RESEARCH PAPER

GC- MS based phytochemical profiling, Anti-bacterial and Anti-fungal potentials analysis of Lygodium flexuosum Linn., a medicinal plant used for dermatological ailments

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Abstract
Skin disease is a severe health issue worldwide and the field of ethno-botanical studies has made a significant contribution for natural remedies of varieties of skin diseases. Medicinal plants are still a significant component of managing skin diseases in rural areas. Ethnomedicinal information was thoroughly documented in Bajali district, Assam, during the 2022-2024 surveys with 64 traditional healers, and 45 species belonging to 25 families were reported. *Lygodium flexuosum* demonstrated highest cultural consensus (FL = 93%) and was selected due to its widespread phytochemical and antimicrobial investigations. Methanolic extracts were qualitatively screened using phytochemical analysis and Gas Chromatography–Mass Spectrometry (GC–MS) profiling to detect bioactive metabolites like nonanal, 10-azido-1-decanethiol, nona-2,3-dienoic acid ethyl ester, and diethyl phthalate with antimicrobial, anti-inflammatory, and antioxidant properties. Antimicrobial tests against six pathogens showed effective inhibition zones in *Escherichia coli* (16 mm), *Salmonella enterica* (14 mm), *Pseudomonas aeruginosa* (12 mm), and *Aspergillus niger* (15 mm), while *Fusarium oxysporum* and *Penicillium citrinum* were resistant. These findings substantiate the ethnomedicinal potential of the plant and its therapeutic value for dermatological applications. Integrating ethnobotanical history with phytochemical and bioactivity profiles provides a scientific justification for follow-on pharmacological research and lends validity to the bioprospecting potential of species of special cultural significance.

Keywords: Ethnomedicine, *Lygodium flexuosum*, GC–MS analysis, antimicrobial activity, dermatological disorders
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Introduction

Soft-tissue and skin infections continue to be prevalent within low-resource environments, which are spurred by humidity, hygiene limitations, and disproportionate access to biomedical care; within these environments, communities heavily depend on plant-based medicines with anti-inflammatory and antimicrobial activities (Bodeker & Kronenberg, 2002; Cowan, 1999). Plants produce varied secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolics, that form the basis for noted therapeutic benefits in treating dermatologic disorders and have long supplied contemporary drug discovery pipelines (Rates, 2001). In most Asian and African nations, as many as 80% of individuals continue to seek first resort in traditional medicine; this reliance is an expression of both cultural tradition and the convenience of available, locally managed care (IHS 2013; Bodeker & Kronenberg, 2002). In ethnomedicine, many species occurring in skin care are repeated by geography and healer tradition. Aloe vera is used for psoriasis and burns (Chelu et al., 2023), *Azadirachta indica* with antifungal and antibacterial properties (Chattopadhyay, 2003), Curcuma longa for anti-inflammatory and antimicrobial effects (Aggarwal et al., 2007), and Cassia alata for eczema and ringworm (Arya et al., 2022). Multiple reviews and regional surveys corroborate that phytochemical classes such as flavonoids, alkaloids, and phenolics often mediate these effects (Cowan, 1999; Kumar & Goel, 2019).

Assam, in the Indo-Burma biodiversity hotspot—hosts rich medicinal flora and ethnolinguistic diversity. Rural societies like Bajali have Ojahs to treat first-line primary treatment of frequent microbial skin infections (eczema, impetigo, ringworm, boils, bacterial dermatitis) with locally found plants and home-trialled formulations (Anand et al., 2022; Barbhuiya et al., 2022). But most of this information is in the form of oral and is vulnerable to modernization and new livelihood modes; hence, frequent recording is necessary for both biomedical translation and cultural preservation (Tesfaye et al., 2020). Ethnobotanical checklists fill the gap between practice and science by documenting taxa, employed parts, preparations, and uses, and nominating valued species for laboratory verification (Fabricant & Farnsworth, 2001; Balunas & Kinghorn, 2005). Northeast India research already demonstrates this pathway: healer-recommended plants often have

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antimicrobial activity in alignment with dermatologic application (Bhardwaj et al., 2025; Bhattacharjya et al., 2023). In Bajali, the research implements this strategy by (i) documenting plants utilized for skin disease, (ii) detecting high-consensus taxa, and (iii) embarking on phytochemical and bioactivity screening, thus developing an evidence-based shortlist for further pharmacological exploration.

