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Original Article

Phytochemical Profiling and Bioactivity Assessment of Traditional Medicinal Plants as Innovative Therapeutics for Wound Care Applications

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ABSTRACT

Background: The worldwide healthcare system faces significant challenges due to prevalence of chronic wounds worsen by antimicrobial resistant bacteria and polymicrobial biofilm. **Objectives:** This research aims to unravel antibacterial and antibiofilm potential of bark of Lodhra, Katphal, Bijak and heartwood of Khadir, mentioned in Ayurveda. **Materials and Methods:** Extracts were prepared by maceration method using ethanol, methanol and water as solvents followed by phytochemicals analysis with qualitative tests and mass spectroscopy. Antibacterial activities of each extract were tested against *Staphylococcus aureus, Staphylococcus haemolyticus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* commonly associated with wound using agar well diffusion method. Crystal violet staining method was used to analyse the biofilm formation and eradication and MTT assay for cytotoxicity study. **Results:** All extracts have shown significant antibacterial activity against *S. aureus, S. haemolyticus, P. aeruginosa, K. pneumoniae.* Combination with the conventional antibiotics revealed synergistic effects with addition of Lodhra (0.5 FIC) and additive with Katphal (0.4 FIC). Ethanolic Katphal and methanolic Khadir extract has shown maximum antibiofilm efficacy against *S. aureus* with inhibition of 67.85% and 83.75% respectively. IC_{50} values of ethanol extract shows non-toxic effects on fibroblast cells. **Conclusion:** By bridging the gap between traditional knowledge and modern scientific exploration, this study aspires to pave the way for innovative wound care solutions.

Keywords: Antibacterial, Antibiofilm, MTT Assay, Phytochemicals, Synergistic.

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INTRODUCTION

Chronic wounds impact a significant portion of world population. The global occurrence of these wounds is predicted to be 1 to 2% in developing countries with the incidence expected to increase with increase in age (Abebia *et al*., 2025). The biofilm presence is accredited as a major factor driving chronicity and are responsible for over 65% of all chronic bacterial infections (Regulski *et al*., 2023). In India, community-based epidemiological study reported that the frequency of chronic wounds to be 1.89 per 1000 population. Chronic wounds often lead to significant discomfort and adversely affect a patient's quality of life. They also place a substantial economic burden on both patients and healthcare systems (Sharma *et al*., 2024). Antimicrobial agents are the keys of modern medicine (Jangra *et al*., 2025), Yet the rise of resistant bacteria have become a severe and growing global threat. Beyond antibiotic resistance, biofilm formation is another survival strategy used by bacteria. Biofilm is the aggregation of microorganisms encased in a slimy matrix called Extracellular Polymeric Substances (EPS) (Goswami *et al*., 2023; Kuschmierz *et al*., 2022). Strategies to manage biofilm infection involves some conventional methods like cleaning, debridement, wound dressing, bandaging, use of antibiotics etc. Emerging strategies includes grafting, negative pressure therapy, stem cell therapy, bioengineered cellular therapy, microbubbles, nanomaterial, nano-emulsion use (Advancements, 2022). The complexity of biofilm and their increased resistance become more challenging to the current treatments (Goswami *et al*., 2023). EPS hinder antibiotic penetration, allows evasion of the host immune system and facilitates gene evolution through genetic material exchange, thereby contributing to the inefficacy of antimicrobial drugs (Di, 2018). Despite their benefits and applications, existing treatment processes (including both surgical and medical) facing restrictions such as short duration of effectiveness, high costs, high toxicity, low efficacy, and increased infection threat (Abebia

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et al., 2025). In conclusion, biofilm have a significant impact on clinical outcomes by fostering chronic infection, persistence and antibiotics resistance (Sharif and Yadav, 2025). Their capacity to evade both host immune response and standard therapeutic methods emphasizes need for innovative approaches for prevention, diagnosis and treatment of chronic wound associated with biofilm (Dwivedi *et al.*, 2017; Karan *et al.*, 2024).

Addressing such challenges turns the way towards traditional medicinal systems, many medicinal plants have antioxidant, angiogenic, anti-inflammatory, and cell modulating properties (Kadam *et al.*, 2020; Sharma *et al.*, 2021). Traditional medicine practices have been identified as significant source of novel antimicrobial agents (Abdallah *et al.*, 2023). However, the mechanism of action of phytochemicals at the tissue level and their antibacterial activity at molecular level are not well documented (Kadam *et al.*, 2020; Shoaib *et al.*, 2016). This work aims to assess the effectiveness of the traditional medicines on biofilm mediated infection. Although there has been recent progress, literature is mainly based on *in vivo* and *in vitro* case studies of small animals only, highlighting the need of extensive research to fill this gap (Kadam *et al.*, 2020). Due to their great chemical diversity, plants are promising sources for developing drugs or strategies for the wound treatments, contributing the development of new low cost, accessible strategies for the treating bacteria- infected wounds (Mandrika *et al.*, 2021). Traditional plant-based remedies are having great significant as a novel antibacterial agent. To investigate this potential, it is essential to incorporate them into present scientific research. Various phenolic compounds, terpenoids, alkaloids, and other plant-derived substances may have the potential to address wound biofilm due to their antioxidant, angiogenic, anti-inflammatory, and cell synthesis-modulating properties (Kadam *et al.*, 2020). However, research in this area is limited and further investigation and development are necessary.

Therefore, the work aims to investigate potential mechanism of action of four medicinal plants using the reference from Ayurvedic literature. The bark of *Symplocos racemosa* (commonly known as Lodhra), *Pterocarpus marsupium* (commonly known as Bijak), *Myrica esculenta* (commonly known as Katphal), and the heartwood of *Senegalia catechu* (commonly known as Khadir) to were used for the study. The bark of *Symplocos racemosa* is extensively used in Ayurvedic and Unani preparation from long time because of its antibacterial, anti-inflammatory effects (Maitra and Satardekar, 2017; Sood *et al.*, 2020). The whole plant of *Myrica esculenta* have nutritional and therapeutic importance (Don and Baghel, 2019). Phenolic nanoparticles of *Pterocarpus marsupium* were showing inhibition action against *Staphylococcus aureus* (Suparno *et al.*, 2024). *Senegalia catechu* has been extensively used in management of various problems because of its antibacterial, anti-inflammatory, antioxidant, antipyretic activities (Tree, 2025). This ancient remedy continues to be an essential herbal resource both for curative and preventive healthcare. The present work revealed antibacterial and antibiofilm properties of these four plants extracts. They were tested against Gram-positive bacteria i.e. *Staphylococcus aureus* and *Staphylococcus haemolyticus* and Gram-negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which are commonly associated with chronic wounds.

MATERIALS AND METHODS

Extraction of phytoconstituents by maceration technique

The bark of *Symplocos racemosa* (Symplocaceae, common name: Lodhra), *Pterocarpus marsupium* (Fabaceae, common name Bijak), *Myrica esculenta* (Myricaceae, common name: Katphal), and the heartwood of *Senegalia catechu* (Fabaceae, common name: Khadir) were used for the study. They were named as L, Bj, Kt, and Kh for Lodhra, Bijak, Katphal and Khadir respectively.

