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Original Paper

Pharmacological Evaluation and Prediction of Pharmaceutical Potential of *Lasiococca comberi* Haines: A Threatened Medicinal Tree

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Antimicrobial resistance (AMR) is a critical health issue that requires the exploration of unexplored plants for the identification of new therapeutic agents. This study examined the leaves of *Lasiococca comberi*, a threatened Indian tree species, focusing on its phytochemical content, toxicity, antibacterial activity, and antioxidant potential. Aqueous extract of the leaves showed the highest presence of secondary metabolites, while the methanol and ethanol extracts demonstrated the lowest minimum inhibitory concentration (MIC). Thin-layer chromatography (TLC) analysis of the ethanol extract revealed seven distinct spots and confirmed its antioxidant activity. The quantification of secondary metabolites highlights the potential of *L. comberi* leaves as a valuable source of bioactive compounds to combat AMR. These findings suggest that *L. comberi* leaves could be a promising source of new antimicrobial agents, warranting further pharmacological investigation.

Keywords: antimicrobial activities, *Artemia salina*, pharmacological values, phytochemical screening, Thin Layer Chromatography, threatened

1 Introduction

India is one of the 12 megadiverse countries in the world, holding 11% of the planet's wild flora. Approximately 28% of the total flora and 33% of the angiosperms in India are endemic (Chitale et al., 2014). This data highlights the country's rich floral wealth, which includes a variety of medicinal plants (Ahmad et al., 2021). However, both globally and particularly in India, numerous threats to biodiversity have been observed and reported by researchers (Das et al., 2025), resulting in the rapid loss of plant species with medicinal importance (Sharma and Thokchom, 2014). Due to these threats, many plant species are classified as threatened according to the IUCN (Chitale et al., 2014). Barik et al. (2018) reported that there are 2,740 threatened plant species belonging to 1031 genera and 217 families. Approximately 13% of the vascular plants in India are threatened (Kumar et al., 2013). Orchids represent the most valuable group of plants, comprising 23% of the total threatened species in India (Kumar et al., 2024). Among the threatened plants, trees also make up a significant portion, with many species increasingly categorized as threatened due to several factors (Silva et al., 2022). In Odisha state, India, about 211 species are threatened, belonging to 69 families (Barik et al., 2018). Among these, *Lasiococca comberi* (Figure 1), is a tree species from the Euphorbiaceae family, has a very low population in limited area of Odisha state. Locally known as Kukudahadi, has medicinal properties and is commonly found in semi-evergreen forests near to perennial rivers and nalas (Saxena and Brahmam, 1995). In contemporary times, the global population is facing numerous health problems, including antimicrobial resistance (AMR),

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which necessitates the discovery of new antimicrobial agents. In this context, *L. comberi* could serve as a potential source of new antimicrobial compounds, which would also support the propagation of this threatened species for restoration efforts and the formulation of future drugs from its plant parts. Considering the challenges this species faces, its habitat and its significance, an attempt has been made to evaluate the pharmacological potential of *L. comberi* leaves. There has previously been no report on its phytochemistry, antimicrobial properties, toxicity to *Artemia salina*, thin-layer chromatography (TLC) studies and antioxidant activities. Therefore, the present study is a novel initiative aimed at drawing attention to its propagation, restoration, and the isolation of active antimicrobial agents.

2 Material and Methods

Leaves of *Lasiococca comberi* were collected from the Khordha district of Odisha state, India. The leaves were air-dried in shaded areas, powdered and stored in a closed container for further experimental works (Kumar et al., 2013). The Soxhlet extraction method was used to obtain extracts with three different solvents; aqueous (RFCI; 00655), methanol (SRL; 59029), and ethanol (CSS-AR; 215522) for phytochemical screening. A voucher specimen of *L. comberi* (APRFH 97) has been deposited to the Herbarium unit of the Biodiversity and Conservation Lab., at Ambika Prasad Research Foundation, Odisha, India (Figure 2). The detection of nine secondary metabolites was conducted using standard method (Jena et al., 2025).

2.1 Quantification of Bioactive Compounds

2.1.1 Estimation of Tannin

A solution was prepared by mixing 200 mg of tannic acid (Tannic acid, T11417, NICE) with 20 ml of distilled water. From this solution, aliquots of 5, 15, 25, 35 and 50 µl were transferred into separate test tubes, and the volume in each tube was adjusted to 1 ml. Next, 0.5 ml of Folin reagent (Phenol reagent, Folin & Ciocalteu's, 29058, S.D. FINE-CHEM Ltd.) and 2.5 ml of 20% sodium carbonate (Sodium Carbonate, CAS: 497-19-8, Cero Care Products LLP) were added to each test tube. The mixtures were shaken for 5 minutes in dark and then allowed to stand for 40 minutes. After this period, the absorbance of each solution was taken at 720 nm to develop a standard curve. For the extraction of tannin, 200 mg of sample was transferred to a 100 ml conical flask, and 75 ml of distilled water was added. The mixture was boiled for 30 minutes, then centrifuged at 2000 rpm for 20 minutes. The supernatant was collected in a 100 ml volumetric flask and the volume was adjusted accordingly. From this volumetric flask, 1 ml of sample was taken and mixed with 75 ml of distilled water, 5 ml of folin reagent, and 10 ml of 20% sodium carbonate. After this, the volume was adjusted to 100 ml. Following another 5 minutes of shaking, the absorbance was again measured at 720 nm and the amount of tannin was calculated using the standard curve (Sadasivam and Manickam, 2019). Triplicates were mentioned and average was taken.

