

Research

Bioactive compounds and antioxidant activity in various parts of *Morus alba* L. Cv. ichinose: a comparative analysis

Lubna Altaf¹ · Shabir Ahmad Wani¹ · Peerzada Rashid Hussain² · Prashant Suradkar² · Mohmmad Farooq Baqual¹ · Aabid Ahmad Bhat¹

Received: 3 May 2024 / Accepted: 16 July 2024

Published online: 30 July 2024

© The Author(s) 2024 [OPEN](#)

Abstract

Present investigation was undertaken to assess the composition of bioactive compounds within various parts of methanolic extracts from mulberry plants utilizing both High-Performance Liquid Chromatography (HPLC) and spectrophotometric methodologies. Furthermore, the antioxidant potential was evaluated through assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), β -carotene bleaching (BCBA), and Hydroxyl Radical Scavenging Activity (HRSA). This research incorporated the analysis of the fruit, root, shoot, and leaf of the mulberry (*Morus alba* L.) Cv. Ichinose. Findings from the investigation revealed that levels of total phenols (533.8 ± 15.6 mg/100 g) and flavonoids (232.3 ± 7.1 mg/100 g) were notably higher in fruit compared to root, while total ascorbic acid content (70.9 ± 3.1 mg/100 g) was significantly elevated in the leaf. The fruit exhibited a significantly higher concentration of total anthocyanins (142.5 ± 3.1 mg/100 g), whereas the leaf contained a substantial amount of total carotenoids (5.8 ± 0.3 mg/100 g). Predominant phenolic acids detected in mulberry included ferulic acid, m-coumaric acid, syringic acid, caffeic acid, chlorogenic acid, and gallic acid. Quercetin and isoquercetin were identified as the major flavonoids in mulberry fruits. In terms of anthocyanins, cyanidin-3-rutinoside (25.6 ± 2.3 mg/100 g) and cyanidin-3-glucoside (16.4 ± 2.1 mg/100 g) were the primary anthocyanins present in the fruit. The main carotenoid found in the leaf was beta-carotene, with levels reaching 2.1 ± 0.3 mg/100 g. Fruit displayed the highest antioxidant activity among all parts examined. Comparison of the EC₅₀ values of fruit (EC₅₀ = 174.2 ± 2.2 μ g/mL), root (EC₅₀ = 179.6 ± 2.3 μ g/mL), and shoot (EC₅₀ = 189.4 ± 2.1) suggested that these segments of the mulberry plant could potentially function as antioxidants and could exhibit a greater hydroxyl radical scavenging effect than standard antioxidants.

Keywords *Morus alba* · Bioactive compounds · HPLC analysis · Antioxidant activity · Phenolic composition

1 Introduction

Plant and plant products owing to their plethora of secondary metabolites have emerged as the epicentre in the development of alternate products with improved functionalities. Studies have shown that fruits, vegetables and other plant materials possess antioxidant properties mainly due to their bioactive compounds particularly the 'phenolic compounds'. In addition to antioxidant potential, plants and their product also assist in reducing the occurrence of

✉ Lubna Altaf, lubnaaltaf@skuastkashmir.ac.in | ¹College of Temperate Sericulture, Mirgund, Sher-E-Kashmir University of Agricultural Sciences & Technology Kashmir, Srinagar, Kashmir (J & K) 191121, India. ²Astrophysical Sciences Division, Nuclear Research Laboratory, Bhabha Atomic Research Centre, Zakura, Srinagar, Kashmir (J&K) 190024, India.



chronic diseases like diabetes, cancer, cardiovascular and neurodegenerative diseases. These beneficial health effects are achieved by cumulative biological exposure to antioxidants which are able to neutralize the proliferation of reactive oxygen species (ROS) and reactive nitrogen (RNS) species which are implicated in the pathology of these diseases [1, 2]. Mulberry (*Morus*) is a typical East Asian plant belonging to family Moraceae. It is widely distributed in varied ecological and geographical zones from intensive cultivation in temperate, sub-tropical and tropical areas to natural occurrence in forests throughout the world. Major *Morus* species include *Morus alba* L., *Morus nigra*, *Morus rubra*, *Morus indica*, *Morus laevigata*, etc. Among these species, *Morus alba* L. is mostly used for feeding of silkworm owing to nutritious, palatable and non-toxic nature of the leaves. Beside the sericulture potential, plants of the genus *Morus* are a rich source of flavonoids including rutin, isoquercetin, cyanidin 3-rutinoside and cyanidin 3-glucoside and contain significant amounts of ascorbic acid, total carbohydrate, beta-carotene, folic acid, protein, minerals and crude fibre [2]. The Union Territory of Jammu and Kashmir (India) offers a fertile environment for growth and development of silkworm and cultivation of mulberry. Kashmir is known for producing high quality silk from indigenous varieties of *Morus alba* owing to its suitable agro-climatic condition [3].

