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INVESTIGATION OF FUNCTIONAL AND STRUCTURAL ATTRIBUTES OF MULBERRY LEAVES AND THEIR RELEVANCE TO NUTRACEUTICAL AND INDUSTRIAL APPLICATIONS

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Abstract
This study explores the functional and structural properties of white Morus alba and blackMorus nigra mulberry leaves to assess their potential applications in nutraceutical and industrial fields. Leaves phenolics were extracted using conventional and ultrasonic-assisted methanolic methods and analyzed for total phenolics, flavonoids, anthocyanins, carotenoids, and antioxidant activity (DPPH assay). Results showed that black mulberry exhibited significantly higher antioxidant activity (IC_{50} = 109.55 µg/mL) than white mulberry (IC_{50} = 211.82 µg/mL), corresponding to its greater total phenolic (861.23 mg/100 g DM) and flavonoid contents (621.17 mg/100 g DM). HPLC analysis indicated elevated quercetin, rutin, catechin, and caffeic acid in black mulberry, while white mulberry was richer in chlorogenic and gallic acids. SEM revealed a more porous structure in black mulberry, favoring solvent penetration and compound extraction, and FTIR confirmed the presence of hydroxyl and carbonyl functional groups associated with phenolic compounds. Overall, black mulberry demonstrated superior phytochemical and antioxidant potential, emphasizing its promising role in developing nutraceutical and sustainable bio-based products.
Keywords: Antioxidant Activity, Morus Alba, SEM, FTIR

1. INTRODUCTION

Mulberry (*Morus spp*. L), a member of the Moraceae family, is a versatile plant with significant economic, nutritional, and ecological importance across Asia and other parts of the world. Traditionally cultivated for sericulture, mulberry leaves serve as the exclusive food source for the silkworm (Bombyx mori), directly influencing silk yield and quality (Tuigong et al., 2015). Beyond its vital role in the silk industry, recent research has highlighted mulberry leaves as a rich source of functional biomolecules, including flavonoids, phenolic acids, alkaloids, and the unique antidiabetic compound 1-deoxynojirimycin (1-DNJ), which exhibits strong inhibitory effects on carbohydrate-digesting enzymes (Sarkhel et al., 2020). These bioactive compounds contribute to the plant's demonstrated antioxidant, anti-inflammatory, antidiabetic, and lipid-lowering properties, making it a promising candidate for nutraceutical and pharmaceutical applications (Jan et al., 2021). Studies further reveal that mulberry leaves are a sustainable source of essential minerals such as iron, zinc, and calcium, vital for human nutrition and suitable for use in functional food fortification (Huria & Saraf, 2024).

In addition to their biochemical richness, the structural and physiological features of mulberry leaves play an essential role in determining their functional and industrial potential. Variations in leaf morphology, protein content, and phenolic composition among mulberry species have been linked to differences in antioxidant capacity and digestibility, factors that directly influence both sericulture efficiency and food processing suitability (Ugulin et al., 2015). Recent advances have demonstrated that environmental conditions such as light spectra and growth media can significantly alter the accumulation of nutraceutical compounds in leaves, enhancing their functional quality (Win et al., 2022). Moreover, mulberry leaf proteins have gained recognition as an emerging source of sustainable plant protein with excellent functional and nutritional attributes suitable for large-scale food applications (Xue & Chen, 2025). This growing body of evidence positions mulberry leaves as a multipurpose bioresource whose structural and functional properties have wide-ranging implications for nutrition, health, textile production, and sustainable agricultural systems.

Mulberry leaves possess an extensive range of pharmacological activities that make them valuable for therapeutic and health-promoting applications. Rich in phenolic acids, flavonoids, and alkaloids, they exhibit strong antioxidant, anti-inflammatory, antidiabetic, and lipid-lowering effects, which contribute to their efficacy in preventing metabolic and cardiovascular disorders (Jan et al., 2021). In addition, bioactive compounds such as oxyresveratrol and gallic acid have demonstrated immunomodulatory effects, reducing pro-inflammatory cytokines and enhancing antioxidant

defense mechanisms (Abbas et al., 2024). The pharmacological diversity of mulberry is further supported by studies on Morus nigra, which exhibit antimicrobial, antidiabetic, and anticancer activities, highlighting the genus's therapeutic potential (Lim & Choi, 2019). Furthermore, historical and contemporary research confirms that various parts of Morus alba, including its leaves, bark, and fruits, have been utilized in traditional medicine for treating ailments such as diabetes, hypertension, and hepatic dysfunction, supporting its recognition as a multifunctional medicinal plant (Kadam et al., 2019).

