In Vitro Antimicrobial and Antibiofilm Efficacy of Medicinal Plant Extracts Against Clinical MDR Isolates from Scalp Infection Cases

Minakshi Rajput, Navneet Kumar

Abstract: The present study aimed to evaluate the antimicrobial and antibiofilm potential of three medicinal plants extracts (*Hemidesmus indicus*, *Hydnocarpus laurifolia* and *Pongamia pinnata*) against ten multidrug resistance bacteria [*Staphylococcus haemolyticus* (MN658867), *Staphylococcus hominis* (MN689675), *Enterobacter xiangfangensis* (MN689678), *Pseudomonas alcaligenes* (MN689677), *Staphylococcus epidermidis* (MN689679), *Bacillus megaterium* (MK466362), *Staphylococcus epidermidis* (MN689682), *Enterobacter kobei* (MN689685), *Bacillus paralicheniformis* (MN689683) and *Bacillus cereus* (MN689684)] isolated from patients suffering from scalp infections. Among all the eight plant extracts, ethyl acetate extract of *H. indicus* (HI-ETAC), methanol extract of *H. laurifolia* (HL-MeOH) and ethyl acetate extract of *P. pinnata* (PP-ETAC) were found to be the most potential extracts against all the MDR strains tested but *P. alcaligenes* (41.00±0.57 mm), *E. xiangfangensis* (38.83±0.45 mm) and *B. paralicheniformis* (40.50±0.66 mm) respectively were found the most sensitive strains. These extracts have high activity index (AI) when compared to reference antibiotics and evaluation of percent activity (PA) revealed that all the G^+ and G^- isolates were 100% sensitive to these three extracts. Spectral intensity index (SSI) clearly showed that the HI-ETAC possesses the maximum SII which was 28.51, followed by PP-ETAC (25.02) and HL-MeOH (20.23) which indicated that the HI-ETAC was the most potent extract. The time-kill curves described the bacteriostatic potential against MDR bacteria at lower concentration (½×MIC) and bactericidal potential at MIC and higher concentrations of the extracts. HI-ETAC, HL-MeOH and PP-ETAC extract exhibited significant antibiofilm potential against *P. alcaligenes, E. xiangfangensis* and *B. paralicheniformis* biofilms respectively and a dose dependent increase in antibiofilm efficacy was observed. The FT-IR analysis of these extracts indicated towards various biologically-active functional groups as O-H, N-H, C=O, COOH, C-OH, OCH_3, P-OR and PO_2, etc. The present investigation showcase that the root extracts of *H. indicus*, seed extracts of *H. laurifolia* and *P. pinnata* have a potential antibacterial effect on MDR bacteria as compared to modern antibiotics, as they are very effective antimicrobial as well as antibiofilm agents. It opens up a new avenue for its phytoconstituents as a therapeutic agent and it can further be applied in pharmaceutical compositions or precursors for the synthesis of new antibiotics.

Key words: *Hemidesmus indicus*, *Hydnocarpus laurifolia, Pongamia pinnata,* MDR bacteria, Antimicrobial, Antibiofilm, Time kill, MIC/MBC, FTIR

1. INTRODUCTION

Hair/scalp and skin affect the confidence and self-esteem in social life and looks of hair play an essential role in personality development and sexual signalling. The scalp represents a unique ecological niche to the several opportunistic bacteria as it is very favorable for several microbial infections due to the presence of differential conditions as high sweat and sebum production, high humidity, high follicular density, dark and warm surface further such environmental conditions distinguish scalp from other body parts. Hair and scalp in routine life, regularly come into contact with combs, fingers, styling implements etc. which could introduce microbial infections [1]. The scalp condition folliculitis is caused by numerous infective pathogens but the major bacteria are *Staphylococcus* spp., *Streptococcus* spp.*, Pseudomonas* spp., *Proteus* spp.*,* and coliform bacilli as *Escherichia coli, Enterobacter* spp., *Klebsiella* spp. and *Serratia* spp. which may cause superficial or deep folliculitis [2, 3]. Conventional antibiotics sometimes fail to cure such infections due to the resistance of microbial strains and inappropriate excessive consumption of antimicrobials stems resistance. The emerging and re-emerging multidrug resistant (MDR) bacteria posed several serious problems in the treatment of various infections which further continued to be clinical as well as public health concern. Such bacteria have been developing resistance rapidly for most of the currently available antimicrobial drugs either by the exchange of genetic material, mutation and via several other resistance mechanisms that are widespread in the bacterial population [4, 5]. Hyper-mutability, multidrug efflux and plasmid addiction are the recently discovered factors for the emergence, dissemination and maintenance of resistance further, such factors compromise the potential of majority of the drugs, therefore, the need for new better and affordable antimicrobials have been dramatically increasing. Medicinal plants and herbs are considered as one of the most promising sources for novel bioactive compounds which could further be utilized as potential alternatives or complementary treatment for drug resistant pathogens [5, 6]. Secondary metabolites and bioactive compounds of plants exhibited remarkable bacteriostatic and bactericidal properties and their mode of action includes various targets to kill bacterial cells so have been investigated as therapeutic agents. Some of them involve partition in cell membrane lipids which resulted in leakage of cell contents [7, 8], inhibition of protein synthesis, DNA replication and cell cycle (S-phase) etc. therefore, they are supposed as promising agents to combat MDR bacteria [9, 10]. In the present piece of work, three medicinal plants namely *H. indicus*, *H. laurifolia* and *P. pinnata* (Table 1) were studied for their antimicrobial and antibiofilm potential against MDR bacteria isolated from patients suffering from scalp infection.

Table 1: Medicinal plants studied in the present research work.

Botanical name	Form	Family	Common name	Used parts
Hemidesmus indicus	Shrub	Periplocaceae	Sariba/Anantmula/Indian Sarsaparilla	Roots
Hydnocarpus laurifolia	Tree (Medium sized)	Flacourtiaceae	Tuvaraka/Chaulmogra/ Soorty oil tree	Seeds
Pongamia pinnata	Tree (Medium sized large)	Fabaceae	Karanja/Malvanut/Indian Beach Tree	Seeds

- *Minakshi Rajput, Department of Botany and Microbiology, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand, India E-mail: m.rajput1991@gmail.com*
- *Navneet Kumar, Department of Botany and Microbiology, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand, India*

2. MATERIAL AND METHODS

2.1 Materials

Nutrient Agar, Mueller Hinton agar, Blood Agar were purchased from HiMedia (Mumbai, India). Petroleum ether, ethyl acetate were obtained from Central Drug House (CDH) (New Delhi, India) and methanol was purchased from Merck Life Science (Mumbai, India). DMSO (Dimethyl Sulphoxide) was obtained from Rankem, Avantor Performance Materials (Maharashtra, India). All the chemicals/reagents utilized in the present investigation were of analytical grade and used without further purification. Ampicillin (A 10 µg), Ciprofloxacin (CF 10 µg), Colistin (CL 10 µg), Co-trimoxazole (CO 25 µg), Gentamicin (G 10 µg), Nitrofurantoin (NF 300 µg), Streptomycin (S 10 µg), Tetracycline (T 30 µg), Bacitracin (B 8 U) Cefazolin (CZ 30 µg), Clindamycin (CD 2 µg), Doxycycline (DO 30 µg), Erythromycin (E 15 µg), Neomycin (N 10 µg) disks for Antimicrobial Susceptibility Testing purchased from HiMedia (Mumbai, India).

2.2 Isolation, Antibiotic Susceptibility Profile and Identification of MDR Bacteria

Swab cultures were taken from seven patients suffering from different scalp infections from Advanced Medical Care Center, Haridwar, Mela Hospital, Haridwar, BHEL Hospital, Haridwar in 2017. Swabs were then transferred on nutrient agar plates and incubated at 37°C for 24 h. All the bacterial colonies were then purified on NAM and sub-cultured on blood agar and chocolate agar plates, which further aerobically incubated for 24-48 h at 37°C in the BOD incubator for the screening of pathogenic bacterial strains. Gram staining was performed to check the morphological characteristics of all the isolates. Antibiotic susceptibility profile of the isolated bacterial strains was determined by the disk diffusion technique (Kirby-Bauer) [11] on Mueller-Hinton agar using 14 antibiotic disks. The interpretative criteria of the zone of inhibition size were based on standards provided by the European Committee on Antimicrobial Susceptibility (EUCAST, 2015) [12]. The preliminary identification of MDR strains was done biochemically by HiMedia kits (KB003, KB009). Further, these isolates were then identified by 16S rRNA gene sequencing services provided by Humanizing Genomics Macrogen Inc. (Seoul, South Korea) in order to verify the results of the biochemical analysis for bacterial identification at the species level. Further, 16S rDNA sequences of these 10 MDR strains were submitted in NCBI to get accession numbers.