Berries, due to their extensive historical utilization in medicinal applications, owe a substantial portion of their therapeutic properties to the antioxidant characteristics bestowed upon them by their distinct composition. The overall antioxidant capacity of plant-based edibles arises from the combined effects of the singular antioxidant components, a factor of utmost significance. Consequently, the clarification of the mechanisms that underlie the antioxidant properties of berries provides invaluable insights into their therapeutic capabilities and functional attributes [3]. The assortment of these antioxidant compounds varies in both quantity and quality across various plant tissues. Owing to the abundance of phytonutrients, different segments of the mulberry plant, including its fruit, roots, leaves, and shoots, have been known to exhibit advantageous effects and have been traditionally employed as remedies for conditions such as joint ailments, hyperglycemia, hypertension, tumors, asthma, cough, diarrhea, dyspepsia, and wounds [1]. Mulberry leaves and fruits boast a high content of phenolic compounds, namely flavonoids, anthocyanins, and phenolic acids, which are responsible for their potent antioxidant properties. These substances aid in the elimination of free radicals, thus safeguarding the body against oxidative stress and associated ailments. The mulberry fruit comprises diverse bioactive substances such as quercetin and kaempferol, which exhibit anti-inflammatory properties. These substances contribute to the reduction of inflammation and have the ability to shield against chronic illnesses like cardiovascular diseases. Furthermore, the consumption of mulberry has been associated with enhanced lipid profiles and minimized formation of arterial plaque, both of which are critical for cardiovascular well-being. Several research studies propose that the phytochemicals present in mulberry, especially the anthocyanins and flavonoids, possess anti-cancer attributes. These substances can prompt apoptosis (controlled cell death) in cancerous cells and impede their growth. The Nutraceutical potential of mulberry is becoming increasingly acknowledged due to its abundant phytochemical composition, highlighting its potential in the development of functional foods. These food products not only offer nourishment but also provide health advantages that can prevent or manage various health conditions [4]. Mulberries possess clinical potential and molecular mechanisms due to their high content of vitamins A and E, as well as carotenoids and other antioxidants. These bioactive compounds have the capacity to safeguard the skin from oxidative stress, diminish the visibility of imperfections, and counteract premature skin aging. Studies indicate that extracts from mulberries have the ability to reduce DNA damage associated with skin cancer and enhance overall skin well-being [5].

Further, due to shift in food consumption, there is an increasing demand for the products with enhanced functionality. In this context, studies are being undertaken to identify the varieties rich in bioactivities and to investigate how the content of these phytonutrients vary in plant tissues. Some studies have been published on the chemical and physical properties of mulberry fruits and its products [1, 6, 7]. However, to our best knowledge; there is hardly any baseline study available on the bioactive composition and variation of antioxidant compounds and activity among different parts in *Ichinose* variety of mulberry grown in North India; which is mainly used in silkworm rearing. Therefore, the present study was aimed at comparing the profiles of bioactive compounds and evaluating the antioxidant activity in different parts of *Morus alba* L. Cv. *Ichinose*. The results of the present study will prove helpful in understanding the variability and accumulation of bioactive compounds and antioxidant activity among different parts of *Ichinose* variety so as to make a favourable choice about which plant part to be used in designing the novel food and pharmaceutical formulations with improved health benefits.

2 Materials and methods

2.1 Preparation of sample

In preparation for analysis, approximately 2 kg of various plant tissues—namely leaves, fruits, bark, and roots—were procured in triplicate from the germplasm bank housed at the College of Temperate Sericulture, Mirgund associated with Sher-e-Kashmir University of Agricultural Sciences and Technology-Kashmir. The collection period extended from mid-June of the year 2021 through to the same interval in 2022. Subsequent to their collection, these samples underwent a cleaning regimen involving a rinse in potable water to remove any residual particulate matter and extrinsic contaminants.

