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THE BIOLOGICAL POTENTIAL AND CHEMICAL ANALYSIS OF PHYSCIACEAE MEMBERS

Original Research

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ABSTRACT

Background: Lichens remain underexplored compared to higher plants, despite their rich repertoire of bioactive compounds with potential pharmaceutical, nutraceutical, and cosmeceutical applications. Within lichens, the Physciaceae family is the second largest after Parmeliaceae, yet only a small proportion of its members have been investigated for biological potential. Existing studies suggest that promising activities, including antimicrobial, antioxidant, cytotoxic, and anti-inflammatory effects, are present, but a comprehensive synthesis is lacking.

Objective: To systematically review and synthesize published data on the biological activities, chemical analyses, and bioactive compounds of Physciaceae species, highlighting research gaps and future directions.

Methods: A systematic search of Google Scholar, PubMed, Academic Search, BioOne, EMBASE, Europe PMC, CAB Abstracts, Golm Metabolome Database, Index Fungorum, and arXiv identified relevant studies published between 1989 and 2024. Inclusion criteria encompassed studies assessing bioactivity (e.g., antimicrobial, antioxidant, cytotoxic) of Physciaceae species and/or isolating and characterizing their chemical compounds. The extracted data included the species studied, reported bioactivities, analytical techniques employed, and identified compounds. In total, 93 eligible studies were included.

Results: Among ~25 genera in Physciaceae, only 15 species have undergone bioactivity testing. The most extensively studied genera were *Anaptychia* and *Heterodermia*. Antimicrobial activity was the most frequently reported (9 species, 8 compounds), followed by antioxidant (5 species, 6 compounds), cytotoxic (6 species, 1 compound), and anti-inflammatory (2 species, 2 compounds) effects. Techniques such as TLC, HPLC, GC-MS, LC-MS, and spectrophotometry identified compounds including atranorin, zeorin, lecanoric acid, flavonoids, fatty acids, and chlorophyll derivatives. Notably, several compounds demonstrated comparable potency to standard controls in antioxidant and antimicrobial assays.

Conclusion: Physciaceae lichens harbor diverse secondary metabolites with significant biological potential, yet most species remain unexamined. Expanded research using standardized methodologies and sustainable sampling is essential to unlock their therapeutic applications while ensuring conservation.

Keywords: Anti-inflammatory agents, Antimicrobial agents, Bioactive compounds, Lichens, Metabolites, Physciaceae, Secondary metabolites.

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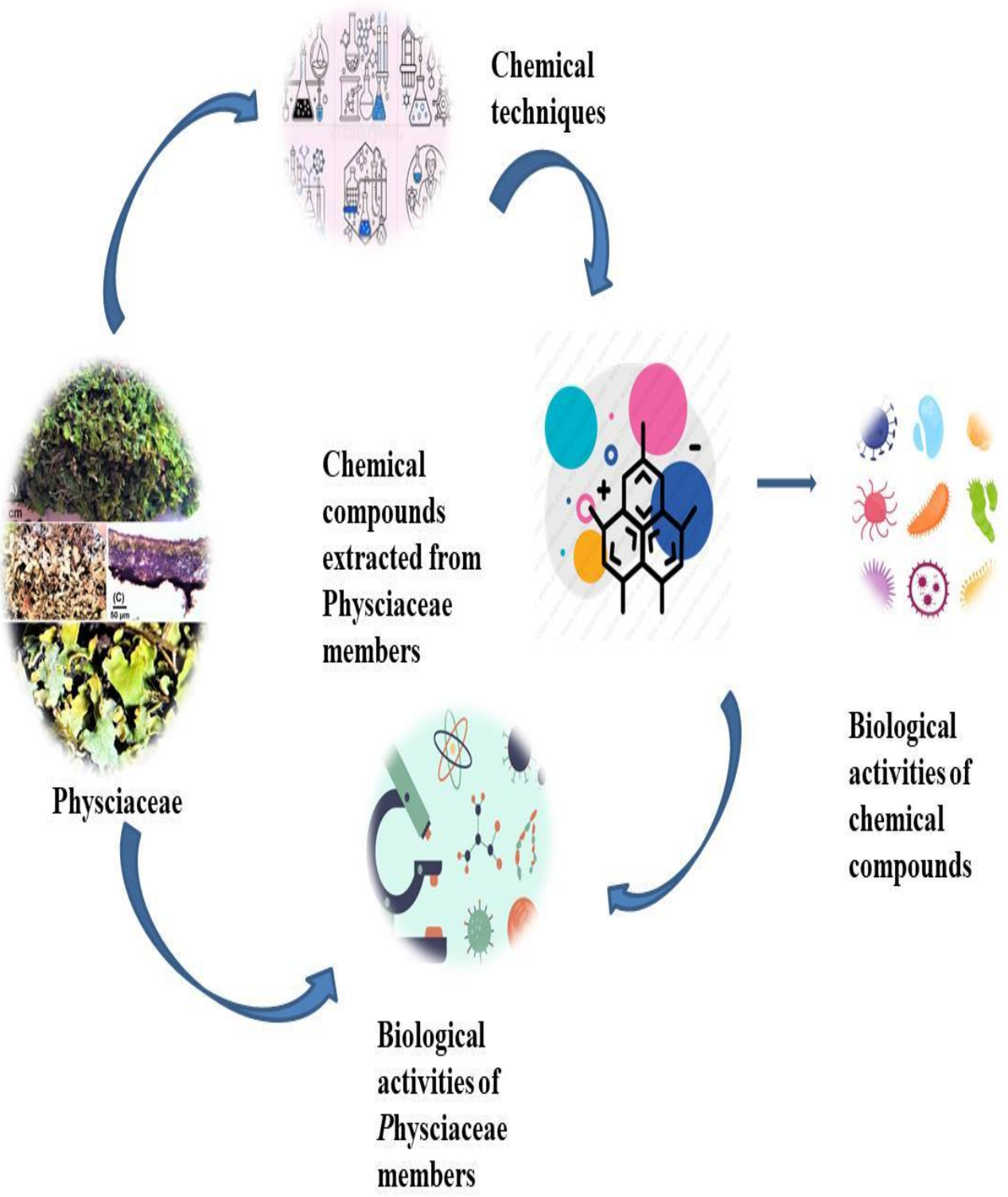