2.1.2 Estimation of Saponins

The determination of total saponin was performed using the method outlined by Obadoni and Ochuko, (2001), with some minor modifications. First, 2 g of the sample was combined with 30 ml of 20% aqueous ethanol and was kept in a flask on stirrer for half an hour. This mixture was then heated at 45 °C for four hours. After heating, the mixture was filtered using Whatman filter paper No. 1. The residue was then extracted again with an additional 20 ml of 25% aqueous ethanol. The combined extracts were then concentrated. The concentrate was then transferred to a seperatory funnel and was extracted twice with 10 ml diethyl ether (Diethyl Ether, CAS: 60-29-77, LOBA CHEMIE PVT. LTD.). The ether layer was discarded, while the aqueous layer was retained and then re-extracted with15 ml of n-butanol (n-butanol, B60679, NICE). The n-butanol extract was washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was then evaporated. Once the solution was evaporated, the sample was dried in the hot air oven at 40 °C until a constant weight was achieved (Jena et al., 2025). Triplicates were mentioned and average was taken. Finally, the saponin content was calculated using the below mentioned formula:

$$\%\ of\ saponins = \left(\frac{final\ weight\ of\ sample}{initial\ weight\ of\ extracts} \right) \times 100$$

2.1.3 Estimation of Total Phenol

Three replicates of 0.5 g of sample were taken and crushed with 60% methanol using a mortar and pestle. The samples were centrifuged five times at 5000 rpm for 20 minutes (Swain and Hillis, 1959). Seven test tubes are taken including blank and 2 test tubes for each replica. 0.1 and 0.2 ml of sample is taken in each test tube except blank. 60% methanol is added to each test tube to make volume up to 1ml. 1 ml of 0.1 N HCl is added and allowed to stand for a few minutes. 1 ml of sodium nitrite molybdate mixture is added, shaken well, and allowed to stand for a few minutes, diluted with 5 ml of distilled water. After dilution, 2 ml of 1 N NaOH is added and allowed to stand for 20 minutes. Readings are taken at 515 nm. Triplicates were mentioned and average

Figure 1 Different plant parts of *L. comberi*
A – whole plant, B – buds, C – inflorescence, D – early stage, E – mature stage of fruits

