.206	20.206	660.3938			
Cpd 69: 20.224	20.224	660.4271			
Cpd 70: Digitoxigenin bisdigitoxoside	20.322	634.3769	$C_{35}H_{54}O_{10}$	-8.22	1
Cpd 71: 20.322	20.322	616.438			
Cpd 72: PHORBOL MYRISTATE ACETATE	20.325	616.4027	$C_{36}H_{56}O_8$	-8.36	3
Cpd 73: C16 Sphinganine	20.378	273.2637	$C_{16}H_{35}NO_2$	11.38	1
Cpd 74: Coenzyme Q6	20.446	590.421	$C_{39}H_{58}O_4$	21.18	1
Cpd 75: LASALOCID	20.447	590.3863	$C_{34}H_{54}O_8$	-7.52	2
Cpd 76: 16-Glutaryloxy-1alpha,25-dihydroxyvitamin D3	20.563	546.3598	$C_{32}H_{50}O_7$	-7.66	2
Cpd 77: (Z)-2-hexacos-17- enamidoethanesulfonic acid	20.879	501.3738	$C_{28}H_{55}NO_4S$	22.76	1
Cpd 78: 20.918	20.918	147.9941			
Cpd 79: O- Desmethylquinidine	20.938	310.1695	$C_{19}H_{22}N_2O_2$	-4.4	15
Cpd 80: 21.060	21.06	315.273			
Cpd 81: GPGro(14:0/14:0) [U]	21.231	666.4367	$C_{34}H_{67}O_{10}P$	15.69	4
Cpd 82: 1,25- ihydroxyvitamin D3 3-	21.486	578.3861	$C_{33}H_{54}O_8$	-7.32	3
Cpd 83: Fusidic acid	21.629	516.3489	$C_{31}H_{48}O_6$	-7.36	2
Cpd 84: Quinine	21.691	324.1852	$C_{20}H_{24}N_2O_2$	-4.47	15
Cpd 85: 1alpha,25-dihydroxy 2alpha-(3- hydroxypropoxy) vitamin D3	21.754	490.3671	$C_{30}H_{50}O_5$	-2.59	14
Cpd 86: 25-hydroxy-1beta- hydroxymethyl-26,27- dimethyl-24a-homo-22,23,24,24a-tetradehydro 3- epivitamin D3 / 2	21.759	468.3547	$C_{31}H_{48}O_3$	11.96	15

Continued...

Table 2: **Cont'd.**

Cpd 87: 26,27-dinor-3alpha,6alpha,12alpha- trihydroxy-5beta-cholestan-24-one	21.891	406.318	$C_{25}H_{42}O_4$	-23.8	15
Cpd 88: 1alpha,25-dihydroxy 26,27-dimethyl-20,21- didehydro-23-oxavitamin D3 / 1alpha,25-dihydroxy-26,27- dime	21.891	446.3393	$C_{28}H_{46}O_4$	0.78	15
Cpd 89: C17 Sphinganine-1- phosphate	21.975	384.279	$C_{17}H_{41}N_2O_5P$	-9.62	2
Cpd 90: 11beta-PGF2alpha- d4	22.094	358.2641	$C_{20}H_{30}D_4O_5$	4.42	15
Cpd 91: Anandamide (18:3, n-6)	22.169	321.2707	$C_{20}H_{35}NO_2$	-12.18	12
Cpd 92: 22.637	22.637	438.3662			
Cpd 93: 27-nor-5b- cholestane-3a,7a,12a,24,25- pentol	22.638	438.3382	$C_{26}H_{46}O_5$	-8.28	6
Cpd 94: Gln Ile Thr	23.45	360.1987	$C_{15}H_{28}N_4O_6$	6.08	15
Cpd 95: Nuatigenin	24.891	430.315	$C_{27}H_{42}O_4$	-15.66	15
Cpd 96: Testosterone isocaproate	25.059	386.292	$C_{25}H_{38}O_3$	-25.63	1
Cpd 97: 27.402	27.402	337.3252			
Cpd 98: 27.515	27.515	352.9191			
Cpd 99: Procainamide	27.586	235.1663	$C_{13}H_{21}N_3O$	9.4	1
Cpd 100: 27.594	27.594	524.5037			

Table 3: **Zone of inhibition and MIC values of LCME against pathogenic bacterial strains.**

