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Screening of *Parmelia parlata* extract for *in vivo* antifungal activity

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Parmelia parlata is recognised for its varied secondary metabolites that have considerable antibacterial and antifungal properties. This study aims to assess the antifungal efficacy of extracts from certain *Parmelia parlata* species *in vivo*. Lichen specimens were gathered, verified, and extracted utilizing solvent methanol. Initial phytochemical analysis indicated the presence of phenolics, depsides, and depsidones, which are recognised for their antifungal characteristics. Wistar rats infected with *Candida albicans* were utilised for *in vivo* assessment to determine the therapeutic effectiveness of the most potent extract. Treatment with *Parmelia perlata* extract significantly reduced fungal burden compared with the infected control group. This research illustrates the remarkable antifungal capabilities of *Parmelia parlata* extracts and proposes their use in formulating alternative treatments for fungal infections. Additional research on the mechanism of action and formulation development is advised.

Keywords: *Parmelia parlata*, Antifungal activity, *In vivo* screening, *Candida albicans*, Natural bioactive compound

INTRODUCTION

Fungi are non-photosynthetic, vegetative organisms that are present in unicellular yeasts or multicellular molds composed of hyphae. Common species including *Aspergillus* and *Candida* are ubiquitously distributed in the environment and can cause infections ranging from superficial to systemic infection (Fig. 1) in immunocompromised individuals[1]. Superficial infections such as dermatophytosis and candidiasis exist globally, while subcutaneous and systemic mycoses exist in tropical and subtropical regions[2,3]. The pathophysiology of fungal infections consists of adhesion to host tissues through surface adhesions, and their survival is enhanced by biofilm formation, capsule production, and melanin synthesis[1,4].

The synthetic antifungal agents are associated with resistance and toxicity and thus increasing the demand for natural antifungal alternatives derived from plants, lichens, and probiotics. The essential oils derived from *Syzygium aromaticum*, *Thymus vulgaris*, and *Melaleuca alternifolia* contains eugenol and thymol[5,6]. Contributing to their antifungal properties similarly Lichens, particularly Lichens belonging to the family Parmeliaceae, particularly *Parmelia perlata*, are rich sources of biologically active secondary metabolites like usnic acid, salazinic acid, and stictic acid contributing their role as antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities[7,8]. The diverseantimicrobial and anticancerous pharmacological activities of *Parmelia parlata* highlight their potential as therapeutic agents in combating fungal and oxidative stress-related diseases[9-11].

MATERIAL AND METHODS

Collection and authentication of Lichen

The lichen was collected from the local market in Prayagraj in November and air-dried at 40°C. The collected material was authenticated by the taxonomist Dr. S. Muthu

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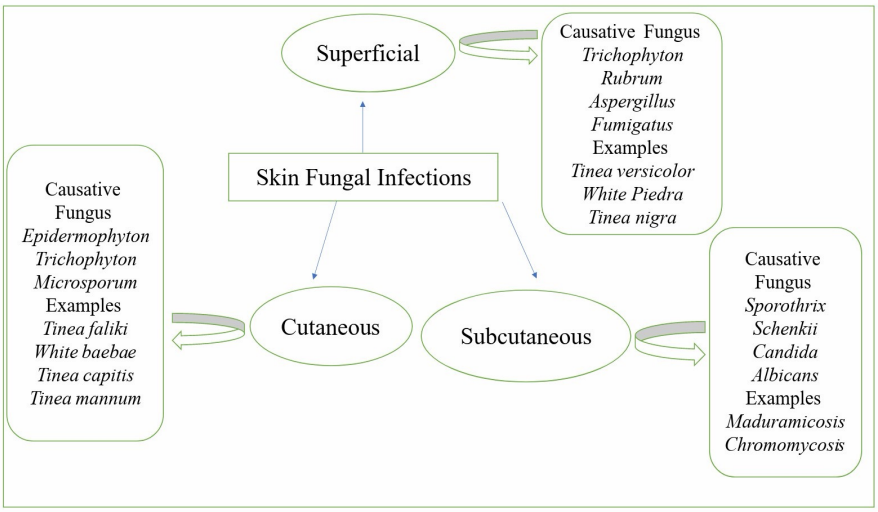

Figure 1. Types of fungal infections

Kumar of the Botanical Survey of India. Ref. No. B.S.I/ C.R.C./2024&25/570.