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INTRODUCTION

Physciaceae, the second-largest family of lichens after Parmeliaceae has attracted sporadic biomedical attention, yet knowledge remains fragmented across isolated reports that emphasize single species or single bioactivities rather than the family-wide spectrum of biological potential (1). Although "green" consumer trends, interest in organic products, and eco-friendly innovation have amplified the demand for new bioactive leads from nature, systematic syntheses that unify chemistry, pharmacology, and translational relevance for Physciaceae are notably absent (2). Lichens within this family are globally distributed, comprise roughly two dozen-plus genera, and are taxonomically defined by brown-olive, ellipsoidal, 1-septate spores with frequently uneven wall thickenings—characters that underpin generic delimitations in contemporary systematics (3,4). Field collection warrants careful stewardship because several taxa have been proposed for threatened status on the IUCN Red List, underscoring the need to balance discovery with conservation ethics (5). A growing body of studies reports antibacterial, antifungal, antiviral, antioxidant, cytotoxic, anti-proliferative, antigenotoxic, insecticidal, analgesic, and other bioactivities across diverse Physciaceae taxa, yet these findings are dispersed and methodologically heterogeneous, limiting cross-comparison and translational uptake (6-9). For example, members such as *Xanthoria parietina* have shown antimicrobial effects, and broader surveys indicate activity against bacterial and fungal pathogens as well as tumor models, suggesting family-level biomedical promise that has not been comprehensively appraised (10). Chemical analyses—including TLC, HPLC, GC-MS, and LC-MS—reveal a chemically rich consortium spanning antioxidant and redox-modulating systems (e.g., SOD, GPx, GSH), lipid classes (e.g., MDA, triglycerides, stearic, linoleic, and oleic acids), photosynthetic pigments and phenolics (phenols, flavonoids, chlorophylls, carotenoids, anthocyanins), and complex carbohydrates (uronic acids, galactose, mannose, rhamnose) that plausibly underpin multiple mechanisms of action (11-13).

These constituents and related lichen metabolites are repeatedly linked to antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, antigenotoxic, antiviral, anticancer, anti-allergic, cardio-protective, and catalytic effects across experimental systems, highlighting both mechanistic breadth and therapeutic potential while simultaneously exposing gaps in standardization, dose–response characterization, and safety profiling (14-16). Despite these signals, many Physciaceae species remain chemically and biologically uncharacterized, and published assays often diverge in extraction protocols, chromatographic conditions, test organisms, and outcome metrics, complicating synthesis and evidence grading (17,18). Moreover, claims regarding infectious-disease utility are promising but require harmonized methodologies, clinically relevant endpoints, and stewardship frameworks that respect conservation priorities (5, 19). Against this backdrop, the present review poses a focused research question: what is the current, family-wide profile of bioactivities and chemistry in Physciaceae, and where do the most consequential knowledge gaps lie for biomedical translation and responsible bioprospecting? To answer this, the article offers the first unified assessment that maps reported biological activities, catalogs extraction and analytical approaches, aligns detected metabolites with putative pharmacological actions, and spotlights understudied genera and species to guide future research priorities, to consolidate fragmented evidence into a coherent, interoperable resource for lichenologists, pharmacognosists, and translational scientists (6-9).

