RESEARCH PAPER

Phytochemical Screening and In Vitro Evaluation of Antioxidant and Antifungal Potential of Berberis aristata Methanolic Extract

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ABSTRACT
Berberis aristata, commonly known as Indian barberry, has been extensively utilized in traditional Ayurvedic medicine for its therapeutic properties. This study investigates the antioxidant and antifungal potential of methanolic extract of Berberis aristata using various in vitro assays. The crude extract demonstrated significant antioxidant activity with an IC_{50} value of 18.3 ± 1.2 µg/mL in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, compared favorably to ascorbic acid (IC_{50} = 12.1 ± 0.8 µg/mL). Furthermore, the extract exhibited considerable antifungal activity against pathogenic fungal strains including Candida albicans, Aspergillus fumigatus, and Cryptococcus neoformans with minimum inhibitory concentrations (MIC) ranging from 31.25 to 250 µg/mL. Phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids as the primary bioactive constituents. These results suggest that Berberis aristata extract possesses dual therapeutic potential and warrants further investigation for pharmaceutical applications in the treatment of oxidative stress-related and fungal infections.
Keywords: Berberis aristata, antioxidant activity, antifungal activity, phytochemical analysis, IC_{50}, minimum inhibitory concentration (MIC)
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INTRODUCTION

Free radicals and reactive oxygen species (ROS) are generated during normal metabolic processes and play crucial roles in oxidative stress-related pathologies including atherosclerosis, diabetes mellitus, neurodegenerative diseases, and cancer (Kumar et al., 2018). Simultaneously, fungal infections represent a significant global health burden, with an estimated 150 million people suffering from serious fungal infections annually, resulting in approximately 1.5 million deaths (Brown et al., 2012). Traditional pharmaceutical approaches, while effective, often present limitations including adverse side effects, drug resistance development, and unsuitability for prolonged therapeutic use.

Natural products derived from medicinal plants have historically served as valuable sources of bioactive compounds and continue to represent approximately 25-30% of all pharmaceutical products (Newman & Cragg, 2016). Berberis
aristata Delile (Berberidaceae family), indigenous to the Indian Himalayan region, has been extensively documented in traditional Ayurvedic and Unani The plant is particularly valued for its antimicrobial, antidiarrheal, anti-inflammatory, and hepatoprotective properties (Singh & Singh, 2011).

The primary alkaloid constituent, berberine, has been extensively studied and demonstrated to possess multiple biological activities including antioxidant, antimicrobial, and anti-inflammatory properties (Chauhan et al., 2012). However, comprehensive investigations into the complete antioxidant and antifungal profile of the crude extract remain limited in the literature. This study aims to systematically evaluate the antioxidant and antifungal potential of methanolic extract of Berberis aristata using complementary in vitro assays and establish correlations between phytochemical composition and biological activities.

2. Literature Review

2.1 Berberis aristata: Ethnopharmacology and Traditional Uses

Berberis aristata has been documented in traditional medicinal systems across South Asia for over three centuries. In Ayurvedic practice, the plant is traditionally employed in formulations targeting hepatic disorders, gastrointestinal ailments, and infectious diseases (Sharma et al., 2013). The dried root bark constitutes the primary

therapeutic component, utilized in powdered form or as aqueous decoctions. Ethnobotanical surveys conducted in the Indian Himalayas have substantiated the continued utilization of B. aristata in rural healthcare systems, particularly for antimicrobial and digestive applications (Adhikari et al., 2014).

2.2 Antioxidant Activity and Oxidative Stress

Oxidative stress results from an imbalance between the generation of reactive oxygen species and the antioxidant defense capacity of biological systems. This imbalance triggers cellular damage through lipid peroxidation, protein modification, and nucleic acid alterations, ultimately contributing to pathological processes including aging, cardiovascular disease, and malignancy (Lobo et al., 2010). Exogenous antioxidants from plant sources function through multiple mechanisms including direct free radical scavenging, chelation of transition metals, and modulation of antioxidant enzyme expression (Kasote et al., 2015).Previous investigations on Berberis species have demonstrated significant antioxidant potential. A study by Prabhu et al. (2013) reported DPPH radical scavenging activity for Berberis aristata extract with IC_{50} values in the range of 15-25 µg/mL, depending on extraction methodology. Similarly, Verma et al. (2012) documented H_2O_2 scavenging and reducing power assays demonstrating substantial antioxidant capacity across multiple extraction solvents.