In the industrial and agricultural context, mulberry plays an irreplaceable role in the sericulture sector as the sole food source for Bombyx mori, the silkworm responsible for silk production. The nutritional composition of mulberry leaves, including high protein and mineral content, directly determines silk yield and quality (Tuigong et al., 2015). Recent advances in cultivation techniques, such as the development of high-yielding, stress-tolerant cultivars and the application of biotechnology, have significantly enhanced leaf quality and resilience, ensuring sustainable silk production (Sarkar et al., 2017). Beyond silk, mulberry cultivation has broader environmental significance. Its extensive root system prevents soil erosion, rehabilitates degraded lands, and supports water retention, making it a strategic species for soil and ecosystem conservation (Wani et al., 2017). Moreover, mulberry's adaptability to varied climatic conditions and tolerance to salinity and drought position it as a suitable crop for sustainable agriculture and biodiversity preservation (Rahman & Islam, 2021). Recent innovations, such as integrating mulberry cultivation into controlled environments like hydroponic and aeroponic systems, further highlight its potential contribution to sustainable food systems (Baciu et al., 2023).

In conclusion, the functional and structural versatility of mulberry leaves underscores their vast potential across pharmacological, industrial, and environmental domains. Their bioactive compounds support diverse health benefits, while their agronomic adaptability and role in sericulture contribute to economic and ecological sustainability. Integrating biochemical, structural, and environmental insights into mulberry leaf research offers a promising pathway for developing new nutraceuticals, sustainable farming models, and bio-based industrial applications. Consequently, understanding the interrelationship between the functional and structural attributes of mulberry leaves and their end-use potential is vital for maximizing their contribution to global health, industry, and sustainability.

MATERIALS AND METHODS

The present study was carried out at the Department of Food Science, Government College University, Faisalabad. Moreover, extraction procedures, including conventional solvent extraction, ultrasound-assisted extraction were carried out at the Innovative technologies lab Department of Food science, Government College University, Faisalabad (GCUF). Nutritional profiling of the extracts was performed at GCUF, along with Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and High-Performance Liquid Chromatography (HPLC) analyses conducted at the Hi-Tech Laboratory, GCUF.

2.1 Raw Material

Black and white mulberries were collected at the commercially ripe stage (April- May, 2024) in the campus area of Government college University Faisalabad. The leaves are washed with distilled water and then dried in a hot air oven at 55 °C for 7-9 h. The dried material was ground into powder with a blender, sieved through an 80-mesh sieve and stored in an air-tight container at 4 °C until use. All chemicals were supplied by Sigma Aldrich, Germany.

2.2 Extraction Methods

2.2.1 Conventional method

The 70 grams of distilled both black and white mulberry were dissolved in 300 ml of methanol via continues string on the hot magnetic plate for about 45 minutes at 800 rpm and at temperature. The resulting solution were then allowed to cool down and then centrifuged at 4500 rpm for 10 minutes. The residue was discarded and the supernatant were then collected and kept at 4 ℃ until future analysis.

2.2.2 Ultrasonic Extraction

The ultrasonic prob enter into the mixture containing same concentration as used for conventional method (70 ml of powder in 300 ml of methanol) and the solution were sonicated at amplitude 45 for 25 minutes. The mixture then allowed to cool down and then centrifuged at 4500 rpm for 10 minutes. The residue was discarded and the supernatant were then collected and kept at 4 ℃ until future analysis.

2.3 Estimation of Total Phenolic Constituents

Total phenolic content of extracts was determined using the Folin & Ciocalteu phenol reagent method (Ajatta et al., 2016). One hundred microliters of the mulberry leaf extract was mixed with 1.7 mL of Folin & Ciocalteu reagent and after 3 min with 1.2 mL of 2% aqueous sodium carbonate solution. Spectrophotometric reading (760 nm) was taken after 15 min of incubation at room temperature performed with shaking. The calibration curve was obtained with gallic acid, and the results were expressed as equivalents micrograms of gallic acid per milliliter of black mulberry extracts.