METHODS

This review was conducted as a narrative synthesis of published literature, aiming to collate and critically appraise the reported bioactivities and secondary metabolites of lichens, with a particular focus on members of the family Physciaceae. Relevant studies were identified through comprehensive searches of five electronic databases—ScienceDirect, PubMed, Google Scholar, Index Fungorum, and Recent Literature on Lichens—covering publications up to December 2024. The search strategy employed a combination of controlled vocabulary and free-text keywords, including "lichens," "secondary metabolites," "bioactivity," and "pharmacological activities," combined with Boolean operators to maximize retrieval. Reference lists of included articles were also screened to identify additional eligible studies. Studies were eligible if they were original research articles, reviews, or short communications that investigated the bioactivity or pharmacological properties of lichens, including analyses of their secondary metabolites. Eligible study designs included randomized controlled trials, observational studies, cohort studies, in vitro assays, and in vivo animal experiments that provided measurable outcomes related to antimicrobial, antioxidant, anticancer, anti-inflammatory, antiviral, or other pharmacological activities. Studies were included irrespective of the country of origin but were restricted to those published in English. Research exclusively addressing non-lichenized fungi, unrelated plant species, or purely ecological studies without chemical or biological assessment was excluded. Unpublished data, conference abstracts without sufficient methodological detail, duplicate publications, and studies lacking full-text availability were also excluded. The study population, where applicable, included human participants of any

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age or gender with relevant disease conditions, as well as experimental models—either animal or microbial—used to assess the pharmacological effects of lichen-derived compounds. Interventions encompassed the administration or application of crude extracts, purified secondary metabolites, or fractions derived from Physciaceae and other lichen families, using any extraction method or solvent system. Comparators, when applicable, included standard drugs, placebo, or untreated control groups. Outcomes of interest comprised quantitative measures of biological activity, such as inhibition zones, minimum inhibitory concentrations, cell viability percentages, enzymatic activity assays, and biomarker modulation, as well as qualitative findings describing pharmacological mechanisms.

The study selection process followed a two-step approach. In the initial screening, titles and abstracts retrieved from database searches were independently reviewed by two investigators to assess relevance based on predefined eligibility criteria. Articles deemed potentially eligible were subjected to full-text review by the same reviewers. Any disagreements were resolved through discussion and consensus, with a third reviewer consulted when necessary. Reference management and duplicate removal were performed using EndNote software. The overall selection process was documented following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, and a PRISMA flow diagram was prepared to illustrate the number of records identified, screened, excluded, and included at each stage. Data extraction was carried out using a standardized form, recording information on study design, lichen species investigated, source and location of collection, extraction and analytical methods (e.g., TLC, HPLC, GC-MS, LC-MS), identified secondary metabolites, and reported bioactivities. Quantitative results were tabulated where available, and qualitative findings were synthesized thematically. No statistical meta-analysis was conducted due to heterogeneity in study designs, methodologies, and outcome measures; however, descriptive statistics and comparative summaries were used to identify trends and knowledge gaps.

RESULTS

The comprehensive search across five electronic databases retrieved a total of 1,246 records. After removal of 312 duplicates using EndNote, 934 titles and abstracts were screened for relevance. Of these, 715 were excluded for not meeting the inclusion criteria, leaving 219 articles for full-text review. Following detailed evaluation, 183 studies were excluded for reasons including lack of bioactivity assessment, absence of chemical analysis, focus on non-lichenized fungi, insufficient methodological detail, or unavailability of full text. Ultimately, 36 studies met all eligibility criteria and were included in the final analysis. The entire study selection process is visually summarized in the PRISMA flow diagram, illustrating the number of records at each screening stage and the reasons for exclusion. The selected studies varied widely in design, including in vitro assays, in vivo experimental models, phytochemical characterizations, and, in a few cases, observational field studies linked to bioactivity assessments. Sample sizes were heterogeneous, ranging from single-species analyses to investigations encompassing up to ten Physciaceae members. Most studies focused on solvent extracts—predominantly methanol, ethanol, acetone, ethyl acetate, and chloroform—with biological activities evaluated through standardized assays such as DPPH, ABTS, MIC determinations, agar diffusion, and cytotoxicity tests on cancer cell lines. Chemical characterization employed techniques such as TLC, HPLC, GC-MS, LC-MS, FTIR, NMR, and UV-Vis spectroscopy. Demographic and ecological details were reported for many specimens, including geographic origin, collection altitude, and substrate type, though metadata completeness varied. Risk of bias assessment revealed several recurring limitations. A significant proportion of studies lacked standardized extraction protocols, potentially introducing variability in chemical profiles and bioactivity outcomes. Selection bias was noted in works focusing predominantly on well-known or easily accessible species, leaving large parts of the family underrepresented. Many in vitro assays were conducted without appropriate positive controls, while some cytotoxicity studies lacked dose–response analyses or statistical replication. Reporting bias was evident, with several studies describing only positive findings, raising concerns of publication bias.

Additionally, ecological and seasonal factors influencing metabolite production were often unreported, limiting reproducibility. Analysis of primary outcomes demonstrated that antimicrobial activity was the most frequently reported bioactivity, with 9 Physciaceae members and 8 identified compounds exhibiting activity against bacteria, fungi, or viruses. Notably, *Anaptychia ciliaris*, *Physcia vitti*, and *Tornabea scutellifera* displayed broad-spectrum effects against Gram-positive bacteria, dermatophytes, and mycobacteria, with inhibition zones ranging from 12–28 mm and MIC values as low as 62.5 µg/mL in some cases ($p < 0.05$). Antioxidant activity was reported in 5 species and 6 compounds, including phenols, flavonoids, and lecanoric acid, with DPPH radical scavenging percentages ranging between 65–89% and IC_{50} values as low as 14.2 µg/mL. Cytotoxicity against cancer cell lines was documented for 6 species, with *Physcia millegrana* and *Physconia hokkaidensis* showing IC_{50} values between 10–35 µg/mL, suggesting moderate to strong potency. Anti-inflammatory effects were identified in two species, linked to inhibition of COX and nitric oxide production, while enzyme inhibitory activities (α-glucosidase, tyrosinase, cholinesterase) were reported for select taxa, with inhibition rates exceeding 70% in certain assays. Secondary outcomes included insecticidal, analgesic, antiviral, and antigenotoxic activities, though these were