2.3 Plant Material Collection and Identification

Roots of *H. indicus* were collected near the hilly areas of village Javan (Pune, Maharashtra) between the months of April and July 2016, seeds of *P. pinnata* were collected from Kankhal, (Haridwar, Uttarakhand) between the months of February and March 2016, packed immediately and stored until processed. Herbariums of plant specimens were prepared then identified and authenticated by BSI (Botanical Survey of India), Dehradun and voucher specimen (Accession No. 118140 for *H. indicus* and 118139 for *P. pinnata*) were deposited in the herbarium of BSI, Dehradun for future reference. The certified seed material of *H. laurifolia* was purchased from Herbal Health Research Consortium Pvt. Ltd., Amritsar, Punjab in March 2016.

2.4 Extraction of Plant Material

Plant materials were cleaned thoroughly with fresh tap water then with distilled water to remove foreign particles as dust etc. and air-dried. The dried materials were grounded into fine homogeneous powder using an electric grinder and 200 g of each sample was sequentially extracted in 600 ml of four different solvents (low to high polarity) namely petroleum ether (PET), ethyl acetate (ETAC), methanol (MeOH), and distilled water (H_2O) using Soxhlet apparatus until the solvent turns clear. The crude extract was filtered, solvent was evaporated by vacuum rotary evaporator and stored at 4°C in sterile vials until further use. The percent yield of extraction was expressed as:

$$\textbf{Extraction yield}\ (\%) = \frac{\text{Weight of dry extract (g)}}{\text{Weight of the sample used for the extraction (g)}} \times 100$$

2.5 Antimicrobial Activity and Quantitative Estimation of Antimicrobial Efficacy

In vitro antimicrobial efficacy of extracts were evaluated by agar well diffusion method [13]. Stock cultures of all the bacterial isolates maintained at 4°C on the slants, transferred in Mueller-Hinton broth (MHB) and incubated at 37°C for 24 h for the preparation of active cultures. To standardize the inoculum density, a 0.5 McFarland standard (1.5×10^8 CFU/ml) was prepared to maintain the turbidity of the bacterial inoculum. The final concentration (200 mg/ml) of all the extracts were prepared in dimethyl sulfoxide (DMSO). Pre-solidified Mueller-Hinton agar plate was inoculated by spreading 100 µl of bacterial culture through a swab over the entire agar surface and 50 µl of different plant extracts were dispensed into the wells of 6 mm diameter and plates were incubated at 37°C for 24 h. DMSO served as a negative control and all the experiments were performed under aseptic conditions in triplicates. Antimicrobial efficacy of extract was measured in the form of the diameter of inhibition zones (mm) and the mean value of the inhibition zones of the triplicates was taken as the final result. The antimicrobial activity was quantitatively accessed in the form of various indices which were as follows:

Activity index (AI): AI was calculated to express the relation between the zone of inhibition of extract to that of reference antibiotic [14, 15].

$$\textbf{AI} = \frac{\text{Diameters of the inhibition zone of extract}}{\text{Diameters of the inhibition zone of the reference antibiotic}}$$

Percent Activity (PA): It demonstrates the total anti-microbial efficacy of a particular extract, or shows the number of microbial strains susceptible to a particular extract. It was expressed as % G^+ for percent activity against Gram's positive bacteria, % G^- for percent activity against Gram's negative bacteria and %T for percent total activity against both types of bacteria [16].

$$\textbf{PA} = 100\left(\frac{\text{No. of susceptible strains to a specific extract}}{\text{Total no. of tested microbial strains}}\right)$$

Spectral Intensity Index (SII): SII is used to express the combination of both spectral range and magnitude of effectiveness (in terms of diameter of inhibition zones) of a specific extract against all sensitive bacteria [16].

$$\textbf{SII} = \frac{\text{Mean of diameters of all sensitive bacterial strains to a specific extract} \times \%T}{100}$$

Bacterial Susceptibility Index (BSI): BSI is used to compare the relative susceptibility between all the bacterial strains tested. The values of BSI ranges between 0 (resistance to all extracts) to 100 (susceptible to all extracts) [16].

$$\mathbf{BSI} = 100\left(\frac{\text{No. of extracts effective against each bacterial strain}}{\text{No. of total extracts}}\right)$$

2.6 Determination of MIC, MBC Values

Minimum inhibitory concentrations (MIC) of crude extracts were determined by the Broth Microdilution method [17]. Stock solutions of different extracts having a range of concentrations (200, 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 mg/ml) were prepared through two-fold serial dilution in sterilized broth. 100 µl of broth having different concentrations of plant extracts were transferred into the microtiter plate followed by the addition of freshly prepared 100 µl of test strain broth and titer plates were then incubated at 37°C for 24 h. The lowest concentration of extract that could inhibit the bacterial growth was believed as MIC. The loopful culture from the wells of the MIC titer plate with no visible growth was sub-cultured on freshly prepared MHA plates and incubated at 37°C for 24 h to evaluate the minimum bactericidal concentrations (MBC) of the plant extract. The lowest concentration with no visible growth on the MHA plate was considered as MBC. Bacitracin (range: 5-0.039 mg/ml) was used as a positive control, whereas broth with DMSO was taken as a negative control, all the dilutions of crude extracts were also cultured on plates for the sterility test.

2.7 Time Kill Assay

The time kill assay was performed with some minor alterations according to Latha et al. and Sheh-Hong and Darah [18, 19]. 1 ml of the bacterial inoculum (1.5×10^8 CFU/ml) was transferred to 50 ml of nutrient broth which was already supplemented with different concentrations (1/2×, 1×, and 2× MIC) of plant extracts and incubated at 37°C, in shaker incubator at 150 r/min for 48 h. Extract free medium (nutrient broth + DMSO + bacterial inoculum) considered as negative control. Bacterial inoculum free medium (extract + nutrient broth) used to recognize the turbidity of the extract at each concentration. At every 4 h intervals, around 4 ml of the sample portion was withdrawn and bacterial growth was monitored as optical density (OD) at 540 nm. Each assay was performed in triplicates and the growth profile curves were plotted to evaluate the antibacterial efficacy over time.

2.8 Antibiofilm Assay

Microtiter plate method was applied to determine the antibiofilm potential or biofilm inhibitory concentration (BIC) of the extracts [20]. Wells of microtiter plate were filled with 180 µl of MH broth and inoculated with 10 µl of bacterial culture (1.5×10^8 CFU/ml). Then 10 µl of varying concentrations (0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 mg/ml) of extracts were added to wells and plates were incubated at 37 °C for 48 h. After incubation, planktonic bacterial cells were discarded and weakly adherent bacterial cells were gently rinsed by washing with de-ionized water and plates were then dried before staining. Well surface adhered biofilm cells were stained for 2-3 min with crystal violet stain (0.1% w/v) and subsequently, excess crystal violet was rinsed off twice and plates were kept for drying. 95% (v/v) ethanol was added to the wells of the microtiter plate for the solubilization of stain and OD was taken at 620 nm by ELISA reader. The biofilm inhibition (%) was calculated using the formula:

$$\%\ \mathbf{Biofilm\ Inhibition} = \frac{(\text{OD of non treated control} - \text{OD of extract treated sample})}{(\text{OD of non treated control})} \times 100$$

2.9 Fourier Transform Infrared (FTIR) Spectroscopic Analysis

Infra-red spectra of the crude plant extracts were screened for the presence of various functional groups responsible for biological activities. The crude extract was placed on the sample chamber of FT-IR spectrophotometer and their spectra were noted down in the range of 4000-600 cm^{-1} on Bruker's FTIR spectrometer. Important absorption peaks appeared in the functional group region as well as in the fingerprint region of the spectra were further studied for the identification and assignment of the types of functional groups/chemical bonds present in the extracts.

2.10 Statistical Analysis

Statistical data, values of inhibition zones (diameter in mm) were analyzed using SPSS, version 20. Each experimental value was expressed as mean ± SE. Statistical significance of the data was determined by Student's t-test and values at the level of $p<0.05$ were considered significant.