2.4 Estimation of total flavonoids

The total flavonoid content was determined according to the aluminium chloride ($AlCl_3$) colorimetric method described by (add citation). Rutin was used to make a calibration curve. Briefly, aliquots of 100 µl of extract were

completed with distilled water to 1 ml, followed by 1 ml of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 15 min in the dark, the absorbance of the reaction mixture was measured at 430 nm.

2.5 Estimation of total anthocyanins

1 ml of leaf extracts were mixed with 12 ml of 1% (w/v) HCl in methanol for 2 days at 3-5 °C with continuous shaking. The extracts are filtered and centrifuged at 9000 g. The assay was carried out in triplicate. The samples absorbance was measured at 530 and 657 nm and anthocyanin concentrations are calculated using the following equation:

$A = A_{530\ nm} - (A_{657\ nm}/3)$.

2.6 Estimation of total carotenoids

1ml of extracts were mixed in 80% acetone, centrifuged at 2500 g for 5 min and then the supernatant was filtered with Whatman paper no. 1. The samples absorbance was measured at 470, 663 and 647 nm and carotenoids concentrations are calculated using the following equation: carotenoid concentrations (µg/100 g) = $5 \times DO_{470\ nm} + 2{,}846 \times DO_{663\ nm} - 14{,}876 \times DO_{647\ nm}$.

2.7 DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined (21). One hundred microliters of leaf extract were mixed into 1.9 mL of 0.025 g L-1 DPPH methanol solution in disposable spectrophotometer cuvettes. Remaining purple color was measured by using a spectrophotometer at 520 nm after 15 min of incubation in darkness. Radical scavenging power (RSP) was calculated by the following equation:

$RSP = (1 - A_{s:15}/A_{s:15}) \times 100$

Where is absorbance of the sample and is absorbance of the blank at 15 min. The kinetic behavior of the mulberry extracts and DPPH mixture was also observed using a spectrophotometer. For this purpose, the same amount of mulberry extract and DPPH solution (as described above) were mixed in quartz cuvettes and absorbance changes were monitored at 30 s intervals at 520 nm for 20 min during which the reaction almost reached a plateau.

2.8 Reducing power

The assay was performed according to the method described by (add citation). Mulberries leaves extracts were diluted to 1 mL with distilled water and 2.5 mL of the 0.2 M phosphate buffer (pH: 6.6) and 2.5 mL of 1% potassium ferricyanide solution were added and vortexed. The mixtures were left to incubate at 50 °C for 20 min in a water bath. The tubes were cooled to room temperature and 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 6000 rpm for 10 min. Two and a half milliliters of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous ferric chloride. Absorbance of the final solution was recorded at 700 nm.

2.9 Structural Elucidation

The characterization of phenolic compounds in white and black Morus alba leaf extracts was performed using HPLC–PDA, where samples were separated on a C18 column and detected at 280, 320, and 370 nm to quantify major phenolic classes using external calibration standards. To complement chemical quantification, FTIR spectroscopy was used to identify key functional groups associated with phenolics, flavonoids, and other bioactive constituents, providing an overview of the extracts' chemical profile. Additionally, SEM analysis was conducted to observe particle morphology, surface texture, and structural features that may influence extract solubility and bioavailability. Together, these analytical techniques offered a general assessment of the chemical composition and structural properties of both white and black mulberry leaf extracts**.**

2.10 Statistical analysis

Experimental data were evaluated by using analysis of variance (ANOVA) and significant differences among means from triplicate analysis at $P < 0.05$ were determined by Duncan's multiple range test, using SPSS 9.0 for Windows.

3. RESULTS AND DISCUSSION:

The antioxidant and phytochemical composition of white and black mulberry leaves showed significant variation across most parameters. Black mulberry exhibited markedly higher antioxidant potential, as indicated by its lower DPPH IC50 value (109.55 ± 28.54 µg/mL) compared to white mulberry (211.82 ± 17.29 µg/mL). Total phenolic content was also substantially higher in black mulberry (861.23 ± 283.12 mg/100 g DM) than in white mulberry (589.47 ± 319.32 mg/100 g DM). A similar pattern was observed for total flavonoids, which were greater in black mulberry (621.17 ± 271.31 mg/100 g DM) relative to white mulberry (437.61 ± 121.51 mg/100 g DM). Total carotenoids were slightly higher in black mulberry (95.92 ± 23.42 mg/100 g DM) than in white mulberry (81.46 ± 52.56 mg/100 g DM). Anthocyanin levels were comparable between the varieties, with white mulberry showing 7.89 ± 1.42 mg/100 g DM and black mulberry showing 7.11 ± 1.16 mg/100 g DM.