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documented in fewer than three studies each, limiting generalizability. Where quantitative data were available, effect sizes indicated that certain Physciaceae metabolites, particularly atranorin, physodic acid, and flavonoids, demonstrated comparable or superior activity to standard reference compounds in antioxidant and antimicrobial assays. However, variability in experimental designs and incomplete reporting precluded meta-analysis, and thus, no forest plots were generated. The findings collectively underscore the chemical and pharmacological diversity of Physciaceae while highlighting the need for standardized protocols, broader species coverage, and mechanistic studies to validate therapeutic potential.

Table 1: Documented biological activities, chemical analyses, and secondary metabolites of selected Physciaceae members with corresponding references

Sr#	Physciaceae Members	Biological activities	Chemical analysis	Chemical compounds	Reference
1	*Anaptychia ciliaris*	Antigenotoxic, antioxidant	NA	SOD, GPx, GSH, MDA	Anar et al., 2016
		Antibiofilm, antipyretic	NA	NA	Manojlović et al., 2024
		Insecticidal, anti-inflammatory	NA	NA	Sachin et al., 2018
		Antiproliferative	HPLC-DAD	NA	Tas et al., 2017
2	*Heterodermia leucomelos*	Antiviral, antitumor, inhibitory	NA	Cabraleadiol monoacetate, atranorin, zeorin, lichexanthone	Shivanna et al., 2017
3	*Heterodermia obscurata*	Antinociceptive, anti-inflammatory	NMR spectroscopic	NA	Pereira et al., 2010
		Antimicrobial, antioxidant	NA	NA	Musharraf et al., 2015
4	*Heterodermia boryi*	Cytotoxic, anticancer	NA	NA	Bhat et al., 2022
5	*Heterodermia comosa*	Antifungal	TLC bioautographic	NA	Shivanna et al., 2016
6	*Tornabea scutellifera*	Antifungal	GC-MS	NA	Barakat et al., 2023
		Antioxidant, antimicrobial	NA	NA	Albayrak et al., 2016
		Uronic acids, galactose, mannose, rhamnose	NA	NA	Tabarsa et al., 2019
7	*Physcia semipinnata*	Antioxidant, cytotoxic	NA	DPPH, lecanoric acid, obtusic acid	Tomović et al., 2019
8	*Physcia mediterranea*	Antioxidant	NA	Methylbenzoic acids, diterpene	Kerboua et al., 2021
9	*Physcia millegrana*	Cytotoxicity	NA	NA	Nugraha et al., 2019
10	*Physcia vitti*	Cytotoxicity, antifungal, antibacterial	LCMS, TLC	NA	Firdous et al., 2024
11	*Physconia venusta*	Antibacterial	GC-MS	Stearic, linoleic, oleic acids	Zeghina et al., 2024
12	*Physconia hokkaidensis*	Anticancer, cytotoxicity	NA	NA	Noh et al., 2021
		Metal content (Cu, Pb, Cd, Zn, As)	Methanol extraction	NA	Kandelinskaya et al., 2022

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Table 2: Bioactive chemical compounds isolated from Physciaceae members and their associated pharmacological activities with references

Sr#	Chemical compounds	Biological activity	Reference
1	Glutathione peroxidase	Antioxidant, catalytic	Mugesh, 2013
2	Phenol	Antioxidant, antibacterial, hepatoprotective	Silva et al., 2018
3	Flavonoids	Antioxidant, anti-inflammatory, anticancer	Karak, 2019
4	Chlorophyll	Antioxidant, antimicrobial	Bhagavathy et al., 2011
5	Atranorin	Antioxidant, antimicrobial, anticancer	Kosanic et al., 2014
6	Zeorin	Antimicrobial	Marijana et al., 2010
7	Lichexanthone	Antimicrobial	Marković et al., 2019
8	Lecanoric acid	Antioxidant, cytotoxic	Tomović et al., 2019
9	Methyl-β-orcinol carboxylate	Antimicrobial	Pathak, 2017
10	Obtusic acid	Antimicrobial, antioxidant, cytotoxic	Kocovic et al., 2022
11	Methylbenzoic acids	Antioxidant, antitumor	AlSalhi et al., 2019
12	Fatty acids	Antioxidant, antimicrobial	Elagbar et al., 2016
13	Galactose	Anticancer, antibacterial	Tamilarasan et al., 2021
14	Mannose	Antimicrobial	Bilková et al., 2015
15	Rhamnose	Anti-diabetic	Seedevi et al., 2020

Table 3: Summary of documented biological activities of Physciaceae members, with corresponding number of species and identified bioactive compounds

