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ORIGINAL ARTICLE

Phytochemical Screening and Evaluating Antimicrobial Potentials of Bioactive Compounds Derived from *Phyllanthus niruri* Against Multidrug Resistant Bacteria

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ABSTRACT
Multidrug resistance looming as an "imminent pandemic" posing a grave threat to therapeutic strategies and limiting the effective life span of conventional antibiotics. Medicinal plants are the source of diverse and structurally complex bioactive compounds that plays a vital role in drug development. Plant extract and their bioactive compounds poses antimicrobial and antibiofilm properties paving a way towards the innovation of novel antimicrobial agent alternative to commercial drugs. The presents study deals with the phytochemical screening of the methanolic leaf extract of the Phyllanthus niruri to identify the hidden treasure of bioactive compounds using HRMS and FTIR analysis along with their antimicrobial and antibiofilm activities against the clinically isolated pathogenic strains. This study reveals the existence of two prime biologically active compounds i.e Lintetralin and Mupirocin in the methanolic leaf extract of P. niruri. In vitro antimicrobial assay of leaf extract, Lintetralin and Mupirocin showed significant antibacterial activities against P. mirabilis and E. faecalis, with Lintetralin exhibited relatively reduced antibacterial activities compared other two. Methanolic leaf extract of P. niruri as well as bioactive components at sub-MIC level significantly mitigated the biofilm formation in the pathogenic strains. Mupirocin showed relatively higher inhibitory effect towards biofilm formation in P. mirabilis (63.37 ± 4.58) % and E. faecalis (67.76 ± 6.52) % compared to Lintetralin. Overall, the leaf extract of P. niruri with novel bioactive molecules have varying degree of antibacterial and antibiofilm potency that might be used to develop novel antimicrobial compound to fight against the multidrug resistance infections.
Keywords: *Antibioflim, Bioactive, Lintetralin, Mupirocin, Phyllanthus niruri.*

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INTRODUCTION

Multidrug resistance is continuously evolving and looming as an "imminent pandemic" posing a grave threat to mankind, complicating therapeutic strategies and rendering treatment more difficult [1]. Increased multidrug resistance leads to higher rates of mortality, morbidity, health care costs and failure of conventional antimicrobial agents [2]. As a result, multidrug resistance demands research of new pipeline for the introduction of newer and alternative antimicrobial agents to combat MDR infections [3]. Research aimed at developing novel pharmaceutical formulations incorporates medicinal plants as crucial components. The exploration of medicinal plant is steadily rising for the creation of natural and alternative medicines. Medicinal plants are not only the source of potential antimicrobial crude drugs but also produces natural products that act as new anti-infection agents in modern pharmacotherapy [4]. Natural products are highly diverse and structurally complex that plays a vital role in drug development, especially for infectious diseases, cancer, cardiovascular disease and multiple sclerosis [5,6]. Further, plant derived bioactive compounds have great therapeutic significance due to their high antimicrobial and antibiofilm activities with minimal side effects that can serve as a novel template for the development of antimicrobial agents against the pathogenic strains [7]. *Phyllanthus* is the largest genera of within the

Phyllanthaceae family, with an impressive diversity of 1,314 species [8]. *Phyllanthus* has drawn plenty of curiosity due to a multitude of variables. Firstly, its widespread distribution throughout tropical and subtropical areas globally [9]. Secondly, the large number and diverse *Phyllanthus* species offers a vast pool of genetic diversity, that underscore its significance in botanical studies and its potential application in various sector like therapeutics, ecology and horticulture. Lastly, the greater variety of specialized metabolites present in plants, provides opportunities for discovering novel and potent bioactive compounds for new pharmaceuticals and medical interventions [8]. As a consequence, *in vivo* and *in vitro* studies, along with clinical investigations have made great progress in uncovering the chemical and pharmacological properties of distinct *Phyllanthus* species [8]. Different *Phyllanthus* species (such as *P. niruri, P. amarus, P. fraternus, P. emblica, P. debilis*) have been used for the effective remedies of hepatopathy, hypertension, fever, diabetes and regarded as bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic [10, 11]. Different parts (fruit, bark, leaves, stem, seeds, root) are ethnobotanically reported to have therapeutic activities [12]. Out of these, *Phyllanthus niruri* is a rich repertoire of phytochemicals which have significant pharmacological properties an [11, 13]. The diverse phytochemicals isolated and identified from *P. niruri* includes flavonoids, terpenoids, alkaloids, lignans, tannins, polyphenols, coumarins and saponins. The flavonoids (quercein, catechin, rutin) are the major components identified in *P. niruri* and reported to have beneficial activities like anti-inflammatory, antimutagenic, antileishmanial and antiviral activities [14]. Further, the derived bioactive lignans (lintetralin, phyltetralin, phyllanthin, niranthin) proven to have intriguing therapeutic properties like anticancer, anti-HIV, anti-hepatitis B, antioxidant, anti-lithic, antimutagenic and antimicrobial [8, 14] and saponins exhibit wound healing effect [9]. Indeed, in-depth studies pertaining to bioactive phytochemicals found in *P. niruri* holds great promise for advancing research in various field including medicinal sector, since several phytochemicals have demonstrated preclinical therapeutic efficacies for a wide range of infectious diseases. Keeping in view, the present research deals with identification and exploration bioactive metabolites derived from methanolic leaf extract of *P. niruri* followed by their characterization by FTIR (Fourier transform infrared spectroscopy) and identification through high resolution mass spectroscopy (HRMS). Further, the efficacy evaluation of identified phytochemicals was determined based on the antimicrobial activities and quantitative as well as qualitative antibiofilm assays. The proposed study can be valuable to explore natural and novel antimicrobial agents as an alternative to conventional antibiotics especially for combating MDR mediated infections.