Phenolic acid profiling revealed substantial differences among compounds. Black mulberry contained significantly higher concentrations of quercetin (0.19456 mg/g), rutin (1.3204 mg/g), catechin (0.05968 mg/g), o-coumaric acid (0.05213 mg/g), and caffeic acid (0.1269 mg/g) than white mulberry ($p < 0.05$). In contrast, white mulberry exhibited markedly greater levels of chlorogenic acid (2.18947 mg/g), ferulic acid (0.12454 mg/g), syringic acid (0.09850 mg/g), gallic acid (0.19219 mg/g), and p-coumaric acid (0.06122 mg/g) compared to black mulberry. Vanillic acid did not differ significantly between samples ($p > 0.05$).

Black mulberry showed stronger antioxidant activity (lower IC50) than white mulberry, indicating a higher free radical neutralization capacity. This trend aligns with earlier findings where black mulberry leaves exhibited stronger antioxidant and antiradical activity due to higher polyphenol richness (Kutlu et al., 2011; Arfan et al., 2012). The enhanced scavenging ability of black mulberry may be attributed to higher flavonoid and phenolic contents, particularly quercetin, rutin, catechin, and caffeic acid compounds known for potent hydrogen-donating abilities (Chan & Lim, 2006). The difference may also relate to pigmentation and secondary metabolite concentration contributed by the darker variety, which usually accumulates higher concentrations of radical-stabilizing molecules (Chen et al., 2006).

Black mulberry recorded a significantly higher TPC (861.23 mg/100 g) compared to white mulberry (589.47 mg/100 g). Phenolics are major contributors to antioxidant potential, which explains the improved DPPH activity observed in black mulberry. Previous studies similarly reported higher phenolic levels in darker mulberry varieties due to increased biosynthesis of phenolic acids under oxidative stress (Awad et al., 2012).

Phenolic compounds such as gallic acid, caffeic acid, and quercetin contribute to anti-inflammatory, antioxidant, and antihyperglycemic properties of mulberry leaves (Bae & Ye, 2010). The elevated phenolic content in black mulberry strengthens its nutraceutical potential.Black mulberry also possessed higher flavonoid content (621.17 mg/100 g) relative to white mulberry (437.61 mg/100 g). Flavonoids like rutin, quercetin, and catechin showed significantly higher concentrations in black mulberry, supporting the TFC results.

Rutin and quercetin are reported as dominant flavonoids in mulberry species and are responsible for enzymatic antioxidant defense, metal chelation, and free radical scavenging (Chan et al., 2013). Andallu et al. (2009) also found high flavonoid accumulation in mulberry leaves linked to antihyperglycemic effects.

Anthocyanin content did not differ significantly between white and black mulberry leaves. This is expected because leaf tissues in Morus species have substantially lower anthocyanin accumulation compared to fruits. Several studies (Arfan et al., 2012; Chen et al., 2006) confirm that mulberry leaves contain limited anthocyanins because they primarily accumulate in fruit skin or colored fleshy tissues. The minor differences observed may arise from slight variation in environmental conditions or leaf maturity but are not biologically substantial.

Carotenoid content was slightly higher in black mulberry (95.92 mg/100 g) compared to white mulberry (81.46 mg/100 g). Carotenoids are significant non-enzymatic antioxidants related to photoprotection and oxidative stress tolerance in leaves. Bickford et al. (2006) and Chan & Lim (2006) reported that mulberry species accumulate carotenoids as part of their adaptive antioxidant defense. While the difference here is modest, it may contribute synergistically to the stronger antioxidant activity observed in black mulberry.

The concentrations of quercetin and rutin were nearly double in black mulberry. These flavonols are well-known for strong antioxidant, anti-inflammatory, and glucose-modulating properties (Ali & Ali, 2012; Andallu et al., 2003). Higher levels in black mulberry corroborate previous reports showing darker mulberry species accumulate more quercetin derivatives (Awad et al., 2012). Catechin content was significantly greater in black mulberry, aligning with findings by Bozin et al. (2006) and Arfan et al. (2012), who reported catechins as major contributors to free radical scavenging in Morus species.