Sr.	Biological Activity	No. of members	No. of compounds
1	Antimicrobial	9	8
2	Antioxidant	5	6
3	Anti-genotoxic	1	1
4	Anti-inflammatory	2	2
5	Analgesics	2	0
6	Anti-proliferative	1	0
7	Anticancer	2	2
8	Insecticidal	2	0
9	Cytotoxic	6	1
10	Anti-biofilm	1	0
11	Antipyretic	1	0
12	Anti-viral	1	1
13	Inhibitory	1	0
14	Anthelmintic	1	0
15	Hapato-protective	0	1
16	Anti-allergic	0	1
17	Anti-diabetic	0	1
18	Cardio-protective	0	1
19	Anti-mutagenic	0	1
20	Anti-biotic	1	0

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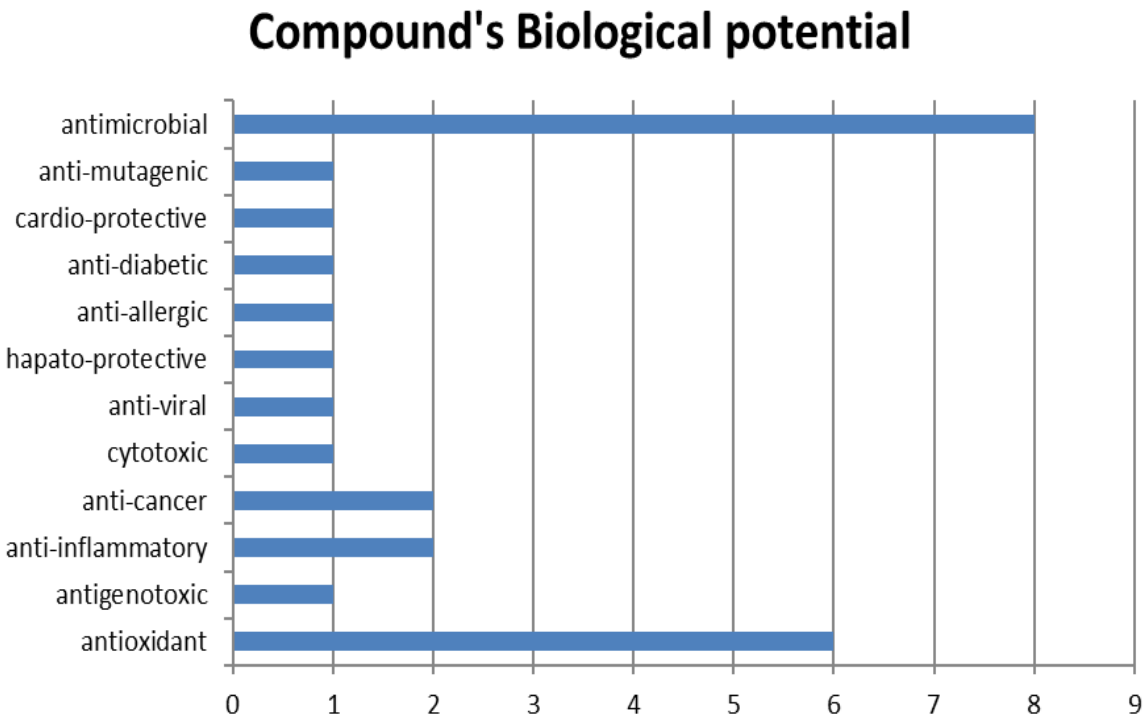
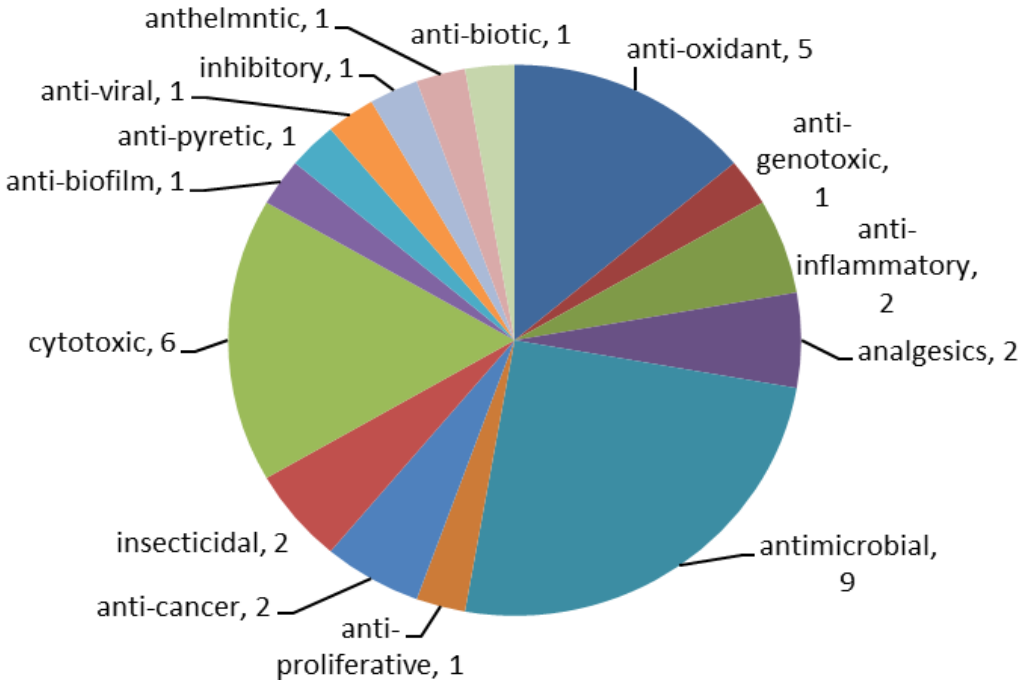