MATERIALS AND METHODS

Collection and preparation of plant extract

The leaf samples of *Phyllanthus niruri* (Family: *Euphorbiaceae*) was gathered from different regions of Gandhamardhan hill range (Geographic location: 20°42'-21°00' North latitude and 82°41'-83°05' East longitude), situated in Bargarh and Bolangir districts, Odisha, India. Leaves were chopped into pieces, dried and ground to fineness. About 500 gm of leaf sample was soaked into methanol for 72 hours at room temperature. Then, the extract was subjected to filtration followed by rota-evaporation, air dried and stored at 4°C for further analysis.

High resolution mass spectroscopy analysis

About 50 mg of the methanolic leaf extract of *P. niruri* was weighed and 25 ml of methanol was added and sonicated for 30 min. About 5 ml solution was diluted to 20 ml with methanol and filtered using 0.22 µm PTFE syringe filter. The analysis was performed using Xevo G3 QToF Waters Corporation with Acquity UPLC I Class Plus and MassLynx software and processed using Progenesis QI software. Separation was done using Acquity UPLC HSS T3 column (100 x 2.1 mm, 1.8 µm). Column was maintained at 40°C and sample was kept at 15°C during analysis. Basic working parameters includes: ionization type: ESI; mode: MSE; acquisition time: 25 min; mass range (50–1200) *m/z*; low collision energy 6 eV; high collision energy (10-40) eV ramp (Positive mode) and 10-30 eV ramp (Negative mode); cone voltage: 40 V (Positive mode) and 30 V (Negative mode); capillary voltage: 3.0 kV (Positive mode) and 2.5 kV (Negative mode); source temperature 130°C; desolvation temperature 500°C; cone gas flow 50 L/h; desolvation gas flow 750 L/h. Mass was corrected by external reference (Lock–spray) consisting of 200 pg/mL solution of leucine enkephalin (Waters Corporation , USA) infused at a flow rate of 10 µL/min *via* a lock–spray interface called Zspray, generating the reference ion for the positive ion mode [(M+H) *m/z* 556.2771] and negative ion mode [(M-H) *m/z* 554.2615] to ensure mass correction during mass spectrometry analysis. Lock–spray scan time was set at 0.5 s with an interval of 10 s. Elution was carried out in positive mode [ES+] at a flow rate of 0.4 ml/min using gradient mobile phase, 0.1% formic acid in water (solvent A), and 0.1% formic acid in acetonitrile (solvent B). Volume ratio of solvent-B was changed as follows: 5% solvent-B for (0-1) min, (5-25)% solvent-B for (1–5) min, (25–35)% solvent-B for (5–8) min, (35–45)%

solvent-B for (8–11) min, (45–55)% B for (11–14) min, (55–90)% solvent-B for (14–20) min, (90–95)% solvent-B for (20–20.1) min and 5% solvent-B for (20.1–25) min. Then, 5 µl of test solution was injected for screening and chromatographs were recorded for 25 min. Elution was carried out in negative mode [ES-] at a rate of 0.4 ml/min using gradient mobile phase, 1mM ammonium formate in water (solvent-A) and acetonitrile (solvent-B). Similarly, 3 µl of test solution was injected for phytochemical screening in negative mode and chromatographs were recorded for 25 min. Molecules were ionized by electrospray ionization at 3eV potential using nitrogen gas and run for 15 min and then ions were detected both in positive and negative polarity. Mass peak intensities were examined against five standard compounds within specified mass range of (50-500) m/z to translate peak intensities into concentration. Mass peaks were annotated using python in-house program by parsing with PubChem library with error range of (± 0.01) m/z.

Fourier transformed infrared spectroscopy (FTIR) analysis

Methanolic leaf extract of *Phyllanthus niruri* was analyzed by FTIR analysis to determine the existence of functional groups solely based on the absorption precisely corresponding to the bonds within molecule. Pellet was prepared using solid powder sample using hydraulic press, introduced into the forced-air oven and dried at 60°C for 96 hr, which were subsequently tested in permeation mode compared to KBr pellets (used as blank) within spectral range (400-4000) cm^{-1} using Bruker (Alpha II). Resolution was set at 4 cm^{-1} with KBr beam splitter and DTGS detector using HGTR assembly.

Microbes and culture media

The clinically isolated bacterial strains, one gram-negative (*Proteus mirabilis*) and one gram positive (*Enterococcus faecalis*) were used in screening the antimicrobial activity of *P. niruri*. Their reference strains (MTCC 425 and MTCC 439) were also procured from Chandigarh. All the microbes were maintained using nutrient agar (Hi-Media).

Antibacterial activity

Minimum inhibitory concentration (MIC)

MIC value of methanolic leaf extract of *P. niruri* and derived compounds (Lintetralin and Mupirocin) were determined by microdilution method against bacterial strains (*P. mirabilis* and *E. faecalis*) and their reference strains following CLSI guidelines (CLSI, 2011). Exponential growth phase culture in Muller-Hinton (MH) broth were serially diluted to obtain 0.5 McFar`land equivalents. Serially diluted samples [Concentration range: (3-0.023) % in 2-fold dilution for methanolic leaf extract of *P. niruri*; (30-0.234) mM in 2-fold dilution for Lintetralin; and (30-0.234) mM in 2-fold dilution for Mupirocin)] were prepared using MH broth followed by the inoculation of bacteria and incubated at 37°C for (14-16) hrs. Bacterial culture without sample was taken as negative control. About 5 µl aliquot of 0.125% TTC (2,3,5-triphenyltetrazolium chloride) was added to each well of 280exadecane plate and incubated at 37°C for 15 min. Development of pink colour implies bacterial growth, whereas the absence of colour indicates growth inhibition [15]. The experiments were conducted in triplicates to validate the findings.