Overall, black mulberry demonstrated stronger antioxidant potential, which correlates with its significantly higher flavonoid and phenolic content. White mulberry, although lower in antioxidant strength, showed superior chlorogenic, ferulic, gallic, and syringic acid levels, suggesting potential health benefits in glucose modulation and anti-inflammatory applications.

These findings align with previous literature supporting the diverse nutraceutical properties of mulberry species.

Table 1. Phytochemical Composition and Antioxidant Activity of White and Black Mulberry Varieties

Varieties	DPPH˚IC 50 (µg/ml)	Total anthocyanins (mg/100g DM)	Total Flavonoids (mg/100g DM)	Total Phenolic (mg/100g DM)	Total carotenoids (mg/100g DM)
White Mulberry	211.82 ± 17.29	7.89 ± 1.42	437.61 ± 121.51	589.47 ± 319.32	81.46 ± 52.56
Black mulberry	109.55 ± 28.54	7.11 ± 1.16	621.17 ± 271.31	861.23 ± 283.12	95.92 ± 23.42

All values are expressed as *mean* ± *SD* (n = 3).

Table 2. Quantitative Analysis of Phenolic and Flavonoid Compounds in White and Black Mulberry Fruits

Chemical acid (mg g^{-1})	White mulberry	Black Mulberry	t value	Probability
Quercetin	0.11050 ± 0.0018	0.19456 ± 0.0014	15.96	0.000
Rutin	0.69752 ± 0.0031	1.3204 ± 0.0053	59.23	0.000
Vanillic acid	0.08124 ± 0.0021	0.07841 ± 0.0027	0.19	0.826
p-Coumaric acid	0.06122 ± 0.0018	0.02051 ± 0.0021	-18.12	0.000

o-Coumaric acid	0.04100 ± 0.0011	0.05213 ± 0.0031	4.21	0.021
Chlorogenic acid	2.18947 ± 0.0029	0.86700 ± 0.0039	-249.9	0.000
Catechin	0.02991 ± 0.0021	0.05968 ± 0.0019	13.71	0.000
Ferulic acid	0.12454 ± 0.0042	0.05900 ± 0.0046	-13.67	0.000
Syringic acid	0.09850 ± 0.0023	0.05800 ± 0.0019	-12.47	0.000
Gallic acid	0.19219 ± 0.0027	0.12700 ± 0.0026	-18.31	0.000
Caffeic acid	0.08124 ± 0.0022	0.1269 ± 0.0019	20.44	0.000

3.1 Scanning Electron Microscopy (SEM):

The surface morphology of the white and black mulberry (*Morus alba* and *Morus nigra*) leaf powders was analyzed by Scanning Electron Microscopy to assess structural characteristics that may influence extraction efficiency and bioactive compound yield. Figure 1 shows the micrograph of *white mulberry* leaf powder, which exhibits a compact and smooth surface with fewer visible pores and limited cell wall rupture. The uniform and dense morphology suggests that the plant cell walls remained relatively intact after drying and grinding, reducing solvent penetration during extraction. This structural integrity could be associated with the comparatively lower phenolic (589.47 mg/100 g DM) and flavonoid contents (437.61 mg/100 g DM) obtained from white mulberry extracts. Similar compact microstructures have been observed in other plant matrices with reduced extraction efficiency (Liu et al., 2016).

In contrast, Figure2 illustrates the *black mulberry* leaf powder surface, which displays a rough, irregular, and porous morphology with clearly visible ruptures and microfractures. These structural disruptions indicate greater cell wall breakdown, likely resulting from higher pigment and phenolic acid content that may weaken the cuticular and parenchymal cell layers. The presence of cracks and pores increases the specific surface area, facilitating solvent diffusion and enhancing the release of phenolic and flavonoid compounds during extraction. Consequently, black mulberry showed significantly higher total phenolic (861.23 mg/100 g DM) and flavonoid (621.17 mg/100 g DM) contents compared with white mulberry.

These findings are in agreement with previous SEM-based investigations on *Morus* species and other phenolic-rich leaves, where cell wall porosity was strongly correlated with extraction efficiency and antioxidant potential (Arfan et al., 2012); (Chan & Lim, 2006).

Furthermore, studies have shown that ultrasonic or solvent-intensive extraction methods cause visible micro-fracturing of leaf surfaces, enhancing antioxidant recovery by disrupting cellulose–lignin networks (Hossain et al., 2012).