3. RESULTS

3.1 Isolation, Screening and Identification of MDR Bacteria

From seven specimens collected, 33 bacterial colonies were isolated from mixed populations and sub-cultured on nutrient agar plates in pure form. The distribution of isolates from scalp infections was as follows: *N*=20/33 (60.61%) Gram's positive cocci (GPC), *N*=10/33 (30.30%) Gram's positive bacilli (GPB) and *N*=3/33 (09.09%) Gram's negative bacilli (GNB) (Fig. 1). *N*=4/33 (12.12%) bacteria were found α hemolytic, *N*=8/33 (24.24%) β hemolytic and *N*=21/33 (63.64%) γ hemolytic (Fig. 2). Heatmap showed the antibiotic susceptibility pattern of all the isolates and it could be seen undoubtedly that 10 out of 33 isolates (PB16, PB17, PB18, PB31, PB32, PB38, PB41, PB51, PB61, PB65) were found resistant to more than two groups of antibiotics (Fig. 3). Out of these 10 MDR isolates, PB18 was found to be the most resistant strains as it showed resistance in 7 antibiotics namely ampicillin, nitrofurantoin, tetracycline, bacitracin, cefazolin, clindamycin and erythromycin. The preliminary identification of these 10 MDR strains was done through biochemical characterization and carbon utilization test which were given in (Fig. 4, 5). The MDR isolates were then subjected to 16S rDNA sequencing in order to confirm the data of phenotyping for the identification of isolates at the species level. The nucleotide BLAST search data gave around 98-100% identity score to the sequences in the database. The sequence data of bacterial isolates were submitted in GenBank and accession numbers have been provided to each strain as *Staphylococcus haemolyticus* (MN658867), *Staphylococcus hominis* (MN689675), *Enterobacter xiangfangensis* (MN689678), *Pseudomonas alcaligenes* (MN689677), *Staphylococcus epidermidis* (MN689679), *Bacillus megaterium* (MK466362), *Staphylococcus epidermidis* (MN689682), *Enterobacter kobei* (MN689685), *Bacillus paralicheniformis* (MN689683) and *Bacillus cereus* (MN865574).

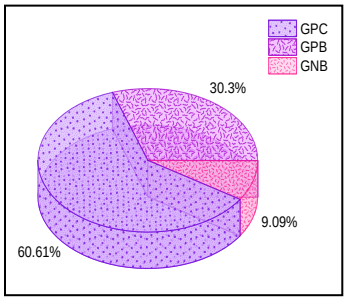


Fig. 1: Gram's Positive Cocci (GPC), Gram's Positive Bacilli (GPB) and Gram's Negative Bacilli (GNB) Isolates (N = 33).

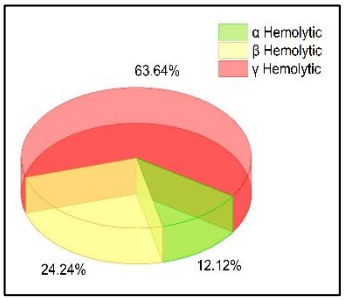


Fig. 2: α, β, γ hemolytic Isolates (N = 33).

3.2 Plant Extraction

Extraction yield (%), pH and the physical appearance of all three medicinal plant extracts are summarized in Table 2. It was observed that in the case of *H. indicus,* HI-MeOH extract gave the maximum yield (17.25%) and the pH of the extract was 6 i.e. acidic. The HL-ETAC extract of *H. laurifolia* gave the highest yield of phytochemicals viz. 34.81% with pH 6. The PP-PET extract of *P. pinnata* gave the maximum extraction yield (26.78%) with oily extract and slightly acidic pH (6.5).

Table 2: Extraction yield, pH, color and consistency of the extracts.

Extracts	Extraction yield (%)	pH	Color	Consistency
HI-PET	7.01	6	Yellowish-brown	Semi solid
HI-ETAC	14.08	6	Light Brown	Semi solid
HI-MeOH	17.25	6	Brown	Semi solid
HI-H_2O	15.42	5	Brown	Semi solid
HL-PET	19.34	6.5	Cream	Liquid (Oily)
HL-ETAC	34.81	6	Brown	Gummy
HL-MeOH	6.49	5	Brown	Semi solid
HL-H_2O	7.74	5	Brown	Semi solid
PP-PET	26.78	6.5	Yellow	Liquid (Oily)
PP-ETAC	5.72	7	Brown	Semi solid (Oily)
PP-MeOH	12.04	5.5	Brown	Semi solid
PP-H_2O	8.53	6	Brown	Gummy

HI-PET: Petroleum ether extract of *H. indicus*; HI-ETAC: Ethyl acetate extract of *H. indicus;* HI-MeOH: Methanol extract of *H. indicus;* HI-H_2O: Water extract of *H. indicus;* HL-PET: Petroleum ether extract of *H. laurifolia;* HL-ETAC: Ethyl acetate extract of *H. laurifolia;* HL-MeOH: Methanol extract of *H. laurifolia;* HL-H_2O: Water extract of *H. laurifolia;* PP-PET: Petroleum ether extract of *P. pinnata*; PP-ETAC: Ethyl acetate extract of *P. pinnata;* PP-MeOH: Methanol extract of *P. pinnata*; PP-H_2O: Water extract of *P. pinnata.*

3.3 Antimicrobial Efficacy of Plant Extracts

In vitro antibacterial activity of different extracts of *H. indicus, H. laurifolia* and *P. pinnata* against MDR isolates was evaluated by the presence or absence of diameter of inhibition zones. The results of the antimicrobial efficacy of different plant extracts and reference antimicrobials are summarized in Table 3. The data of inhibition zones showed a huge variation in antimicrobial potential of different plant extracts. In case of root extracts of *H. indicus,* out of four different solvent extracts, HI-ETAC was found the most potent extract against MDR isolates and *P. alcaligenes* was considered the most sensitive strain with 41.00±0.57 mm inhibition zone followed by *B. cereus* (39.83±0.44 mm), *B. paralicheniformis* (37.00±0.57 mm), *E. kobei* (33.00±0.57 mm), *E. xiangfangensis* (29.33±0.33 mm), *S. epidermidis* (23.66±0.33 mm), *B. megaterium* (22.83±0.44 mm), *S. hominis* (21.00±0.57 mm), *S. epidermidis* (20.66±0.33 mm) and *S. haemolyticus* (16.83±0.44 mm). Similarly, out of four solvents, the ethyl acetate extract of *P. pinnata* seeds (PP-ETAC) was found to be the most effective extract against all the isolates and *B. paralicheniformis* was the most sensitive strain with inhibition zone of 40.50±0.66 mm which further followed by *P. alcaligenes* (38.50±0.33 mm), *E. kobei* (28.50±0.45 mm), *B. megaterium* (24.50±0.33 mm), *S. hominis* (22.50±0.33 mm), *S. epidermidis* (21.00±0.57 mm), *B. cereus* (20.66±0.57 mm), *S. epidermidis* (20.50±0.45 mm), *S. haemolyticus* (17.00±0.45 mm) and *E. xiangfangensis* (16.55±0.44 mm). In case of *H. laurifolia,* HL-MeOH out of four seed extracts was observed the most potential extract and *E. xiangfangensis* was found to be the most sensitive MDR strain with 38.83±0.45 mm zone of inhibition followed by *P. alcaligenes* (25.50±0.44 mm), *S. haemolyticus* (20.33±0.33 mm), *B. paralicheniformis* (20.00±0.33 mm), *E. kobei* (18.50±0.50 mm), *B. cereus* (17.66±0.45 mm), *S. hominis* (16.66±0.66 mm), *B. megaterium* (15.83±0.45 mm), *S. epidermidis* (14.50±0.66 mm) and *S. epidermidis* (14.00±0.33). Among all the eight plant extracts, HI-EAC, HL-MeOH and PP-ETAC were found to be the most potential extracts against all the MDR strains tested. These plant extracts showed comparatively higher antimicrobial potential against almost all the strains then reference antibiotics and statistically significant ($P<0.05$).

The antimicrobial sensitivity patterns of each plant extracts are shown in Table 4. The high activity index (AI) reveals the comparatively high antimicrobial efficacy of plant extract than reference antibiotic. According to the results, it was clearly understood that HI-ETAC possess the highest activity index or very effective against *P. alcaligenes* when compared to bacitracin, HL-MeOH possesses the highest activity index against *E. xiangfangensis* when compared to erythromycin and PP-ETAC had the highest activity index against *P. alcaligenes* when compared to bacitracin. Percent activity (PA) is considered as the total antimicrobial potential of a particular extract. All the G^+ and G^- isolates were 100% sensitive to the tested concentration of HI-PE, HI-ETAC, HI-MeOH, HL-PE, HL-ETAC, HL-MeOH and PP-ETAC extracts. The results of spectral intensity index (SSI) clearly showed that the HI-ETAC possesses the maximum SII which was 28.51, followed by PP-ETAC (25.02) and HL-MeOH (20.23) which indicated that the HI-ETAC was the most potential extract among all the eight extracts against all the MDR isolates. Considering bacterial susceptibility index (BSI), *P. alcaligenes* and *S. epidermidis* were observed the most susceptible strains having BSI 100 to the extracts of *H. indicus,* similarly, *E. xiangfangensis* and *P. alcaligenes* were most susceptible (BSI 100) to the extracts of *H. laurifolia,* in this regard, BSI 100 indicate towards the all four plant extracts were effective against these isolates. In the case of *P. pinnata* extracts, there was no strain having BSI 100, but *S. haemolyticus, S. hominis, E. kobei* and *B. cereus* having BSI 75 which means that 3 out of 4 extracts were effective against these strains (Table 5).