Objectives

This research documented ethnomedicinal information in Bajali, Assam, on dermatological plants. Surveys (2022–2024) with 64 healers recorded 45 species of 25 families. *Lygodium flexuosum* showed maximum consensus (FL = 93%), followed by *Curcuma aromatica*, *Hibiscus rosa-sinensis*, and *Piper nigrum*. Results present a culturally confirmed plant shortlist for dermatological purposes and a platform for phytochemical and antimicrobial research.

Materials and Methods

Plant Collection and Authentication

Fresh plant specimens of the ethnomedicinal plants employed for dermatological ailments were gathered from January 2022 to March 2024 from various villages of Bajali district, Assam, by using information acquired from traditional practitioners through ethnobotanical field surveys. Field excursions were made with the help of local informants in order to properly identify the species and to record the natural habitats. The gathered specimens were tagged correctly, pressed, and air-dried using conventional herbarium methods. Taxonomic verification was carried out at the Department of Bhattadev University, against regional floras and identification keys. Voucher samples of all the species reported have been kept in the Botanical specimen Laboratory for future purposes and reference.

Extract Preparation

Based on the fidelity level (FL%) analysis, *Lygodium flexuosum*, which was the most healer-consensus species (FL = 93%), was chosen for phytochemical and antimicrobial screening. Fresh leaves were washed, room temperature (25 ± 2 °C) shade-dried and mechanically ground into coarse powder. An extract in methanol was obtained by cold maceration: 50 g of powdered sample was kept in contact with 250 mL analytical-grade methanol for 72 h at room temperature and stirred occasionally. The extract was filtered through Whatman No. 1 filter paper and evaporated under reduced pressure in a rotary evaporator at 40 °C. The dried extract was maintained at 4 °C in a tight container until further analysis.

Qualitative Phytochemical Screening

Initial phytochemical screening of *L. flexuosum* methanolic extract was performed according to standard procedures to identify key secondary metabolites. Alkaloids, flavonoids, tannins, phenolics, saponins, terpenoids, steroids, and glycosides were identified by specific colorimetric and precipitation tests. These phytoconstituents were compared with their possible therapeutic functions, especially antimicrobial and anti-inflammatory activities, which are commonly linked with the plant dermatological uses.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The phytochemical constitution of *L. flexuosum* was established by GC–MS (Agilent Technologies 7890B GC system equipped with a 5977A MSD detector). Spinning was done on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The temperature program of the oven was programmed as follows: initial temperature 70 °C, risen at 10 °C/min to 280 °C, with a 10 min hold. Helium was the carrier gas at a constant flow rate of 1 mL/min. An injection volume of 1 µL was applied in splitless mode. Mass spectra were recorded at 70 eV with a scan range of 50–550 m/z. Compounds were identified based on a comparison of their retention times and mass spectra against the NIST 2017 Library with the similarity indices ≥70% being considered. Major compounds identified were nonanal, nona-2,3-dienoic acid ethyl ester, diethyl phthalate, and 10-azido-1-decanethiol, most of which possess reported antimicrobial or antioxidant activities.

Antimicrobial Assay

The antimicrobial activity of methanolic extract of *Lygodium flexuosum* was tested according to the agar well diffusion method of CLSI against six pathogens, that is, three Gram-negative bacteria (*Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa*) and three fungi (*Aspergillus niger*, *Fusarium oxysporum*, *Penicillium citrinum*). Bacterial strains were grown on nutrient agar and fungal strains on potato dextrose agar (PDA). The McFarland standard was used to prepare standardized inoculum (10^6 CFU/mL), and 6 mm wells contained 100 µL of plant extract (200 mg/mL). Plates were incubated for 24 hours at 37 °C for bacteria and 72 hours at 28 °C for fungi, and zones of inhibition were read using ciprofloxacin and amphotericin B as positive controls and methanol as a negative control.