The plant extracts were prepared by using a maceration technique using 10 g of plant material (Figure 1) dissolved in with 100 mL of absolute ethanol, methanol, and distilled water separately for 72 hr. Extracts were filtered and then concentrated using rotary evaporator at 40°C. Dried extracts were then stored in cool and dry place (Article, 2020). Extracts were subjected to phytochemical analysis and antibacterial, anti-biofilm evaluation. For each sample, amount of dried extract mass was used for calculation yield was calculated (Ngamkhae *et al.*, 2022) as,

$$\text{Yield percentage} = \frac{\text{mass of dried plant extract}}{\text{mass of plant sample used}} \times 100$$

Phytochemical profiling by LC MS

In accordance with the established protocols, a qualitative analysis of phytochemicals was conducted to estimate the presence of saponins, tannins, alkaloids, flavonoids, steroids, terpenoids, and phenol in plants extracts (Al-Deen *et al.*, 2019; Madhu *et al.*, 2016; Pooja *et al.*, 2022). Ethanol extracts of plant material were subjected to LC- Mass Spectroscopy for identification of the phytochemicals based on molecular formula, mass and retention time. Phytochemicals separation was carried out in positive ion mode using 6200 series TOF/6500 series Q-TOF B.09.00 (B9044.0) mass spectrometer with electrospray ionization source.

Antibacterial and anti-biofilm activity of plant extracts

Two Gram-positive bacteria, *Staphylococcus aureus* DSM 3463(A) and *Staphylococcus haemolyticus* 10024 CHB (B) and two Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 THL (C) and *Klebsiella pneumoniae* ssp. o*zaenae* 9295(D) were used for the study. The bacteria used for the study are predominantly present in wound and all of these bacteria were biofilm forming ability. The identity of the bacterial strains was further validated by MALDI-TOF MS. The results are

documented in our publication. (Shinde *et al.*, 2025). All the bacteria were maintained on TSA (Tryptic Soy Agar) plates till further use and incubated at 37°C for 24 hr before use.

Plant extracts were screened for antibacterial activity using the agar diffusion technique. The study was performed as per the procedure mentioned by CLSI guidelines (Clinical and Laboratory Standard Institute). Bacterial cultures were inoculated in TSB (Tryptic Soy Broth) and incubated at 37°C for 24 hr.

This inoculum was diluted to adjust the OD between 0.08 and 0.1, maintaining the turbidity approximately 1.5×10^8 CFU/mL (0.5 McFarland standard). These suspensions were then spread on MHA (Mueller Hinton agar) plates using sterile cotton swabs (Untachai *et al.*, 2018). Aseptically, in each well 100 µL of plant extract was added with concentration of 50 mg/mL. The isolates were also tested with different antibiotics that are often used in clinical trials to assess their resistance levels (Nath *et al.*, 2019). Antibacterial activity of plant extract was then evaluated by measuring the zone of growth inhibition surrounding the wells. Subsequently, the MIC (Minimum Inhibition Concentrations) of plant extracts was determined by broth dilution method. A sterile 96 well plate was supplemented with 100 µL of TSB with 5 µL of bacterial culture and different concentrations of plant extracts (20, 10, 5, 2.5, 1.25 mg/mL). The plates were incubated at 37°C for 24 hr. The absorbance was measured at 600 nm. The MIC was determined as the lowest plant extract concentration in the wells that shows no significant difference in absorbance compared to the blank control wells. The experiment was conducted in triplicates and the findings are shown as the average of the three measurements ± standard deviation.

Fractional Inhibitory Concentration (FIC) assay

Bacterial suspension with 0.5 McFarland standard were spread on MHA plates using sterile cotton swabs. Antibacterial activity of antibiotics disc viz. Aztreonam - AT (30 mcg/disc), Co-Trimoxazole-COT (25 mcg), Linezolid- LZ (30 mcg), Tobramycin- TOB (10 mcg), Tetracyclin - TE (30 mcg), Gentamicine - GEN (10 mcg), Amikacin-Ak (30 mcg), Piperacillin PIT (100 mcg) were done and zone of inhibition was measured. Same antibiotics discs were placed on the plate and 5 µL of plant extract were added to each disc and plates were incubated. FIC, i.e. Fractional inhibitory concentration was calculated with the formula (Nath *et al.*, 2019).

$$\text{FIC} = \text{extract FIC} + \text{antibiotics FIC}$$

Where,

$$\text{FIC extract} = \frac{\text{MIC of plant extract in combination}}{\text{MIC of plant extract only}}$$

Figure 1: Parts of selected plants (Bark of Lodhra, Bijak, Katphal and heartwood of Khadir).

$$\text{FIC antibiotics} = \frac{\text{MIC of antibiotics in combination}}{\text{MIC of antibiotics only}}$$

The FIC index was interpreted as synergy; FIC≤ 0.5, additive; 0.5 ≥ FIC ≤ 1, indifference; 1 ≥ FIC ≤ 4; and antagonism; FIC < 4 (Chauhan *et al*., 2023; Shafiei *et al*., 2016).

Antibiofilm assay

Assessment of biofilm inhibition efficacy of plants extracts

All the bacterial strains were reported to produce biofilm as documented in our earlier work (Shinde *et al*., 2025). The concentrations of plant extracts showing significant antibacterial activity was used for antibiofilm study. 100 µL plant extract diluted with TSB was added to 96 well plates. Overnight grown culture of bacteria diluted to 0.5 McFarland standards in fresh medium was added. Bacterial culture and media with antibiotics is use as positive control, negative control was prepared by adding bacterial culture with TSB media. Equal volume of only media was added as media control, well with media plus plant extract was used as extract control (Bazargani and Rohloff, 2016). Plate was kept at 37°C for 24 hr. Aseptically, wells were aspirated and planktonic cells were removed by washing three times with Phosphate Buffer Saline (PBS). The attached biofilm was fixed by adding 150 µL of 96% methanol for 15 min. Methanol was removed and stained the wells with 200 µL of 1% crystal violet for 20 min. Then wells were cleaned with sterile distilled water and air dried. The stain from adhered biofilm was extracted by addition of 150 µL of 33% glacial acetic acid and absorbance was noted at 630 nm. The experiment was performed in triplicate and percentage of biofilm inhibition was determined by using following formula:

% of Biofilm inhibition = (OD negative control- OD of media control) - (OD test - OD extract control)/ (OD negative control- OD media control) × 100

Biofilm reduction assay

For the biofilm reduction assay, 100 µL of overnight-grown bacterial culture in TSB was added to a 96-well microtiter plate and incubated at 37°C for 24 hr to allow biofilm formation. After incubation, the planktonic medium was carefully removed, and the wells were gently washed three times with PBS. Different concentrations of plant extracts diluted with media were added to the respective wells and incubated at 37°C for an additional 24 hr. Control wells were maintained under the same conditions, and biofilm quantification was performed using the crystal violet staining method (Sood *et al*., 2020).