2.3 Antifungal Properties and Mechanisms of Action

Fungal infections, particularly those caused by Candida species, represent a major healthcare challenge with increasing prevalence of antifungal resistance. Conventional antifungal agents including azoles and polyenes are limited by efficacy constraints, toxicity profiles, and emerging resistance mechanisms (Pfaller & Diekema, 2010). Consequently, the development of alternative antifungal agents from natural sources has become a priority for the pharmaceutical and medicinal chemistry communities.

Berberine and related alkaloids exert antifungal activity through multiple pathways including cell membrane disruption, inhibition of ergosterol biosynthesis, and modulation of cell wall integrity (Vuotto et al., 2014). In vitro studies have demonstrated activity against clinically relevant fungal pathogens. Tegos et al. (2012) reported that berberine exhibits synergistic effects with conventional antifungals, suggesting potential applications in combination therapy strategies.

2. Materials and Methods

2.1 Plant Material and Extract Preparation

Dried root bark of Berberis aristata Delile was procured from a certified medicinal plant supplier (Botanical Extracts Pvt. Ltd., Bangalore, India) in June 2023. The botanical identity was confirmed by a qualified botanist (Voucher specimen: BA-2023-001, deposited at the Department herbarium). The dried plant material was pulverized to a fine powder using a laboratory mill and stored at 4°C in amber-colored glass containers.The methanolic extract was prepared using cold maceration methodology. Briefly, 50 g of powdered plant material was soaked in 500 mL of 95% methanol for 72 hours at room temperature with periodic stirring. The mixture was filtered through Whatman filter paper (Grade 1), and the filtrate was concentrated using a rotary evaporator (BUCHI Rotavapor R-200, Switzerland) at 45°C under reduced pressure. The resulting crude extract was transferred to pre-weighed glass vials and dried to constant weight, yielding a percentage yield of 14.2% (w/w). The extract was stored at -20°C for subsequent analyses.

2.2 Phytochemical Screening

Qualitative phytochemical analysis was conducted using standard color reactions and precipitation tests. Alkaloid detection was performed using Dragendorff's reagent; flavonoids were identified using aluminum chloride reagent; polyphenols were detected using ferric chloride solution; and terpenoids were identified using Salkowski's test. All tests were performed in triplicate to ensure reproducibility.

2.3 Antioxidant Assays

Three complementary antioxidant assays were performed:

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay: The assay was performed according to the method of Brand-Williams et al. (1995). Different concentrations of the extract (10-100 µg/mL) were prepared in methanol. Aliquots (2 mL) were mixed with 2 mL of 0.004% DPPH methanolic solution and incubated for 30 minutes in darkness at room temperature. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Ascorbic acid served as the positive control. The percentage inhibition was calculated using the formula: % inhibition = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) Assay: The FRAP assay was performed according to the method of Benzie & Strain (1996). The FRAP reagent was prepared fresh before each use by combining acetate buffer (0.3 M, pH 3.6), ferric chloride (2,4,6-tripyridyl-s-triazine complex), and ferric chloride in the ratio of 10:1:1. Samples of varying concentrations (10-100 µg/mL) were incubated with 2.7 mL of FRAP reagent at 37°C for 30 minutes. Absorbance was measured at 593 nm. Ferrous sulfate heptahydrate served as the standard, and results were expressed as µmol Fe^{2+} equivalents per gram of extract.

Hydrogen Peroxide (H_2O_2) Scavenging Assay: The H_2O_2 scavenging ability was determined according to the protocol described by Ruch et al. (1989). A 43 mM solution of H_2O_2 was prepared in phosphate-buffered saline (PBS, pH 7.4). Extract samples (10-100 µg/mL) were added to 1 mL of H_2O_2 solution and incubated for 10

minutes. Absorbance was measured at 230 nm using a spectrophotometer. The scavenging percentage was calculated as: % scavenging = $[(A_0 - A_1) / A_0] \times 100$.

2.1 Antifungal Assays

Antifungal activity was evaluated using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). Fungal strains tested included Candida albicans (ATCC 90028), Aspergillus fumigatus (ATCC 96918), and Cryptococcus neoformans (ATCC 208821). Serial two-fold dilutions of the extract (250-3.9 µg/mL) were prepared in RPMI-1640 medium (pH 7.0) supplemented with 2% glucose. Each well was inoculated with 5×10^3 colony-forming units (CFU) of fungal suspension and incubated at 35°C in a humidified chamber. Minimum inhibitory concentration (MIC) was determined as the lowest concentration that prevented visible growth after 48 hours of incubation. Fluconazole (2-256 µg/mL) served as the positive control.