Physicochemical analysis

The physicochemical investigation of the powdered lichen material, including moisture content (loss on drying), total ash, acid-insoluble ash, water-soluble ash, sulphated ash, alcohol-soluble extractive value, water-soluble extractive value, and fluorescence analysis, were performed as per the procedure described in the Ayurvedic Pharmacopoeia of India and WHO guidelines for quality control of herbal materials, and results were expressed as mean ± SD[12].

Extract preparation

The extraction of lichen was performed using a Soxhlet apparatus. Coarse lichen powder (50 g) was placed in a thimble, and for the removal of the fatty compounds, petroleum ether was used at 45°C, followed by extraction with methanol. After the completion of the extraction, the solvent was evaporated using a rotary evaporator and the collected extract was allowed to dry in a desiccator and kept in an air tight container.

Phytochemical analysis

Preliminary phytochemical investigation was performed to detect secondary metabolites by using standard qualitative methods like carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, phenolic compounds, glycosides, saponins, and steroids[12].

LC-MS (MS Q-TOF)

MS Q-TOF component model G6550A system (HRLCMS-Q-TOF-Agilent Technologies, USA) was used to identify and quantify lichen methanolic extract metabolites. The liquid chromatography apparatus includes an autosampler HiP model G4226A, binary pump model G4220B, ion source dual AJS ESI, Column Comp. model G1316C, DAD model G4212B. Acetonitrile is the solvent B, while 0.1% formic acid in Milli-Q water is the solvent A. The following parameters were measured: MS capillary voltage 3500 V, nebulizer pressure 35 psig, injection volume 5 µL, drying gas temperature 250°C, and drying gas flow rate 13 L/min.Each component's flow injection research was used to optimize the mass spectrometry settings[13].

Preparation and characterization of *P. parlata* suspension

The suspension was prepared using carboxymethyl cellulose (CMC) as a suspending agent. The lichen extract was dissolved in a small volume of alcohol and incorporated into the aqueous phase containing CMC, followed by volume adjustment with purified water. The formulation was evaluated for sedimentation volume, redispersibility, viscosity, crystal growth, and rheological behaviour using standard pharmaceutical evaluation methods[14,15]**.** The prepared suspension was applied topically once daily throughout the treatment period.

Pharmacological evaluation

Animals and ethical approval

Healthy adult Wistar albino rats (100-200 g) of either sex were procured and acclimatized for 14 days before experimentation. Animals were housed under standard laboratory conditions (temperature 26 ± 2°C, relative humidity 44-56%, and 12 h light/dark cycle) with free access to standard pellet diet and water *ad libitum*. All experimental procedures were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Approval No. UIP/IAEC/Feb.-2024/03.

Experimental design and sample size

A total of 24 rats were randomly allocated into four experimental groups (n = 6 per group). The sample size was selected based on previous *in vivo* antifungal studies employing similar infection models and was considered adequate to detect biologically significant differences among treatment groups while minimizing animal use in accordance with the 3Rs principle (Replacement, Reduction, and Refinement).

Skin irritation study

The dermal irritation potential of the lichen formulation was evaluated according to OECD Guideline 404. Animals were randomly divided into two groups: a control group and a treatment group. A measured quantity (0.5 g) of the *P. perlata* suspension was applied to the shaved dorsal skin of rats and observed for signs of erythema, edema, or irritation at 24, 48, and 72 h after application. Dermal reactions were assessed using the Draize scoring system.