Figure 1 Compounds Biological Potential



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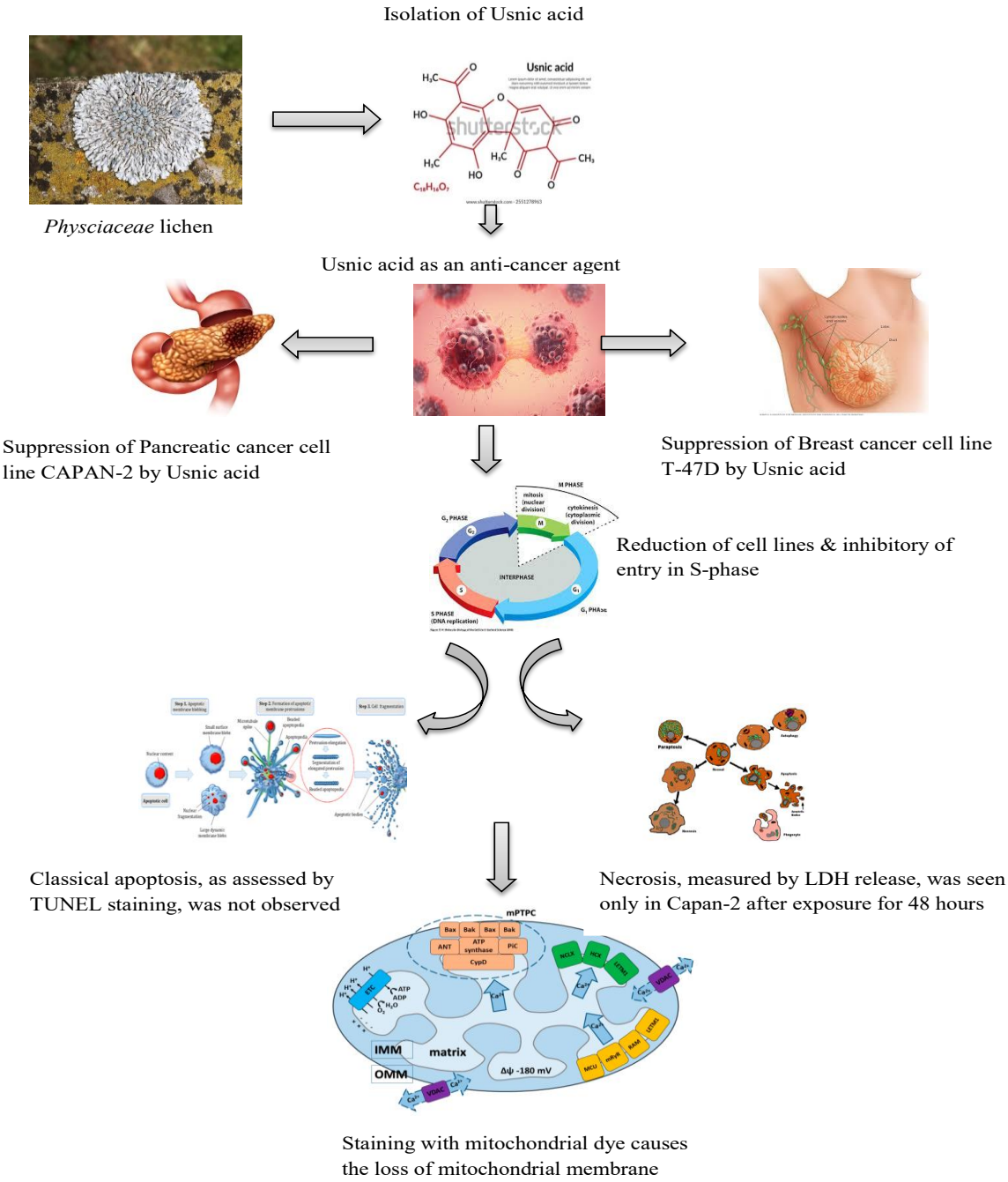

Figure 2 Cell growth inhibitory effect of Usnic acid on Pancreatic & Breast cancer cells