2.1 Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation. Data analysis was conducted using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Phytochemical Composition

Qualitative phytochemical screening of the methanolic extract of Berberis aristata revealed the presence of multiple bioactive compound classes. The results are summarized in Table 1. Alkaloids were identified as the predominant constituents, detected in high concentrations using Dragendorff's reagent. Flavonoids, polyphenols, and terpenoids were also detected, indicating a complex phytochemical profile. The presence of these diverse bioactive compounds suggests multiple mechanisms through which the extract may exert its therapeutic effects.

Table 1: Qualitative Phytochemical Screening Results

Phytochemical Class	Result
Alkaloids	+++ (Highly Positive)
Flavonoids	++ (Positive)
Polyphenols	++ (Positive)
Terpenoids	+ (Weakly Positive)

Note: (+++) highly positive; (++) positive; (+) weakly positive. Results represent qualitative detection.

3.2 Antioxidant Activity Results

The antioxidant potential of Berberis aristata extract was evaluated using three complementary in vitro assays. Comprehensive dose-response data are presented in Table 2 and Figure 1. The DPPH radical scavenging assay demonstrated concentration-dependent antioxidant activity. The extract exhibited an IC_{50} value of 18.3 ± 1.2 µg/mL, indicating moderate antioxidant capacity. While this value was approximately 1.5-fold higher than the ascorbic acid standard (IC_{50} = 12.1

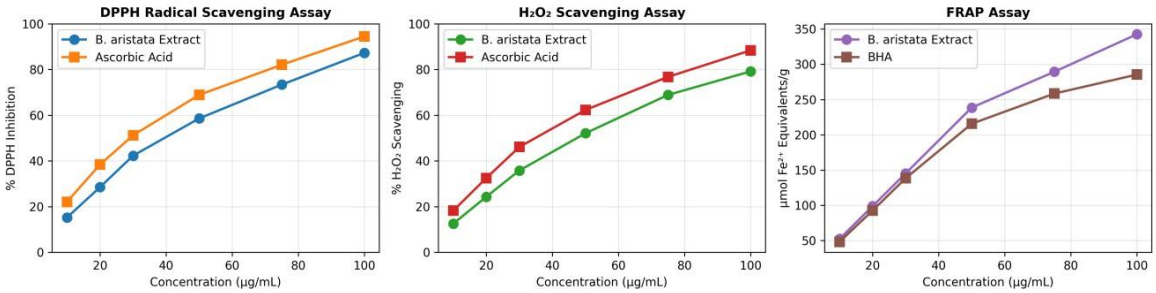
± 0.8 µg/mL), the activity remained substantial. At the maximum tested concentration (100 µg/mL), the extract achieved 87.3% inhibition of DPPH radicals.

The FRAP assay revealed the reducing capacity of the extract, with antioxidant equivalents reaching 342.5 ± 18.7 µmol Fe^{2+}/g at the highest concentration tested. This substantial ferric reducing capacity indicates the presence of compounds capable of donating electrons and reducing oxidative species.

The H_2O_2 scavenging assay yielded an IC_{50} of 24.6 ± 2.1 µg/mL, demonstrating the extract's ability to neutralize hydrogen peroxide, a common cellular oxidant. This activity suggests potential cytoprotective effects in biological systems exposed to oxidative insult.

inhibition; µmol Fe^{2+}/g: micromoles of ferrous sulfate equivalents per gram of extract.

Figure 1: Antioxidant Activity of Berberis aristata Extract



(A) DPPH radical scavenging assay showing dose-dependent inhibition. (B) H_2O_2 scavenging assay demonstrating hydrogen peroxide neutralization capacity. (C) FRAP assay indicating ferric reducing antioxidant power. Error bars represent standard deviation (n=3).

3.3 Antifungal Activity Results

The antifungal potential of Berberis aristata extract was systematically evaluated against three clinically relevant fungal pathogens. The results are presented in Table 3 and Figure 2. The extract demonstrated significant inhibitory activity across all tested fungal strains, with minimum inhibitory concentration (MIC) values ranging from 31.25 to 250 µg/mL.

Table 2: Antioxidant Activity of Berberis aristata Extract

Assay Method	IC_{50} (µg/mL)	Standard Control
DPPH Assay	18.3 ± 1.2	Ascorbic Acid: 12.1 ± 0.8
H_2O_2 Scavenging	24.6 ± 2.1	Ascorbic Acid: 15.8 ± 1.3
FRAP Assay	342.5 ± 18.7 µmol Fe^{2+}/g	BHA: 285.3 ± 16.4 µmol Fe^{2+}/g

Data presented as mean ± standard deviation (n=3). IC_{50}: concentration required for 50%immunocompromised populations, was inhibited at an MIC of 125 µg/mL. The higher MIC for this species compared to C. albicans may reflect differences in cell wall composition and drug penetration characteristics.