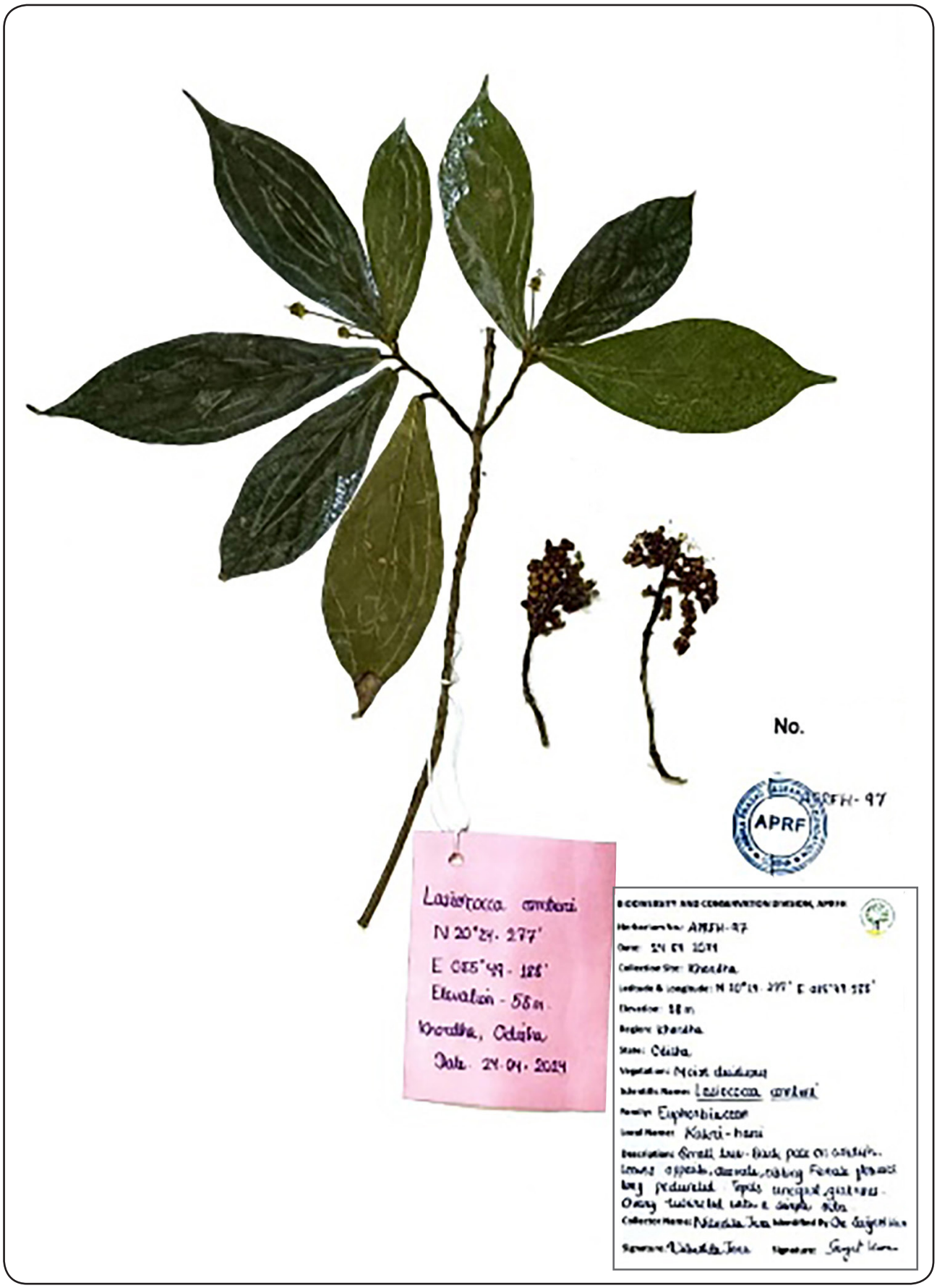

Figure 2 Herbarium specimen of *L. comberi*

was taken. The amount of phenol present in the sample is calculated from the standard graph (Swain and Hillis, 1959).

2.1.4 Estimation of Flavonoids

Aqueous extract of leaves (100 µl) was mixed 100 µl of 5% sodium nitrite and allowed to stand for 5 minutes. After this, 150 µl of aluminium chloride was added, and the mixture was incubated for 6 minutes. Following incubation, 200 µl of 1 M sodium hydroxide was added. The total volume of the mixture was made up to 3 ml with distilled water and incubated for 15 minutes at room temperature in the dark. Absorbance was measured at 510 nm. Triplicates were mentioned and average was taken. The flavonoid content in the sample was calculated using the standard graph prepared for quercetin (Shirazi et al., 2014).

2.2 Antibacterial Activities

The antimicrobial activity of different solvents extracted from *L. comberi* leaves was studied against three gram-positive bacteria; *Streptococcus pneumonia*e (MTCC 2672), *Streptococcus pyogenes* (MTCC 442), *Streptococcus mutans* (MTCC 497), as well as one gram-negative bacteria, *Shigella flexneri* (MTCC 1457). These bacteria were obtained from the Institute of Microbial Technology in Chandigarh. The plant extract was prepared using Soxhlet extraction method and was then diluted using 10% dimethyl sulfoxide (DMSO) to make a stock solution with a concentration of 1,000 mg.ml^{-1}. Various concentrations of the plant extracts were prepared from this stock solution. For the antibacterial activity analysis, fresh inoculum was prepared from cultures that had been grown for 24 hours. A loopful of each pure microbial culture was suspended in nutrient broth, and the turbidity of the suspension was adjusted to match the standard McFarland turbidity standard (Gayathiri et al., 2018).

2.2.1. Agar Well Diffusion (AWD) Assay

An agar well diffusion assay was conducted using plant extracts with three solvents (aqueous, methanol and ethanol), following the guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS). First, an inoculum was spread on nutrient agar petri plates using sterile swabs dipped in bacterial suspension. Six-millimeter wells were then punctured in the agar. Different concentrations of the plant extracts were prepared from a stock solution of 1,000 mg.ml^{-1}, specifically at concentrations of 25 mg.ml^{-1}, 50 mg.ml^{-1}, 100 mg.ml^{-1} and 200 mg.ml^{-1} as shown in Table 2. A volume of 100 µl from each concentration of the plant extracts was added to the respective wells. As a positive control, ampicillin at a concentration of 5 mg.ml^{-1} was also included. The plates were left to diffuse in a refrigerator for 30 mins before being incubated at 37 °C for 24 hours. After incubation, the zone of inhibition was measured and compared to the positive control. The results were expressed as the mean ±standard deviation of triplicate (Kumar et al., 2017).

2.2.2. Disc Diffusion Assay

The disc diffusion assay of *L. comberi* leaf extracts was carried out following the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). In the Agar-Well Diffusion method, a bacterial suspension was evenly spread across sterile nutrient agar plates using sterile swabs.

Various concentrations of the plant extracts (25 mg.ml^{-1}, 50 mg.ml^{-1}, 100 mg.ml^{-1} and, 200 mg.ml^{-1}) were prepared from a stock solution of 1,000 mg.ml^{-1} for each of the different solvents used for the plant extracts. Whatman filter paper No. 1 was cut into 6 mm disc pieces, which were then suspended in the different concentration of the solvent extracts. These discs were placed on the nutrient agar plates that contained the test organism, using sterile forceps to ensure an approximately equal distance between them. The plates were then incubated at 37 °C for 24 hours. As a positive control, an Ampicillin disc, suspended in a 5 mg.ml^{-1} ampicillin solution, was also added to the plates. The results were expressed as the mean ±standard deviation of triplicates (Kumar et al., 2017).