Well diffusion assay

Antibacterial activities of methanolic leaf extract of *P. niruri* and bioactive compounds (Lintetralin and Mupirocin) were determined by well diffusion assay against bacterial strains (*P. mirabilis* and *E. faecalis*) and their reference strains following CLSI guidelines (CLSI, 2011). For the purpose, overnight bacterial strains were swabbed on MH agar followed by preparation of 8 mm wells. Then, 100 µl aliquots of samples at MIC value were added to wells, incubated at 37°C for 24 hrs and diameter of zones of inhibition were measured and recorded [16]. The experiments were conducted in triplicates to validate the findings.

Biofilm formation assay

Qualitative methods

Congo red agar (CRA) method, a qualitative approach used for detection of biofilm producing microbes that relies on the colour change of bacterial colonies inoculated on CRA medium was performed [17]. Bacterial culture treated with sub-MIC level of methanolic leaf extract of *P. niruri*, and bioactive compounds (Lintetralin and Mupirocin) were streaked onto Congo red agar with following media supplements: 0.8 mg/ml congo red dye (w/v)); sterile medium brain heart infusion (BHI) agar (37 mg/ml), sucrose (50 mg/ml) and agar (10 mg/ml). CRA plates were incubated aerobically at 37°C for (24-48) hr for colony development. Bacterial culture without treatment was regarded as the positive control.

Quantitative methods

Polystyrene based 24-MTP (microtiter plate) was used for quantitative biofilm formation assay. Bacterial culture was inoculated into MH broth supplemented with or without sub-MIC level of the methanolic leaf extract of *P. niruri* and bioactive compounds (Lintetralin and Mupirocin) and subjected to incubation at 37°C for 24 hrs. Thereafter, culture media was discarded and biofilm matrix attached to wall was washed

twice for complete separation of cell debris and air dried. The attached biofilm matrix was stained with 0.1% crystal violet (w/v) for 20 mins followed by washing stained biofilm matrix twice with PBS buffer to discharge excessive strain and air dried. Subsequently, biofilms lining the wall of microplate was resolubilized with 95% ethanol and the absorbance was measured at 540 nm using microplate reader [18].

RESULTS

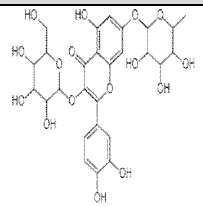
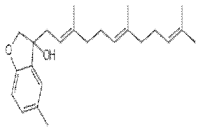
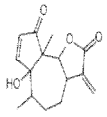
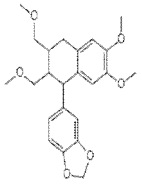
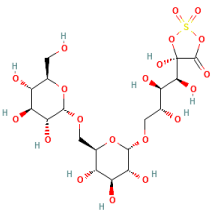
Phytochemical screening of methanolic leaf extract of *P. niruri* by HRMS revealed the occurrence of 10 bioactive natural compounds of therapeutic importance like petiolaroside, bufadienolide, parthenicin, lintetralin, dextransulfat, portulacaxanthin III, borreline, hexadecatrienoic acid, rosmarinic acid and mupirocine (Table 1). Details of the identified compounds along with chemical profiling including retention time, molecular formula, neutral mass (Da), mode, molecular structure and fragmentation (m/z) were collated (Table 1). Plant derived phytochemicals were identified based on fragmentation patterns, total ion chromatogram in positive mode and negative mode (Figure 1 and 2), combined spectra of ion chromatogram, and direct comparison with chemical profiles using NIST library and published mass spectra. Further, HRMS analysis reveals two prime biologically active compounds (Lintetralin and Mupirocin) present in methanolic leaf extract of *P. niruri* which may be responsible for effective antimicrobial activities. FTIR analysis of methanolic leaf extract of *P. niruri* identifies the existence of different chemical moieties with characteristic IR fingerprints ranging from 3278 cm^{-1} to 1029 cm^{-1} (Table 2). This study depicts the presence of different function groups of bioactive constituents through the FTIR spectrum (Figure 3). MIC value of methanolic leaf extract of *P. niruri* and two bioactive phytochemicals (Lintetralin and Mupirocin) were determined. Eight different concentrations of samples were added with culture medium individually against bacterial isolates (*P. mirabilis* and *E. faecalis*) and their reference strains (MTCC 425 and MTCC 439). MIC value of methanolic leaf extract of *P. niruri* was found to be 3% against clinical isolates (*P. mirabilis* and *E. faecalis*) and their reference strains (Figure 4a). Besides, MIC of lintetralin was found to be 30 mM against bacterial isolates and their reference strains of *E. faecalis* and *P. mirabilis* (Figure 4b). Further, MIC exhibited by mupirocin against *P. mirabilis* and its reference strain was found to be 25 mM, whereas 30 mM against *E. faecalis* and its reference strain (Figure 4c). Well diffusion assay of methanolic leaf extract of *P. niruri* exhibited strong antibacterial activities against *P. mirabilis* and its reference strain (MTCC 425) with zone of inhibition of 17 mm and 21 mm at the MIC level respectively. Similarly, leaf extract showed clear zone of inhibition of 15 mm and 18 mm at MIC level against *E. faecalis* and its reference strain (MTCC 439). Lintetralin exhibited relatively reduced antibacterial activities compared to methanolic leaf extract of *P. niruri* and mupirocin against the tested bacterial strains. Besides, study evident that mupirocin showed promising antibacterial properties at the MIC level with zone of inhibition 16 mm and 19 mm against the reference strains MTCC 425 and MTCC 439 respectively. CRA method used for biofilm formation assay revealed the formation of crystalline black colonies in untreated strains of *P. mirabilis* and *E. faecalis* and their reference strains, which indicated profuse production of exopolysaccharide matrix for biofilm production (Figure 5). In contrast, there was significant decline in biofilm matrix formation in clinically strains and their reference when treated with sub-MICs level of the methanolic leaf extract, lintetralin and mupirocin, which indicates their ability to inhibit the biofilm formation in the pathogenic strains (Figure 5). Decline in exopolysaccharide matrix production indicates the antibiofilm activities of the plant samples. Quantitative biofilm formation assay based on crystal violet staining revealed significant inhibition in biofilm formation in both clinical strains (*P. mirabilis* and *E. faecalis*) and their reference strains (MTCC 425 and MTCC 439). The study suggested that bioactive compounds (Lintetralin and Mupirocin) showed relatively higher inhibitory effects on biofilm formation compared to methanolic leaf extract of *P. niruri* (Figure 6). Relatively higher inhibitory effect was exhibited by mupirocin towards biofilm formation in *P. mirabilis* (63.37 ± 4.58) % and its reference strain MTCC 425 (65.51 ± 5.23) % respectively (Figure 5a). Besides, mupirocin also exhibited relatively higher inhibitory effects on biofilm formation in *E. faecalis* (67.76 ± 6.52) % and its reference strain MTCC 439 (71.56 ± 6.37) % respectively (Figure 6b).