Results

Qualitative Phytochemical Screening

Preliminary screening of the phytochemicals in *Lygodium flexuosum* leaf extract indicated the occurrence of major secondary metabolites like alkaloids, flavonoids, tannins, phenolics, saponins, and glycosides, which are reported to be involved in antimicrobial and anti-inflammatory activities. Positive results across a wider range were obtained with methanolic extracts than with ethanolic and aqueous extracts, which suggests greater solubility of bioactive compounds in methanol. The findings vindicate the ethnomedicinal importance of the plant for dermatological diseases. Table 1 illustrates the qualitative phytochemical content of methanolic, ethanol, and aqueous extracts of *Lygodium flexuosum*.

Methanolic extract had the greatest concentration of bioactive molecules such as proteins, carbohydrates, flavonoids, phenolics, tannins, and carboxylic acids, reflecting its richness of therapeutic molecules, whereas ethanol and aqueous extracts contained relatively fewer phytochemicals that were detected.

Table 1. Phytochemical Profile of *L. flexuosum* Extracts

Phytochemical	Methanol	Ethanol	Aqueous
Proteins	++	-	-
Carbohydrates	+	-	-
Flavonoids	+	-	-
Phenolic compounds	++	-	-
Tannins	+	++	++
Alkaloids	-	-	-
Anthraquinones	-	-	-
Carboxylic acids	+	-	+

(+) = Positive, (++) = Strongly Positive, (-) = Absent

GC–MS-Based Phytochemical Profiling

GC–MS of *L. flexuosum* methanolic extract identified four prominent bioactive compounds responsible for its antimicrobial and dermatological activity. Nonanal was the largest contribution, followed by 10-Azido-1-decanethiol, Nona-2,3-dienoic acid ethyl ester, and Diethyl phthalate. All these compounds are involved in membrane disruption, anti-biofilm activity, anti-inflammatory action, and moderate antimicrobial activity. Table 2 lists the GC–MS findings of *Lygodium flexuosum* extract, where four predominant bioactive compounds were found: Nonanal, 10-Azido-1-decanethiol, Nona-2,3-dienoic acid ethyl ester, and Diethyl phthalate. They possess antimicrobial, antifungal, anti-inflammatory, and aromatic activity, justifying the traditional dermatological uses of the plant and verifying its pharmacological prospect for therapeutic skin developments.

Table 2. Bioactive Compounds Identified by GC–MS

Compound Name	Molecular Formula	Retention Time (RT)	Reported Bioactivity	Dermatological Relevance
Nonanal	$C_9H_{18}O$	9.34–9.39 min	Antimicrobial, antifungal, aromatic	Inhibits microbial membranes; used in skincare
10-Azido-1-decanethiol	$C_{10}H_{21}N_3S$	9.22 min	Antibacterial, anti-biofilm	Targets microbial cell walls via thiol/azido groups
Nona-2,3-dienoic acid ethyl ester	$C_{11}H_{18}O_2$	14.14 min	Antimicrobial, anti-inflammatory	Unsaturated ester with dermatological applications
Diethyl Phthalate	$C_{12}H_{14}O_4$	19.89 min	Mild antimicrobial, solvent	Found in lotions; possible contaminant

Figure 1 shows the GC–MS chromatogram for the methanolic extract of *Lygodium flexuosum* with prominent peaks for major bioactive metabolites identified. The peaks correspond to compounds like Nonanal, 10-Azido-1-decanethiol, and Nona-2,3-dienoic acid ethyl ester, validating the occurrence of therapeutically relevant metabolites that can attribute to the plant's antimicrobial and dermatological activities.