MTT assay for toxicity

Cytotoxicity of ethanolic plant extracts was studied using MTT (3, 4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide) assay (Dandannavar *et al*., 2019; Sci *et al*., 2021). Here, MTT, yellow coloured tetrazolium dye is reduced to insoluble formazan crystals by mitochondrial lactate dehydrogenase. For this assay, mouse fibroblast cell line L929 was cultured in MEM (Minimum Essential Media) supplemented with 10% FBS (Fetal Bovine Serum), 1% antibiotics and 1% NEAA (Non-essential amino acids) and incubated at 37°C under 5% CO_2. At 80% cell density, the culture was trypsinized, stained with Tryphan blue and counted using hemocytometer. Here, dead cells are appearing blue in colour. Seed 100 µL of L929 cell suspension (1×10^4 cells/mL) in a 96 well plate and cultured overnight at 37°C, 5% CO_2. After incubation, the cells were treated with different concentration of plant extracts (10, 20, 40, 60, 80,100 ug/mL) keeping media control (medium without cells), cell control (medium with cells and no plant extract) and vehicle control (medium with plant extract and no cells) and further kept at 37°C for 24 hr. Add 20 µL of MTT solution in each well (10% of total volume) and after 2 to 4 hr of incubation add 100 µL solubilizing solution and stir for 30 min to enhance dissolution of formazan crystals. Read the absorbance on ELISA reader at 570 nm and percentage of cell viability was calculated as:

$$\text{Percentage of cell viability} = \frac{\text{OD of treated cells} - \text{OD of blank}}{\text{OD of untreated cells} - \text{OD of blank}} \times 100$$

Statistical analysis

All experiments were run in triplicate, and the results are reported as the mean±SD standard deviation). To assess statistical significance among multiple groups, ANOVA (A One-way Analysis of Variance) was employed. Results were considered statistically significance if $p<0.05$.

RESULTS

Extraction of phyto-constituents

The highest quantity of extract was obtained from Khadir using all three solvents peaking with 66.7% with ethanol, 63.4% with methanol followed by 52.1% with water. Lodhra demonstrated highest yield with aqueous extraction at 41% while 36.8% with ethanol and 33.7% with methanol. Katphal produces 14.4% with methanol, 12% with water and 7.4% with ethanol. Bijak resulted in 6.4% with ethanol followed by 4.6% and 5.2% with methanol and water respectively.

Phytochemicals analysis

Qualitative phytochemical analysis indicated positive results for flavonoids, tannins, terpenoids, anthraquinone, glycosides and alkaloids. Anthraquinone test was negative for Bj, results were shown in Table 1A. The LC-MS analysis of ethanolic extracts of Bj, L, Kt and Kh in positive ion mode displayed several distinct peaks, signifying the presence of a various phytochemicals with differing polarities. Peaks that appear early in the chromatogram indicate polar phenolic compounds, whereas those that appear in the middle and later stages are associated with flavonoids, alkaloids, and terpenoids-like substances. List of phytochemicals reported by LS-MS analysis along with their molecular mass and retention time are mentioned in Table 1B.

Antibacterial and anti-biofilm assay of plant extracts

Antibacterial assay of plant extracts

Ethanol, methanol and aqueous extracts of four plant samples were analysed for their antibacterial activity against four different bacteria by agar well diffusion method and results are tabulated in Table 2. Among all extracts, Katphal was showing significant zones of inhibition ranged between 12 mm to 25 mm for Gram positive and 10 mm to12 mm for Gram negative bacteria. Ethanolic, methanolic and aqueous extract of Lodhra exhibited significant antibacterial activity against all four tested microorganisms, with the effectiveness in the following order: *S. aureus* > *S. haemolyticus* > *P. aeruginosa* > *K. pneumoniae*. The zone of inhibition was in the range of 11-17 mm for Gram-positive bacteria and 10-13 mm for Gram-negative bacteria. Ethanolic extract of Bijak indicated good activity against four bacteria, with zone of inhibition in the range of 10 mm to 15 mm. Khadir also exhibited inhibition against *S. aureus* and *S. haemolyticus* was in the range of 10 mm to 13 mm. Methanolic and aqueous extract of Bijak, Lodhra and Khadir was not showing significant activities against *P. aeruginosa* and *K. pneumoniae.* Two-way ANOVA observed significant main effect of plants, types of extracts and microorganisms with $p<0.05$.

Fractional Inhibitory Concentration (FIC) assay

Since ethanolic extract of Lodhra and Katphal demonstrated notable antibacterial property, these extracts were combined with conventional antibiotics: Aztreonam - AT (30 mcg/disc), Co-Trimoxazole-COT (25 mcg), Linezolid- LZ (30 mcg), Tobramycin- TOB (10 mcg), Tetracyclin - TE (30 mcg), Gentamicine - GEN (10 mcg), Amikacin - Ak(30 mcg), Piperacillin PIT (100 mcg) to evaluate their effectiveness against *Staphylococcus aureus* (Gram positive, Figure 2A) and *Pseudomonas aeruginosa* (Gram negative, Figure 2B). The zone of inhibition of combination of conventional antibiotics and ethanolic extract of Lodhra and Katphal with the corresponding FIC index values for each antibiotic combination is presented in Table 3. The result revealed synergistic effects with addition of Lodhra and Katphal.

Antibiofilm activity

Biofilm inhibition assay of plant extracts

The antibiofilm potential of plant extracts against four bacterial strains was evaluated using crystal violet staining method. Ethanolic (Et), Methanolic (Mt), and aqueous (D/W) extracts of Katphal (Kt), Khadir (Kh), Lodhra (L), and Bijak (Bj) was used to assess the anti-biofilm potential and result are mentioned in Table 4A. For *S. aureus*, the ethanolic extract of Katphal exhibited the highest antibiofilm activity with 67.85% reduction, while methanolic extract of Khadir indicated 83.57% reduction. Conversely, aqueous extract of Khadir and ethanolic extract of Bijak, have shown minimal or no inhibition. Methanolic extract of Lodhra exhibited variable activity with inhibition up to 46.28% against *K. pneumoniae*. Bijak extracts have shown the least antibiofilm potential, indicating possible no inhibition (Figure 3A).

Overall, ethanol and methanol extracts exhibited higher antibiofilm activity across the tested bacterial strains. These findings suggest the potential of plant extracts, especially Katphal and Khadir, as sources of anti-biofilm agents against clinically relevant bacteria.

Biofilm reduction assay

The results from the antibacterial and antibiofilm assays prompted us to further investigate the effect of the ethanol extracts of these plants on pre-formed biofilms. The ability of plant extracts to inhibit biofilm, which was already formed was determined and presented in Table 4B. Khadir was showing higher percentage of inhibition with *S. aureus, S. hemolyticus* and *K. pneumoniae*, except *P. aeruginosa* showing much lower inhibition. Katphal has shown the highest biofilm inhibition of *K. pneumoniae*. Lodhra was showing significant effectiveness against *S. hemolyticus*, and Bijak against *K. pneumoniae.* Overall, the data reflect significant variability in inhibitory effects (Figure 3B).

Cell toxicity assay

Figure 4 indicates the dose dependent viability of mouse fibroblast cells after 24 hr treatment with plants extracts using MTT assay. Here cell viability affected as increasing the extract concentration for all plants except Katphal. IC_{50} values for B and Kh is between 50 to 60 ug/mL, whereas L is more robust crossing it closure to

Table 1A: Qualitative phytochemical analysis.