Post-cleansing, the non-fruit tissues were individually encased within aluminium foil to ensure a preservation environment free from moisture and other potential degradants. Fruits, on the other hand, were allocated to transparent glass containers to safeguard their integrity and prevent loss of analytes. These preparations were then subjected to a stabilizing cryogenic condition at a temperature of negative 20 degrees Celsius until the time of their respective analyses. Prior to undergoing analytical procedures, each sample was methodically tempered to reach ambient room temperature, facilitating accurate and consistent assay results.

2.2 Analysis of bioactive compounds

In the present study, the total chlorophyll, phenolic, flavonoid, and carotenoid contents of the samples were determined using established protocols and a UV–Visible Spectrophotometer (Hitachi 330, Germany).

2.3 Total chlorophyll

The chlorophyll content was assessed following the protocol [8] wherein 3 g of the sample, in triplicate, was extracted with 80% acetone until the residue was colorless. The combined extracts were centrifuged, and the supernatants were made up to 100 mL with 80% acetone. Absorbance was measured at 645 and 663 nm. **Formula:**

$$\text{Total Chlorophyll Content (mg/100g)} = \frac{[20.2(A_{645}) + 8.02(A_{663})] \times V \times 100}{(1000 \times W)}$$

Here,

A = Absorbance at specific wavelength.

V = Volume made-up of chlorophyll extract in 80% acetone.

W = Weight of sample taken.

2.4 Total phenols and flavonoids

For the total phenolic content, 3 g of the homogenized sample was extracted three times with 80% ethanol, centrifuged, and the supernatants dried under nitrogen gas. The residue was dissolved in distilled water, reacted with Folin-Ciocalteu reagent, and after treatment with sodium carbonate, absorbance was measured at 650 nm. Total phenolics were expressed as mg Gallic Acid Equivalents (GAE) per 100 g [9]. Similarly, for total flavonoid content, extracts obtained from 3 g of sample with 80% ethanol were processed, dried, and then reacted sequentially with NaNO₂, AlCl₃, and NaOH, followed by absorbance measurement at 510 nm. Flavonoid content was expressed as catechin equivalents per 100 g [9].

2.5 Total anthocyanins

The total anthocyanin content was determined utilizing the pH-differential method outlined in reference [10]. A 1 mL aliquot of clarified mulberry extract was transferred to a 25 mL volumetric flask and brought to volume using a pH 1.0 buffer solution (comprising 1.49 g KCl per 100 mL water and 0.2 N HCl, mixed in a 25:67 ratio). Concurrently, a separate 1 mL sample was placed in a 25 mL volumetric flask and diluted to volume with a pH 4.5 buffer solution

(consisting of 1.64 g sodium acetate per 100 mL water, pH adjusted to 4.5 using 0.2 N HCl). Absorbance was determined using the formula: $\Delta A = (A_{510 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 1.0 - (A_{510 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 4.5$. The total anthocyanin concentration, expressed as mg of cyanidin-3-glucoside equivalents per 100 g of sample, was calculated according to the following equation:

$$\text{Total Anthocyanin Content (mg/100 g)} = (A/\epsilon L) \times \text{MW} \times D \times (V/G)$$

where:

- A = Absorbance
- ϵ = molar extinction coefficient = 26,900
- L = path length = 1 cm
- MW = Molecular weight of anthocyanins = 449.2
- D = Dilution factor
- V = Final volume (mL)
- G = Sample weight (g)

2.6 Total carotenoids

To determine total carotenoid content, the extracts were combined and filtered through Whatman filter paper. A 25 mL aliquot of the filtered acetone extract was transferred to a separating funnel, and 20 mL of petroleum ether was added. After standing for 15 min, 100 mL of 5% sodium chloride solution was added to the separating funnel, and the contents were thoroughly mixed and allowed to separate into two phases. The aqueous phase was discarded, and the organic phase was collected and washed four times with 100 mL of distilled water to remove residual acetone. The petroleum ether phase was then passed through a funnel containing anhydrous sodium sulfate to remove residual water and collected in a 100 mL volumetric flask. The contents were brought to volume with petroleum ether, and the absorbance was measured at 450 nm using petroleum ether as a blank. The total carotenoid content, expressed as mg beta-carotene equivalents per 100 g, was calculated as under.