Therefore, the SEM images confirm a clear structure–function relationship: The denser surface of white mulberry restricts solvent access, leading to lower extraction yield, whereas the porous and fractured surface of black mulberry enhances the diffusion of methanol solvent, contributing to its higher antioxidant and phytochemical concentrations.

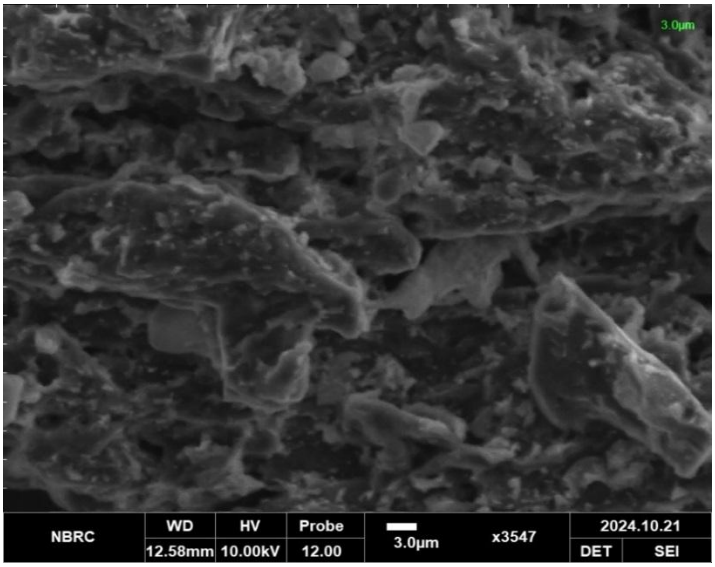

Figure 1. SEM micrograph of *Morus alba* (white mulberry) leaf powder showing compact and less porous surface morphology (Magnification: 1000×).

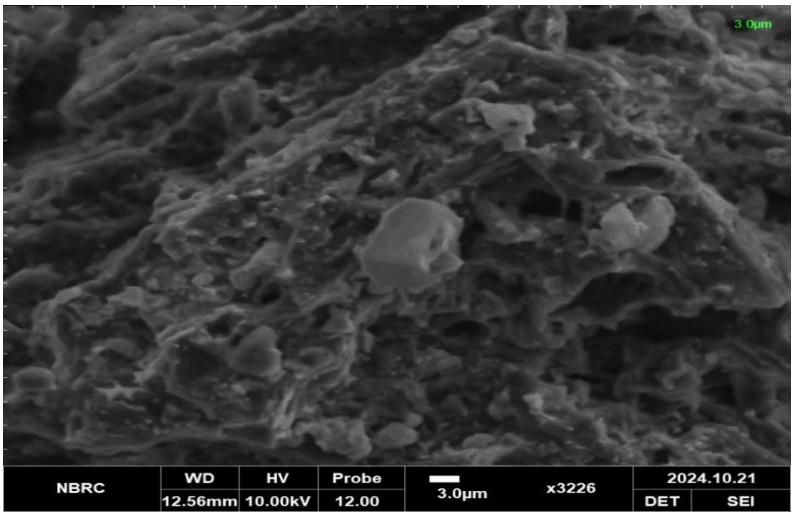

Figure 2. SEM micrograph of *Morus nigra* (black mulberry) leaf powder showing porous and disrupted cell wall structure (Magnification: 1000×).

3.2 Fourier Transform Infrared Spectroscopy (FTIR):

Fourier Transform Infrared (FTIR) spectroscopy was conducted to identify the major functional groups and chemical constituents present in the extracts of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves. The FTIR spectra recorded in the range of 4000–650 cm^{-1}, exhibited several characteristic absorption bands corresponding to hydroxyl, carboxyl, alkane, and polysaccharide functional groups. The major observed peaks and their corresponding functional group assignments are summarized in Figure 02.

A broad absorption band observed around 3400 cm^{-1} was attributed to O–H stretching vibrations, confirming the presence of hydroxyl groups associated with phenolic and flavonoid compounds. This broad band reflects strong hydrogen bonding typical of polyphenolic substances in mulberry leaves, which are known for their antioxidant potential (Walkowiak-Bródka et al., 2022). Peaks at approximately 2920 cm^{-1} and 2850 cm^{-1} corresponded to C–H stretching vibrations of aliphatic compounds, indicating the presence of lipidic and waxy constituents commonly found in leaf tissues.

