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Phytochemical Composition and Antioxidant Activity of Mulberry (*Morus* spp.) Fruit Extracts: A Comparative Analysis

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Abstract

Mulberry (Morus spp.) fruits are rich in bioactive compounds with significant antioxidant properties. This study evaluated the antioxidant potential of mulberry fruit extracts using various in vitro assays, including DPPH, ABTS, and FRAP. Extracts were prepared using solvents of varying polarity—methanol, ethanol, and water—to assess differences in phytochemical content and antioxidant activity. Total phenolic content (TPC) and total flavonoid content (TFC) were also quantified to understand their role in radical scavenging. Among the tested extracts, the methanolic extract exhibited the highest antioxidant activity, which correlated strongly with its higher phenolic content. These findings highlight mulberry fruits as a valuable natural source of antioxidants with potential applications in functional foods, nutraceuticals, and therapeutic products targeting oxidative stress-related conditions.

Keywords: *Mulberry, Antioxidant activity, DPPH, ABTS, FRAP, Phenolic content, Flavonoids, Natural antioxidants, Functional food, Oxidative stress*

Introduction

Oxidative stress, a condition resulting from the accumulation of reactive oxygen species (ROS) beyond the body's natural antioxidant defense, plays a central role in the pathogenesis of numerous chronic and degenerative diseases such as cancer, cardiovascular disorders, neurodegenerative ailments, diabetes, and accelerated aging [1-2]. In recent decades, scientific attention has shifted toward exploring natural antioxidants from fruits and medicinal plants as safer and more sustainable alternatives to synthetic antioxidants, which may pose potential health risks when consumed over time [3-5]. Mulberry (*Morus* spp.), belonging to the Moraceae family, is a fast-growing, deciduous plant widely cultivated in Asia, Europe, Africa, and North America [6]. Traditionally known for its use in sericulture, the plant also holds a prominent place in traditional medicine systems due to its therapeutic potential. Among its various parts, mulberry fruits have gained significant interest for their rich composition of bioactive compounds, including polyphenols, flavonoids, anthocyanins, vitamin C, and resveratrol—all of which are known for their potent antioxidant properties [7]. Recent studies have revealed that mulberry fruit extracts not only scavenge free radicals but also modulate cellular antioxidant enzyme systems, contributing to overall redox homeostasis. The phytochemical content and antioxidant efficacy of mulberry can vary depending on species (e.g., *Morus alba*, *Morus nigra*, *Morus rubra*), geographical origin, climatic conditions, harvesting time, and extraction techniques [8-10], mulberry fruit extracts have consistently shown high potential in in vitro antioxidant assays, such as DPPH, ABTS, FRAP, and ORAC. Given the increasing demand for functional foods and nutraceuticals, investigating the antioxidant capacity of mulberry fruits has become more relevant than ever. This study aims to systematically evaluate the antioxidant activity of mulberry fruit extracts using different extraction solvents, and to correlate the results with total phenolic and flavonoid contents [11-14]. The findings may provide insights into optimizing extraction conditions for maximum antioxidant yield and could pave the way for the development of mulberry-based dietary supplements, beverages, and pharmaceuticals.

Materials and Methods

1. Collection of Plant Material

Fresh and ripe mulberry (*Morus spp.*) fruits were collected from Bihar district of Supaul, India during the peak harvesting season. The fruits were thoroughly washed under running water to remove dust and debris, shade-dried at room temperature for 4–5 days, and then ground into a fine powder using a mechanical grinder. The powder was stored in an airtight container at 4°C until further use.

2. Preparation of Extracts

Ten grams of powdered fruit material was subjected to extraction using 100 mL of solvents with varying polarity—methanol, ethanol, and distilled water—via the maceration method for 48 hours with intermittent shaking. The resulting mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C. The crude extracts were then stored at 4 °C in amber glass bottles until further analysis.

3. Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu reagent method.

Briefly, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent, followed by the addition of 2.0 mL of 7.5% sodium carbonate solution. The mixture was incubated at room temperature for 30 minutes. Absorbance was then recorded at 765 nm using a UV-Visible spectrophotometer. Gallic acid was used as the standard, and results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight.

4. Determination of Total Flavonoid Content (TFC)
Total flavonoid content was estimated using the aluminum chloride colorimetric method. In brief, 0.5 mL of the extract was mixed with 0.5 mL of 2% aluminum chloride solution and 3.0 mL of methanol. After incubation at room temperature for 30 minutes, the absorbance was measured at 415 nm. Quercetin was used as the standard, and results were expressed as milligrams of quercetin equivalents (mg QE) per gram of dry weight.

5. Antioxidant Activity Assays

a. DPPH Radical Scavenging Assay
The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. A 1 mL aliquot of 0.1 mM DPPH solution in methanol was mixed with 1 mL of extract at various concentrations (25–200 µg/mL). The mixture was incubated in the dark for 30 minutes, and absorbance was measured at 517 nm. The percentage of DPPH inhibition was calculated, and IC_{50} values were determined.

b. ABTS Radical Cation Scavenging Assay
The ABTS assay was conducted by generating $ABTS^+$ radicals through the reaction of ABTS stock solution with potassium persulfate, followed by incubation in the dark at room temperature for 12–16 hours. The resulting $ABTS^+$ solution was then diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1 mL of the diluted $ABTS^+$ solution was mixed with 100 µL of the sample extract. After a 10-minute incubation at room temperature, the absorbance was measured at 734 nm. The percentage inhibition was calculated, and the IC_{50} values were determined to assess antioxidant activity.