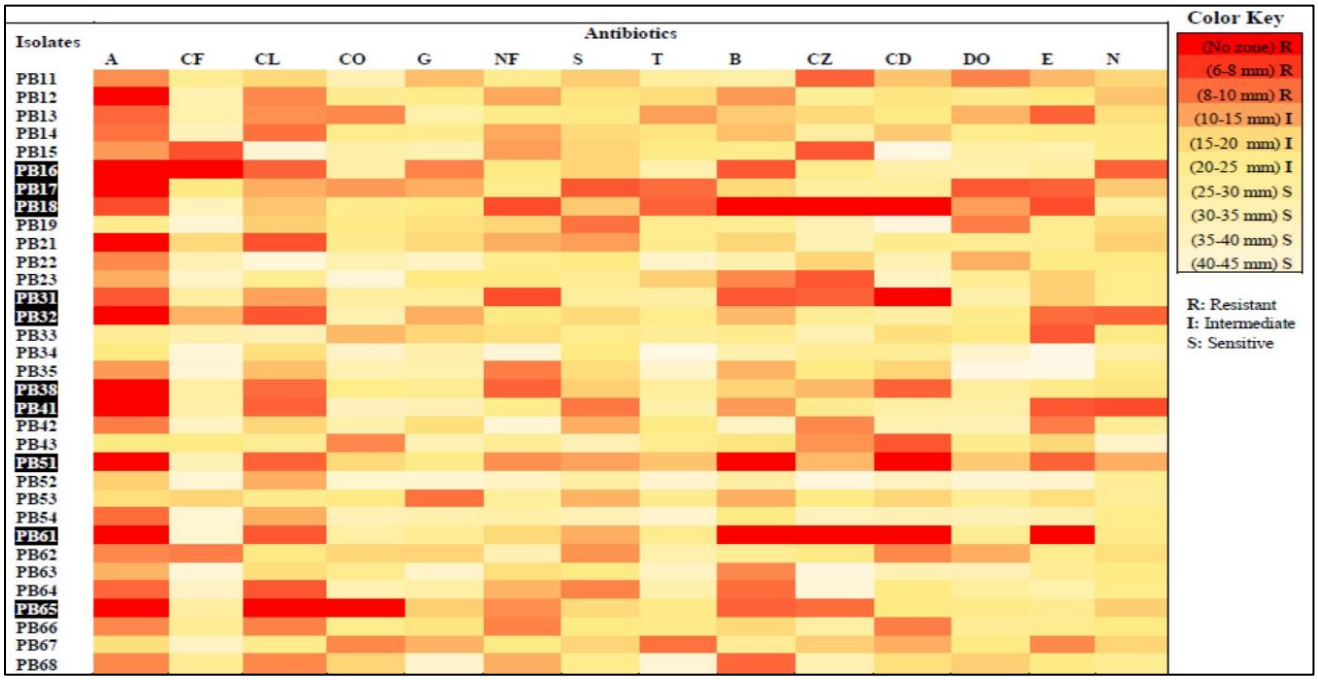


Fig. 3 : Heat map showing antibiotic susceptibility profile (in terms of inhibition zones in mm) of isolates. Black background highlighted isolates were found MDR bacteria.

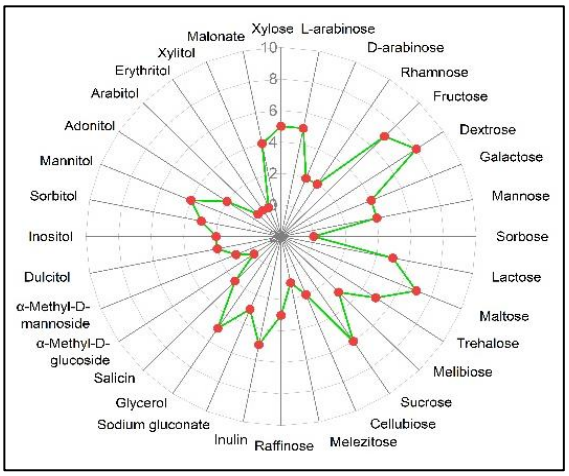


Fig. 4: Utilization of carbon sources by MDR bacterial isolates.

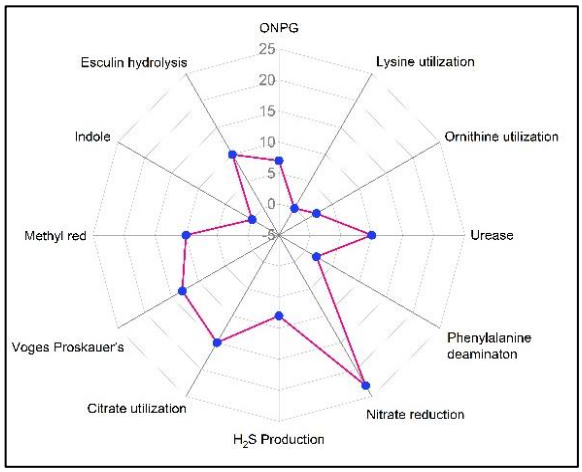


Fig. 5: Biochemical characterization of MDR bacterial isolates.

3.4 Determination of MIC and MBC

The MIC and MBC of root extracts of *H. indicus,* seed extracts of *H. laurifolia* and *P. pinnata* are shown in Fig. 6 A, B, C. In case of *H. indicus,* the lowest MIC/MBC values were observed with HI-ETAC extract which ranged from 1.25-12.5 mg/ml for MIC and 3.12-25 mg/ml for MBC for all the isolates. The minimum MIC/MBC values were observed for *P. alcaligenes* and *B. cereus* which were 1.25/3.12 mg/ml. The lowest MIC/MBC values in the case of *H. laurifolia* were given by HL-MeOH extract which ranged from 1.25-25 mg/ml for MIC and 3.12-50 mg/ml for MBC against all the bacterial isolates. HL-MeOH extract presented the lowest MIC/MBC values against *E. xiangfangensis* with 1.25/3.12 mg/ml. Out of four extract ethyl acetate extract of *P. pinnata* (PP-ETAC) posed the lowest MIC/MBC range against all the MDR isolates which was 1.25-3.12 mg/ml for MIC and 3.12-12.5 mg/ml for MBC. *P. alcaligenes, E. kobei* and *B. paralicheniformis* were found the most sensitive strain as they were inhibited by the lowest concentration of PP-ETAC extract which was 1.25 mg/ml (MIC) and 3.12mg/ml (MBC).

3.5 Time Kill Assay

According to the results of antimicrobial activity, out of all 8 extracts, HI-ETAC, HL-MeOH and PP-ETAC were found the most potential plant extracts against *P. alcaligenes, E. xiangfangensis* and *B. paralicheniformis* respectively, the time kill assay was only performed with these plant extracts and isolates. The time kill curves of *P. alcaligenes* exposed to ½×MIC, MIC and 2×MIC of HI-ETAC; *E. xiangfangensis* exposed to ½×MIC, MIC and 2×MIC of HL-MeOH; and *B. paralicheniformis* exposed to ½×MIC, MIC and 2×MIC of PP-ETAC were shown in Fig. 7 A, B, C. At the exposure of ½×MIC

Table 3: Inhibition Zones (mm) by extracts of *H. indicus, H. laurifolia, P. pinnata* and reference antimicrobials against isolated MDR bacteria.

Extract/ Standard	Isolates									
	S. haemolyticus	*S. hominis*	*E. xiangfangensis*	*P. alcaligenes*	*S. epidermidis*	*B. megaterium*	*S. epidermidis*	*E. kobei*	*B. paralicheniformis*	*B. cereus*
HI-PET	12.50±0.28	12.00±0.50	09.00±0.57	35.33±0.33	15.00±0.57	14.33±0.33	13.00±0.57	19.83±0.44	10.66±0.33	13.00±0.57
HI-ETAC	16.83±0.44	21.00±0.57	29.33±0.33	41.00±0.57	23.66±0.33	22.83±0.44	20.66±0.33	33.00±0.57	37.00±0.57	39.83±0.44
HI-MeOH	11.83±0.44	15.00±0.28	16.16±0.44	24.16±0.44	13.66±0.66	16.00±0.57	12.66±0.33	20.33±0.33	15.33±0.33	22.50±0.76
HI-H_2O	-	-	-	12.16±0.44	-	-	08.50±0.28	-	-	-
HL-PET	21.50±0.57	12.00±0.57	26.50±0.33	17.50±0.57	12.50±0.44	11.50±0.33	11.00±0.44	14.66±0.33	22.00±0.57	13.00±0.50
HL-ETAC	14.66±0.33	12.50±0.44	23.66±0.57	20.00±0.33	10.00±0.57	12.50±0.66	12.00±0.33	10.50±0.57	15.66±0.44	14.00±0.33
HL-MeOH	20.33±0.33	16.66±0.66	38.83±0.45	25.50±0.44	14.50±0.66	15.83±0.45	14.00±0.33	18.50±0.50	20.00±0.33	17.66±0.45
HL-H_2O	-	-	24.50±0.57	12.66±0.76	-	-	-	-	-	-
PP-PET	10.50±0.33	08.50±0.50	08.00±0.45	-	-	-	-	09.00±0.33	-	09.50±0.45
PP-ETAC	17.00±0.45	22.50±0.33	16.55±0.44	38.50±0.33	20.50±0.45	24.50±0.33	21.00±0.57	28.50±0.45	40.50±0.66	20.66±0.57
PP-MeOH	12.00±0.57	14.50±0.45	-	20.50±0.57	13.55±0.50	15.00±0.33	13.25±0.44	17.55±0.50	-	21.55±0.33
PP-H_2O	-	-	-	-	-	08.50±0.33	-	08.00±0.47	-	-
E	28.50±0.66	09.00±0.33	09.00±0.56	19.00±0.45	11.00±0.66	23.00±0.66	08.00±0.33	10.00±0.45	-	25.25±0.33
N	10.00±0.57	18.50±0.66	-	24.00±0.44	10.00±0.33	21.00±0.44	07.00±0.66	16.00±0.57	23.00±0.44	19.00±0.66
B	09.00±0.33	20.00±0.45	28.50±0.66	08.00±0.57	17.00±0.45	19.50±0.66	14.25±0.44	-	-	10.00±0.33
DMSO	-	-	-	-	-	-	-	-	-	-