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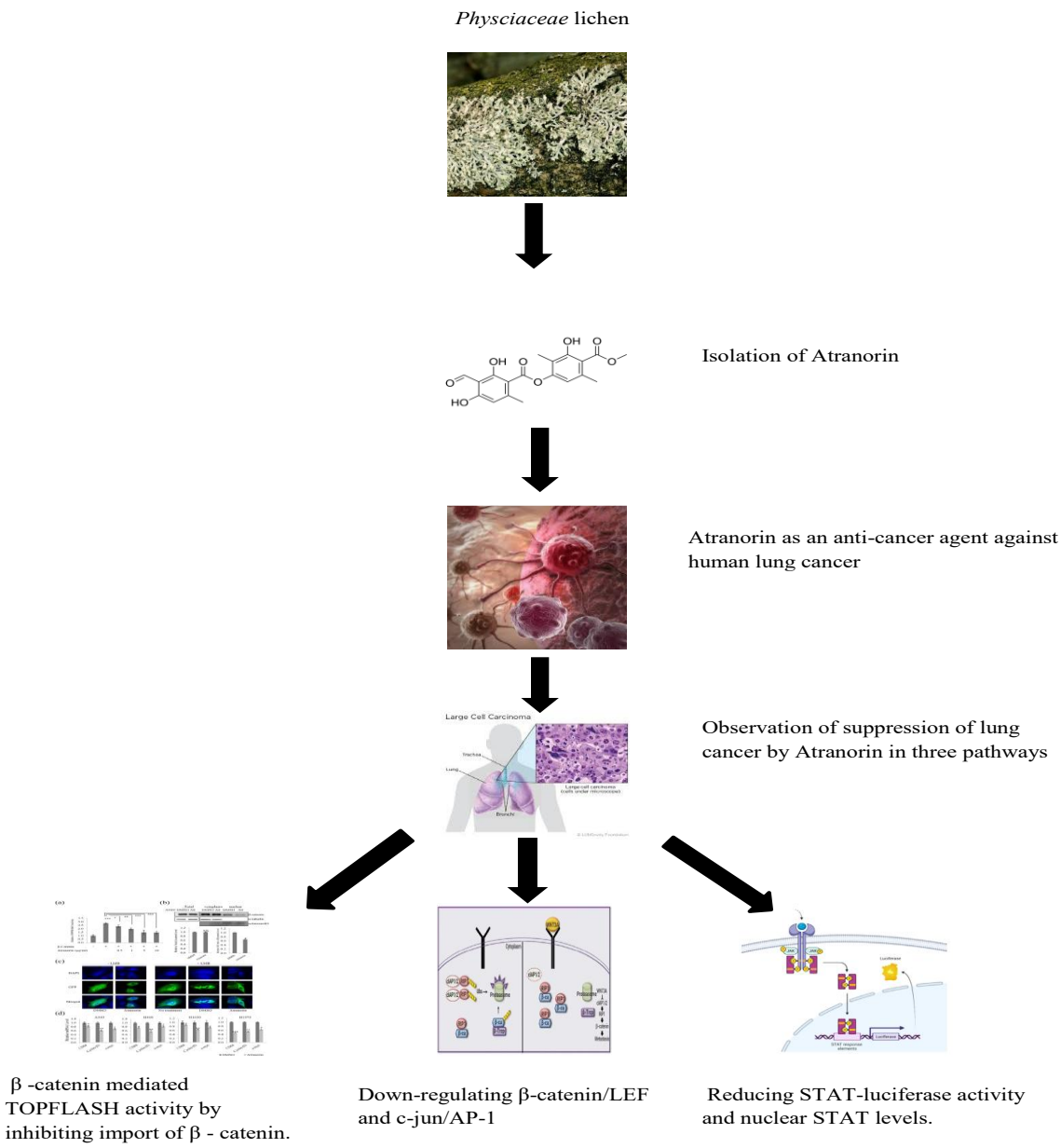

Figure 3 Atranorin inhibited lung cancer invasion and tumor growth in vitro and in vivo

DISCUSSION

The present review synthesized the available evidence on the biological activities, chemical analyses, and secondary metabolites of members of the Physciaceae family, providing an integrated perspective on their pharmacological potential. Findings indicated that antimicrobial and antioxidant activities remain the most extensively documented properties, with several species—most notably *Anaptychia ciliaris*, *Physcia vitti*, and *Tornabea scutellifera*—demonstrating broad-spectrum antimicrobial efficacy against clinically relevant bacteria, fungi, and mycobacteria. These results are consistent with previous literature describing the antimicrobial potential of lichen-derived metabolites, particularly depsides and depsidones, which have been repeatedly linked to strong antibacterial and

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antifungal effects (18,19). The reported inhibitory activity against multidrug-resistant pathogens such as *Staphylococcus aureus* and *Mycobacterium tuberculosis* reinforces the relevance of Physciaceae metabolites as potential scaffolds for novel antimicrobial drug development, an area of increasing urgency in light of global antimicrobial resistance. Antioxidant activity was also frequently reported, primarily assessed through radical scavenging assays including DPPH, ABTS, and FRAP, and was often correlated with phenolic content, such as atranorin, lecanoric acid, and flavonoids. These findings align with prior evidence suggesting that phenolic-rich lichens exhibit significant free-radical neutralising capacity, supporting potential nutraceutical or cosmeceutical applications in oxidative stress-related conditions (20-22). However, it was noted that Physciaceae species generally demonstrated slightly lower antioxidant activity compared with certain Parmeliaceae representatives, suggesting possible differences in metabolite concentration or composition that warrant further comparative chemical profiling (23).