Fungal strain preparation, infection induction, treatment, and evaluation

Candida albicans (MCCB 0280) was obtained from the Department of Industrial Microbiology, SHUATS, Prayagraj, India, and cultured on potato dextrose medium at 30-35°C for 48 h. In Wistar rats, cutaneous candidiasis was induced by introducing 100 µL of a *Candida albicans* suspension (3×10^7 CFU/mL) onto a dorsally abraded superficial skin (2.5 × 2.5 cm).

Twenty-four animals were randomly assigned to four groups (n = 6):

- Group I: Healthy control (non-infected, untreated)
- Group II: Infected control (*Candida albicans* infected, untreated)
- Group III: Standard treatment (*Candida albicans* infected and treated with luliconazole)
- Group IV: Test treatment (*Candida albicans* infected and treated with *P. parlata* suspension

Treatments were administered topically for 14 days. Antifungal efficacy was evaluated microbiologically by determining the fungal burden (log CFU/site) from infected skin homogenates cultured on Sabouraud Dextrose Agar (SDA), followed by colony enumeration after incubation at 37 ± 1°C for 48 h[16-19].

Statistical analysis

All quantitative data were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett post hoc multiple comparison test. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 9.0.

Results

Physiochemical analysis

The physicochemical evaluation of *P. parlata* powder confirmed its quality and purity. Parameters such as loss on drying, total ash value, extractive values, and other quality control measures were found to be within acceptable limits (Table 1), indicating the absence of excessive moisture and inorganic contaminants. These findings support the suitability of the raw material for further pharmacological investigations as shown in Table 1.

Fluorescence investigation

Fluorescence characteristics of *P. parlata* powder were examined under visible light and UV radiation (254 and 366 nm). Distinct color changes were observed following treatment with different chemical reagents (Table 2), providing characteristic fluorescence profiles useful for authentication and quality control of the lichen material.

Preliminary phytochemical analysis

Qualitative phytochemical analysis revealed the presence of several bioactive constituents, including flavonoids, alkaloids, tannins, phenolic compounds, carbohydrates, glycosides, and steroids (Table 3). These phytochemicals are known to possess antimicrobial, antioxidant, and anti-inflammatory activities and may contribute to the observed biological effects.

Evaluation of phytochemicals by HRLCMS

HRLCMS-QTOF analysis identified several metabolites in the methanolic extract of *P. parlata* (Fig. 2; Table 4). Major compounds detected included usnic acid, lecanoric acid, glutathione, and folic acid. Usnic acid and lecanoric acid are well-documented lichen secondary metabolites with reported antimicrobial properties, suggesting their potential contribution to the antifungal activity observed in this study. The compounds were tentatively identified based on accurate mass measurements and comparison with available mass spectral databases. Further confirmation by MS/MS fragmentation analysis and authentic reference standards is required.

Table 1. Physicochemical parameters of *P. parlata* powder

S. No.	Parameters	Values (% w/w)
1	LOD	6%
2	Total ash value	7%
3	Acid insoluble ash value	1%
4	Water soluble ash value	0.5%
5	Alcohol soluble extractive value	14%
6	Water soluble extractive value	4%

Table 2. Fluorescence observation of *P. parlata* powder

S. No.	Reagents	UV Light (254 nm)	UV Light (365 nm)	Visible light
1	H_2SO_4	Black	Black	Black
2	Acetic acid	Green	Brown	Brownish green
3	Methanol	Brownish green	Brown	Light yellow
4	NaOH	Olive	Black	Green
5	$FeCl_3$	Olive	Green	Olive

Table 3. Phytochemical observation of *P. parlata* extract

S. No.	Test	Result
1	**Carbohydrates**	
	Fehling's test	+
	Benedict test	+
2	**Proteins & amino acid**	
	Biuret test	-
	Millon's test	-
3	**Glycosides**	
	Baljet test	+
	Legal test	+
	Keller Kiliani test	+
	Anthraquinone test	-
4	**Tannins**	+
5	**Alkaloids**	
	Mayer's test	+
	Hager's test	+
6	**Steroids**	
	Salkowski test	+
	Liebermann Burchard test	+
7	**Saponins**	-
8	**Flavonoids**	
	Shinoda test	+

Characterization of *P. parlata* extract suspension

The prepared suspension exhibited acceptable pharmaceutical characteristics (Table 5). Both formulations showed good sedimentation behavior, satisfactory viscosity, neutral pH, and absence of crystal growth during storage. These findings indicate good physical stability and suitability for topical administration.