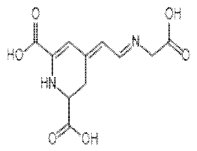
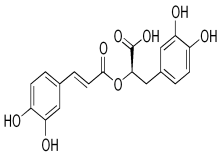
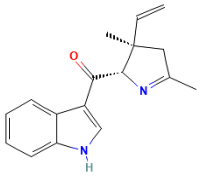
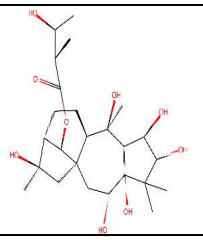
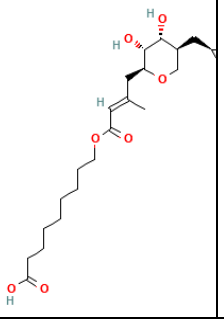
DISCUSSION

Medicinal plants used as folk herbal medicines are the source of new biologically active compounds [19]. *Phyllanthus species* has extensive medicinal properties and has long history in the health care sector. Researcher have reported *Phyllanthus niruri* to have diverse pharmacological activities like antibacterial, anti-inflammatory, anti-fungal, antiviral, anti-plasmodial, anticancer and anti-oxidant [20, 21]. The phytochemical analysis indicated the existence of diverse array of bioactive secondary metabolites including Lintetralin and Mupirocin in the methanolic extract of *P. niruri* by FTIR and HRMS analysis [9,

22, 25]. The *in vitro* study reveals antibacterial activities of the plant extract and bioactive metabolites that are effective against the pathogenic multidrug resistant clinical bacteria and have been previously reported [23, 24]. Besides, researcher reported *P. niruri* methanolic leaf extract as potent antibacterial agents inhibiting tested gram positive and gram-negative bacteria [26]. Further, the study also indicates the antibiofilm activities of the methanolic leaf extract, Lintetralin and Mupirocin that significantly mitigates the formation of the exopolysaccharide matrix in the pathogenic clinical strains and holds the promise to destroy the biofilm formation in the pathogens. Moreover, the pure bioactive compounds individually showed efficient antimicrobial and antibiofilm activities and might be considered as natural antibiotics for developing new therapies [27].

.Table 1. Bioactive phytochemical constituents identified in the methanolic leaf extract of *Phyllanthus niruri* through HRMS analysis.

Sl.	Name of the compound	R.T. (min)	M.F	m/z	N. mass (Da)	Mode	Structure	Fragmentation *(m/z)*
1.	2-(3,4-dihydroxyphenyl)-5-hydroxy-3-{[(2s,5s)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-7-{[(2s,4s,5r)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy}chromen-4-one **(Petiolaroside)**	5.17	$C_{27}H_{30}O_{16}$	611.1633	610.1561	+ve		$[C_{27}H_{30}O_{16}]^{+H}$, 465.1028, 303.0475, 85.0285, 71.0492
2.	5-methyl-3-(3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-2h-1-benzofuran-3-ol **(Bufadienolide)**	15.91	$C_{24}H_{34}O_2$	355.2634	354.2562	+ve		$[C_{24}H_{34}O_2]^{+H}$, 217.1224, 203.1067, 177.0910, 165.0910, 151.0754, 147.0805, 131.0492, 107.0492, 103.0543, 91.0543
3	[3aS-(3aalpha,9aalpha,9bbeta)]-3,3a,4,5,9a,9b-Hexahydro-9a-hydroxy-6,9-dimethyl-3-methylene-azuleno[4,5-b]furan-2,7-dione **(Parthenicin)**	16.56	$C_{15}H_{18}O_4$	263.2879	262.2808	+ve		$[C15H18O4]^{+H}$, 243.1016, 231.1016, 229.0859, 216.0625, 201.0911, 187.0754, 173.0961, 158.0726, 156.0570, 145.0648
4.	5-[(1R,2S,3S)-6,7-dimethoxy-2,3-bis(methoxymethyl)-1,2,3,4-tetrahydronaphthalen-1-yl]-1,3-benzodioxole **(Lintetralin)**	16.86	$C_{23}H_{28}O_6$	401.1971	400.1899	+ve		$[C23H28O6]^{+H}$, 369.1697, 354.1462, 193.0859, 151.0454, 137.0597
5.	(2R,3R,4S)-2,3,4-Trihydroxy-4-[(4R)-4-hydroxy-2,2-dioxido-5-oxo-1,3,2-dioxathiolan-4-yl] butyl 6-O-alpha-D-glucopyranosyl-alpha-D-glucopyranoside **(Dextransulfat)**	4.15	$C_{18}H_{30}O_{20}S$	633.0772	598.0792	-ve		$[C_{18}H_{30}O_{20}S]^{+Cl}$, 463.0399, 300.9871, 272.9921