A sharp absorption peak near 1730 cm^{-1} was assigned to C=O stretching vibrations of carbonyl groups present in carboxylic acids and esters. This band indicates the existence of compounds such as chlorogenic acid derivatives and flavonoid esters, which are abundant in *Morus* species (Chen et al., 2018). Another distinct band observed around 1620 cm^{-1} corresponded to C=C stretching in conjugated alkenes and aromatic rings, characteristic of phenolic and flavonoid compounds. These functional groups are consistent with the phenolic fingerprint typically found in antioxidant-rich mulberry extracts.

In the fingerprint region, multiple absorption bands were detected between 1450–1040 cm^{-1}, corresponding to C–H bending, C–O stretching, and C–O–C linkages. These vibrations are characteristic of alkanes, esters, and polysaccharides, indicating the presence of cellulose, hemicellulose, and secondary alcohol groups in the leaf matrix (Shao et al., 2023). Weak absorption peaks around 870 cm^{-1} and 720 cm^{-1} were attributed to C=C bending vibrations, suggesting the presence of unsaturated alkenes and other aromatic compounds.

Comparative analysis of the spectra revealed that black mulberry extract exhibited stronger O–H and C=O peaks than white mulberry, suggesting a higher concentration of phenolic and flavonoid compounds, which correlates with its higher antioxidant potential measured in chemical assays. Conversely, white mulberry showed relatively higher intensity in polysaccharide-related regions, indicating a slightly more carbohydrate-rich composition.

Overall, the FTIR analysis confirmed that both white and black mulberry leaves contain complex mixtures of hydroxyl, carbonyl, and ether-containing bioactive compounds, consistent with their rich polyphenolic composition and strong antioxidant potential. These results agree with previous FTIR characterizations of mulberry leaf materials, which revealed similar functional group profiles associated with secondary metabolites and phenolic compounds (Walkowiak-Bródka et al., 2022; Chen et al., 2018).

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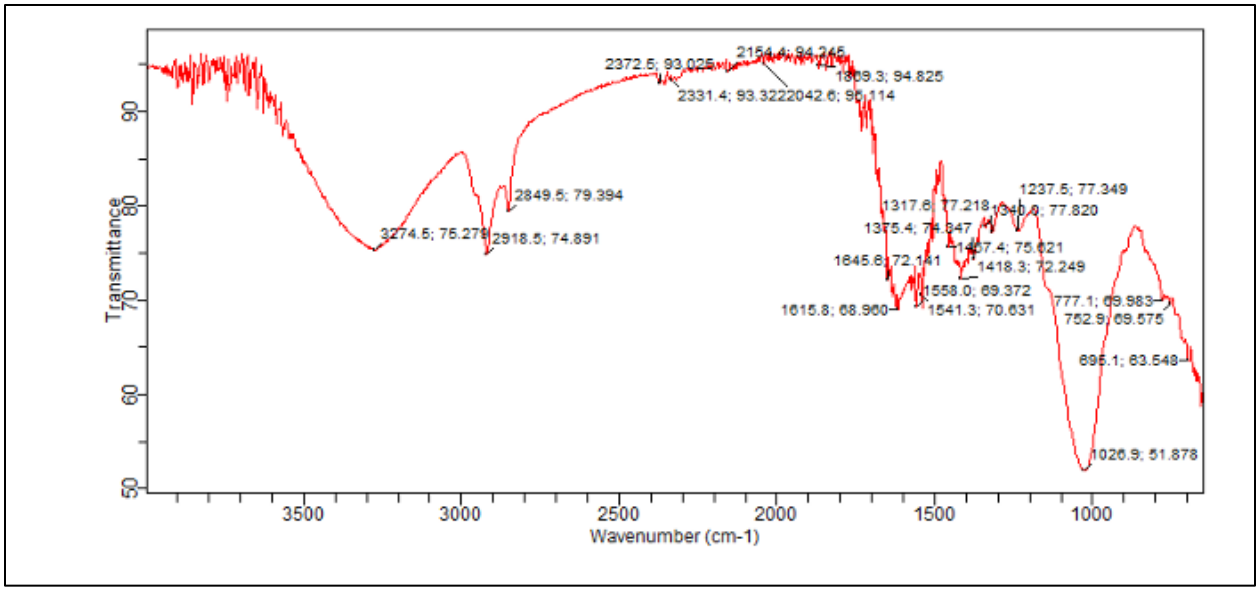