Phytochemicals	Bj	L	Kt	Kh
Flavonoids	+	-	-	-
Tannins	+	+	+	+
Anthraquinone	-	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	+	+	+
Alkaloids	+	+	+	+

Table 1B: Phytochemicals identified from ethanol extract using Mass spectroscopy.

Lodhra	RT	Mass	Khadir	RT	Mass
Miserotoxin	3.233	267.0941	Niaziminin A	11.028	399.135
Edetate	14.011	292.0917	Sirodesmin H	11.511	454.1403
Methyl 3,4,5-	14.084	252.0996	Disperse Blue 1	12.191	268.0948
trimethoxycinnamate			Trinexapac-ethyl	12.38	252.1003
Aurasperone E	15.649	588.1642	Asticolorin C	12.919	568.1731
(E,E)-4,4''-Bi(N-4-hydroxycinnamoylseroto nin)	16.359	642.2501	Asparaginyl-Histidine	13.441	269.1123
2-Phenylethyl beta-Dglucopyranoside	16.854	284.1251	Decarbamoylsaxitoxin	13.566	256.1289
(S)-Rutaretin	17.776	262.0836	Spaglumic Acid	13.6	304.0917
N-(2-Methoxyphenyl)-N'-	17.993	292.1201	Methyl 3,4,5-	14.646	252.0998
(2-naphtyl)urea			trimethoxycinnamate		
Glutaminyl-Tryptophan	18.457	332.149	6-(1,2,3,4-Tetrahydro-6-		
8-Epideoxyloganin	19.031	374.1584	hydroxy-2-naphthyl)-	15.629	241.11
Poncirin	19.096	594.1958	2(1H)-pyridone		
O-Isopentenylhalfordinol	19.407	306.1366	Hamaudol	17.315	276.0991
Coniferin	19.789	342.1327	Mitoxantrone	23.018	444.1996
			4,4-Difluoro-	25.909	338.2059
			17betahydroxy-		
			17alpha-methylandrost-		
			5-en-3-one		
			9-Fluoro-	26.918	360.2102
			2alphamethylpregn-		
			4-ene-		
			3,11,20-trione		
			7-Methylinosine	27.146	283.1043
Bijak	RT	Mass	Katphal	RT	Mass
N-2-Methylpropyl) acetamide	2.237	115.0992	4-tert-Butylphenyl salicylate	14.202	270.1254
Hesperetin 3'-O-sulfate	3.667	382.0366	Prosulfuron	3.027	419.0879
Penicillin O	6.821	330.0708	Miserotoxin	3.398	267.0944
Podolactone B	10.213	394.125	Midodrine	10.488	254.1263
Afzelechin	10.215	274.0835	Phaseolic acid	12.625	296.0523
7-beta-Dglucopyranosyloxybutylid enephthaide	10.213	366.1297	Dinocton 6	13.229	354.1432
Aspergillic acid	10.309	224.1524	Arctiopicrin	13.231	350.1716
Ptelatoside A	10.740	414.1518	beta-Rhodomycin	13.493	543.2117
Tutin	10.749	294.1097	Orizabin	13.636	366.1664
ECG / Epicatechin gallate	10.891	442.0905	Prosolanapyrone III	14.202	302.151
(S)-2,3-Dihydro-3,5-dihydrxy-2-oxo-3-i ndoeacetic acid 5- glucoside	14.011	385.101	Allamandin	13.764	308.0886
(S)-N-[3-(3,4-Methylenedioxyphenyl)-2-(acetylthio)methyl-1-oxoprolyl)glycine benzyl ester	14.919	486.1455	Sambucus nigra degraded cyanogenic glycosides(2'-epimer)	14.413	353.1111
Coixinden B	17.232	234.0895	Na-Hexanoyl-Nbinosityltryptophan	20.684	464.2164
Coriandrone E	17.489	248.068	Zearalanone	14.203	320.162

Lodhra	RT	Mass	Khadir	RT	Mass
(Z)-1,5-Tridecadiene	19.450	180.1884	Karwinaphthol B	14.204	288.1353
2-Tridecanone	19.453	198.19859	Aziridyl benzoquinone	14.206	338.1481
Diisopropyl phthalate	19.490	250.1201	3-(2-methypropanoyloxy)-8-(2-methibutanoyloxy)-9, 10-epoxy-p-mentha-1,3,5-triene	14.635	334.1769
DHAP(6:0)	20.360	268.0712	Gibberellin A51	15.594	332.1618
Strigolactone ABC-rings	23.558	234.125	N2-Malonyl-D-tryptophan	15.594	290.0913
Cynaroside A	23.851	444.1996	Diphenolic acid	15.594	286.1198
			Musca-aurin-VII	16.03	349.1133
			Oxocamphor	17.273	166.0999
			Microcystin YR	18.996	1044.528
			Etoxazole	19.793	359.1693
			Stachyoside A	20.231	526.191
			Acremoauxin A	21.938	323.1373
			Brevetoxin A	23.473	866.4838
			5-Nonyltetrahydro-2-oxo-3-furancarboxylic acid	28.353	256.1668
			7-Methylinosine	28.375	283.104

Table 2: Antibacterial activity of plant extracts measured as mean zones of inhibition (mm) in Agar well diffusion assay.

Bacterial strains	Ethanol Extract				Methanol Extract				Aqueous Extract			
	L	Bj	Kt	Kh	L	Bj	Kt	Kh	L	Bj	Kt	Kh
S. aureus	17	15	25	13	13	11	18	13	13	10	18	10
S. hemolyticus	16	10	21	11	11	10	12	11	12	10	12	10
P. aeruginosa	13	11	11	10	10	NA	11	NA	12	NA	11	NA
k. pneumoniae	11	13	12	12	NA	NA	10	NA	10	NA	NA	NA

Kt- Katphal, Kh- Khadir, L-Lodhra, Bj-Bijak, The values are mean of three experiments.

80-90 ug/mL, Katphal showed induce proliferation up to 137% to 160% respectively.

DISCUSSION

The traditional medicinal system, significantly influences the modern medicines (Venugopal and Shukla, 2025). Single or polyherbal formulation may offer broad spectrum of bioactive molecules (Jangra *et al*., 2025). Phytochemical profiling along with antibacterial and antibiofilm activity of the bark of *Symplocos racemosa* (Lodhra), *Pterocarpus marsupium* (Bijak), *Myrica esculenta* (Katphal), and the heartwood of *Senegalia catechu* (Khadir) was performed against *Staphylococcus aureus, Staphylococcus haemolyticus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which are frequently associated to chronic wounds.

The highest yield of extraction was observed with Khadir across all three solvents indicating its rich phytochemical content. Qualitative phytochemical estimation revealed the occurrence of tannins, flavonoids, terpenoids, anthraquinone, alkaloids and glycosides which have been reported to employ varying degree of action against bacteria. LC-MS analysis further confirmed the diverse range of phytochemicals, highlighting the complexity and therapeutic potential of these extracts.