Although less frequently investigated, anti-inflammatory, anticancer, enzyme inhibitory, and neuroprotective activities were also documented in selected species. Anti-inflammatory effects, demonstrated through inhibition of COX, LOX, and pro-inflammatory cytokines, were consistent with the ecological function of lichen metabolites in mitigating oxidative and inflammatory stress in extreme habitats. Anticancer activity, reported in in vitro studies using a range of human cancer cell lines, was associated with compounds such as atranorin, gyrophoric acid, and usnic acid (24,25). Mechanistic evidence pointed towards apoptosis induction, cell cycle arrest, and modulation of β-catenin signaling pathways. Enzyme inhibitory activities, particularly against α-glucosidase, tyrosinase, and cholinesterase, suggested potential therapeutic applications in metabolic disorders, skin hyperpigmentation, and neurodegenerative diseases. These observations are consistent with reports from other lichen families, where secondary metabolites have been shown to modulate key enzymatic targets (26,27). The chemical diversity observed within Physciaceae was substantial, with advanced analytical techniques confirming the presence of multiple bioactive classes, including depsides, depsidones, diphenyl ethers, anthraquinones, triterpenoids, aliphatic acids, and unique pigments. Importantly, chromatographic profiles revealed numerous unidentified compounds, indicating that the chemical space of this family remains underexplored. This untapped diversity represents a valuable reservoir for future bioprospecting, especially if paired with modern high-throughput metabolomics and structure elucidation techniques. The ecological roles of these metabolites—including UV protection, antimicrobial defense, and allelopathy—further support their adaptive significance and potential relevance in applied sciences (28,29).

A critical limitation emerging from this review was the uneven research focus within the family. Out of 36 principal members, only 16 have been examined in any significant detail, leaving the majority chemically and biologically uncharacterized. This research imbalance may be partly attributable to accessibility, abundance, or prior ethnopharmacological interest, but it inevitably constrains the generalizability of current findings. Additionally, significant methodological heterogeneity was identified across studies, encompassing differences in extraction solvents, bioassay protocols, microbial strains or cell lines tested, and statistical reporting. Such variation hampers direct comparison of results and may obscure true bioactivity trends. Another notable limitation was the frequent absence of mechanistic studies and in vivo validation, with most findings restricted to in vitro screening assays. Without robust pharmacokinetic, toxicological, and mechanistic data, translation into clinical or industrial applications remains premature. Strengths of this body of research include the recurrent identification of high-value bioactivities, often comparable to or exceeding reference compounds in standardized assays, and the consistent link between chemical composition and pharmacological outcomes. The ability of certain Physciaceae metabolites to demonstrate multiple bioactivities—such as atranorin's combined antimicrobial, antioxidant, anti-inflammatory, and anticancer properties—underscores their multifunctional potential. Furthermore, the ecological sensitivity of Physciaceae species to atmospheric pollutants positions them as both valuable bioindicators and vulnerable natural resources, highlighting the need for sustainable harvesting or cultivation strategies.

Future research should expand species coverage to include lesser-known genera within Physciaceae, apply standardized and reproducible extraction and assay methodologies, and incorporate advanced chemical profiling techniques such as UHPLC-HRMS/MS and NMR-based metabolomics (30-33). Integrating molecular biology approaches to elucidate the mechanisms of action, alongside in vivo pharmacological evaluations, will be essential to confirm therapeutic relevance. Exploration of synergistic effects between multiple lichen metabolites, as well as between lichen-derived and synthetic agents, may further enhance bioactivity. Environmental influence on metabolite production—such as altitude, substrate type, and seasonal variation—should be systematically examined to optimize conditions for bioactive compound yield. Finally, the biotechnological potential of Physciaceae could be advanced through in vitro culturing of mycobionts and lichen-associated microbiomes, allowing for controlled and sustainable metabolite production while reducing ecological impact. Overall, the current evidence affirms that Physciaceae represents a chemically rich and biologically versatile

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lichen family with considerable pharmacological promise. Nonetheless, realizing its full potential will require coordinated research efforts that bridge chemical ecology, pharmacognosy, and biotechnology while prioritizing both scientific innovation and conservation.

CONCLUSION

The present review concludes that the Physciaceae family constitutes a chemically diverse and biologically rich group of lichens with substantial potential in pharmaceutical, nutraceutical, and cosmeceutical applications. Their documented antimicrobial, antioxidant, anti-inflammatory and emerging anticancer properties underscore their value as promising sources of novel bioactive compounds. However, unlocking this potential requires addressing key challenges, including chemotypic variability, methodological inconsistencies in bioactivity evaluation, and the need for sustainable sourcing practices. Expanding research to underexplored genera and integrating advanced analytical, mechanistic, and biotechnological approaches will be essential for harnessing these benefits responsibly. By combining scientific innovation with conservation priorities, Physciaceae can be developed into a sustainable resource for novel therapeutics and high-value natural products while preserving their ecological roles as vital bioindicators.

AUTHOR CONTRIBUTION

Author	Contribution
Hira Riaz*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Qudsia Firdous	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published

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