3 Formula:

$$\text{Total carotenoids (mg/100g)} = (\Delta A/eL) \times \text{DF} \times \text{MW} \times (V/G)$$

Here, ΔA = Absorbance.. e = Beta-carotene molar extinction coefficient = 2590.

L = Cell path length = 1 cm; MW = Molecular weight of beta-carotene = 536.8 DF = Dilution factor; V = Final volume in ml and G = Weight of the sample in gm [11].

3.1 Extraction and HPLC analysis of phenolic compounds

A mass of 50 g of mulberry tissue was subjected to triple extraction with an 80% methanol solution, maintaining a sample-to-solvent ratio of 1:2. The resultant extracts were amalgamated and subsequently condensed through evaporation at a controlled pressure and a temperature not surpassing 40 degrees Celsius, yielding a product hereafter termed the Whole Concentrate (WC). A specified portion of the WC was further processed to isolate phenolic compounds using ethyl acetate as the extraction solvent. The ethyl acetate phase was collected, dried over anhydrous sodium sulfate, and then clarified using Whatman filter paper No. 42. The consolidated ethyl acetate fraction was then evaporated under a reduced pressure regime, also respecting the maximum temperature threshold of 40 degrees Celsius, and preserved within a desiccator set at 4 degrees Celsius, awaiting High-Performance Liquid Chromatography analysis. For the chromatographic identification and quantification of phenolic constituents within the mulberry extract, a solution at a concentration of 1.0% (w/v) in HPLC-grade methanol was prepared. Prior to HPLC, sample filtration was executed sequentially using Whatman filter paper No. 42 and then 0.22 micron membrane filters. The HPLC–DAD analysis was performed using an Agilent Technologies instrument, 1100 Series [12].

3.2 Extraction and HPLC analysis of anthocyanins and carotenoids

Anthocyanin content was determined using a high-performance liquid chromatography system equipped with a diode-array detector. The detection wavelength was set to 520 nm. Anthocyanins were identified by comparing their retention times to those of known standards, and quantification was performed using a standard curve.

For anthocyanin extraction, a 3 g sample of homogenized mulberry was mixed with a solution of ethanol and 1.5 N HCl (85:15, v/v) at a 1:3 sample-to-solution ratio. The mixture was kept in a dark environment overnight to ensure complete extraction. After extraction, the extracts were combined and concentrated at 40 °C under reduced pressure using a rotary vacuum evaporator. Before HPLC analysis, the extracts were centrifuged at 14,000 rpm for 15 min at low temperature, and a 20 µL aliquot of the supernatant was injected into a C-18 column. The column was prewashed with methanol and equilibrated with the mobile phase for 1 h. The mobile phase consisted of 100% methanol (A) and 10% formic acid (B), and the elution program was as follows:

- 0–20 min: 9–35% A
- 20–30 min: 35% A
- 30–40 min: 35–50% A
- 40–55 min: 50–9% A

The mobile phase flow rate was maintained at 1 µL/min.

Carotenoid content was also determined using HPLC. For this, a 1.0% (w/v) solution of the mulberry sample in petroleum ether was prepared and filtered through a 0.22 µm 270 membrane filter. A 20 µL sample was injected into the HPLC system equipped with a C18 column. The column was prewashed with methanol and equilibrated with the mobile phase for 1 h. The mobile phase consisted of acetonitrile, dichloromethane, and methanol in a ratio of 70:20:10. The analysis was performed under isocratic elution at a flow rate of 1 µL/min, and the detector wavelength was set to 450 nm. Carotenoids were identified by comparing their retention times and UV–visible spectra to those of known standards. Quantification was performed using a standard curve generated by plotting the peak area percentage against the concentration of the standards [08], [11].

3.3 Antioxidant assays

The antioxidant activities of mulberry extracts were evaluated using several assays.