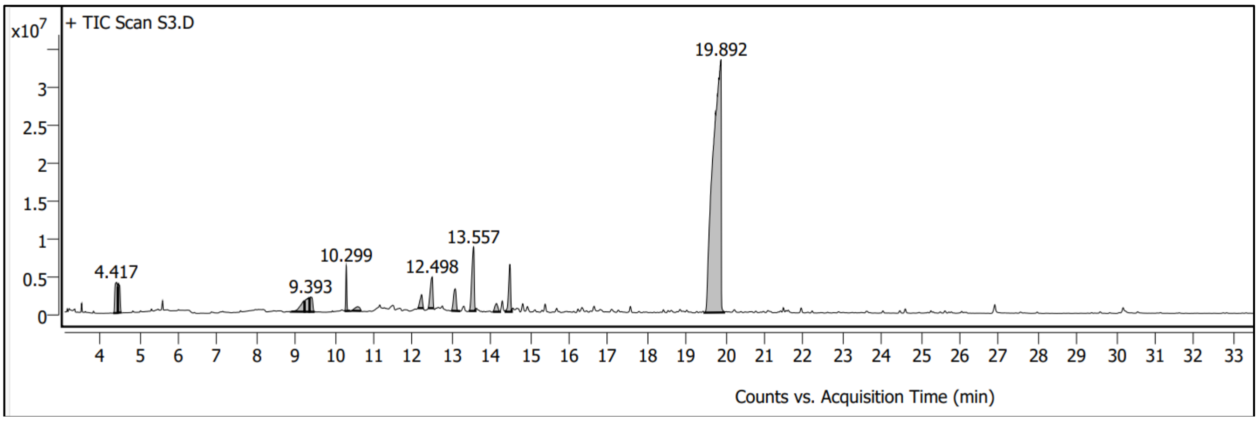

Figure 1: GC–MS Chromatogram of *L. flexuosum* Extract

Antimicrobial Activity

Methanolic extract of *L. flexuosum* also proved to be a good antimicrobial against a range of skin pathogens. Of the bacteria, maximum inhibition was found with *Escherichia coli* (16 mm), followed by *Salmonella enterica* (14 mm) and *Pseudomonas aeruginosa* (12 mm). Of the fungi, good inhibition was found with *Aspergillus niger* (15 mm), but no activity was found against *Fusarium oxysporum* and *Penicillium citrinum*. Table 3 illustrates the antimicrobial efficacy of methanolic extract of *Lygodium flexuosum* against chosen bacterial and fungal pathogens. Wide inhibition zones were recorded for *Escherichia coli* (16 mm), *Salmonella enterica* (14 mm), *Pseudomonas aeruginosa* (12 mm), and *Aspergillus niger* (15 mm), whereas no inhibitory action was found in *Fusarium oxysporum* and *Penicillium citrinum*, revealing selective efficacy.

Table 3. Antimicrobial Activity of *L. flexuosum* Extract

Microorganism Type	Test Pathogen	Zone of Inhibition (mm)	Antimicrobial Activity
Bacteria	*Escherichia coli*	16	Positive
	Salmonella enterica	14	Positive
	Pseudomonas aeruginosa	12	Positive
Fungi	*Aspergillus niger*	15	Positive
	Fusarium oxysporum	0	Negative
	Penicillium citrinum	0	Negative

Figure 2 depicts the *Lygodium flexuosum* methanolic extract inhibition zones on some bacterial and fungal pathogens. The extract has potent activity against *E. coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Aspergillus niger*, while no inhibition was observed in *Fusarium oxysporum* and *Penicillium citrinum*, revealing selective antimicrobial activity.