Values are the mean of three replicates; Significant at $P \leq 0.05$ level of analysis of variance; Diameter of well: 6 mm HI-PET: Petroleum ether extract of *H. indicus*; HI-ETAC: Ethyl acetate extract of *H. indicus;* HI-MeOH: Methanol extract of *H. indicus;* HI-H_2O: Water extract of *H. indicus;* HL-PET: Petroleum ether extract of *H. laurifolia;* HL-ETAC: Ethyl acetate extract of *H. laurifolia;* HL-MeOH: Methanol extract of *H. laurifolia;* HL-H_2O: Water extract of *H. laurifolia;* PP-PET: Petroleum ether extract of *P. pinnata*; PP-ETAC: Ethyl acetate extract of *P. pinnata;* PP-MeOH: Methanol extract of *P. pinnata*; PP-H_2O: Water extract of *P. pinnata;* E: Erythromycin; N: Neomycin; B: Bacitracin; DMSO: Dimethyl Sulfoxide.

Table 4: Antimicrobial susceptibility patterns of different extracts of *H. indicus, H. laurifolia* and *P. pinnata* against isolated MDR bacteria.

Antimicrobial Susceptibility Patterns			HI-PET	HI-ETAC	HI-MEOH	HI-H_2O	HL-PET	HL-ETAC	HL-MEOH	HL-H_2O	PP-PET	PP-ETAC	PP-MEOH	PP-H_2O
Activity Index (AI)	*S. haemolyticus*	E	0.43	0.59	0.41	-	0.75	0.51	0.71	-	0.36	0.59	0.42	-
		N	1.25	1.68	1.18	-	2.15	1.46	2.03	-	1.05	1.70	1.20	-
		B	1.38	1.87	1.31	-	2.38	1.62	2.25	-	1.16	1.88	1.33	-
	S. hominis	E	1.33	2.33	1.66	-	1.33	1.38	1.85	-	0.94	2.50	1.61	-
		N	0.64	1.13	0.81	-	0.64	0.67	0.90	-	0.45	1.21	0.78	-
		B	0.60	1.05	0.75	-	0.60	0.62	0.83	-	0.42	1.12	0.72	-
	E. xiangfangensis	E	1.00	3.25	1.79	-	2.94	2.62	4.31	2.72	0.88	1.83	-	-
		N	R	R	R	-	R	R	R	R	R	R	-	-
		B	0.31	1.02	0.56	-	0.92	0.83	1.36	0.85	0.28	0.58	-	-
	P. alcaligenes	E	1.85	2.15	1.27	0.64	0.92	1.05	1.34	0.66	-	2.02	1.07	-
		N	1.47	1.70	1.00	0.50	0.72	0.83	1.06	0.52	-	1.60	0.85	-
		B	4.41	5.12	3.02	1.52	2.18	2.50	3.18	1.58	-	4.81	2.56	-
	S. epidermidis	E	1.36	2.15	1.24	-	1.13	0.90	1.31	-	-	1.87	1.23	-
		N	1.50	2.36	1.36	-	1.25	1.00	1.45	-	-	2.06	1.35	-
		B	0.88	1.39	0.80	-	0.73	0.58	0.85	-	-	1.21	0.79	-
	B. megaterium	E	0.62	0.99	0.69	-	0.50	0.54	0.68	-	-	1.06	0.65	0.36
		N	0.68	1.08	0.76	-	0.54	0.59	0.75	-	-	1.16	0.71	0.40
		B	0.73	1.17	0.82	-	0.58	0.64	0.81	-	-	1.25	0.76	0.43
	S. epidermidis	E	1.62	2.58	1.58	1.06	1.37	1.50	1.81	-	-	2.62	1.65	-
		N	1.85	2.95	1.80	1.21	1.57	1.71	2.07	-	-	3.00	1.89	-
		B	0.91	1.44	0.88	0.59	0.77	0.84	1.01	-	-	1.47	0.92	-
	E. kobei	E	1.98	3.30	2.03	-	1.46	1.05	1.85	-	0.90	2.85	1.75	0.80
		N	1.23	2.06	1.27	-	0.91	0.65	1.15	-	0.56	1.78	1.09	0.50
		B	R	R	R	-	R	R	R	-	R	R	R	R
	B. paralicheniformis	E	R	R	R	-	R	R	R	-	R	R	R	-
		N	0.46	1.60	0.66	-	0.95	0.68	0.86	-	-	1.76	-	-
		B	R	R	R	-	R	R	R	-	R	R	R	-
	B. cereus	E	0.51	1.57	0.89	-	0.51	0.55	0.69	-	0.37	0.81	0.85	-
		N	0.68	2.09	1.18	-	0.68	0.73	0.92	-	0.50	1.07	1.13	-
		B	1.3	3.98	2.25	-	1.30	1.40	1.76	-	0.95	2.05	2.15	-
Percent Activity (PA)		% G^+	100	100	100	14.28	100	100	100	-	42.85	100	85.71	14.28
		% G^-	100	100	100	33.33	100	100	100	66.66	66.66	100	66.66	33.33
		% T	100	100	100	23.80	100	100	100	33.33	54.75	100	76.18	23.80
Spectral Intensity Index (SSI)			15.46	28.51	16.76	02.45	16.21	14.54	20.23	06.19	04.98	25.02	12.17	01.96

HI-PET: Petroleum ether extract of *H. indicus*; HI-ETAC: Ethyl acetate extract of *H. indicus;* HI-MeOH: Methanol extract of *H. indicus;* HI-H_2O: Water extract of *H. indicus;* HL-PET: Petroleum ether extract of *H. laurifolia;* HL-ETAC: Ethyl acetate extract of *H. laurifolia;* HL-MeOH: Methanol extract of *H. laurifolia;* HL-H_2O: Water extract of *H. laurifolia;* PP-PET: Petroleum ether extract of *P. pinnata*; PP-ETAC: Ethyl acetate extract of *P. pinnata;* PP-MeOH: Methanol extract of *P. pinnata*; PP-H_2O: Water extract of *P. pinnat;* E: Erythromycin; N: Neomycin; B: Bacitracin; % G^+: Percent activity against Gram positive bacteria; % G^- : percent activity against Gram negative bacteria; % T: Percent total activity against both type of bacteria; - : No inhibitory activity; R: Strain resistant to reference antimicrobial.

Table 5: Bacterial Susceptibility Index (BSI) of different extracts of *H. indicus, H. laurifolia* and *P. pinnata* against isolated MDR bacteria.

BSI (%)	Isolates									
	S. haemolyticus	*S. hominis*	*E. xiangfangensis*	*P. alcaligenes*	*S. epidermidis*	*B. megaterium*	*S. epidermidis*	*E. kobei*	*B. paralicheniformis*	*B. cereus*
H. indicus	75	75	75	100	75	75	100	75	75	75
H. laurifolia	75	75	100	100	75	75	75	75	75	75
P. pinnata	75	75	25	50	50	50	50	75	25	75

Bacterial Susceptibility Index (BSI).