SL. No	Inhibition zone diameter (mm) and MIC (mg/ml^{-1})				
	Microorganism	ZI of LCME (1500 µg/well)	Activity index	MIC	ZI of Ciprofloxacin (20 µg/well)	MIC
1	*S.aureus*	17.67±0.88	0.697	$1.10±0.15×10^{-2}$	25.33±0.67	$3.16±0.10×10^{-3}$
2	*S.pneumoniae*	13.67±0.33	0.494	$7.12±0.01×10^{-2}$	27.67±0.33	$3.16±0.10×10^{-3}$
3	*V.cholerae*	15.33±0.33	0.605	$3.10±0.15×10^{-2}$	25.33±0.88	$5.82±0.10×10^{-3}$
4	*P.aeruginosa*	16.33±0.67	0.620	$6.30±0.10×10^{-2}$	26.33±0.33	$0.60±0.30×10^{-3}$
5	*S.typhi*	17.67±0.33	0.757	$1.20±0.01×10^{-2}$	24.33±0.33	$1.56±0.10×10^{-3}$

Values are mean±standard error (n=3) of three different samples, analyzed individually in triplicate, ZI, the diameter of inhibition zone (mm) including well diameter of 6 mm, MIC, minimum inhibitory concentration (mg/ml). AI (Activity Index) = ZI of Test/ZI of Standard.

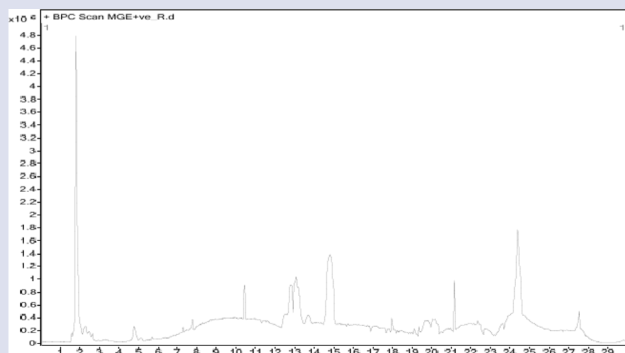

Figure 2: HR-LCMS Chromatograph of *B. scandens* leaf calli methanol extract

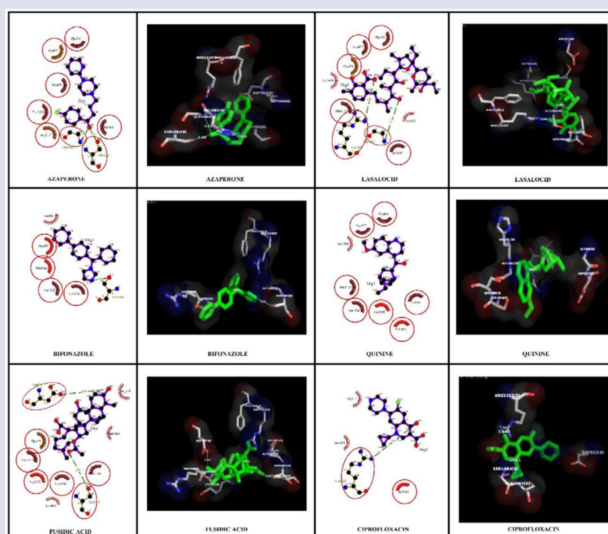

Figure 3: 2D and 3D protein-ligand interaction DNA gyrase with the ligands azaperone bifonazole, fusidic acid and lasalocid.

Table 4: ***In silico*** **ADMET and drug likeness prediction using data warrior.**

Compounds	cLogP	CLogS	H-Acceptors	H-DONORS	TPSA	Drug likeness
Azaperone	2.76	-3.42	4	0	36.44	5.409
Bifonazole	4.757	-6.844	2	0	17.82	2.0429
Fusidic acid	5.823	-5.363	6	3	104.06	0.050
Lasalocid	5.363	-6.118	8	4	133.52	2.5589
Quinine	2.61	-3.09	4	1	45.59	0.878

Table 5: Molecular docking values of LCME compounds obtained from LCMS analysis.