2.2.3 Broth Dilution Assay for MIC (Minimum Inhibitory Concentration)

Serial dilutions were prepared from selected solvents of the plant extracts or stock solution (1,000 mg.ml^{-1}). Various concentrations of the plant extracts were generated from the stock solution (500 mg.ml^{-1}, 300 mg.ml^{-1}, 400 mg.ml^{-1}, 200 mg.ml^{-1}, 100 mg.ml^{-1}, 50 mg.ml^{-1} and 25 mg.ml^{-1}) were prepared from the stock solution of the different solvents of leaves extracts respectively. For the inoculation process, 500 µl of the bacterial inoculum and 500 µl of the leaves extract was added to 2 ml of the sterile Nutrient Broth. This inoculation procedure was followed for each concentration of the leaves extract using selected solvents. For the positive control, the inoculum was placed in a sterile nutrient broth, while the negative control consisted of only sterile nutrient broth without the inoculum. All the samples were incubated at 37 °C for 24 hours. After this incubation period, turbidity was assessed, compared and, recorded (Kumar et al., 2017).

2.3 Toxicity to Artemia salina

For toxicity analysis, the initial process involved hatching of Brine Shrimp cysts (MAF Peqon Artemia). The optimum salinity for proper hatching of brine shrimp (*Artemia salina*) cysts was standardized using saline concentrations of 2.0%, 3.5%, and 5.0% (Kumar et al., 2012). The brine shrimp cysts were then incubated in 3.5% saline water with adequate aeration and kept at room temperature, which ranged from 28 °C to 35 °C under light for 48 hours. Hatching could typically be observed between 24 and 48 hours, depending on the quality of the cysts, the level of aeration, and the light provided. Different concentrations of the *L. comberi* leaves extract were prepared (15 mg.ml^{-1}, 31 mg.ml^{-1}, 47 mg.ml^{-1}, 63 mg.ml^{-1} and, 79 mg.ml^{-1}), using 3.5% saline water to maintain a total volume of 2 ml in each test tube. A 1% DMSO solution was used to dissolve the crude extracts. Ten nauplii were selected and introduced into five test tubes containing the extracts. Positive and negative controls were prepared using 3.5% saline water and vincristine sulphate (5 mg.ml^{-1}) respectively, also with a volume of 2 ml. The live larvae demonstrated high motility, making it possible to differentiate them from the unhatched cysts. The surviving nauplii were counted to analyze the mortality rate (Kumar et al., 2012).

2.4 TLC analysis

TLC plates were prepared on 6 cm glass slides using silica gel powder (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd.). The slides were washed with a clinical laboratory detergent and then dried. To remove surface contaminants, clean and dried slides were wiped with ethyl acetate. A slurry was prepared by mixing 3 g of silica with 20 ml of distilled water while stirring constantly. The slurry was poured over the slides, which were then left undisturbed until the silica layer dried (Kumar et al., 2013). The slides were then activated at 50 °C for 20 minutes before running (Mishra and Bhatnagar, 2024). A mobile phase of methanol: chloroform in a ratio 3 : 2 was used. Finally, the spots were visualized, and the retention factor (Rf) values were recorded (Kumar and Jena, 2014).

2.5 Antioxidant activity

For the detection of antioxidant activity, a assay using 2, 2 diphenyl-1-picrylhydrazyl (DPPH) was performed (Bhatnagar et al., 2013). The DPPH used was of anlytical grade (2,2-Diphenyl-1-Picrylhydrazyl DPPH Extrapure, 95%, 29128 [1898-66-4], Sisco Research Laboratories (SRL) Pvt. Ltd.). Initially, the TLC plates were air-dried, and then the chromatograms were sprayed with 0.2% solution of DPPH in methanol as an indicator. The presence of antioxidant compounds was identified by observing yellow spots against a purple background on the TLC plates after spraying with the DPPH solution. Qualitative screening of the constituents in the leaf extracts of *L. comberi* for antioxidant activity was carried out using TLC sheet. The chromatograms were developed in the following solvent systems:

a) ethyl acetate : methanol : water (40 : 5.4 : 4);
b) chloroform : ethyl acetate : formic acid (5 : 4 : 1);
c) n-hexane : petroleum ether : ethanol (90 : 10 : 1).

3 Results and Discussion

The phytochemical analysis of *L. comberi* leaf extracts revealed the presence of several bioactive compounds (Table 1 and Figure 3). The aqueous extract contained tannins, saponins, terpenoids, phenolic compounds, reducing sugars, and alkaloids, indicating its medicinal potential. These compounds are associated with various pharmacological activities, including antibacterial properties (Kovac et al., 2022), cytotoxic effects,

Table 1 Screening of secondary metabolites of *L. comberi* leaves using different extracts

Bioactive compounds	Solvents		
	aqueous	methanol	ethanol
Tannins	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Flavonoids	-ve	-ve	-ve
Terpenoids	+ve	-ve	-ve
Phenolic compounds	+ve	+ve	+ve
Reducing sugars	+ve	+ve	+ve
Steroids	-ve	-ve	-ve
Alkaloids	+ve	+ve	+ve
Carbonyl compounds	-ve	-ve	-ve

+ve – positive indicates the presence of the bioactive compound; -ve – negative indicates the absence of that bioactive component