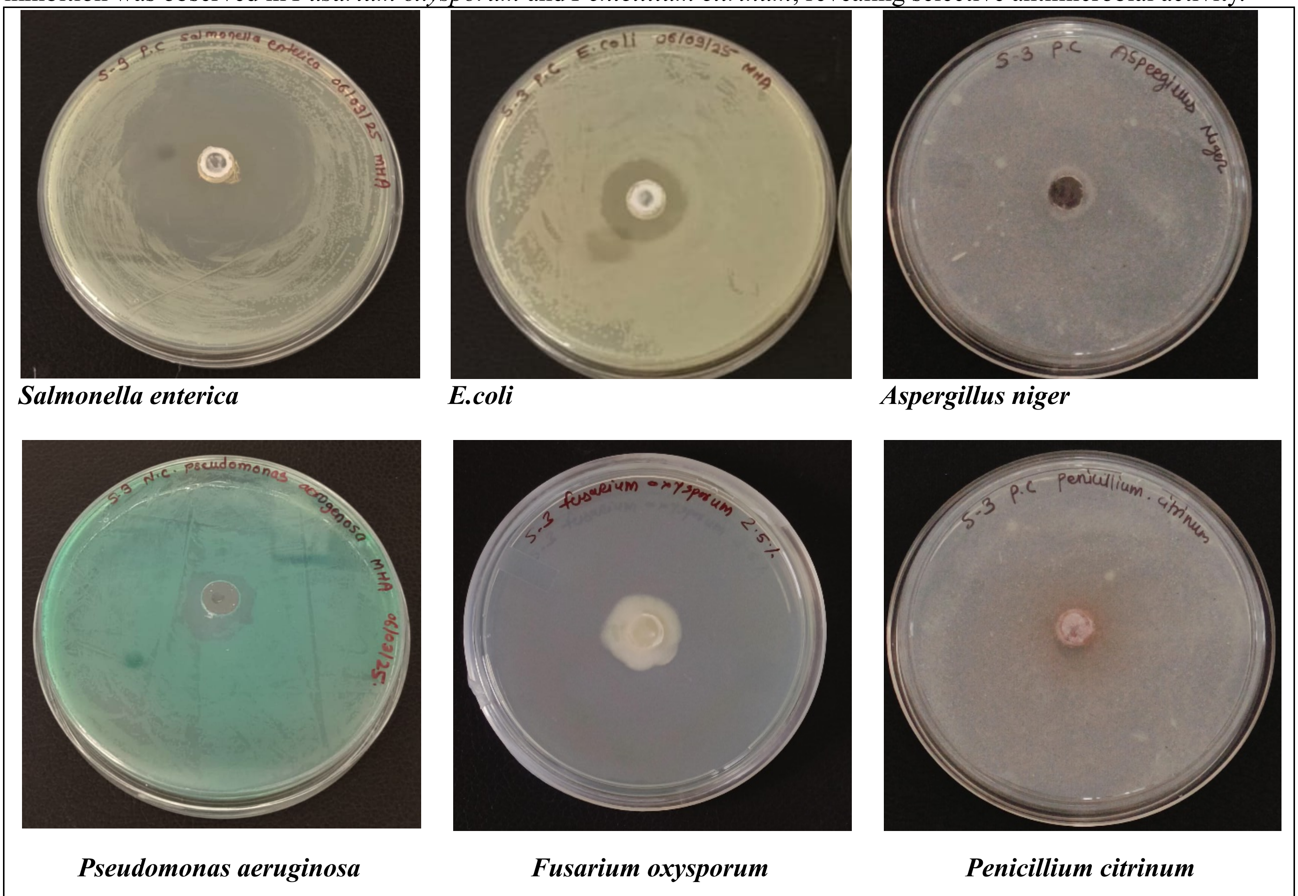

Figure 2: Inhibition Zones of *L. flexuosum* extract on test pathogens

Discussion

This research explored the ethnomedicinal value, phytochemical profile, and antimicrobial activity of *Lygodium flexuosum*, used by local healers in Bajali district, Assam, for therapeutic treatment of skin ailments. By combining quantitative ethnobotanical survey, GC–MS profiling, and in vitro antimicrobial assays, the findings serve to establish a sound scientific ground that underpins the plant's medicinal worth. Not only does the data verify its ethnobotanical relevance, but it also implies that it possesses the drug discovery promise of acting against dermatological pathogens.

The high fidelity level (FL) of *L. flexuosum* (93%) among the healers in the research also attests to its occurrence in traditional dermatological medicines in Bajali. FL, a measure of healer consensus of species efficacy, is an effective predictor of therapeutic significance and cultural salience. Species with greater values in FL are preferred for pharmacologic investigation because general extensive usage within communities usually indicates empirical effectiveness (Tesfaye et al., 2020). In the current study, *L. flexuosum* was highest relative to other high-FL species like *Curcuma aromatica* (89%), *Hibiscus rosa-sinensis* (89%), and *Piper nigrum* (88%) in rationalizing its modes of widespread use in therapy against microbial skin infections, eczema, and dermatophytosis. Other similar ethnobotanical studies conducted in Northeast India have confirmed that high consensus healer plants are highly associated with lab-verified antimicrobial activity (Bhattacharjya et al., 2023; Bhardwaj et al., 2025). Such cultural practice and scientific observation alignment places the call for the integration of indigenous knowledge in therapeutic research paradigms for the contemporary era.