Pharmacological evaluation

Skin irritation test

No signs of erythema, edema, or other dermal irritation were observed in any treatment group throughout the 72-hour observation period (Table 6). The erythema score remained zero in all animals, indicating that the *P. parlata* suspension was non-irritating and safe for topical application.

In vivo anti-fungal activity

The antifungal efficacy of *P. parlata* suspension was evaluated using a *Candida albicans*-induced cutaneous infection model in Wistar rats. The untreated infected group (Table 7, Figure 3) exhibited the highest fungal burden (4.885 ± 0.075 log CFU/s ite), whereas treatment with the standard drug luliconazole significantly reduced fungal growth to 1.685 ± 0.078 log CFU/site ($p < 0.0001$).

Treatment with *P. parlata* suspension resulted in a dose-dependent reduction in fungal load. The low-dose (5% w/w) formulation reduced fungal burden to 3.230 ± 0.069 log CFU/site, whereas the high-dose (10% w/w) formulation reduced fungal burden to 2.137 ± 0.031 log CFU/site ($p <$ 0.0001 versus infected control).

Compared with the infected control group, the low-dose and high-dose formulations produced approximately 33.9% and 56.3% reductions in fungal burden, respectively, based on log CFU values. Furthermore, only 2 of 6 animals remained infected in the high-dose treatment group compared with 4 of 6 animals in the low-dose group, demonstrating superior therapeutic efficacy of the higher dose formulation.

These findings indicate significant antifungal activity of *P. parlata* extract and support its potential as a topical antifungal agent.

Discussion

The present study investigated the physicochemical characteristics, phytochemical composition, formulation properties, safety, and antifungal efficacy of *P. parlata* extract. The quality and identity of the lichen was confirmed by Physicochemical and fluorescence analyses, while the presence of biologically active metabolites was confirmed by phytochemical screening and HRLCMS-QTOF analysis. Among the identified compounds, usnic acid is recognized as one of the most important lichen-derived secondary metabolites and has been extensively reported for its antimicrobial and antifungal activities. Previous studies have shown that usnic acid inhibits fungal growth by disrupting membrane integrity, interfering with energy metabolism, and impairing cellular respiration. Similarly, lecanoric acid and other phenolic constituents possess antimicrobial properties through oxidative stress induction and disruption of fungal cell-wall synthesis.

The *in vivo* antifungal study demonstrated a significant reduction in *Candida albicans* colonization following