6.	(2s,4z)-4-{2-[(carboxymethyl)imino]ethylidene}-2,3-dihydro-1h-pyridine-2,6-dicarboxylic acid **(Portulacaxanthin III)**	4.54	$C_{11}H_{12}N_2O_6$	305.0171	268.0685	-ve		$[C_{11}H_{12}N_2O_6]^{-2H+K}$, 272.9919, 244.9970, 217.0021
7.	2-(3,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-4-oxo-2,3-dihydro-1-benzopyran-3-yl acetate **(Rosmarinic acid)**	5.87	$C_{18}H_{16}O_8$	395.0526	360.0546	-ve		$[C_{18}H_{16}O_8]^{+Cl}$, 363.0277, 319.0379
8.	14-ethylidene-1,10-diazatetracyclo[11.2.2.0Â³,Â¹Â¹.0â´,â¹] heptadeca-3(11),4,6,8-tetraen-12-one **(Borreline)**	12.71	$C_{17}H_{18}N_2O$	265.1340	266.1413	-ve		$[C_{17}H_{18}N_2O]^{-H}$
9.	(1s,3r,4r,6r,7r,8s,9r,10r,13r,14r,16r)-3,4,6,7,9,14-hexahydroxy-5,5,9,14-tetramethyltetracyclo[11.2.1.0Â¹,Â¹â°.0â´,â¸]283exadecane-16-yl (2s,3s)-3-hydroxy-2-methylbutanoate **(Hexadecatrienoic acid)**	16.31	$C_{25}H_{42}O_9$	485.2776	486.2849	-ve		$[C_{25}H_{42}O_9]^{-H}$, 339.1813
10.	9-[I-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-[[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl]oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid **(Mupirocin)**	16.71	$C_{26}H_{44}O_9$	499.6147	500.622	-ve		$[C_{26}H_{44}O_9]^{+HCOOH-H}$, 267.7904, 260.8822, 248.4511

RT- Retention Time, MF-Molecular Formula

Table 2. Bioactive phytochemical constituents identified through FTIR analysis of the methanolic leaf extract of *Phyllanthus niruri.*

SI.	Peak wave number (cm^{-1})	Types of vibration	Functional group	Bond
1.	3278.84	Stretching	Hydroxyl	O-H
2.	2926.99	Stretching	Alkane	C-H
3.	1714.10	Stretching	Carbonyl	C=O
4.	1610.71	Stretching	Alkene	C=C
5.	1512.80	Stretching	Alkene	C=C
6.	1442.33	Stretching	Alkane	C-H
7.	1336.39	Bending	Hydroxyl	O-H
8.	1205.01	Bending	Alkane	C-H
9.	1029.14	Stretching	Alcohol	C-O

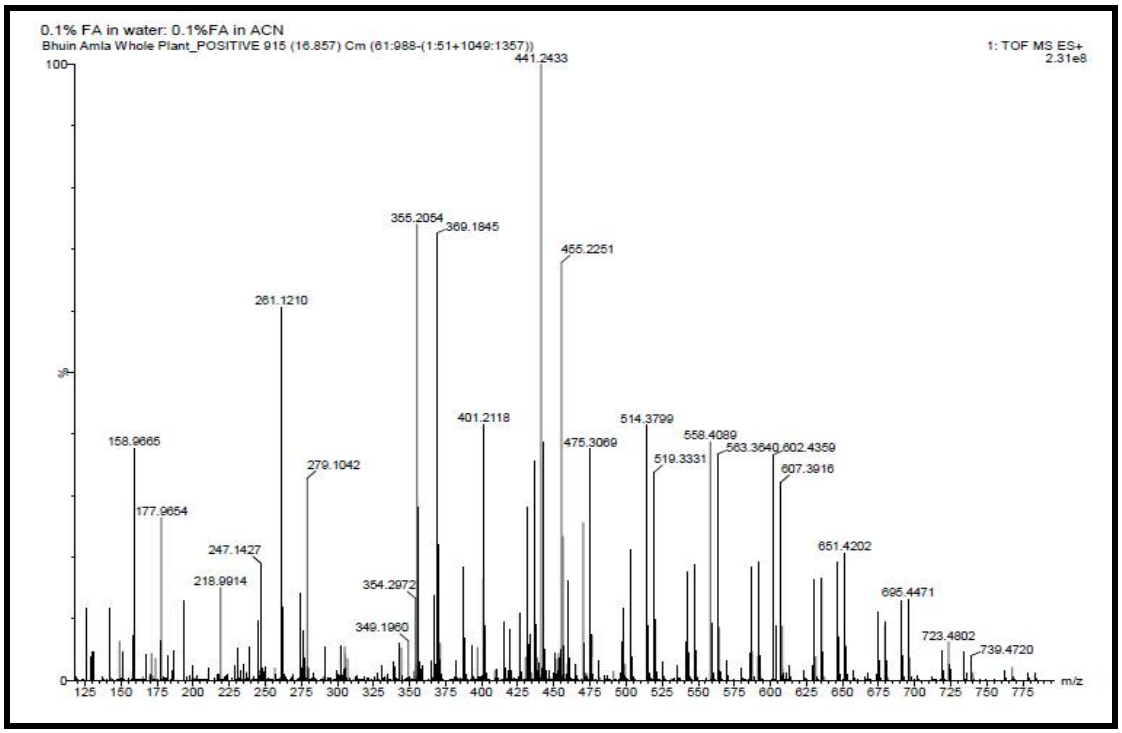


Figure 1. Combined spectra of positive ion chromatogram indicating the molecular mass of phytochemicals present in methanolic leaf extract of *Phyllanthus niruri*.