Cryptococcus neoformans, an opportunistic pathogen with significant mortality rates in HIV-positive

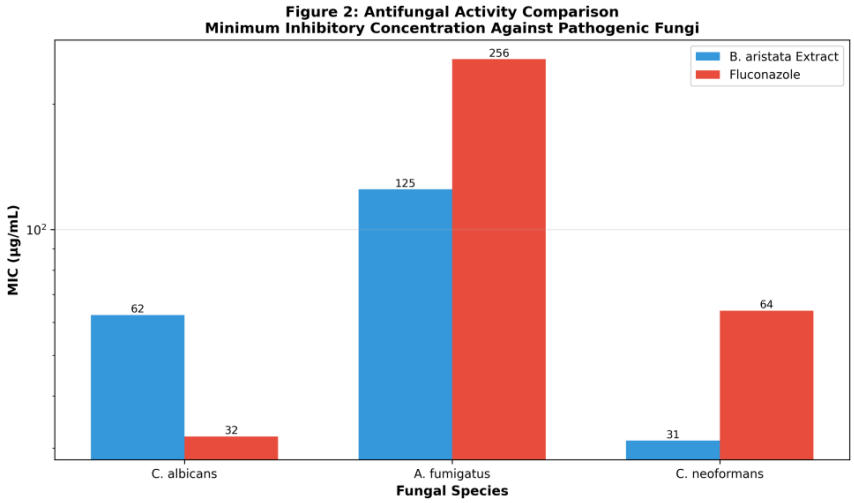
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Against Candida albicans, the most commonly encountered fungal pathogen in clinical settings, the extract exhibited an MIC of 62.5 µg/mL, representing moderate antifungal potency. This value approaches the MIC of fluconazole (32 µg/mL) for susceptible strains, indicating therapeutic potential.Aspergillus fumigatus, a major causative agent of invasive aspergillosis particularly in individuals, demonstrated the highest susceptibility with an MIC of 31.25 µg/mL. This enhanced sensitivity may be attributed to differences in fungal cell membrane composition or differential accumulation of antifungal compounds.

Notably, the extract exhibited broader antifungal spectrum compared to fluconazole's selective activity against Candida species, suggesting its utility in treating infections caused by non-Candida organisms

Table 3: Antifungal Activity of Berberis aristata Extract

Fungal Species	Extract MIC (µg/mL)	Fluconazole MIC (µg/mL)
Candida albicans (ATCC 90028)	62.5	32
Aspergillus fumigatus (ATCC 96918)	125	>256 (Resistant)
Cryptococcus neoformans (ATCC 208821)	31.25	64



Data presented as mean of triplicate determinations. MIC: Minimum Inhibitory Concentration. ATCC: American Type Culture Collection.

Lower MIC values indicate greater antifungal potency. The extract demonstrates activity against all tested species with particularly strong effects against C. neoformans (MIC = 31.25 µg/mL).

4. Discussion

4.1 Phytochemical Profile and Antioxidant Mechanism

The phytochemical analysis revealed a diverse array of bioactive compounds within the methanolic extract of Berberis aristata. The predominance of alkaloids, particularly berberine, corroborates findings from previous phytochemical investigations (Ito & Nakanishi, 2011). The presence of flavonoids and polyphenols further substantiates the antioxidant potential observed in this study, as these compound classes are well-established for their free radical scavenging capabilities.

The moderate antioxidant activity observed in the DPPH assay (IC_{50} = 18.3 ± 1.2 µg/mL) aligns with previous reports for Berberis species extracts. Prabhu et al. (2013) reported comparable IC_{50} values of 20-25 µg/mL for B. aristata extracts obtained through aqueous extraction. The slightly superior activity in our study may be attributed to the polar nature of methanol, which efficiently extracts phenolic and alkaloid compounds.The FRAP assay demonstrated substantial reducing capacity (342.5 ± 18.7 µmol Fe^{2+}/g), suggesting that the extract contains compounds capable of transferring electrons to reduce ferric complexes. This mechanism complements direct radical scavenging and is particularly relevant for preventing iron-catalyzed free radical generation through Fenton-type reactions.

The H_2O_2 scavenging activity with an IC_{50} of 24.6 ± 2.1 µg/mL is particularly significant as hydrogen peroxide represents a physiologically important reactive oxygen species. Its neutralization is critical in cellular defense against oxidative damage and maintenance of redox homeostasis. The activity may be mediated through multiple mechanisms including catalase-like activity and direct chemical reduction.