Antibacterial assay demonstrated that the Lodhra extract showed significant inhibitory action against all tested microorganisms. Bijak extract has shown least antibacterial activity whereas Katphal extract showing highest inhibition especially against Gram positive bacteria. Khadir displayed moderate inhibition activity which was limited to *S. aureus* and *S. haemolyticus*. The observed variations in antibacterial efficacy among the plant extracts with different solvents indicate differential solubility and action of bioactive phytochemicals.

Combination studies with conventional antibiotics paired with ethanol extract of Lodhra and Katphal revealed synergistic effect against *S. aureus* and *P. aeruginosa* enhancing antibacterial efficacy.

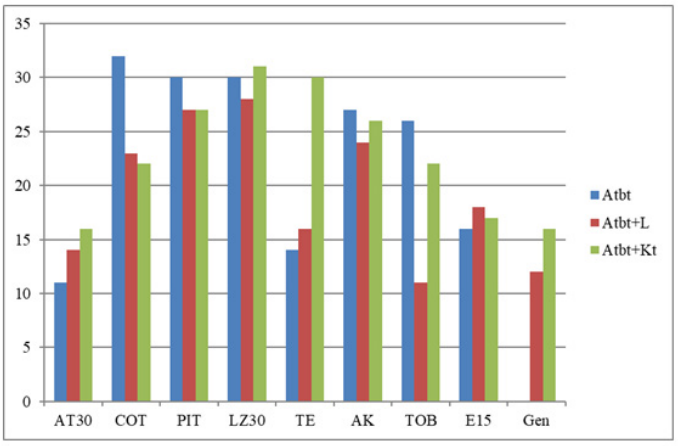

Figure 2A : Showing comparison of action of combination of conventional antibiotics and Plant extracts (Lodhra and Katphal) on *S. aureus*.

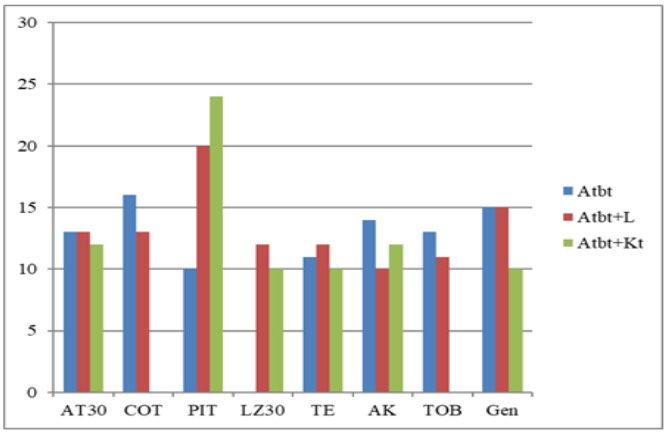

Figure 2B: Showing comparison of action of combination of conventional antibiotics and Plant extracts (Lodhra and Katphal) on *P. aeruginosa*.

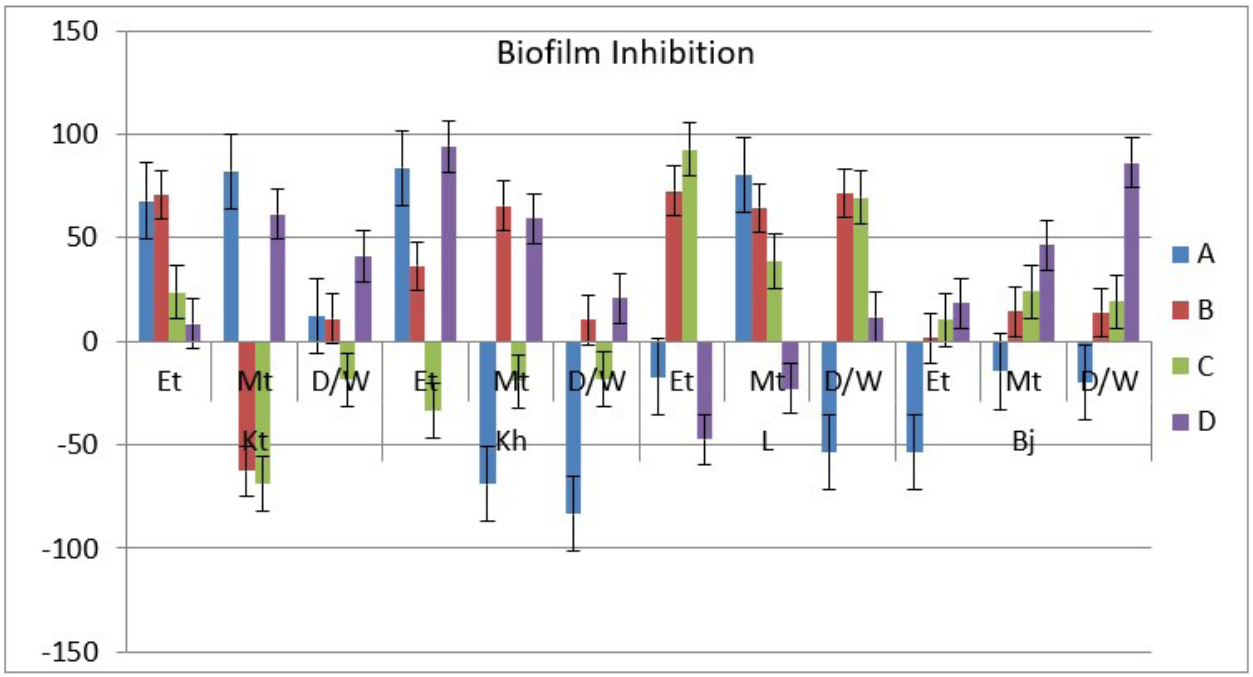

Figure 3A: Percentage of biofilm inhibition using Ethanol (Et), Methanol (Mt) and Water (D/W) extracts of Katphal (Kt), Khadir (Kh), Lodhra (L) and Bijak (Bj) on *S. aureus* (A), *S. hemolyticus* (B), *P. aeruginosa* (C) and *K. pneumoniae*.

The antibiofilm assay results demonstrated that the ethanol and methanol extracts exhibited greater inhibition of biofilm formation compared to the aqueous extracts. Among the tested samples, the ethanol extract of Katphal and the methanol extract of Khadir showed the highest antibiofilm activity against *Staphylococcus aureus*, with inhibition percentage of 67.85% and 83.75%, respectively.

In contrast, the aqueous extracts of Bijak and Khadir, along with methanol extract of Khadir, reported minimum or no inhibition which may be attributed to an insufficient concentration of active phytoconstituents in the extracts. The inhibitory activity of the plant extracts against pre-formed biofilms varied considerably depending on both the type of extract. Khadir extract exhibited a broad spectrum of biofilm inhibition against most of the tested organisms, except *Pseudomonas aeruginosa*, whereas Katphal and Lodhra demonstrated comparatively selective antibiofilm activity.

Antibiotics resistant bacteria have adapted to withstand antibiotics rendering infection, difficult to treat posing a significant global health challenge. Thus, a critical need exists for the development of a novel inhibitory agent targeting these bacteria. Throughout the history, plant and their derivatives have been extensively used to treat such medical conditions. A wealth of research has focused on extracting natural products to evaluate their antimicrobial potential.