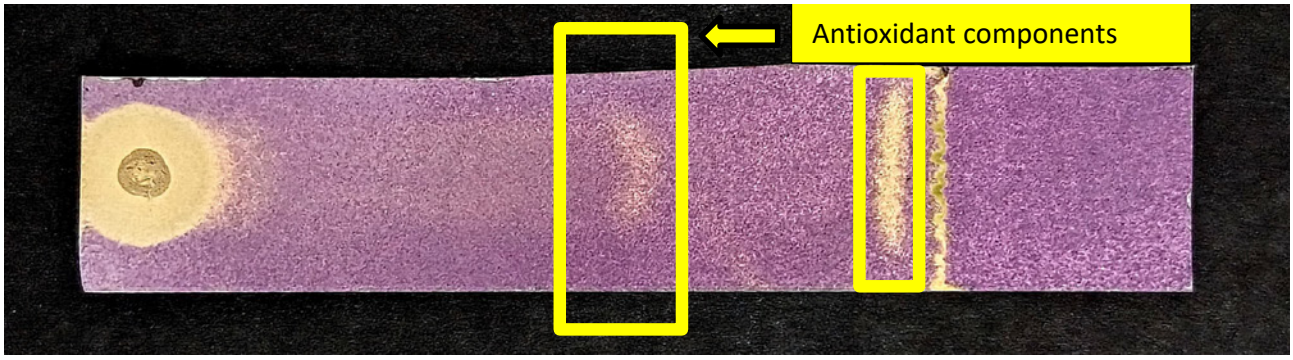

Figure 3 TLC based antioxidant activity of methanolic leaf extract of *L. comberi* in chloroform: ethyl acetate: formic acid (5 : 4 : 1) mobile phase

anticancer activity (Jaeger and Cuny, 2016), antidiabetic properties (Lin et al., 2016), antioxidant activity (Khatri and Chhetri, 2020), and analgesic properties (Aryal et al., 2022). The methanolic extract of *L. comberi* leaves also contained tannins, which have cardioprotective properties (Smeriglio et al., 2017), saponins that possesses immunomodulatory effects, phenolic compounds that exhibit anti-inflammatory activity (Sun and Shahrajabian, 2023), and alkaloids that may help prevent chronic diseases (Khatri and Chhetri, 2020). In addition, the screening of ethanolic extracts revealed the presence of various phytoconstituents, including tannins, saponins, phenolic compounds, reducing sugars, and alkaloids. These compounds are linked to several pharmacological activities, such as wound healing (Pizzi, 2021), acting as an antidote (Shi et al., 2004), anti-aging effects (Rahman et al., 2021), antioxidant properties (Khatri and Chhetri, 2020), and antiviral activities (Ferreira, 2022). The above potentials are validated after quantification of total phenols, tannins, saponins and flavonoids (Table 2). It was noticed that tannins showed high concentration in leaves (Table 2).

Table 2 Quantification of bioactive compounds and extractive yield % in the leaves of *L. comberi*

Parameters	Quantification
Total Phenols (mg.100 g^{-1})	2.45
Tannins (mg.100 g^{-1})	29.11
Saponins %	4.49
Flavonoids (mg.100 g^{-1})	0.12

Phytochemical analysis revealed the presence of secondary metabolites, specifically phenolic compounds, suggesting that the leaf extract may possess cytotoxic activity. Consequently, a cytotoxicity test was conducted using different extracts of *L. comberi* with nauplii of *Artemia salina*, as presented in Table 3 and Figure 4. The results indicated that after 3 hours, the ethanolic extract of the leaves exhibited the highest activity at a concentration of 15 $mg.ml^{-1}$ concentration. The methanolic extract displayed moderate activity, while the aqueous extract demonstrated the lowest activity the same concentration. These findings suggest that the leaf extracts have potential anticancer properties (Ullah et al., 2013).

The antibacterial activity of aqueous, methanolic and ethanolic extracts of *L. comberi* leaves were evaluated using the agar-well diffusion assay against three gram-positive bacteria and one gram-negative bacteria. The results indicated that the aqueous extract of *L. comberi* demonstrated significant antibacterial activity, exhibiting the largest zone of inhibition (ZI) of 24 ±0.1 mm against *S. pyogenes* (Table 4). This was followed by the methanolic extract with a ZI of 21 ±0.13 mm and the ethanolic extract, which also had a ZI of 21 ±0.06 mm (Table 4). These findings auggest that the aqueous extracts has the highest antibacterial activity compared to other extracts. The results have been presented in Table 4.

Table 5 indicated the antibacterial activity of *L. comberi* leaf extracts against four bacterial strains using the disc diffusion assay. The antibacterial activity increases with the concentration for the extracts, with higher concentrations (100 $mg.ml^{-1}$ and 200 $mg.ml^{-1}$) showing greater inhibition compared to lower concentrations (25 $mg.ml^{-1}$ and 50 $mg.ml^{-1}$). At 25 $mg.ml^{-1}$, all extracts exhibited minimal or no inhibition (zone of inhibition; ZI ≤7 mm). In contrast, at 200 $mg.ml^{-1}$, the highest zones of inhibition were observed across all the extracts, indicating enhanced antibacterial activity at higher concentrations. The aqueous extract demonstrated the strongest antibacterial activity, especially at 200 $mg.ml^{-1}$, where it recorded the highest ZI values across all bacterial strains. The methanolic extract has moderate antibacterial activity, slightly lower than of the aqueous extract. The ethanolic extract exhibited the weakest antibacterial activity among the three solvents. Additionally, *S. mutans*, *S. pneumoniae*, and

Table 3 Toxicity analysis using Brine shrimp nauplii of different extracts of *L. comberi*