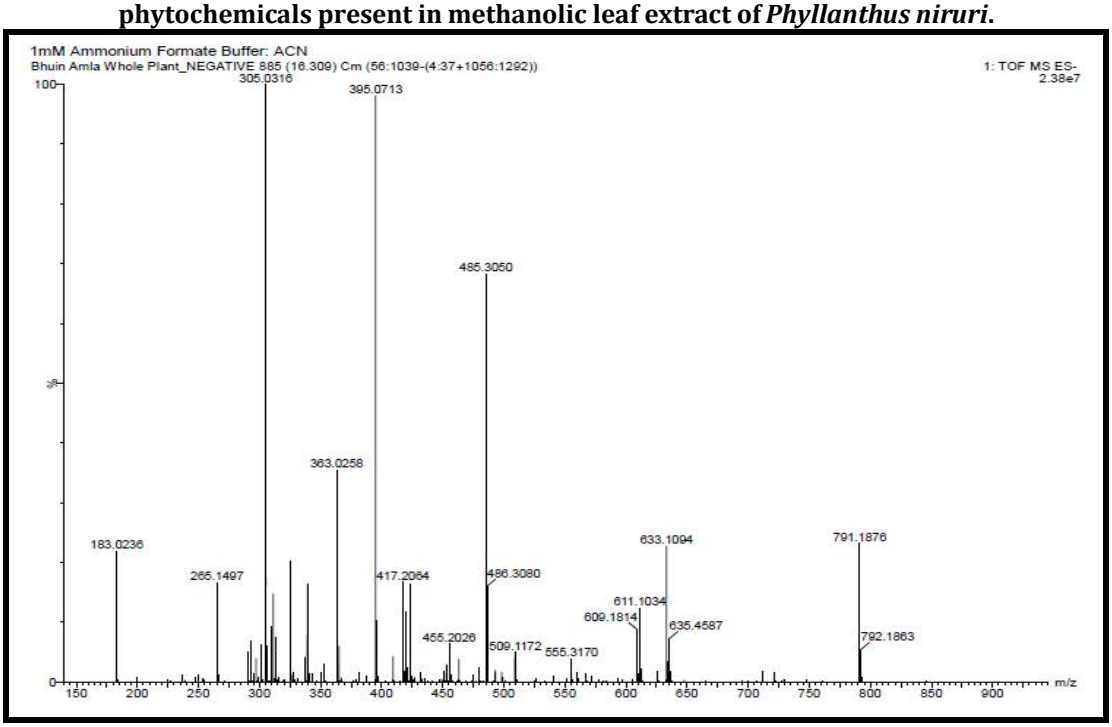


Figure 2. Combined spectra of negative ion chromatogram indicating the molecular mass of phytochemicals present in methanolic leaf extract of *Phyllanthus niruri*.

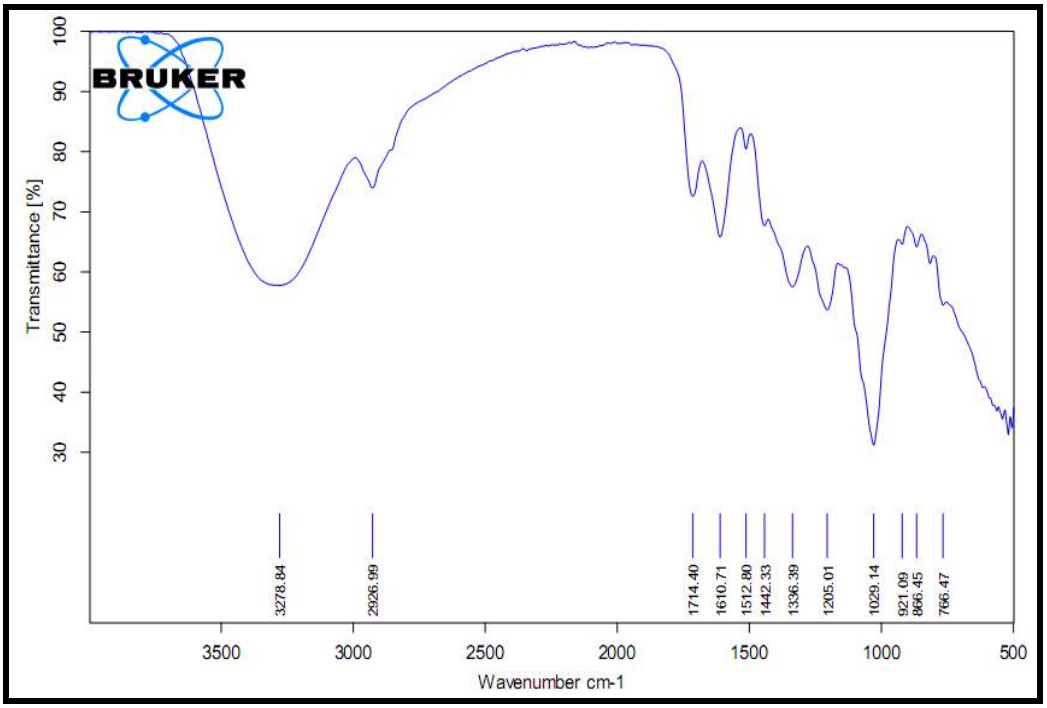


Figure 3. FTIR spectrum of methanolic leaf extract of *Phyllanthus niruri* revealed the different functional groups of bioactive phytochemical constituents.