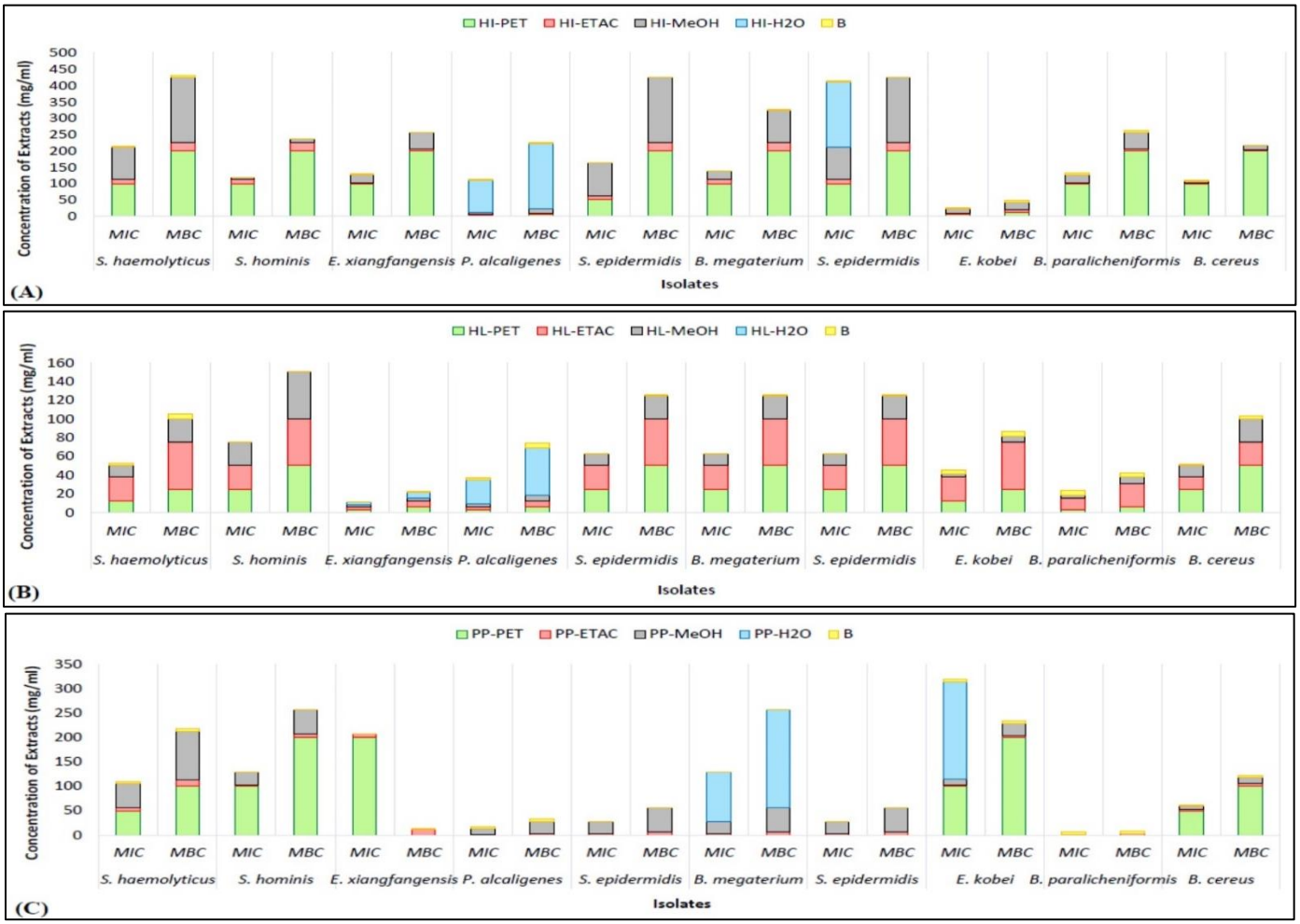


Fig. 6: MIC, MBC/MFC of extracts of **(A)** *H. indicus*, **(B)** *H. laurifolia,* **(C)** *P. pinnata* against isolated MDR bacteria.

of all the 3 extracts, a slight increase in OD was observed after 4 h of exposure but after 20 h, a slight drop in OD then stationary phase was observed in the growth profile of all the 3 bacterial isolates when compared with the control. However, at the exposure of MIC and 2×MIC of all the 3 extracts, there was no increase in OD of bacterial growth observed, else a drop in absorbance was recorded which suggested absolute bacterial eradication just after 12 and 4 h respectively. The time-kill profile of these bacterial isolates explained that the plant extracts have the potential to inhibit the growth of bacterial cells (bacteriostatic) at lower concentrations (½×MICs) and to kill the cells (bactericidal) at MICs and other higher concentrations.

3.6 Antibiofilm Activity

The antibiofilm activity experiment was only performed with HI-ETAC, HL-MeOH and PP-ETAC as these were found to be the most effective plant extracts against *P. alcaligenes, E. xiangfangensis* and *B. paralicheniformis* respectively. When compared to the control (non-treated), a concentration-dependent reduction in biofilm formation was observed by all the three plant extracts. This showed that HI-ETAC, HL-MeOH and PP-ETAC extract exhibited significant antibiofilm potential against *P. alcaligenes, E. xiangfangensis* and *B. paralicheniformis* biofilms respectively and a dose-dependent increase in antibiofilm efficacy was found. This experiment was performed to quantitate the biofilm inhibitory concentration or to evaluate the inhibition in biofilm production by the various concentrations of the extracts. From the results obtained, it was reported that 1.56 mg/ml and above concentrations of HI-ETAC, HL-MeOH and PP-ETAC extracts prevent the biofilm formation almost 90-100%. The exposure of 3.12 mg/ml concentration of HI-ETAC, PP-ETAC extracts, exerted around 98% of biofilm inhibition, so it was taken as Minimum Biofilm Inhibitory Concentration (MBIC) and in case of HL-MeOH, 6.25 mg/ml concentration showed almost 98% inhibition in biofilm, it was considered as MBIC (Fig. 8 A, B, C).

3.7 FT-IR Spectral Analysis

FT-IR spectral analysis helps to identify the various functional groups of the bioactive phytochemicals present in the extract which is based on the peak values of the FTIR spectrum. The data of infrared spectral analysis of different plant extracts revealed the presence of several functional groups in them. The FT-IR spectra of the three most potential plant extracts which were HI-ETAC, HL-MeOH and PP-ETAC are shown in Fig. 9 A, B, C and the major IR absorption frequencies with their respective assignments are tabulated in Table 6. Results indicated the presence of multiple functional groups in different extracts such as alcohol, alkane, alkene, aldehyde, ketone, carboxylic acid, amine, amide and ester etc.

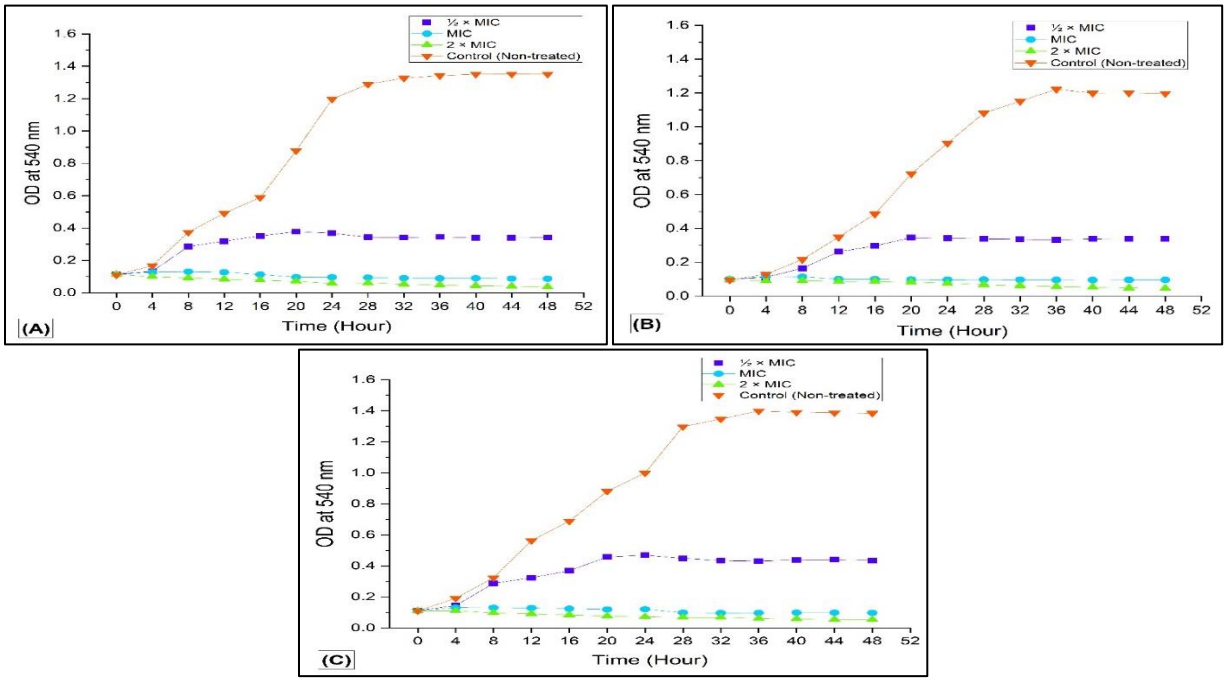


Fig. 7: Growth profile of **(A)** *P. alcaligenes* after exposure to the different concentrations of HI-ETAC, **(B)** *E. xiangfangensis* after exposure to the different concentrations of HL-MeOH **(C)** *B. paralicheniformis* after exposure to the different concentrations of PP-ETAC.