Figure 3. FTIR spectrum of *Morus alba* (white mulberry) leaf extract showing dominant O–H and C=O absorption bands

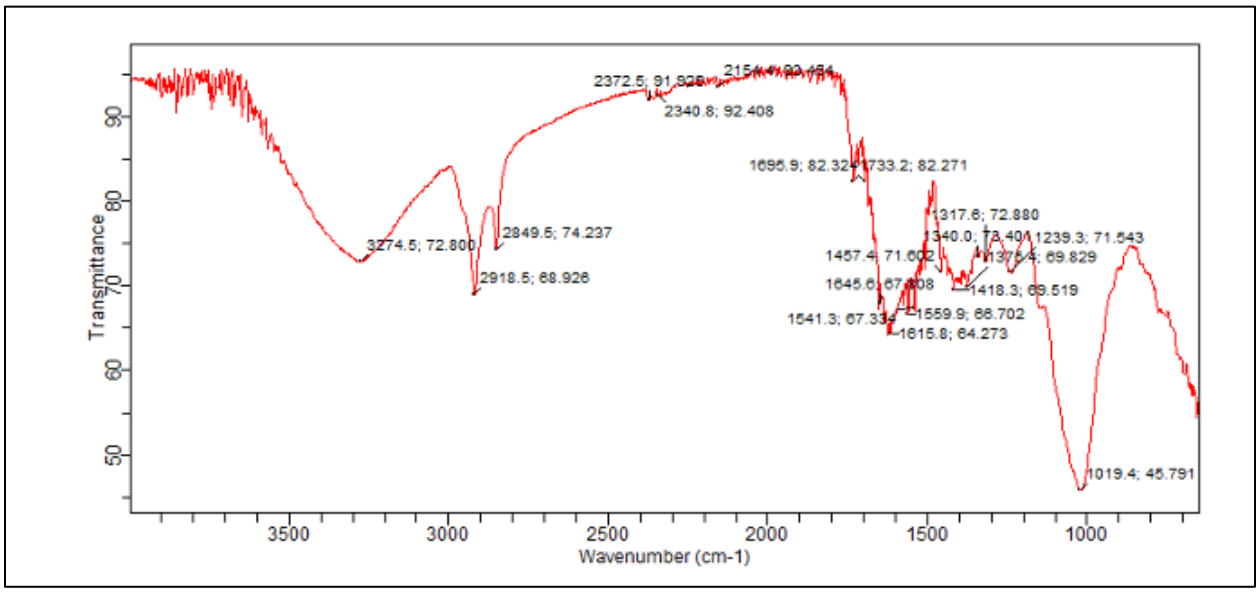

Figure 4. FTIR spectrum of *Morus nigra* (black mulberry) leaf extract highlighting aromatic and polysaccharide-associated C–O–C stretching vibrations.

CONCLUSION

The present study demonstrated that black mulberry (Morus nigra) leaves possess superior functional and structural characteristics compared to white mulberry (Morus alba), highlighting their greater potential for nutraceutical and industrial utilization. Higher levels of phenolic and flavonoid compounds, along with stronger antioxidant activity, were observed in black mulberry extracts, reflecting their richer phytochemical composition. SEM analysis revealed a more porous surface structure in black mulberry, enhancing solvent penetration and extraction efficiency, while FTIR spectra confirmed the abundance of hydroxyl and carbonyl groups typical of phenolic and flavonoid compounds. These findings establish a clear structure function relationship in mulberry leaves, where microstructural features influence bioactive compound yield and antioxidant potential. Overall, black mulberry emerges as a promising candidate for developing natural antioxidant formulations, functional foods, and sustainable bio-based products, whereas white mulberry retains value for moderate antioxidant and medicinal applications. Future work should focus on standardizing extraction processes and assessing bioavailability to optimize industrial and therapeutic applications of mulberry leaf bioactives.

Authors' Contribution

Muhammad Arslan Aslam conceived and designed the study, conducted the experiments, and prepared the initial manuscript draft. Farhan Saeed supervised the research, guided data analysis, and critically revised the manuscript. Ali Imran provided technical expertise in phytochemical and structural analyses and contributed to manuscript review. Bilal Hussain assisted in laboratory work, data processing, and statistical evaluation. All authors read and approved the final version of the manuscript.

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