Extracts	Concentration ($mg.ml^{-1}$)	Initial number of Nauplii	Number of deaths of nauplii (after hours)				Death % (after 3 hours)
			1	2	3	4	
Aqueous	15	10	6	7	7	9	70
	31	10	6	7	8	10	80
	47	10	7	8	8	10	80
	63	10	8	8	9	10	90
	79	10	9	10	10	10	100
	3.5% saline	10	0	0	0	0	0
	vincristine sulphate	10	9	10	10	10	100
Methanolic	15	10	7	8	8	10	80
	31	10	8	9	9	10	90
	47	10	8	9	10	10	100
	63	10	9	10	10	10	100
	79	10	9	10	10	10	100
	3.5% saline	10	0	0	0	0	0
	vincristine sulphate	10	10	10	10	10	100
Ethanolic	15	10	7	7	9	10	90
	31	10	7	8	10	10	100
	47	10	7	8	10	10	100
	63	10	9	9	10	10	100
	79	10	9	10	10	10	100
	3.5% saline	10	0	0	0	0	0
	vincristine sulphate	10	10	10	10	10	100

Table 4 Antibacterial activity (zone of inhibition in mm) of L. comberi leaves using Agar-well diffusion assay

Extract	Concentration ($mg.ml^{-1}$)	Zone of inhibition (mm) (mean ±SD)			
		S. pyogenes	*S. mutans*	*S. pneumoniae*	*S. flexneri*
Aqueous	25	ZI ≤7	ZI ≤7	ZI ≤7	ZI ≤7
	50	10 ±0.35	10 ±0.3	9.8 ±0.05	9.5 ±0.05
	100	23 ±0.3	21 ±0.35	20.5 ±0.2	19 ±0.35
	200	24 ±0.1	22 ±0.2	21.5 ±0.1	21±0.2
Methanolic	25	ZI ≤7	ZI ≤7	ZI ≤7	ZI ≤7
	50	9.8 ±0.02	9.7 ±0.15	9.4 ±0.32	9.2 ±0.43
	100	18.5 ±0.15	18 ±0.2	17.9 ±0.01	17.3 ±0.02
	200	21 ±0.13	19.2 ±0.1	19 ±0.13	19.2 ±0.03
Ethanolic	25	ZI ≤7	ZI ≤7	ZI ≤7	ZI ≤7
	50	8.8 ±0.02	8.6 ±0.03	8 ±0.08	7.9 ±0.04
	100	17 ±0.06	15.8 ±0.04	15 ±0.05	14.9 ±0.04
	200	21 ±0.06	21 ±0.07	20.8 ±0.05	19 ±0.2

ZI – zone of inhibition; SD – standard deviation; $n = 3$

Table 5 Antibacterial activity (Zone of inhibition in mm) of *L. comberi* leaves using Disc diffusion assay

Extract	Concentration ($mg.ml^{-1}$)	Zone of Inhibition (mm) (mean ±SD)			
		S. pyogenes	*S. mutans*	*S. pneumoniae*	*S. flexneri*
Aqueous	25	ZI ≤7	ZI ≤7	ZI ≤7	ZI ≤7
	50	11 ±0.12	10.5 ±0.15	9.8 ±0.03	9.7 ±0.02
	100	14 ±0.08	13 ±0.13	12.8 ±0.03	12.5 ±0.04
	200	15 ±0.11	14 ±0.14	13.8 ±0.03	12.9 ±0.02
Methanolic	25	ZI ≤7	ZI ≤7	ZI ≤7	ZI ≤7
	50	10 ±0.1	9.8 ±0.3	9.4 ±0.3	9 ±0.15
	100	13 ±0.1	12.6 ±0.2	12 ±0.2	11.7 ±0.13
	200	14 ±0.2	13.3 ±0.2	13 ±0.14	12.8 ±0.03
Ethanolic	25	ZI ≤7	ZI ≤7	ZI ≤7	ZI ≤7
	50	10 ±0.1	9.4 ±0.13	9.2 ±0.2	8.8 ±0.2
	100	12 ±0.02	11.8 ±0.12	11 ±0.2	10.7 ±0.2
	200	13 ±0.1	12 ±0.2	11.7 ±0. 05	11 ±0.4

ZI – zone of inhibition; SD – standard deviation; $n = 3$

Table 6 Analysis of minimum inhibitory concentration (MIC) using broth dilution assay of *L. comberi* leaf extracts against *Streptococcus pyogenes*

Extract	Concentration ($mg.ml^{-1}$)							Inoculum control	Broth control	Positive control
	500	400	300	200	100	50	25			
Methanolic	NG	NG	NG	GR	GR	GR	GR	GR	NG	NG
Ethanolic	NG	NG	NG	GR	GR	GR	GR	GR	NG	NG
Aqueous	NG	NG	NG	NG	GR	GR	GR	GR	NG	NG

NG – no growth; GR – growth; positive control – Kanamycin ($12.5\ mg.ml^{-1}$)

Table 7 TLC analysis of *L. comberi* leaves

Mobile phase	Extracts	Rf value of spots (cm)						
		S1	S2	S3	S4	S5	S6	S7
Chloroform : methanol (2 : 3)	aqueous	0.85	0.11	–	–	–	–	–
	methanolic	0.85	0.81	0.78	0.72	–	–	–
	ethanolic	0.94	0.93	0.89	0.86	0.81	0.77	0.72