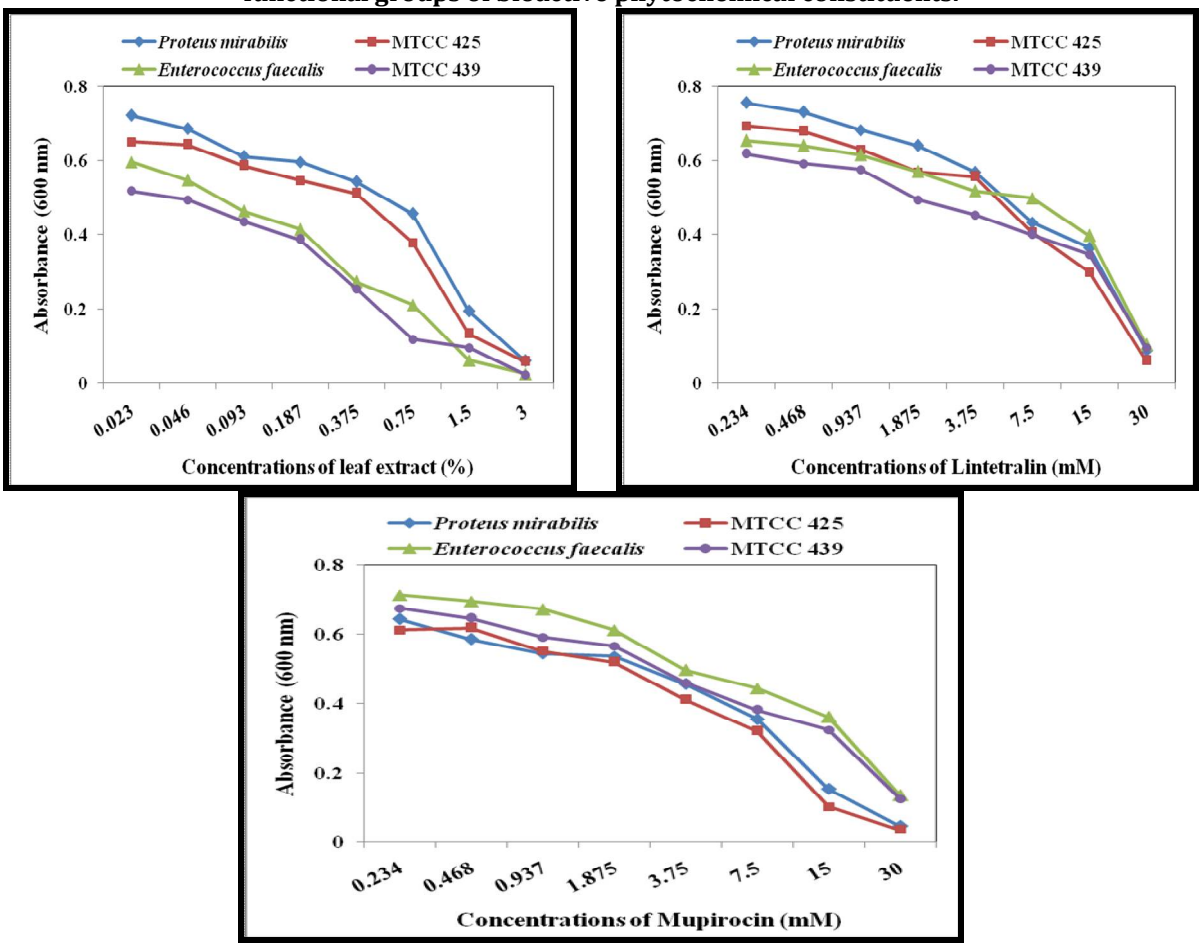


Figure 4. MIC value of (a) the methanolic leaf extract of *Phyllanthus niruri*; the plant derived bioactive compounds such as (b) Lintetralin and (c) Mupirocin against clinically isolated bacterial strains (*Proteus mirabilis* and *Enterococcus faecalis*) as well as their reference strains (MTCC 425 and MTCC 439).

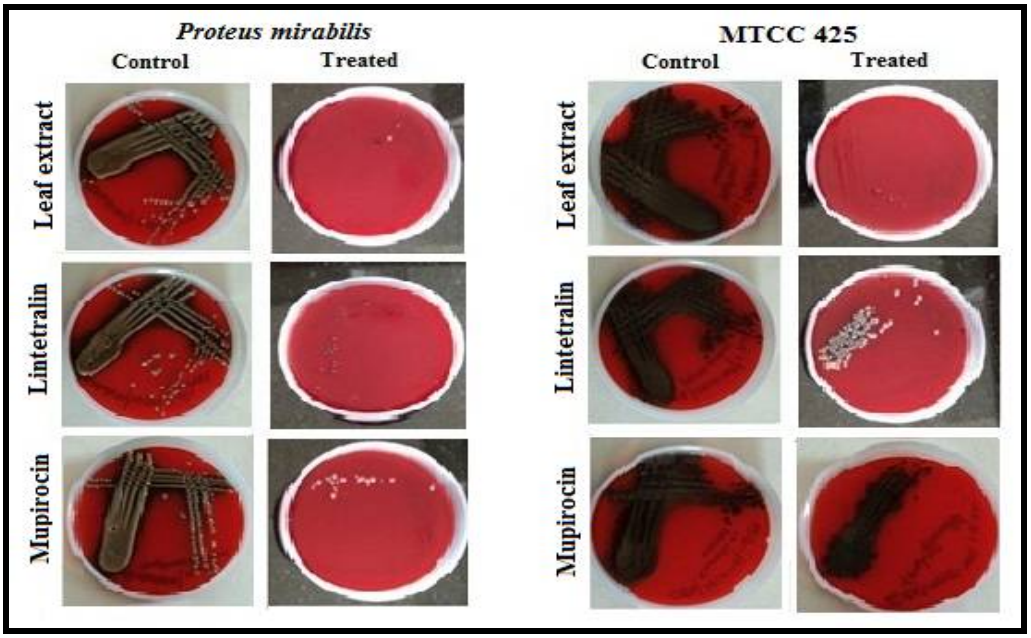


Figure 5a. Effect of sub-MICs level treatment with methanolic leaf extract of *P. niruri* and its bioactive compounds (Lintetralin and Mupirocin) on biofilm formation in the clinically isolated *Proteus mirabilis* and its reference strain (MTCC 425) using Congo red agar method.