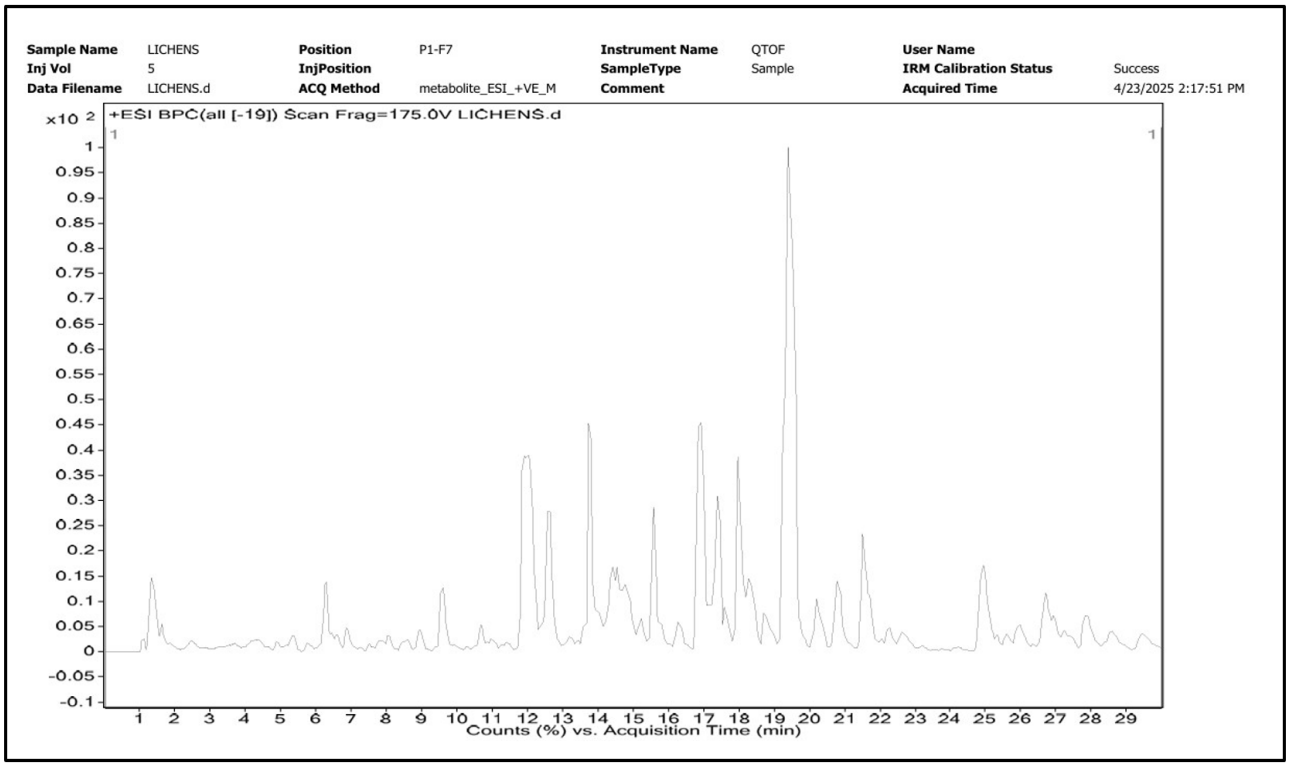

Figure 2. LCMS (MS Q-TOF) showing metabolites identified from *P. parlata* extract

Table 4. Constituents characterized by formula mass, potential compounds, monoisotopic masses, and other chromatographic features

S. No.	Base peak	*m/z*	Mass	Formula	Possible compound
1	397.163	343.0815	344.0884	$C_{18}H_{16}O_7$	Usnic acid
2	167.0347	317.0642	318.074	$C_{16}H_{14}O_7$	Lecanoric acid
3	298.0443	330.071	307.0816	$C_{10}H_{17}N_3O_6S$	GSH / Glutathione
4	151.0369	442.146	441.1385	$C_{19}H_{19}N_7O_6$	Folic acid
5	178.0489	417.1258	416.1184	$C_{10}H_{20}N_6O_{12}$	Fluorodifen

The identified compounds represent tentative assignments based on LC-MS analysis and require further structural confirmation

Table 5. Characterization parameters of *P. parlata* extract suspension

S. No.	Parameter	Batch 01 (Low dose, 5% w/w)	Batch 02 (High dose, 10% w/w)
1	Sedimentation volume	0.8	0.4
2	Flow rate	5 mL / 6.32 sec	5 mL /10.62 sec
3	Viscosity	59.6 cp	60.4 cp
4	pH	7 ± 0.57	7 ± 0.57
5	Crystal growth	No	No

treatment with *P. parlata* suspension. The observed dose-dependent response suggests that increasing concentrations of bioactive metabolites enhanced antifungal efficacy. The high-dose formulation produced a 56.3% reduction in fungal burden and substantially decreased the number of infected animals, indicating marked therapeutic potential.