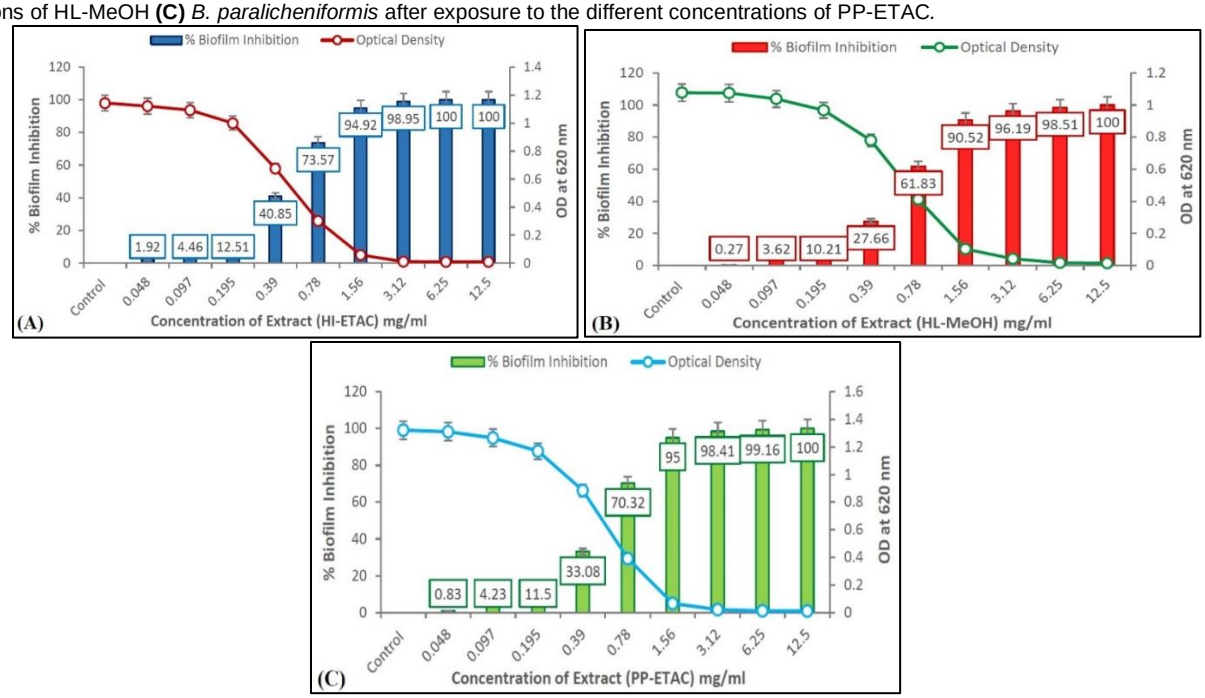
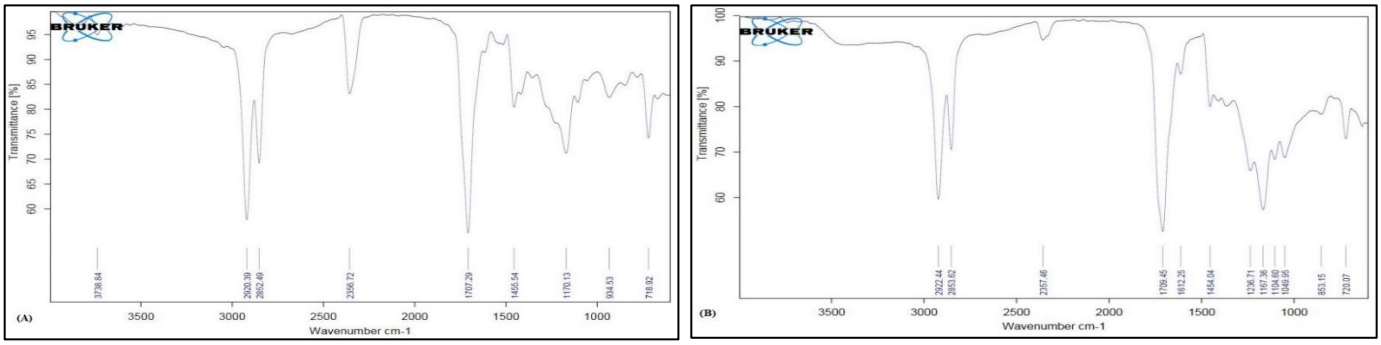


Fig. 8: % inhibition in biofilm formation after exposure to the different concentrations of **(A)** HI-ETAC against *P. alcaligenes,* **(B)** HL-MeOH against *E. xiangfangensis,* **(C)** PP-ETAC against *B. paralicheniformis.*



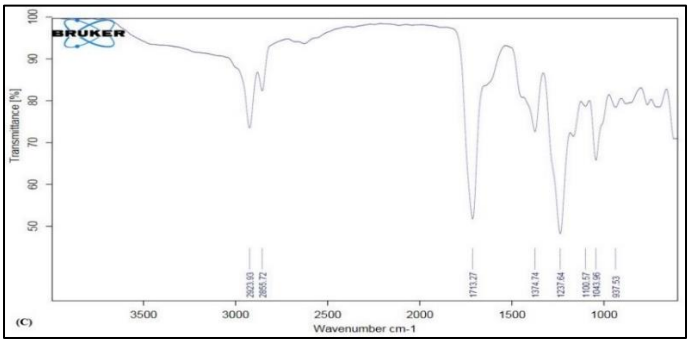


Fig. 9: FTIR spectrum of most potential plant extracts **(A)** HI-ETAC, **(B)** HL-MeOH, **(C)** PP-ETAC

Table 6: Major peaks and vibrational assignments observed in the FT-IR spectra of most potential plant extracts **(A)** HI-ETAC, **(B)** HL-MeOH, **(C)** PP-ETAC

Extract	Wave number (cm^{-1}) and vibration mode	Probable phytochemicals
HI-ETAC	2922.44 (asymmetric stretching of CH_2 of acyl chains), 2853.62 (asymmetric CH_2 stretching of the methylene chains), 2357.45 (O=C=O stretching), 1709.45 (C=O), 1612.25 (C=C stretching), 1454.04 (asymmetric methyl deformation), 1236.71 [amide III and asymmetric phosphodiester stretching (PO^{2-})], 1167.36 (C-O stretching of C-OH groups), 1104.60 (symmetric stretching of P-O-C), 1049.45 (CO-O-C; C-O stretching coupled with C-O bending of the C-OH), 853.15 (CH out-of-plane bending), 720.07 (CH out-of-plane bending)	Alkaloids, flavonoids, lipids, polyphenols, proteins, amino acids, carbohydrate, sterols, tannins, terpenoids, etc.
HL-MeOH	3738.84 (O-H and N-H stretching), 2920.39 (asymmetric stretching of CH_2 of acyl chains), 2852.49 (symmetric stretching vibration of CH_2 of acyl chains), 2356.72 (O=C=O stretching), 1707.29 (C=O), 1455.54 (C-O-H; CH_3 symmetric bending), 1170.13 (asymmetric CO-O-C stretching), 934.53 [phosphodiester stretching (P-OR esters)], 718.92 (CH out-of-plane bending)	Alkaloids, flavonoids, polyphenols, lipids, fatty acids, proteins, carbohydrate, etc.
PP-ETAC	2923.93 (C-H stretching), 2855.72 (asymmetric CH_2 stretching of the methylene chains), 1713.27 (C=O thymine), 1374.74 (C-N stretching), 1237.64 [asymmetric stretching of PO_2^- (phosphate I)], 1100.57 [symmetric stretching of PO_2^- (phosphate II)], 1043.96 (-CH_2OH group and C-O stretching coupled with C-O bending of the C-OH group), 937.56 [carotenoid; phosphodiester stretching (P-OR esters); RCOOH OH bending]	Alkaloids, flavonoids, lipids, polyphenols, carbohydrate, tannins, proteins, terpenoids, carboxylic acid containing phytochemicals, etc.

HI-ETAC: Ethyl acetate extract of *H. indicus;* HL-MeOH: Methanol extract of *H. laurifolia;* PP-ETAC: Ethyl acetate extract of *P. pinnata*.