3.3.1 DPPH

The ability of the extracts to scavenge DPPH radicals was assessed by measuring the decrease in absorbance at 517 nm. A 10 mL aliquot of the extract was combined with 2 mL of 60 mM DPPH solution in ethanol. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 min. The absorbance was measured using a UV–Visible spectrophotometer. The DPPH solution was prepared fresh daily, stored in flasks covered with aluminum foil, and kept in the dark at 4 °C until use. The scavenging activity was expressed as the percentage inhibition of DPPH radicals, calculated using the following formula:

$$\text{Inhibition percentage (IP)} = [(A_{\text{control}} - A_{\text{sample}}) \times 100] / A_{\text{control}} \text{ [13].}$$

3.3.2 FRAP

The FRAP assay measures the reducing capacity of the extracts by monitoring the reduction of ferric to ferrous ions in the presence of antioxidants. A 5 mL aliquot of the mulberry extract was mixed with 2.5 mL of 200 mM phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide solution. The mixture was incubated at 50 °C for 20 min and then rapidly cooled. After adding 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 200 rpm for 10 min. A 5 mL aliquot of the upper layer was then mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance of the resulting solution was measured at 700 nm using a UV–Visible spectrophotometer. A higher absorbance indicated greater reducing power [14].

3.3.3 HRSA

The HRSA assay evaluates the ability of the extracts to scavenge hydroxyl radicals generated through the Fenton reaction. A reaction mixture containing 100 µL of 28 mM 2-deoxyribose, 500 µL of the sample in phosphate buffer, 200 µL of 200 µM FeCl₃ in 1.04 mM aqueous EDTA (1:1, v/v), 100 µL of 1 mM H₂O₂, and 100 µL of 1 mM ascorbic acid was incubated at 37 °C for 1 h. The reaction was stopped by adding 1.0 mL of 2.8% trichloroacetic acid. Then, 1.0 mL of 1.0% thiobarbituric acid was added, and the mixture was incubated in a water bath at 80 °C for 20 min. After cooling, the absorbance was measured at 532 nm against an appropriate blank. Hydroxyl radical scavenging activity, expressed as percentage inhibition, was calculated using the following formula:

$$\text{Inhibition Percentage (IP)} = \{(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}\} \times 100 \text{ [15]}.$$

3.3.4 BCBA

The antioxidant capacity of mulberry extracts was assessed using a beta-carotene bleaching assay. A beta-carotene/linoleic acid emulsion was prepared by initially dissolving beta-carotene (5 mg) in chloroform (50 mL). A 3 mL aliquot of this solution was then added to a round bottom flask containing linoleic acid (40 mg) and Tween 40 (400 mg). The chloroform was subsequently evaporated using a rotary evaporator. Distilled water (100 mL) was gradually added to the mixture with vigorous shaking to create an emulsion.

To perform the assay, aliquots (4.5 mL) of the prepared emulsion were mixed with 0.3 mL of mulberry extract (at a final concentration of 250 µg/mL) in methanol. These mixtures were incubated at 50 °C in a water bath for 100 min. Control samples were prepared identically, but with 0.3 mL of solvent replacing the mulberry extract. A blank solution was also prepared, omitting the beta-carotene.

Oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm every 20 min. The antioxidant activity of the extracts was determined by calculating the percentage inhibition of beta-carotene bleaching using the following formula:

$$AA = 100[1 - (A_0 - A_t)/(A^{\circ}0 - A^{\circ}t)]$$

where:

- A₀ = Absorbance of the sample at the beginning of the incubation
- A_t = Absorbance of the sample after incubation for 120 min
- A[°]₀ = Absorbance of the control at the beginning of the incubation
- A[°]_t = Absorbance of the control after incubation for 120 min [16].

3.4 Statistical analysis

Statistical analysis of the data was performed utilizing a completely randomized design, as per the framework outlined in reference [17]. Analysis of variance (ANOVA) of the data was performed using MINITAB statistical analysis software package (Minitab, version 11.12, 32 bit, Minitab, Inc., State College, PA, USA). Difference between means of data was compared by least significant difference (LSD) and Student's t-test was applied to determine if the difference was statistically significant. Differences at $p \leq 0.05$ were considered to be statistically significant. Coefficient of correlation was determined by Karl Pearson method. Triplet replicates for each measurement were assayed for every treatment, with results being expressed as mean values accompanied by their respective standard deviations.