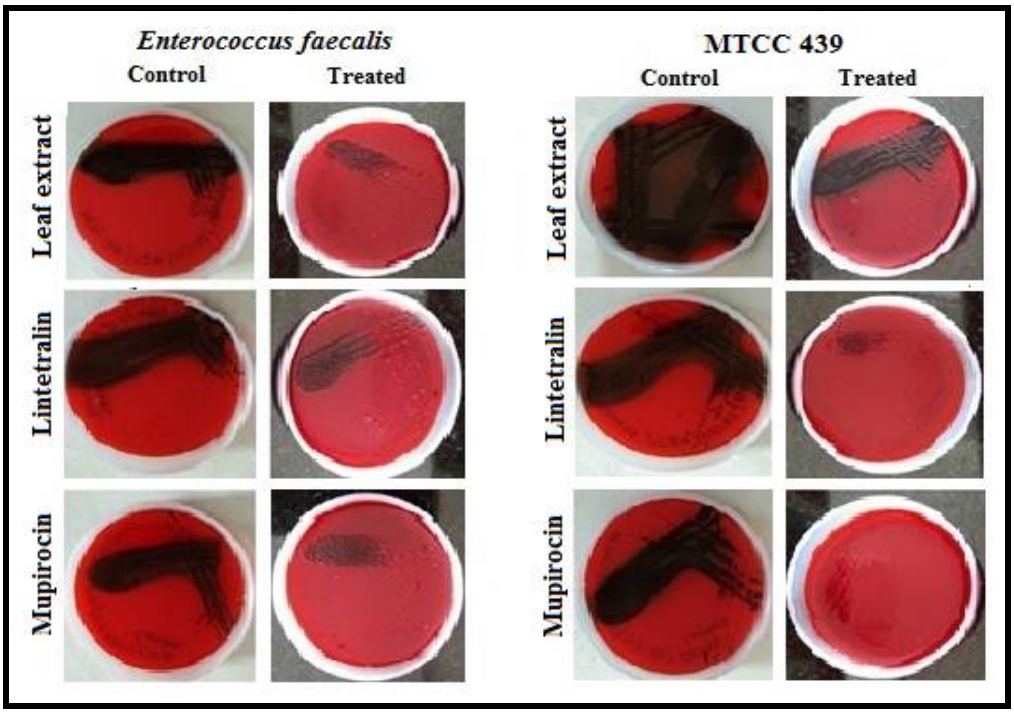
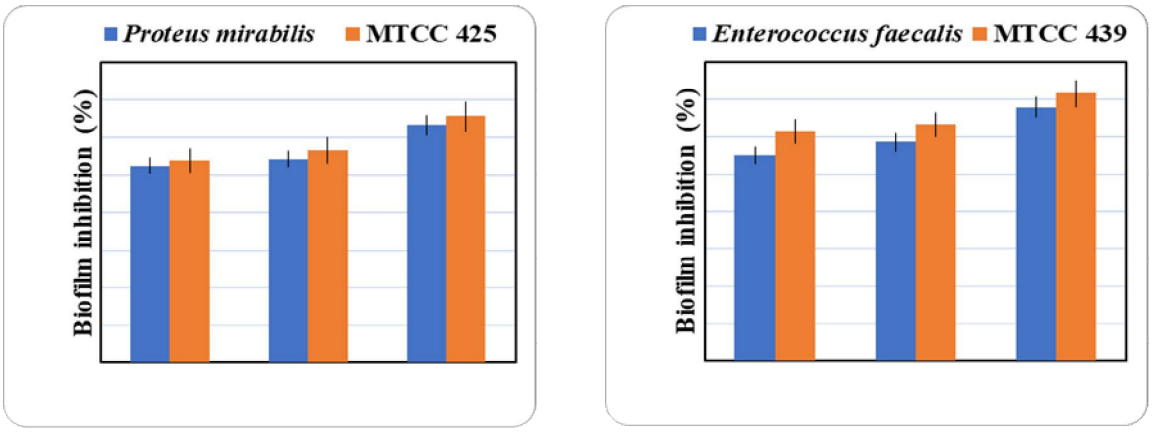


Figure 5b. Effects of sub-MICs level of treatment with methanolic leaf extract of *P. niruri* and bioactive compounds (Lintetralin and Mupirocin) on biofilm formation in the clinically isolated *Enterococcus faecalis* and its reference strain (MTCC 439) using Congo red agar method.



(a) (b)

Figure 6. Effects of sub-MICs level of treatment with the methanolic leaf extract of *Phyllanthus niruri* and plant derived compounds (Lintetralin and Mupirocin) on biofilm formation in the clinically isolated bacterial strains such as (a) *Proteus mirabilis* and its reference strain (MTCC 425); (b) *Enterococcus faecalis* and its reference strain (MTCC 439) using quantitative crystal violet staining method.

CONCLUSION

Natural products with bioactive compounds remain a promising pool for the discovery of scaffolds with enormous structural diversity and functional complexity. The bioactive compounds derived from the medicinal plant can be directly developed or used as starting points for optimization into novel therapeutics. The antimicrobial activity exhibited by plant extract results from the combined action of the multiple compounds with synergistic, additive or antagonistic activity. The target microorganism is less likely to acquire resistance against the plant derived bioactive compounds compared to single bioactive molecule, attracting natural products for combating MDR pathogens. Thus, evaluating antimicrobial activity both plant extract as well as its pure bioactive compounds through microbiological methods and clinical trials is crucial. The study emphasizes isolating pure bioactive metabolites (Lintetralin and Mupirocin) from the methanolic leaf extract of *Phyllanthus niruri* and assessing their antimicrobial potentials against pathogenic bacterial strains (*Proteus mirabilis* and *Enterococcus faecalis*). These finding hold promise for innovating novel antimicrobial agents. Understanding the mode of action and nature of interactions of natural bioactive metabolites will further substantiate the development of new therapies over the long-term. With the advancements in scientific and technological sectors, natural product (NP)-based drug discovery continues to contribute significantly to improve human health and longevity.

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AUTHOR CONTRIBUTIONS

Each author has significantly contributed for literature review, sample collection, experimental design and data interpretation. Dr. Amiya Kumar Patel has completed the final draft including results compilation. All authors have agreed to the publication version of the manuscript.

CONFLICT OF INTEREST

None

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