LIGAND	AFFINITY (kcal/mol)	H-BONDS	H-BOND LENGTH (Å°)	H-BOND WITH	HYDROPHOBIC INTERACTIONS
azaperone	-4.4	2	2.98 3.11	2XCT:Ser1085::AZA:OAB 2XCT:Gly1082::AZA:OAB	Gly436, Asp437, Gly459, His1081, Arg1122, Phe1123
bifonazole	-4.3	1	3.18	2XCT:Ser1084::BIF:NAB	Arg458, Gly459, Gly1082,Arg1122, Phe1123
fusidic acid	-4.7	2	2.89 2.91	2XCT:Asp437::FUS:OAC 2XCT:Ser1085::FUS:OAF	Arg458,Gly459, Lys460, His1081, Gly1082, Ser1084, Arg1122,Phe1123,
lasalocid	-4.9	2	2.86 3.09	2XCT:Arg1122::LAS:OAC 2XCT:Gly1082::LAS:OAE	Gly436, Asp437, Gly459, Asp512, Ser1084, Ser1085, Phe1123,
quinine	-4.6	-	-	-	Cys300, Gly301, Leu484, Glu488, Leu601, Ala602, Lys603, Ser604, Val605
ciprofloxacin	-4.4	1		2XCT:Arg1122::CIP:OAQ	Asp512, Ser1084, Ser1085

S.aureus with a significant MIC value of $1.10\pm0.15\times10^{-2}$. Inhibition of bacterial strains are summarized in Table 3.

Toxicity prediction

Result of pharmacokinetic properties and toxicity analysis of 5 compounds (azaperone, bifonazole, fusidic acid, lasalocid and quinine) identified by HR-LCMS is shown in Table 4. All the 5 compounds obey the Lipinski's 'Rule of 5 limit better LogS values and were free from mutagenic tumorigenic, reproductive and irritant effect. In general, a poor solubility is associated with bad absorption and the aqueous solubility (Log S) of the compound which significantly affects its absorption and distribution characteristics. Based on the results from the DataWarrior, LogP, better LogS, good drug score and less toxicity risk parameters are predicted as shown in the Table 4.

Molecular Docking

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 HR-LCMS identified ligand molecules- azaperone, bifonazole, fusidic acid, lasalocid and quinine with bacterial enzyme DNA gyrase is shown in Figure 3. Among them the compound lasalocid exhibited better docking efficiency with DNA gyrase. It forms two hydrogen bonds with amino acids Arg1122, and Gly1082 in the active site of the target protein with bond length 2.86 and 3.09 respectively, with the least binding affinity -4.9 and hence is considered as the best dock conformation (Table 5). Compound azaperone forms two hydrogen bonds with Gly1082 and Ser1085 amino acids with bond length 3.11 and 2.98 Å. The compound bifonazole forms only one hydrogen bond with the amino acid Ser108 with bond length 3.18 Å and the compound Fusidic acid forms two hydrogen bonding with Asp437 and Ser1085 with bond length 2.89 and 2.91 Å respectively. While compound quinine doesn't form hydrogen bond with the aminoacids of the active pocket. However, all these docked molecules exhibited more hydrophobic interaction than the standard drug ciprofloxacin. The RMSD has often been used to measure the quality of reproduction of a known binding pose by molecules with ligands. All docked molecules have zero RMSD values as shown in the Table 5.

DISCUSSION

Medicinal plants have been used as a source of medicine in all cultures since times immemorial.[18] Even though World Health Organization reported that the primary health care system for the 60% population of the world is represented by the traditional medicines yet a great number of plant species with potential biological activities were unexplored.[19] The extracts of several medicinal plants are very effective against microbial as well as parasitic infections.[20] Although synthetic or chemical drugs as compared to herbal medicines can have greater or quicker effects, but they possess many adverse effects and risks.[21] Herbal medicines are generally less expensive as compared to synthetic ones.

The continuous exploitation of several medicinal plant species from the wild.[22] and substantial loss of their habitats during past 15 years have resulted in population decline of many high value medicinal plant species over the years. The primary threats to medicinal plants are those that affect any kind of biodiversity used by humans.[23,24] Attempts are

being made by different organizations to cultivate various medicinal plant species, including rare and endangered categories.[25] Evidence that plant cell cultures are able to produce secondary metabolites came quite late in the history of *in vitro* techniques.[26]

Callus culture is very useful to obtain commercially important secondary metabolites or drugs can be directly extracted from the callus tissues without scarifying the whole plant. In the present study, HR-LCMS analysis of LCME showed the presence of various compounds. Among them the compounds azaperone, bifonazole, fusidic acid, lasalocid and quinine are reported as good antibacterial agents[27-30] As compared to *in vivo* plant parts the *de novo* synthesis of secondary metabolites takes place in the *in vitro* derived calli due to the influence of hormones supplemented in the media. The standardized technique can be explored commercially for the mass production of compounds.