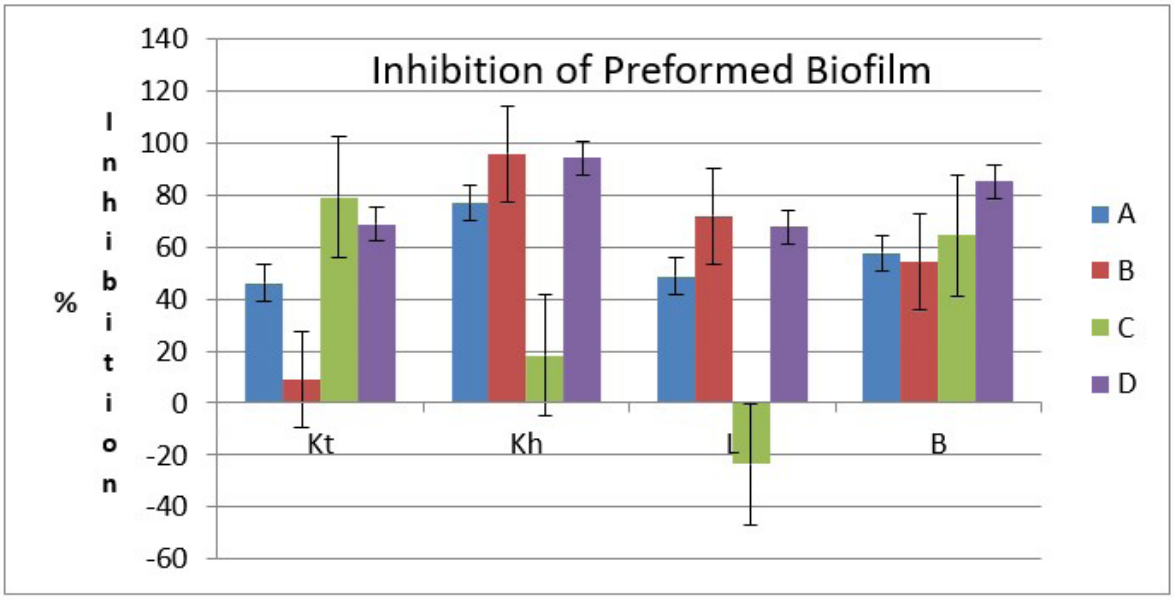

Figure 3B: Showing % of inhibition of preformed biofilm using ethanol Extract of Kt- Katphal, Kh- Khadir, L- Lodhra, Bj- Bijak on A- *S. aureus*, B- *S. hemolyticus*, C- *P. aeruginosa*, D- *K. pneumoniae*.

Table 3: Mean zones of inhibition of combination of conventional antibiotics and Plant extracts in well diffusion assay with the corresponding FIC index values for each antibiotic combination.

Zone of Inhibition (mm)								
S. aureus				*P. aeruginosa*			FIC index	
Name of Atbt	Atbt	Atbt+L	Atbt+Kt	Atbt	Atbt+L	Atbt+Kt	Atbt+L	Atbt+Kt
AT30	11	14	16	13	13	12	0.53	0.43
COT	32	23	22	16	13	0	0.525	0.425
PIT	30	27	27	10	20	24	0.51	0.41
LZ30	30	28	31	0	12	10	0.53	0.43
TE	14	16	30	11	12	10	0.53	0.43
AK	27	24	26	14	10	12	0.53	0.43
TOB	26	11	22	13	11	0	0.51	0.41
Gen	0	12	16	15	15	10	0.51	0.41

Atbt - Antibiotics alone, Atbt+L - antibiotics with Lodhra extract, Atbt+kt- antibiotics with Katphal extract.

Table 4A: Antibiofilm potential (in %) of plant extracts against four bacteria using crystal violet staining assay.

Bacteria	Kt			Kh			L			Bj		
	Et	Mt	D/W	Et	Mt	D/W	Et	Mt	D/W	Et	Mt	D/W
A	67.85	82.14	11.85	83.57	-69.07	-83.57	-17.14	80	-53.57	-53.57	-14.64	-19.85
B	70.64	-62.8	10.9	36.42	65.48	10.2	72.58	64.51	71.32	1.45	14.32	13.96
C	23.61	-68.8	-18.6	-33.4	-19.25	-18.3	92.53	38.54	69.47	10.41	24.06	19.2
D	8.3	61.14	41.02	93.98	59.28	20.89	-47.36	-22.75	11.43	18.34	46.28	86.06

The values are mean of three different experiments. Kt- Katphal, Kh- Khadir, L- Lodhra, Bj- Bijak, Et -ethanol extract, Mt- methanol extract, D/W- water extract, A- *S. aureus*, B- *S. hemolyticus*, C- *P.aeruginosa*, D- *K. pneumoniae*.

Table 4B: Inhibition (in %) of preformed biofilm using ethanol extract of Kt, Kh, L and B.

Bacteria	% of inhibition of preformed biofilm			
	Kt	Kh	L	Bj
A	46.17	76.92	48.71	57.70
B	8.93	96.00	72.00	54.30
C	79.00	18.20	-23.40	64.49
D	68.79	94.20	67.90	85.39

Kt- Katphal, Kh- Khadir, L-Lodhra, Bj-Bijak, *S. aureus* (A), *S. hemolyticus* (B), *P. aeruginosa*(C) and *K. pneumonia*.

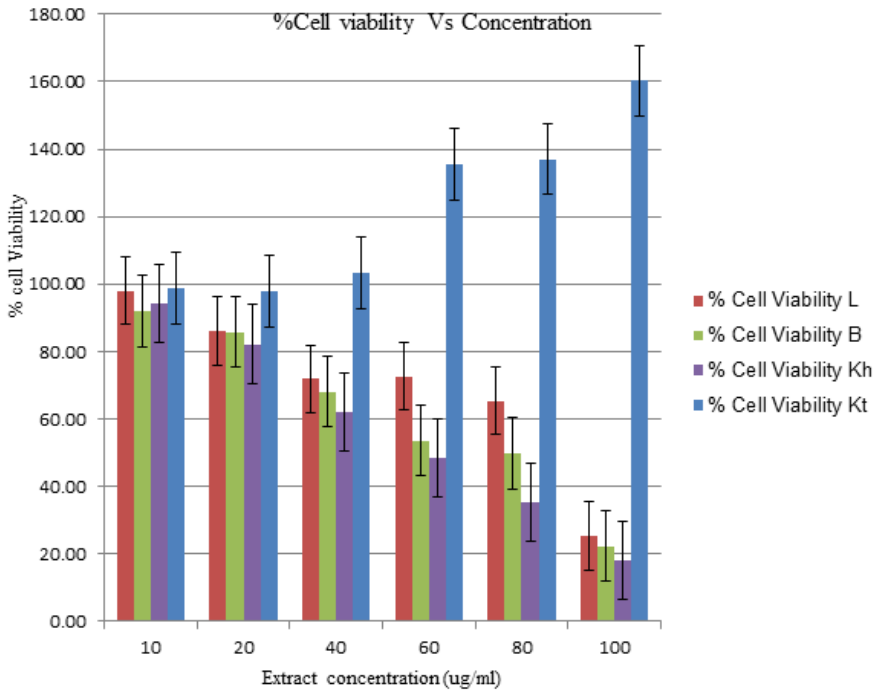

Figure 4: % viability of mouse fibroblast cell line L929 with different concentration of ethanol extract of L, B, Kh and Kt. The values are expressed as ± SD.