4. DISCUSSION

The MDR strains of bacteria have been developing genes or gene products that could enable them to resist against the tested antimicrobial drugs. In this regard, the treatment with most of the antibiotics prescribed by several clinicians has been questionable as there is no drug susceptibility testing facilities in the majority of hospitals. Unless and otherwise, novel or modified currently available antibiotics will be revolutionized, MDR strains impose potentially large socioeconomic and health burden on our society as well as threat future provision of health care services [21]. The present finding substantiates many previous studies that many clinically isolated bacterial strains showed resistance to the several currently available antibiotics. Resistance to most of the antimicrobials is due to either intrinsic properties (natural phenotypic traits) or mutation in indigenous genes or through the acquisition of resistance genes by mobile genetic elements as transposons and plasmids. Such intrinsic and acquired properties could make the pathogens capable of rapid inactivation of antimicrobials by means of exportation or degradation of the antibiotics out of the cell through the alteration in antibiotic target site or the efflux system [22]. Another study suggested that the evolution of antibiotic resistance in pathogenic and commensal bacteria may occur due to the interaction between drug exposure and horizontal gene transfer by transformation, conjugation and transduction [4, 5]. As a result, several bacteria resisted for more than two groups of antibiotics due to which classified as MDR bacteria. Those issues have prompted an urgent search for alternatives from natural bioactive compounds [23]. Medicinal plants synthesize secondary metabolites like alkaloid, flavonoids, sterols, terpenes, tannins, saponins, glycoside, resins, lactones and quinines etc. and these compounds exhibited a broad spectrum therapeutic potential [6]. Hence, this study was particularly concentrated on the antibacterial and antibiofilm potential of herbal extracts against clinical MDR bacterial isolates.

The present study reported 33 bacterial isolates from patients suffering with scalp infections and out of 33 isolates, 3.3% bacteria were found MDR and most of them are opportunistic pathogens causing infections in immunocompromised persons, usually hospitalized hosts. These MDR strains were investigated according to the cultural and biochemical properties, along with the amplification of the 16S rRNA gene. *S. haemolyticus* a coagulase-negative staphylococci is generally found as human skin flora and the second most frequently isolated bacteria from human blood cultures. It has the highest level of antibiotic resistance and can generally cause infections by its ability to spread widely in tissues and production of extracellular substances [24]. Similarly, *S. hominis* is also coagulase-negative staphylococci and opportunistic pathogen causing infection in patients whose immune systems are compromised and it is reported as emerging multidrug resistant bacteria, thus leaving narrow therapeutic options [25]. *Enterobacter* spp., are the second most common carbapenem-resistant bacteria which increasingly contribute to the spread of infections [26]. In particular, *E. xiangfangensis* and *E. kobei* are involved in *Enterobacter cloacae* complex (ECC) which are the most common nosocomial bacteria and emerging MDR pathogens capable of producing a wide range of urinary and respiratory tracts infections [27]. *S. epidermidis,* coagulase-negative staphylococci, typically live on the human skin/mucosa and is one of the five most common pathogens that cause nosocomial infections [28]. Putative antibiotic resistant genes

are present in *B. paralicheniformis* cause an array of infections as ear infections, meningitis, urinary tract infections, septicemia etc. and mostly they occur as secondary infections in immunodeficient or otherwise compromised hosts. It may exacerbate previous infections by producing secondary metabolites as toxins (tissue-damaging) that interfere with treatment [29]. *B. cereus* is an emerging antimicrobial resistant strain having increased potential of foodborne infections [30]. *P. alcaligenes*, is a rare opportunistic human pathogen and resistant to broad-spectrum cephalosporins and monobactams [31]. These all isolated strains are opportunistic pathogens causing infections or somehow enhancing the intensity or severity of the infection in immunocompromised persons, cancer patients etc. As the present study reported that these strains are resistant for most of the commonly utilized antibiotics, therefore these are an emerging threat in the treatment of scalp, skin and nosocomial infections.

Present research work evaluated the antimicrobial potential of different extracts of *H. indicus, H. laurifolia* and *P. pinnata* against isolated MDR bacteria from patients suffering from different scalp infections. Different solvent extracts of plants comprise different concentrations of bioactive compounds and on the basis of that, their antimicrobial potential also variates. Many previous studies indicated that medicinal plant extracts contain a number of phytochemicals that showed remarkable antimicrobial properties against MDR bacteria. The present investigation reported that ethyl acetate root extract of *H. indicus* exhibited the best antimicrobial activity among all the plant extracts tested against almost all the MDR bacteria. Methanol, as well as ethyl acetate seed extract of *H. laurifolia* and ethyl acetate seed extract of *P. pinnata* showed high antimicrobial potential against isolated MDR bacteria. Several previous studies indicated the antimicrobial potential of these medicinal plant extracts. *H. indicus* showed a broad spectrum antibacterial activity against MDR bacteria namely *E. coli, B. subtilis, S. aureus, Salmonella paratyphi* and *Shigella dysenteriae* [32]. Saritha et al. reported antibacterial potential and mechanism of action of root extract of *H. indicus* against *E. coli* [33]. Moreover, methanol seed extracts of *H. wightiana* (Syn. *H. laurifolia*) exhibited potent antimicrobial efficacy against *E.coli, S. typhi, Proteus mirabilis* and *Klebsiella pneumoniae* [34]. According to Sajid et al., aqueous methanolic seed extracts of *P. pinnata* exhibited the remarkable antimicrobial activity against several pathogenic microorganisms, including *P. aeruginosa*, *P. stutzeri*, *E. coli, Aspergillus oryzae*, *A. niger* and *Fusarium solani* [35]. Another study reported that methanol seed extracts of *P. pinnata* showed very good antimicrobial potential against clinical bacterial isolates namely *P. aeruginosa*, *S. aureus, S. marcescens, M. luteus*, *P. vulgaris* and *K. pneumoniae* [36].

The time kill assay was performed to confirm the results of MIC experiment and to evaluate the efficacy of three plant extracts namely HI-ETAC, HL-MeOH and PP-ETAC to alter the normal growth profile of the MDR bacteria and to eliminate its growth *in vitro*. Data revealed that the MIC and 2×MIC exposure of the extracts cease the growth of test strains within 8-12 h and no subsequent regrowth was seen which confirmed that these extracts exhibited prolonged antimicrobial potential. The time kill experiment helps in the quantification of pharmacodynamics of an antimicrobial agent by estimating the decrease in growth profile of microorganisms as a function of time and concentration of the antimicrobial agent [37]. The formation of biofilm by bacteria is recognized as one of the major virulence factors during the etiology and treatment of the infection caused by pathogen as it helps to survive in hostile conditions [38]. The present investigation also dealt with the antibiofilm activity of HI-ETAC, HL-MeOH and PP-ETAC extracts against *P. alcaligenes, E. xiangfangensis* and *B. paralicheniformis* biofilm respectively and results displayed that extract has the potential to assuage the MDR bacterial biofilms.

Biological activity of any bio-organic molecule is directly relevant or influenced by the functional groups present in it, as they contribute significantly to its acid-base properties, solubility, partition coefficient, crystal structure and stereochemistry etc. and all these properties are supposed to affect the metabolic extraction, absorption, effectiveness and toxicity of any bioactive compound [39]. Hence, the functional groups analysis plays an essential role in understanding of physicochemical and biological properties of the extracts as well as in the evaluation of the structure of the bioactive compounds. FT-IR analysis of three most potent extracts (HI-ETAC, HL-MeOH and PP-ETAC) revealed the presence of various phytochemicals carrying several functional groups as O-H, N-H, CH-, CH_2-, CH_3-, C=O, COOH, C-OH, P-OR, and PO_2 etc. OH, C=O group is an integral part of most of the phenolics as flavonoids and tannins, terpenoids etc., CH-, CH_2-, CH_3-, C=O, C-OH, P-OR and PO_2 are mainly the part of lipids, fatty acids, phospholipids, carbohydrates etc., CH-, CH_2-, CH_3-, COOH and N-H are present in amino acids, proteins and alkaloids etc. It is noteworthy to mention here that the data of functional groups obtained from FTIR analysis alone is not sufficient to prove the existence of all above mentioned phytochemical classes, especially when it comes to mixtures of many different compounds. This analysis of crude extracts displayed various functional groups which are phytochemical markers and useful analytical tools to check out not only the quality and composition of the extracts but also to identify it as a medicinally important plant. The present work certainly encourages the advanced research activities on chromatographic identification and isolation of phytoconstituents and some new biologically active compounds in their pure state from these extracts.

5. CONCLUSION

The application of herb-based antimicrobials for the bio-control of various infections, as a novel emerging alternative to antibiotic treatments leading to non-toxic and more environmental managing for infectious diseases, is the must. Currently, it has become essential to think over some novel effective therapeutics or substitute to antibiotics like herbal compounds, due to the rapid development of resistance against chemotherapeutic agents. The present study had the potential value to develop herb-based products as antimicrobials agents against MDR bacteria. It supports the significant use of medicinal plant extracts in treating scalp infections caused by bacteria and these active plant extracts will provide new bioactive phytoconstituents with better activity and more effect against MDR as well as susceptible pathogens responsible for several infections than currently available antibiotics. The future aspects of the study are designed as the isolation and identification of the bioactive components of the active crude extracts of the plants and some other *in vivo* investigations are also needed for the better understanding of their action mechanism as an antimicrobial and antibiofilm agent.

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