Table 8 TLC based antioxidant activity of leaves of *Lasiococca comberi* in different mobile phases

Mobile phase	Solvent extract		
	methanol	ethanol	aqueous
Chloroform : ethyl acetate : formic acid (5 : 4 : 1) (CEF)	0.616	0.958	0.967
	0.945	0–0.069 (streak)	0–0.327 (streak)
	0.986		
	0–0.09 (streak)		
n-hexane : petroleum ether : ethanol (90 : 10 : 1) (BEA)	0.142	0.983	0.981
	0.968		0–0.415 (streak)
	0–0.095 (streak)		
Ethyl acetate : methanol : water (40 : 5.4 : 4) (EMW)	0.904	0.847	0.98
	0.952		0–0.672 (streak)

S. flexneri showed inhibition, although at slightly lower levels as compared to *S. pyogenes*. The results have been tabulated in Table 4 and Table 5.

Table 6 presented the results of Minimum Inhibitory Concentration (MIC) assay, which was conducted using the broth dilution method to assess the antibacterial activity of *L. comberi* leaf extracts against *S. pyogenes*. The aqueous extract exhibited the highest effectiveness, with a MIC of 200 $mg.ml^{-1}$ against *S. pyogenes*. In contrast, the methanolic and ethanolic extract showed moderate activity, with a MIC of 300 $mg.ml^{-1}$. The positive control, Kanamycin at 12.5 $mg.ml^{-1}$, exhibited no growth (NG), confirming its strong antibacterial effect. These findings suggest that *L. comberi* leaf extracts, particularly in the aqueous form, possess antibacterial properties against *S. pyogenes*.

Thin-layer chromatography (TLC) was conducted using three different extracts of *L. comberi* leaves to analyze organic compounds and determine the retention factor (Rf) of various compounds. As shown in Table 7, the ethanolic extract of the *L. comberi* leaves exhibited the highest number of spots, with a total of 7, followed by the methanolic extract, which produced 4 spots. The aqueous extract showed just 2 spots. These findings suggest that *L. comberi* has significant potential for analysis and application in novel pharmaceuticals. Additionally, table 8 presents the antioxidant components found in the leaves (Figure 3).

4 Conclusions

Present study provides significant insights into the pharmacological potential of *Lasiococca comberi*, a threatened medicinal tree. The findings suggest that the leaves of this plant possess notable antibacterial and antioxidant activities, attributed to the presence of various bioactive compounds. The results of this research underscore the importance of further exploring and utilizing *L. comberi* as a potential source of new antimicrobial agents. Moreover, the study highlights the need for conservation efforts to protect this threatened species and its habitat. Overall, the pharmacological evaluation of *L. comberi* leaves offers promising prospects for future research and development in the field of natural medicine.

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Conflict of Interest

Authors have no conflict of interest.

Author Contributions

JRJ, JKS and ST written the manuscript; KD proof reading and SK and SS carried out experimental works and supervision.

AI and AI-Assisted Technologies Use Declaration

There is no AI and AI-assisted technologies are used.

References

Ahmad, S., Zahiruddin, S., Parveen, B., Basist, P., Parveen, A., Gaurav, Parveen, R., & Ahmad, M. (2021). Indian Medicinal Plants and Formulations and Their Potential Against COVID-19-Preclinical and Clinical Research. *Frontiers in Pharmacology*, 11, 578970. https://doi.org/10.3389/fphar.2020.578970

Aryal, B., Raut, B.K., Bhattarai, S., Bhandari, S., Tandan, P., Gyawali, K., Sharma, K., Ranabhat, D., Thapa, R., Aryal, D., Ojha, A., Devkota, H.P., & Parajuli, N. (2022). Potential Therapeutic Applications of Plant-Derived Alkaloids against Inflammatory and Neurodegenerative Diseases. *Evidence Based Complementary Alternative Medicines*, 2022, 1–18. https://doi.org/10.1155/2022/7299778

Barik, S.K., Tiwari, O.N., Adhikari, D., Singh, P.P., Tiwary, R., & Barua, S. (2018). Geographic distribution pattern of threatened plants of India and steps taken for their conservation. *Current Science*, 114(3), 470–503. https://doi.org/10.18520/cs/v114/i03/470-503

Bhatnagar, S., Panda, P., & Behera, D.R. (2013). Phytochemical analysis and evaluation of cytotoxic and antioxidant activity of fruit extracts of *Terminalia racemosa*. *International Journal of Pharmacognosy and Phytochemical Research*, 5(3), 151–154. https://doi.org/10.20959/wjpr20242-30967

Das, U., Datta, P., & Behera, B. (2025). Identification of Major Threats of Climate Change, Hazards, and Anthropogenic Activities on Biodiversity Conservation in the Buxa Tiger Reserve, India. *Environmental Management*, 75(1), 3–20. https://doi.org/10.1007/s00267-024-01998-y

Ferreira, M.U. (2022). Alkaloids in Future Drug Discovery. *Molecules*, 27(4), 1347. https://doi.org/10.3390/molecules27041347

Chitale, V.S., Behera, M.D., & Roy, P.S. (2014). Future of endemic flora of biodiversity hotspots in India. *PLoS One*, 9(12). https://doi.org/10.1371/journal.pone.0115264