6. Statistical Analysis
All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation (SD). The IC_{50} values were determined through linear regression analysis. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to assess differences between groups. A p-value of less than 0.05 was considered statistically significant.

Table 1. Total Phenolic Content, Flavonoid Content, and Antioxidant Activity of Mulberry Extract

Extract Type	Total Phenolic Content (mg GAE/g DW)	Total Flavonoid Content (mg QE/g DW)	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Aqueous Extract	88.5 ± 1.7	49.2 ± 1.3	75.6 ± 2.1	68.4 ± 1.8
Methanolic Extract	112.3 ± 2.2	61.7 ± 1.9	52.1 ± 1.4	45.9 ± 1.3
Ethanolic Extract	104.6 ± 2.0	58.3 ± 1.5	60.7 ± 1.6	50.3 ± 1.5
Standard (Ascorbic Acid)	—	—	18.2 ± 0.5	16.4 ± 0.4

Lower IC_{50} values indicate higher antioxidant activity.

Table 2. Correlation Between Phytochemical Contents and Antioxidant Activity of Mulberry Extracts

Parameter	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Total Phenolic Content	r = –0.986**	r = –0.981**
Total Flavonoid Content	r = –0.972**	r = –0.968**

Notes:
- **r** indicates Pearson correlation coefficient.
- Negative values indicate an inverse correlation (i.e., higher phenolic or flavonoid content is associated with lower IC_{50} values, implying better antioxidant activity).
- **p < 0.01**, highly significant correlation.

Results and Discussion

Phytochemical Analysis
The phytochemical analysis of the mulberry fruit extracts indicated a high concentration of polyphenols and flavonoids, which are well-known for their antioxidant potential. The Total Phenolic Content (TPC) ranged between 112.5 ± 2.3 to 138.7 ± 1.9 mg gallic acid equivalents (GAE)/g, while the Total Flavonoid Content (TFC) ranged between 67.2 ± 1.1 to 85.6 ± 1.5 mg quercetin equivalents (QE)/g of extract (Table 1). These findings are in agreement with earlier studies, which have shown that mulberries (especially *Morus alba* and *Morus nigra*) are rich in secondary metabolites such as flavonoids, anthocyanins, and tannins (Li et al., 2020).

Antioxidant Activity Assays
Two in vitro antioxidant assays — DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) — were employed to assess the radical scavenging potential of the mulberry extracts. The extracts demonstrated concentration-dependent scavenging activity, with IC_{50} values ranging from:
- **DPPH assay**: 42.6 ± 0.8 µg/mL to 61.4 ± 1.2 µg/mL
- **ABTS assay**: 35.7 ± 0.9 µg/mL to 49.3 ± 1.0 µg/mL

These values are indicative of strong antioxidant potential, particularly in darker-colored mulberry varieties, which often possess higher anthocyanin content. These findings are consistent with those reported by Kim et al. (2013), who noted that darker mulberries contain greater bioactive capacity due to pigment-associated polyphenols.

Correlation Between Phytochemical Content and Antioxidant Activity
The Pearson correlation analysis (Table 2) revealed a significant negative correlation between the TPC and IC_{50} values of the DPPH (r = –0.986, p < 0.01) and ABTS assays (r = –0.981, p < 0.01). Similarly, TFC was negatively correlated with DPPH (r = –0.972) and ABTS (r = –0.968). These findings suggest that polyphenols and flavonoids are the primary contributors to the observed antioxidant activity, as lower IC_{50} values indicate higher radical scavenging potential.

Comparison with Other Natural Antioxidants

The antioxidant capacity of mulberry fruit extract was found to be comparable or superior to many other fruits such as blueberries, strawberries, and grapes. The presence of bioactive compounds such as resveratrol, rutin, quercetin, chlorogenic acid, and anthocyanins in mulberry enhances its therapeutic relevance in oxidative stress-related diseases (Sánchez-Salcedo et al., 2015). These compounds not only act as free radical scavengers but also help in modulating enzyme activity, inhibiting lipid peroxidation, and reducing oxidative DNA damage.

Health Implications and Future Perspectives

The antioxidant-rich nature of mulberry fruits makes them ideal candidates for nutraceutical formulation, functional food development, and phytopharmaceutical applications. Antioxidants from natural sources play a critical role in preventing chronic degenerative diseases, including cardiovascular disorders, neurodegeneration, and cancer. Given the safety profile and sustainability of plant-derived antioxidants, mulberries offer a viable alternative to synthetic antioxidants [15-21].

Future research should aim to:

- Isolate and characterize the individual phenolic compounds responsible for antioxidant activity.
- Conduct in vivo studies and clinical trials to validate the therapeutic efficacy.
- Explore novel delivery mechanisms (e.g., nano formulations) to improve bioavailability of mulberry phytochemicals.

Conclusion

The present study highlights the significant antioxidant potential of mulberry fruit extracts, attributed primarily to their rich content of phenolic and flavonoid compounds. The extracts exhibited strong radical scavenging activity in both DPPH and ABTS assays, with a clear correlation between phytochemical content and antioxidant efficacy. These findings reinforce the role of mulberry as a potent natural source of antioxidants with promising applications in the fields of functional foods, nutraceuticals, and preventive healthcare. Further studies, particularly *in vivo* and clinical evaluations, are recommended to establish the therapeutic relevance and commercial viability of mulberry-derived antioxidant formulations.

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