4 Results and discussion

4.1 Total phenols and flavonoids

The contents of total phenols and flavonoids in various parts of the mulberry plant are summarized in Table 1. A significant variation in total phenol content was observed among different mulberry parts. Fruit exhibited the highest total phenol content (533.8 mg/100 g), followed by root (368.3 mg/100 g) and shoot (271.7 mg/100 g), with leaf recording

the lowest content (250.9 mg/100 g). This trend was also observed for total flavonoid content, with fruit containing 232.3 mg/100 g compared to 110.2 mg/100 g in leaf. These findings are consistent with previous studies on mulberry phenolics which reported a total phenolic content of 432.50 mg/100 g in *Morus alba* fruits [18]. Another recent study reported total phenolic levels in 19 varieties of mulberry leaves ranging from 11.2 to 31.1 mg/g [6], while a different study found total phenolic content in mature leaves of various mulberry varieties grown in China to range from 27.6 to 37.4 mg/100 g [7]. Additionally, phenolic content in *Morus alba* shoots and roots has been reported at 285.62 mg GAE/g and 165 mg GAE/g, respectively [19]. The total flavonoid content in *Morus alba* fruits has been documented to range from 29.00 to 137.86 mg/100 g [18], and in roots, it ranges from 204.36 to 800 µg/g [20]. Furthermore, studies have reported total flavonoid content in *Morus alba* shoots ranging from 128 to 450 mg/100 g, and in leaves between 193.87 and 398.33 mg rutin equivalents mg/100 g [21].

The observed variations in phenolic content among different parts of the mulberry plant can be attributed to differences in extraction procedures and analytical methods employed. Additionally, phenolic compound levels in different parts, particularly leaves and fruits, may fluctuate in response to environmental factors such as drought, temperature variations, pollution, UV light, and pathogenic attacks. These factors significantly influence the synthesis and accumulation of phenolic compounds in plants.

4.2 Ascorbic acid, dehydroascorbic acid and total ascorbic acid

Table 1. Presents the concentrations of ascorbic acid, dehydroascorbic acid, and total ascorbic acid in various parts of the mulberry plant. The leaf exhibited the highest ascorbic acid content at 68.1 mg/100 g, followed by the fruit (27.2 mg/100 g), shoot (15.7 mg/100 g), and root (11.3 mg/100 g). Similarly, dehydroascorbic acid content was highest in the leaf (2.8 mg/100 g) and fruit (2.5 mg/100 g), compared to the shoot (1.2 mg/100 g) and root (1.1 mg/100 g). Consequently, total ascorbic acid, defined as the sum of ascorbic acid and dehydroascorbic acid, was also highest in the leaf (70.9 mg/100 g), followed by the fruit (29.7 mg/100 g), shoot (16.9 mg/100 g), and root (12.4 mg/100 g).

These findings align with previous research. A study by [22] reported total ascorbic acid levels in *Morus alba* leaves ranging from 160 to 280 mg/100 g. Additionally, [1] documented ascorbic acid content in *Morus alba* fruits at 29.32 mg/100 g. Furthermore, [23] evaluated the ascorbic acid levels in the leaves of three mulberry varieties, reporting a range of 1.66 to 3.84 mg/100 g.

Variations in ascorbic acid levels across different parts of the mulberry plant are attributed to climatic factors, including light intensity and average temperature. The amount and intensity of light during the growing season significantly influence the formation and accumulation of ascorbic acid. Plant parts exposed to maximum sunlight tend to have higher ascorbic acid levels. In general, lower light intensity during growth results in lower ascorbic acid content in plant tissues [23].

Table 1 Content of bioactive compounds in different parts of mulberry (*Morus alba* L). Cv. *Ichinose*