This work has shown the potential of plant extracts evaluating particularly Lodhra, Katphal and Khadir as an antibacterial and antibiofilm agent. Our research findings are generally consistent with existing literature (Acharya *et al.*, 2016; Butala *et al.*, 2017; Tree, 2025). The variation in the activities highlighted the importance of choice of solvent for extraction and the extract composition. The synergistic effect of Lodhra with antibiotics combination suggests a promising adjunctive approach to combat antibacterial resistant strains. Cytotoxicity studies indicating dose dependent responses with L, B and Kh whereas Kt induces proliferation in fibroblast cells. According to the data often cited in literature and National Cancer Institute consider IC_{50}<100 ug/mL to be active and our data showed non-toxicity of ethanol extract of plants and can be used for further studies.

CONCLUSION

In search of better treatment, numerous approaches are available in the literature to deal with bacteria and biofilm associated infections. The limitation of existing procedure and the continuing threat of antimicrobial resistance to world health highlighted the need that traditional practices need to be integrated and validated. Integrating the traditional wisdom with modern scientific approaches, we can potentially enhance health care efficacy and foster the development of novel therapeutic solution.

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ABBREVIATIONS

EPS: Extracellular Polymeric Substances; **L:** Lodhra; **Bj:** Bijak; **Kt:** Katphal; **Kh:** Khadir; **LC MS:** Liquid Chromatography-Mass Spectrometry; **TOF:** Time-of-Flight; **Q-TOF:** Quadrupole Time-of-Flight; MALDI-TOF **MS:** Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry; **TSA:** Tryptic Soy Agar; **CLSI:** Clinical and Laboratory Standard Institute; **TSB:** Tryptic Soy Broth; **OD:** Optical Density; **CFU:**

Colony Forming Units; **MHA:** Mueller Hinton agar; **MIC:** Minimum Inhibition Concentrations; **FIC:** Fractional Inhibitory Concentration; **AT:** Aztreonam; **COT:** Co-Trimoxazole; **LZ:** Linezolid; **TOB:** Tobramycin; **TE:** Tetracyclin; **GEN:** Gentamicine; Ak: Amikacin; PIT: Piperacillin; **PBS:** Phosphate Buffered Saline; **MTT:** 3, 4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide; **IC_{50}:** Half-maximal inhibitory concentration; **ELISA:** Enzyme-Linked Immunosorbent Assay; **SD:** Standard Deviation; **ANOVA:** Analysis of Variance; **Et:** Ethanolic; **Mt:** Methanolic; **D/W:** Aqueous.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUMMARY

The healthcare system faces significant challenges due to prevalence of chronic wounds worsen by antimicrobial resistant bacteria and polymicrobial biofilm. This study investigates antibacterial and antibiofilm potential of extracts of bark of Lodhra, Katphal, Bijak and heartwood of Khadir plants referred from Ayurveda. Maceration technique was used to prepare extract using ethanol, methanol and water followed by phytochemical analysis by qualitative method and MS analysis. Antibacterial activity of extracts were assessed against Staphylococcus *aureus, Staphylococcus haemolyticus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* commonly associated with wound using agar well diffusion method. Biofilm inhibition and eradication was tested using crystal violet assay and cell cytotoxicity with MTT assay. All extracts showed significant antibacterial activity, ethanol extract exhibited maximum antibiofilm activity and non-toxic to fibroblast cells. This study tried to bridge traditional Ayurvedic knowledge with modern sciences, to pave a way for wound care solution.

REFERENCES

Abdallah, E. M., Alhatlani, B. Y., Menezes, R. D. P., Henrique, C., & Martins, G. (2023). Back to Nature: Medicinal Plants as Promising Sources for Antibacterial Drugs in the Post-Antibiotic Era Plants, 12(17), 3077. https://doi.org/10.3390/ plants12173077

Abebia, V., Pinem, B., Perdanakusuma, D. S., Teferdi, M. G., Al-taie, H. A., *et al.* (2025). Chronic wounds: risk factors and evidence-based intervention, 10(1), 56-66. https://doi.org/10.20473/jre.v10i1.66610

Acharya, N., Acharya, S., Shah, U., Shah, R., & Hingorani, L. (2016). A comprehensive analysis on *Symplocos racemosa* Roxb.: Traditional uses, botany, phytochemistry and pharmacological activities. Journal of Ethnopharmacology, 181, 236-251. https://doi.org/10.1016/j.jep.2016.01.043

Advancements, R. (2022). Innovative Treatment Strategies to Accelerate Wound Healing: Trajectory and Recent Advancements.

Al-Deen, A. M. T., ALhaidari, S. A. A., Al-Kaf, A. G., Al-Hadi, F. A., & Mahbashi, A. Al. (2019). Phytochemical Screening and Thin Layer Chromatographic of *Prunus Dulcis* (Almond) Medicinal Plant Leaves Used in Folk Medicine for Treatment of Wounds and Burns in Hufash District Al Mahweet Governorate-Yemen. Universal Journal of Pharmaceutical Research, May. https://doi.org/10.22270/ujpr.v4i2.252

Article, R. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes, *Journal of pharmacy & bioallied* sciences,12(1), *1*. https://doi.org/10.4103/jpbs.JPBS

Bazargani, M. M., & Rohloff, J. (2016). Antibiofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control, 61, 156-164. https://doi.org/10.1016/j.foodcont.2015.09.036

Butala, M. A., Kukkupuni, S. K., & Vishnuprasad, C. N. (2017). Ayurvedic anti-diabetic formulation Lodhrasavam inhibits alpha-amylase, alpha-glucosidase and suppresses adipogenic activity *in vitro*. Journal of Ayurveda and Integrative Medicine, 8(3), 145-151. https://doi.org/10.1016/j.jaim.2017.03.005

Chauhan, N., Khan, A., & Farooq, U. (2023). Synergistic Effect of Combined Antibiotic and Methanolic Extracts of *Withania somnifera* and *Catharanthus roseus* against MDR Salmonella enterica Serovar Typhi. 2023(1), 8836886. https://doi.org/10.1155/2023/8836886

Dandannavar, V. S., Balakrishna, J. P., Dhawale, L., & Joseph, J. P. (2019). Wound-healing potential of methanolic extract of *Rhaphidophora korthalsii* leaves possibly mediated by collagen and fibronectin expression in L929 cell line. 9(8), 813-820. https://doi.org/10.5455/njppp.2019.9.0623404072019

Di Martino, P. (2018). Extracellular polymeric substances, a key element in understanding biofilm phenotype. AIMS Microbiology, 4(2), 274-288. https://doi.org/10.3934/MICROBIOL.2018.2.274

Don, D., & Baghel, U. S. (2019). Antimicrobial Attributes of Di ff erent Solvent Extracts. Biomolecules, 9(357), 15.