Jena, N., Vimala, Singh, B., Patra, A., Sharma, B.P., Hossain, E., & Kumar, S. (2025). Methods for ethnobotanical data collection, phytochemistry, antioxidant, anthelmintic, and antimicrobial activities for pharmacological evaluation of medicinal plants. *Journal of Biodiversity and Conservation*, 9(2), 87–107. https://doi.org/10.5281/zenodo.15368734

Khatri, D., & Chhetri, S.B.B. (2020). Reducing Sugar, total phenolic content, and antioxidant potential of Nepalese plants. *Biomed Research International*. 2020. https://doi.org/10.1155/2020/7296859

Kovac, J., Slobodnikova, L., Trajcikova, E., Rendeková, K., Mučaji, P., Sychrová, A., & Bittner Fialová, S. (2022). Therapeutic

Potential of Flavonoids and Tannins in Management of Oral Infectious Diseases-A Review. *Molecules*, 28(1),158. https://doi.org/10.3390/molecules28010158

Kumar, J., Katoch, D., Thakur, A., Pathania, A., Anand, A., Choudhary, K., & Shelja. (2024). A comprehensive review on threats and conservation status of orchids. *Journal of Applied Biology & Biotechnology*, 12(2), 43–47. https://doi.org/10.7324/JABB.2024.150084

Kumar, S., & Jena, P.K. (2014). Chromatographic, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. *Plant Science Research*, 36 (1 & 2), 83–90.

Kumar, S., Jyotirmayee, K., & Sarangi, M. (2013). Thin Layer Chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. *International Journal of Pharmaceutical Sciences Review and Research*, 18(1), 126–132.

Kumar, S., Mahanti, P., Singh, N.R., Rath, S.K., Jena, P.K., & Patra, J.K. (2017). Antioxidant activity, antibacterial potential and characterization of active fraction of *Dioscorea pentaphylla* L. tuber extract collected from Similipal Biosphere Reserve, Odisha, India. *Brazilian Journal of Pharmaceutical Sciences*, 3(4):e17006. https://doi.org/10.1590/s2175-97902017000417006

Kumar, S., Tripathy, P.K., & Jena, P.K. (2012). Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and its toxicity to *Artemia salina*. *Plant science Research*, 34(1 & 2), 93–96.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules*, 21(10),1374. https://doi.org/10.3390/molecules21101374

Mishra, S., & Bhatnagar. S. (2024). Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. *World Journal of Pharmaceutical Life Science*, 10 (2), 116–118. https://doi.org/10.5281/zenodo.13742393

Obadoni, B.O., & Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8, 203–208. https://doi.org/10.4314/gjpas.v8i2.16033

Pizzi, A. (2021). Tannins medical/ pharmacological and related applications: a critical review. *Sustainable Chemistry and Pharmacy*, 22. https://doi.org/10.1016/j.scp.2021.100481

Rahman, M.M., Rahaman, M.S., Islam, M.R., Rahman, F., Mithi, F.M., Alqahtani, T., Almikhlafi, M.A., Alghamdi, S.Q., Alruwaili, A.S., Hossain, M.S., Ahmed, M., Das, R., Emran, T.B., & Uddin, M.S. (2021). Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. *Molecules*, 27(1), 233. https://doi.org/10.3390/molecules27010233

Sadasivam, S., & Manickam, A. (2009). *Biochemical methods*. (3rd ed.) New Age International Publishers.

Saxena, H.O., & Brahmam, M. (1995). *The Flora of Orissa, Volume 5*. Regional Research Laboratory, Bhubaneswar, Odisha & Orissa Forest Development Corporation Limited., Bhubaneeswar.

Sharma, S. & Thokchom, R. (2014). A review on endangered medicinal plants of India and their conservation. *Journal of Crop and Weed*, 10(2), 205–218.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from edible legumes: chemistry, processing, and health benefits. *Journal of Medicinal Food*, 7(1), 67–78. https://doi.org/10.1089/109662004322984734

Shirazi OU, Khattak MMAK, Shukri NAM and Nasyriq MNA. (2014). Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *Journal of Pharmacognosy and Phytochemistry*, 3(3), 104–108.

Silva, S.V., Andermann, T., Zizka, A., Kozlowski, G., & Silvestro, D. (2022). Global Estimation and Mapping of the Conservation Status of Tree Species Using Artificial Intelligence. Frontiers *in Plant Science*, 13. https://doi.org/10.3389/fpls.2022.839792

Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British Journal of Pharmacology*, 174(11), 1244–1262. https://doi.org/10.1111/bph.13630

Sun, W., & Shahrajabian, M.H. (2023). Therapeutic Potential of Phenolic Compounds in Medicinal Plants-Natural Health Products for Human Health. *Molecules*, 28(4), 1845. https://doi.org/10.3390/molecules28041845

Swain, T., and Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. – the quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 10, 63–68. https://doi.org/10.1002/jsfa.2740100110

Ullah, M.O., Haque, M., Urmi, K.F., Zulfiker, A.H., Anita, E.S., Begum, M., & Hamid, K. (2013). Anti-bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. Asian Pacific Journal of Tropical Biomedicine, 3(1), 1–7. https://doi.org/10.1016/s2221-1691(13)60015-5