Mulberry part	TP	TF	AA	DAA	TAA	Chla	Chlb	TCh	TAn	TCar
Fruit	533.8 ± 15.6 ^d	232.3 ± 7.1 ^d	27.2 ± 2.2 ^c	2.5 ± 0.5 ^b	29.7 ± 2.1 ^c	7.8 ± 0.5 ^a	2.9 ± 0.2 ^a	10.7 ± 1.2 ^a	142.5 ± 3.1 ^b	0.54 ± 0.1 ^a
Leaf	250.9 ± 7.1 ^a	110.2 ± 3.2 ^a	68.1 ± 3.3 ^d	2.8 ± 0.4 ^b	70.9 ± 3.1 ^d	218.2 ± 2.1 ^c	94.1 ± 2.2 ^c	312.5 ± 3.1 ^c	0.01 ± 0.00 ^a	5.8 ± 0.3 ^c
Shoot	271.7 ± 13.3 ^b	147.2 ± 5.6 ^b	15.7 ± 1.3 ^b	1.2 ± 0.3 ^a	16.9 ± 1.6 ^b	21.2 ± 1.3 ^b	8.9 ± 1.3 ^b	30.1 ± 2.3 ^b	0.03 ± 0.00 ^a	0.92 ± 0.2 ^b
Root	368.3 ± 8.9 ^c	179.1 ± 4.2 ^c	11.3 ± 1.1 ^a	1.1 ± 0.1 ^a	12.4 ± 1.1 ^a	Nd	Nd	Nd	0.05 ± 0.00 ^a	0.38 ± 0.1 ^a
LSD	7.7	6.2	3.5	0.7	3.1	5.5	3.7	6.2	0.08	0.3

Values are mean ± SD (n = 3); LSD least significant difference (p ≤ 0.05), nd not detected, TP total phenols, TF total flavonoids, AA ascorbic acid, DAA dehydroascorbic acid, TAA total ascorbic acid, Cha chlorophyll a, Chb chlorophyll b, TCh total chlorophyll, TAn total anthocyanins, TCar total carotenoids

Values with different superscript lowercase letters (a–d) differ significantly (p ≤ 0.05)

All the parameters are expressed as mg/100 g fw. Total phenols are expressed in Gallic acid equivalents; total flavonoids as catechin equivalents; total anthocyanins as cyanidin-3-glucoside equivalents; total carotenoids as beta-carotene equivalent

4.3 Chlorophyll a, chlorophyll b and total chlorophyll

The content of chlorophyll a, chlorophyll b and total chlorophyll is shown in Table 1. Data indicated that chlorophyll a was significantly ($p \leq 0.05$) higher compared to chlorophyll b in all parts of the mulberry except in root which was devoid of chlorophyll. Among the mulberry parts, leaf had significantly higher content of chlorophyll a (218.2 ± 2.1 mg/100 g) followed by shoot (21.2 ± 1.3 mg/100 g). Chlorophyll b was also significantly ($p \leq 0.05$) higher in leaf (94.1 ± 2.2 mg/100 g) followed by shoot (8.9 ± 1.3 mg/100 g). Fruit had the lowest contents of both chlorophyll a (7.8 ± 0.5 mg/100 g) and chlorophyll b (2.9 ± 0.2 mg/100 g). Total chlorophyll taken as sum of chlorophyll a and b was also significantly ($p \leq 0.05$) higher in leaf (312.5 ± 3.1 mg/100 g) followed by shoot (30.1 ± 2.3 mg/100 g). In mulberry fruit, total chlorophyll content was 10.7 ± 1.2 mg/100 g. Total chlorophyll contents reported in the present study are in conformity with the results of [24] on chlorophyll content of *Morus alba* where total chlorophyll ranged from 2.6–4.7 mg/100 g. [25] reported Chlorophyll a, b and total chlorophyll with a value of 94.5–184.3, 60.2–101.5 and 154.6–275.3 mg/100 g, respectively in *Morus alba* L. leaves var. Zolwinska Wielkolistna. Variation in chlorophylls reported in the present study can be explained on the basis that plant or plant parts that grow under sun light or are exposed to higher light intensities have higher proportion of chlorophyll “a” compared to chlorophyll b. Plants or plant parts which grow under shade have higher proportion of chlorophyll “b” compared to chlorophyll a [26].

4.4 Total anthocyanins and carotenoids

The quantitative analysis of anthocyanins and carotenoids in various components of mulberry is presented in Table 1. Within the different segments of the mulberry plant, the concentration of anthocyanins was notably higher in the fruit (142.5 ± 3.1 mg/100 g), whereas the carotenoid levels were significantly elevated in the leaves (5.8 ± 0.3 mg/100 g). Moreover, there was a slight disparity in carotenoid content between roots and fruits. Earlier research studies have also documented comparable findings regarding the overall levels of anthocyanins and carotenoids distributed in various parts of the mulberry plant. A study by [27] indicated that the concentration of anthocyanins and carotenoids in the fruit of seven mulberry varieties ranged from 19.9 to 102.8 mg/g and 0.006 to 0.01 mg/g, respectively. The total carotenoid content found in leaves and roots fell within the range of 1.9–3.7 mg/100 g and 0.45–0.8 mg/100 g, respectively. Additionally, [18] reported that the anthocyanin content in different *Morus* fruits varied from 44.3 to 128.68 mg/100 g, while [28] documented a range of 0.51–28.6 mg/g for anthocyanin content in fruits from twelve mulberry varieties in China. The variability observed in the levels of anthocyanins and carotenoids across various parts of the mulberry plant can be attributed to factors such as climatic conditions, geographical location, and is particularly pronounced in regions exposed to abundant sunlight.