S. aureus bacteremia is a significant cause of morbidity and mortality in neutropenic patients with cancer.[31] In the present study LCME of *B. scandens* exhibited significant inhibitory effect on both gram positive *Staphylococcus aureus, Streptococcus pneumoniae. Pseudomonas aeruginosa* and gram negative *Salmonella typhi*, *Vibrio cholera*, strains which causes pneumonia (lung infection), osteomyelitis (bone infection), endocarditis (heart infection), phlebitis (infection of veins and blood vessels), mastitis (infection of breast and formation of abscesses) and meningitis (brain infections).in humans. Previous investigator Adeeba Anjum.[32] evaluated the antibacterial property of *B. scandens* leaf and stem bark against 13 bacterial clinical isolates both gram positive (*Bacillius cereus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea, Staphylococcus aereus*) and gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibro meniscus, Vibrio parahaemolyticus*) The methanol extract of the leaf exhibited the highest activity against *S. lutea* of 21.6mm and for *S. aereus* 19.1mm ZI. LCME shows 17.6mm ZI. The antibacterial property of LCME is due the cumulative effect of the compounds azaperone, bifonazole, fusidic acid, lasalocid and quinine and it was supported by molecular docking studies. The *in silico* docking of lasalocid with the DNA gyrase showed higher binding affinity as well as hydrogen bonding and good hydrophobic interaction with the receptor. Among these 5 ligands lasalocid showed highest binding affinity and hydrophobic interaction with the amino acids of the active pocket. 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 cleave and religate DNA to regulate DNA topology and are a major class of antibacterial and anticancer drug targets.[33] The 5 ligand molecules exhibited the antibacterial activity by hindering the function of DNA gyrase.

CONCLUSION

Leaf calli methanol extract of *B. scandens* contains good antibacterial compounds azaperone, bifonazole, fusidic acid, lasalocid and quinine. The antibacterial activity was more significant against *S. aureus*. *In silico* docking studies also supported the inhibition of DNA gyras with highest bonding efficiency and hydropobic interaction. Due to unscientific over exploitation many of the medicinal are becoming endangered. The harvesting of antibacterial compunds from the *in vitro* grown leaf calli of *Bridelia scandens* is a better method to combat contagious microbial diseases.

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CONFLICT OF INTEREST

No Conflict of Interest.

ABBREVIATIONS

LCME: Leaf callus methanol Extract; **BAP:** 6-Benzylaminopurine; **2.4-D:** 2,4-Dichlorophenoxyacetic acid; **MS:** Murashige and Skoog; **DMSO:** Dimethyl sulfoxide; **RMSD:** Root Mean Square Deviation; **ZI:** Zone of Inhibition; **MIC:** Minimum Inhibitory Concentration; **HR-LCMS-:** High Resolution Liquid Chromatograph Mass Spectrometer; **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity.

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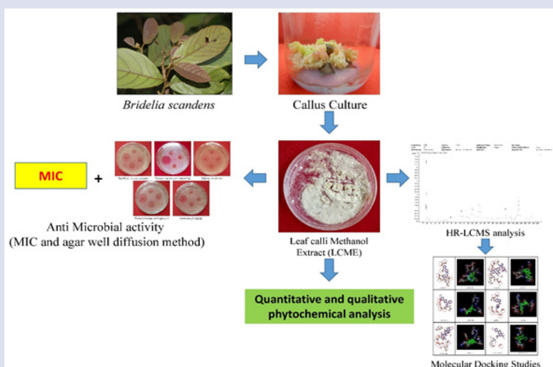
GRAPHICAL ABSTRACT



SUMMARY

- Evaluation of antibacterial activity and molecular docking studies of *B. scandens* leaf Calli extract was performed.
- LCME show significant antibacterial activity against selected human clinical pathogens.
- And the molecular docking shows phyto components obtained from the LCME shows good inhibition against bacterial DNA gyrase.
- The present study shows that the LCME is a good antibacterial agent against human clinical pathogen.

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