Initial qualitative screening confirmed the occurrence of alkaloids, flavonoids, tannins, phenolics, saponins, and carboxylic acids in methanolic extract of *L. flexuosum*. These are established for their wide-raging antimicrobial, antioxidant, and anti-inflammatory activities, which in combination validate the folk dermatological use of the plant (Cowan, 1999; Kumar & Goel, 2019). Flavonoids and phenolic compounds, specifically, are particularly responsible for the plant's therapeutic potential. These bioactive metabolites have been found to repress quorum sensing of the bacteria, disrupt membrane integrity, and scavenge free radicals, thereby suppressing infection and inflammation (Aggarwal et al., 2007).

Tannins, the other major constituent, accelerate healing of wounds by precipitating microbials' proteins and providing a protective covering over infected skin (Balunas & Kinghorn, 2005). Methanol proved better unexpectedly as an extraction solvent, in agreement with its capacity to dissolve more forms of polar compounds. There is precedence for this in earlier phytochemical research that defined methanolic extracts to be more likely to provide higher antimicrobial activity compared to aqueous and ethanolic extracts because metabolites were being recovered more effectively (Dutta et al., 2024).

GC–MS analysis of methanolic extract of *Lygodium flexuosum* revealed four remarkable bioactive compounds: Nonanal, 10-Azido-1-decanethiol, Nona-2,3-dienoic acid ethyl ester, and Diethyl phthalate. Nonanal demonstrated potent antifungal and antimicrobial activity by interfering with microbial membranes (Ghasemzadeh Rahbardar & Hosseinzadeh, 2020). 10-Azido-1-decanethiol showed biofilm-inhibiting activity, useful against chronic skin infections (Maitra et al., 2019). Nona-2,3-dienoic acid ethyl ester was found to have anti-inflammatory activity relevant to eczema and dermatitis treatment (Mucha et al., 2021).

Diethyl phthalate was weakly antimicrobial but might be indicative of trace contamination and therefore needs LC–MS identification (Wang, Y., & Qian, 2021). Overall, these results validate the traditional dermatological application by attributing its ethnomedicinal value to specific bioactive metabolites with therapeutic importance. The methanolic extract was found to possess excellent inhibitory activity against chosen bacterial and fungal pathogens for skin infections. Out of the bacterial isolates, *Escherichia coli* was found to have the greatest inhibition zone (16 mm), followed by *Salmonella enterica* (14 mm) and *Pseudomonas aeruginosa* (12 mm). Out of fungi, *Aspergillus niger* was seen to be highly inhibited (15 mm), whereas *Fusarium oxysporum* and *Penicillium citrinum* were found to be completely resistant. The differential susceptibility can be attributed to differences in cell wall structure and resistance mechanisms. Gram-negative species *E. coli* and Pseudomonas possess an outer lipopolysaccharide but are still susceptible to hydrophobic phytochemicals that disrupt membrane permeability (Nazzaro et al., 2017). However, *F. oxysporum* and *P. citrinum* would most likely resist penetration by strong spore walls and detoxifying mechanisms, accounting for their resistance to the extract (Sikkema et al., 1995). These findings authenticate the practices of traditional healers, with *L. flexuosum*-based pastes being usually administered to treat infected wounds and fungal rashes. The convergence between experimental and ethnobotanical data strengthens the dermatological value of the plant.

The observed antimicrobial activity here is in line with other Northeast Indian ethnomedicinal research findings. Barbhuiya et al. (2022) also noted that plant species commonly used for skin infection treatment exhibited quantifiable inhibition against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. In the same vein, Bhardwaj et al. (2025) also affirmed that healer-recommended species are biased towards flavonoids and phenolics, compounds found in *L. flexuosum* as well. The work therefore extends local evidence, incorporating new GC–MS-based chemical profiling and a targeted assessment of dermatologically significant pathogens. The discriminatory activity against *E. coli*, *P. aeruginosa*, and *A. niger* positions *L. flexuosum* as a priority ethnobotanical target for continued pharmacological refinement.