These findings are consistent with previous reports describing the antifungal activity of lichen-derived compounds against Candida species and other pathogenic fungi. Similar studies have reported that lichen metabolites exhibit fungistatic and fungicidal effects through alteration of membrane permeability, inhibition of ergosterol

Table 6. Test for skin irritation

S. No.	Experimental groups	Erythemal score (n=3)		
		24 hr	48 hr	72 hr
1	Negative control	Nil	Nil	Nil
2	Standard group	Nil	Nil	Nil
3	*P. parlata* suspension treated (Low Dose)	Nil	Nil	Nil
4	*P. parlata* suspension treated (High Dose)	Nil	Nil	Nil

Table 7. Antifungal efficacy of *P. parlata* (CFU in animal skin after 14 days of treatment)

S. No.	Treatment	No. of infected animals/ total no. of animals	Log cfu per infected site
1	Positive control	6/6	4.885 ±0.075****
2	Standard group	1/6	1.685±0.078****
3	*P. parlata* suspension treated (Low Dose)	4/6	3.230 ±0.069****
4	*P. parlata* suspension treated (High Dose)	2/6	2.137 ±0.031****

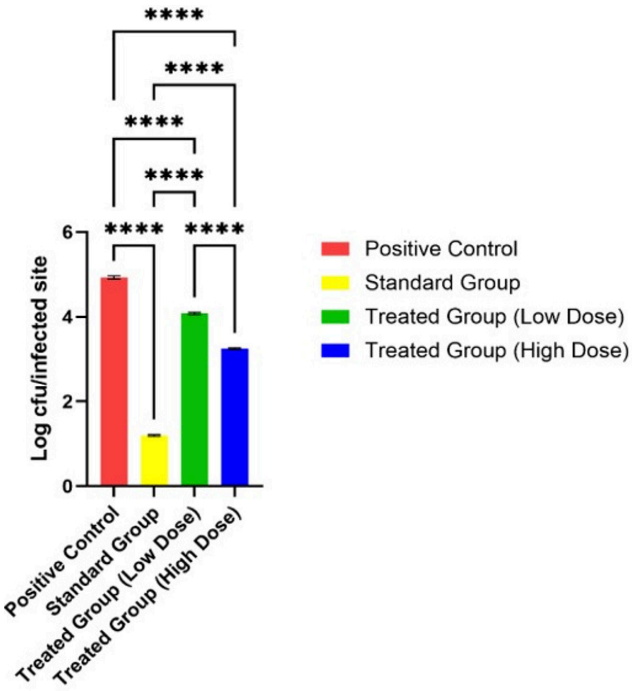

Figure 3. Antifungal efficacy of *P. parlata* (CFU in animals skin after 14 days of treatment) values are expressed as mean ± SEM (n=6), ****p<0.0001 using dunnet post hoc test

biosynthesis, and suppression of fungal biofilm formation. The absence of dermal irritation in treated animals further supports the suitability of the formulation for topical application. Safety is a critical consideration in antifungal therapy, and the lack of erythema or edema observed in the present study suggests good local tolerability.

Overall, the results indicate that *P. parlata* extract possesses promising antifungal activity, likely mediated through the synergistic action of usnic acid, phenolic compounds, flavonoids, and other secondary metabolites. Further studies involving isolation of active constituents, detailed mechanistic investigations, and long-term toxicity evaluation are warranted to support its development as a novel topical antifungal formulation.

Conclusion

The present study demonstrated that the methanolic extract of *P. perlata* possesses significant topical antifungal activity against *C. albicans*-induced cutaneous infection in Wistar albino rats. The observed reduction in fungal burden, together with the absence of dermal irritation, suggests that the formulation is safe and therapeutically promising. The antifungal activity may be attributed to the presence of lichen secondary metabolites such as usnic acid and phenolic compounds. However, further studies involving isolation of active constituents, detailed mechanistic investigations, and clinical evaluation are required before therapeutic application.

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