Dwivedi, D., Dwivedi, M., Malviya, S., & Singh, V. (2017). Evaluation of wound healing, anti-microbial and antioxidant potential of *Pongamia pinnata* in wistar rats. Journal of Traditional and Complementary Medicine, 7(1), 79-85. https://doi.org/10.1016/j.jtcme.2015.12.002

Goswami, A. G., Basu, S., Banerjee, T., & Shukla, V. K. (2023). Biofilm and wound healing: from bench to bedside. European Journal of Medical Research, 28(1), 1-18. https://doi.org/10.1186/s40001-023-01121-7

Jangra, N., Singla, A., Puri, V., & Dheer, D. (2025). Herbal bioactive-loaded biopolymeric formulations, 15(16), 12402-12442. https://doi.org/10.1039/d4ra08604j

Kadam, S., Madhusoodhanan, V., Bandgar, A., & Kaushik, K. S. (2020). From Treatise to Test: Evaluating Traditional Remedies for Anti-Biofilm Potential. Frontiers in Pharmacology, 11(October), 1-11. https://doi.org/10.3389/fphar.2020.566334

Karan, S., Mohan, C., Venkatachalam, L., Senbagaraman, A., Kavitha, S., *et al.* (2024). Siddha Medicine as a Novel Approach to Biofilm Disruption: Current Evidence and Research Prospects. 13(3), 8185-8200.

Kuschmierz, L., Meyer, M., Bräsen, C., Wingender, J., Schmitz, O. J., *et al.* (2022). Exopolysaccharide composition and size in *Sulfolobus acidocaldarius* biofilms. 13, 982745.https://doi.org/10.3389/fmicb.2022.982745

Madhu, M., Sailaja, V., Satyadev, T., & Satyanarayana, M. V. (2016). Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. ~ 25 ~ Journal of Pharmacognosy and Phytochemistry, 5(2), 25-29.

Maitra, S., & Satardekar, K. (2017). Pharmacological Study of *Symplocos racemosa* Roxb. International Journal of Pharmacy & Pharmaceutical Research, 9(1), 106-113.

Mandrika, I., Kumar, S., Zandersone, B., Eranezhath, S. S., Petrovska, R., *et al.* (2021). Antibacterial and Anti-Inflammatory Potential of Polyherbal Formulation Used in Chronic Wound Healing. Evidence-Based Complementary and Alternative Medicine, 2021(1), 9991454. https://doi.org/10.1155/2021/9991454

Nath, K., Talukdar, A. Das, Bhattacharya, M. K., Bhowmik, D., Chetri, S., *et al.* (2019). *Cyathea gigantea* (Cyatheaceae) as an antimicrobial agent against multidrug resistant organisms. BMC Complementary and Alternative Medicine, 19(1), 1-8. https://doi.org/10.1186/s12906-019-2696-0

Ngamkhae, N., Monthakantirat, O., Chulikhit, Y., & Boonyarat, C. (2022). Journal of Applied Research on Medicinal and Aromatic Plants Optimization of extraction method for Kleeb Bua Daeng formula and comparison between ultrasound-assisted and microwave-assisted extraction. Journal of Applied Research on Medicinal and Aromatic Plants, 28, 100369. https://doi.org/10.1016/j.jarmap.2022.100369

Pooja, R., Varsha, S. L., Aliya, M. S., T, C. K., Damini, B. M., *et al.* (2022). Phytochemical screening, GCMS, UV-vis and FTIR analysis of leaf methanolic extract of *Sapindus mukorossi* L. 3(05), 97-104.

Regulski, M., Myntti, M. F., & James, G. A. (2023). Anti-Biofilm Efficacy of Commonly Used Wound Care Products in *In vitro* Settings, *Antibiotics,* 12(3), *536*.

Sci, J. P., Ibrahim, A. M., Hamid, M. A., Althiab, R. A., Husni, A., & Shariff, M. (2021). *In vitro* fibroblasts viability and migration stimulation of *Acalypha indica*: an insight on wound healing activity, 7(1), 183. https://doi.org/10.1186/s43094-021-00333-0

Shafiei, Z., Haji, Z., Rahim, A., & Philip, K. (2016). Antibacterial and anti-adherence effects of a plant extract mixture (PEM) and its individual constituent extracts (*Psidium* sp., *Mangifera* sp., and *Mentha* sp.) on single- and dual-species biofilms. *PeerJ,* 4, *e2519*. https://doi.org/10.7717/peerj.2519

Sharif, S., & Yadav, A. K. (2025). Bacterial biofilm and its role in antibiotic resistance. The Microbe, 7(November 2024), 100356. https://doi.org/10.1016/j.microb.2025.100356

Sharma, A., Khanna, S., Kaur, G., & Singh, I. (2021). Medicinal plants and their components for wound healing applications. Future Journal of Pharmaceutical Sciences, 7(1), 53. https://doi.org/10.1186/s43094-021-00202-w

Shinde, P., Jaiswal, S., & Tale, V. (2025). Temporal Profiling of Extracellular Polymeric Substances Associated with Biofilm Formation in Bacteria Isolated from Wound

Infection. *Journal of Pure & Applied Microbiology,* 19(2), 1174.https://doi.org/10.22207/JPAM.19.2.21

Shoaib, M., Shah, S. W. A., Ali, N., Shah, I., Ullah, S., *et al.* (2016). Scientific investigation of crude alkaloids from medicinal plants for the management of pain. BMC Complementary and Alternative Medicine, 16(1), 1-8. https://doi.org/10.1186/s12906-016-1157-2

Sood, H., Kumar, Y., Gupta, V. K., & Arora, D. S. (2020). Bioprospecting the antimicrobial, antibiofilm and antiproliferative activity of *Symplocos racemosa* Roxb. Bark phytoconstituents along with their biosafety evaluation and detection of antimicrobial components by GC-MS. BMC Pharmacology and Toxicology, 21(1), 1-20. https://doi.org/10.1186/s40360-020-00453-y

Suparno, S., Lestari, E. S. A., & Grace, D. (2024). Antibacterial activity of Bajakah Kalalawit phenolic against *Staphylococcus aureus* and possible use of phenolic nanoparticles. *Scientific Reports,* 14(1), 19734.https://doi.org/10.1038/s41598-024-70799-4

Tree, C. (2025). Journal of Ayurveda and Integrated Sumit R *et al*. An Ayurvedic Tree with Great Potential. 10, 176-187. https://doi.org/10.21760/jaims.10.9.27

Untachai, J., Dodgson, W., Srifa, A., & Dodgson, J. L. A. (2018). *In vitro* antibacterial activities of selected traditional plants. Journal of Pure and Applied Microbiology, 12(2), 265-276. https://doi.org/10.22207/JPAM.12.1.31

Venugopal, S., & Shukla, A. (2025). Ayurvedic medicine as a solution to replace antibiotics in order to combat antibiotic resistance in bacteria. World Journal of Biology Pharmacy and Health Sciences. 21. 405-409. 10.30574/wjbphs.2025.21.2.0087.

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