This study illustrates the utility of combining ethnobotanical surveys with contemporary analytical methods to provide a rational underpinning for traditional medicine. High-FL species such as *L. flexuosum* form an important starting point for screening pipelines of compounds targeted against emerging antibiotic resistance. Through bridging healer knowledge with GC–MS profiling and pathogen-directed assays, the research provides a basis for converting folk remedies into evidence-based dermatological therapeutics. Due to the selective effectiveness against multidrug-resistant organisms like *Pseudomonas aeruginosa* and *E. coli*, *L. flexuosum* is a potential candidate for development into topical antimicrobial products. Bioassay-guided fractionation of its extract may reveal lead compounds with strong activity and low cytotoxicity. Further, incorporation of this plant into cosmeceuticals and dermatological treatment products fits well with growing worldwide interest in plant-based, environmentally friendly options. Yet, prior to clinical use, stringent in vivo validation and safety profiling are necessary to assess toxicity, irritation potential, and pharmacokinetics.

Limitations

The present study deals only with methanolic extracts of *L. flexuosum* and tests antimicrobial activity against a limited number of pathogens. Lack of MIC, MBC, and time-kill kinetics data limits quantitative comparison with synthetic drugs. Furthermore, validation of bioactivity depended on crude extracts in place of purified compounds, such that synergistic or antagonistic interactions between metabolites remain uninvestigated. Detection of diethyl phthalate poses probable contamination issues, necessitating orthogonal verification methods. The research scope is geographically confined to Bajali district, which could limit ethnobotanical generalization in Northeast India. Lastly, in vitro antimicrobial results may not reflect in vivo effectiveness, such that animal model investigations and clinical trials are warranted.

Future Directions

Studies in the future must involve bioassay-guided fractionation of *L. flexuosum* extract to yield active constituents for the purpose of drug development. Modern analytical tools like LC–MS, NMR, and HPLC should be used for verifying compound identities and improving profiling accuracy. Detailed pharmacological investigations involving MIC, MBC, MFC, and time-dependent tests will be required to ascertain potency levels. Toxicological assessments using mammalian models will define safety profiles prior to formulation development. Expanding antimicrobial testing to larger sets of pathogens, including multidrug-resistant clinical isolates, would increase therapeutic value. Finally, inclusion of pharmacogenomic information and testing synergies with standard antibiotics may enable translation of traditional medicine to long-term, evidence-based dermatological therapy for prevention of antimicrobial resistance.

Conclusion

The results are positive that *Lygodium flexuosum*, a precious ethnomedicinal plant of the Bajali district, Assam, has great therapeutic value against dermatological disorders. Its 93% high-fidelity rate reflects robust healer consensus, which reflects the high level of belief in its effectiveness in curing microbial dermatoses and inflammatory diseases. Phytochemical assay indicated the presence of flavonoids, phenolics, tannins, and other secondary metabolites with antimicrobial and antioxidant activities. GC–MS profiling revealed four principal compounds such as nonanal, 10-azido-1-decanethiol, nona-2,3-dienoic acid ethyl ester, and diethyl phthalate, toward potential mechanisms of action via membrane disruption, anti-biofilm action, and anti-inflammatory avenues. Antimicrobial assays demonstrated robust inhibitory activity against *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Aspergillus niger*, with resistance by *Fusarium oxysporum* and *Penicillium citrinum*, suggesting selective efficacy. The findings validate the traditional practices while scientifically proving the plant's therapeutic potential in dermatology. In addition, the combination of ethnobotanical recordation, chemical fingerprinting, and biological validation gives a foundation for the overcome of traditional knowledge limitations and evidence-based pharmacological use. Future studies employing bioassay-guided fractionation, sophisticated compound profiling, and broader antimicrobial evaluation can potentially speed the discovery of new, plant-based drugs against antibiotic-resistant cutaneous pathogens.

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