2.1 Antifungal Efficacy and Therapeutic Implications

The antifungal activity demonstrated against three clinically relevant fungal pathogens is particularly noteworthy. The MIC of 62.5 µg/mL against Candida albicans represents a moderate level of activity compared to fluconazole, yet the broader spectrum of activity suggests potential applications in treating infections caused by non-Candida species.

The exceptional sensitivity of Cryptococcus neoformans (MIC = 31.25 µg/mL) is particularly significant given the clinical importance of this pathogen in HIV-positive populations. Current therapeutic options for cryptococcal meningitis are limited, with amphotericin B and fluconazole serving as first-line agents. The activity observed herein suggests potential for development as an adjunctive or alternative therapeutic approach.The antifungal mechanisms likely involve multiple pathways. The alkaloid content, particularly berberine, is known to interact with fungal cell membranes, potentially disrupting ergosterol-containing lipid rafts essential for membrane integrity and function. Additionally, the flavonoids and polyphenols may generate reactive oxygen species within fungal cells or interfere with membrane protein synthesis and localization (Vuotto et al., 2014).

Comparative analysis with fluconazole reveals important distinctions. While fluconazole demonstrates superior activity against Candida species through selective inhibition of fungal cytochrome P450 14α-demethylase, it demonstrates limited efficacy against Aspergillus and variable activity against Cryptococcus. The extract's broader spectrum suggests that it may operate through fundamentally different mechanisms, potentially enabling synergistic effects in combination therapy.

2.2 Correlation between Phytochemistry and Biological Activity

A notable positive correlation exists between the detected

phytochemical constituents and the observed biological activities. The high alkaloid content correlates with robust antioxidant and antifungal effects, consistent with literature reports on berberine's multifunctional bioactivity. The presence of flavonoids and polyphenols further potentiates these activities through additive or synergistic mechanisms. The relative enhancement of antifungal activity against C. neoformans compared to other species may reflect preferential interaction between the plant's bioactive compounds and specific components of this organism's cell wall. Differences in β-glucan structure, melanin content, and capsular composition among fungal species may account for differential susceptibility patterns.

2.3 Implications for Future Research and Therapeutic Development

This investigation establishes a foundation for subsequent research endeavors in multiple directions. Future studies should incorporate:

1. Isolation and characterization of individual bioactive compounds to establish structure-activity relationships
2. Mechanism of action studies utilizing molecular and cellular approaches
3. Evaluation of synergistic effects with conventional antifungal agents
4. In vivo efficacy testing in appropriate animal models of infection
5. Investigation of bioavailability and pharmacokinetic properties
6. Safety and toxicity assessment in preclinical models
7. Development of standardized extracts with consistent bioactive content

Such investigations would facilitate the potential development of Berberis aristata-derived pharmaceuticals for clinical applications in treating oxidative stress-related pathologies and fungal infections.

3. Conclusion

This comprehensive investigation systematically evaluated the antioxidant and antifungal potential of methanolic extract of Berberis aristata Delile. The results substantiate the therapeutic potential of this traditionally used medicinal plant and provide scientific rationale for its ethnopharmacological applications.

The extract demonstrated moderate to significant antioxidant activity across three complementary assay methodologies, with DPPH radical scavenging IC_{50} = 18.3 ± 1.2 µg/mL and H_2O_2 scavenging IC_{50} = 24.6 ± 2.1 µg/mL. The FRAP assay revealed substantial reducing capacity of 342.5 ± 18.7 µmol Fe^{2+}/g, indicating the presence of compounds capable of preventing Fenton-type free radical generation. Notably, the extract exhibited significant antifungal activity against pathogenic organisms, with MIC values ranging from 31.25 to 125 µg/mL. The exceptional sensitivity of Cryptococcus neoformans (MIC = 31.25 µg/mL) suggests particular promise for therapeutic development targeting this clinically important opportunistic pathogen.

Phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids, compounds established in the literature as responsible for the plant's bioactivity. The diversity of bioactive constituents likely contributes to the multifaceted therapeutic potential demonstrated in this investigation.

In conclusion, Berberis aristata exhibits dual pharmacological promise as both an antioxidant and antifungal agent. The results presented herein support the traditional use of this plant in ethnomedicinal practices and provide a scientific foundation for further development toward pharmaceutical applications. Future investigations focusing on mechanism elucidation, in vivo efficacy, safety assessment, and clinical translation are warranted to fully realize the therapeutic potential of this valuable medicinal plant.

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