4.5 Content of phenolic acids

Table 2 and Fig. 1. Illustrates the phenolic composition and content in various segments of the mulberry plant. Predominant phenolic categories identified encompass phenolic acids, flavonoids, and anthocyanins. Analysis conducted via HPLC unveiled the existence of four hydroxybenzoic acids and seven hydroxycinnamic acids across diverse parts of the mulberry plant. Syringic acid emerged as the primary phenolic acid detected in the fruit, root, and shoot, succeeded by gallic acid found solely in the fruit and root. Protocatechuic acid was exclusively present in the leaves, whereas gentisic acid was identified solely in the fruit and root. Specifically, syringic acid levels were measured at 36.7 ± 3.2 mg/100 g in the fruit, 25.4 ± 2.3 mg/100 g in the root, and 20.2 ± 2.1 mg/100 g in the shoot. Gallic acid was quantified at 15.4 ± 2.2 mg/100 g in the fruit and 11.3 ± 1.1 mg/100 g in the root. Comparatively, the total hydroxybenzoic acid content significantly surpassed that of the root, shoot, and leaf, standing at 53.7 ± 2.1 mg/100 g in the fruit. Previous studies have also highlighted the presence of gallic acid, syringic acid, and protocatechuic acid as primary phenolic acids in mulberries [29]. Among the cinnamic acid derivatives, ferulic acid, m-coumaric acid, caffeic acid, and chlorogenic acid were the key phenolic acids identified in the fruit, root, shoot, and leaf. Within the fruit, ferulic acid was detected at 65.6 ± 5.1 mg/100 g, while m-coumaric acid was measured at 50.2 ± 4.6 mg/100 g. Quantification of the total hydroxycinnamic acids in various parts ranged from 52.2 ± 1.2 to 175.5 ± 2.6 mg/100 g, with the highest levels observed in the fruit (175.5 ± 2.6 mg/100 g) and root (135.4 ± 1.8 mg/100 g) [30]. Previous research has indicated that chlorogenic acid predominates as the hydroxycinnamic acid in mulberries, whereas p-coumaric acid, m-coumaric acid, and caffeic acid are present in lesser quantities. Studies have reported a range of 0.10–0.16 mg/g of chlorogenic acid in white mulberry fruits, with others noting values from 0.1 to 3.1 mg/100 g [29, 31]. Evaluation of nineteen mulberry varieties revealed chlorogenic acid as the primary phenolic acid in mulberry leaves, with concentrations ranging from 2.46 to 10.2 mg/100 g [7].

Table 2 Content of individual phenolic compounds (mg/100 g) in different parts of mulberry (*Morus alba* L.) Cv. *Ichinose*

Phenolic compounds	Fruit	Root	Shoot	Leaf	LSD
<i>a) Hydroxybenzoic acids (HBA)</i>					
i) Gallic acid	15.4 ± 2.2 ^{b,2}	11.3 ± 1.1 ^{b,1}	Nd	Nd	1.1
ii) Protocatechuic acid	Nd	Nd	Nd	2.1 ± 0.5	–
iii) Gentisic acid	1.6 ± 1.1 ^{a,1}	1.5 ± 0.8 ^{a,1}	Nd	Nd	0.4
iv) Syringic acid	36.7 ± 3.2 ^{c,3}	25.4 ± 2.3 ^{c,2}	20.2 ± 2.1 ¹	Nd	1.2
LSD	2.4	2.3	–	–	
Total HBA	53.7 ± 2.1	38.2 ± 1.3	20.2 ± 2.1	2.1 ± 0.5	
% of HBA	10.1	10.4	7.4	0.83	
<i>b) Hydroxycinnamic acids (HCA)</i>					
i) Chlorogenic acid	18.8 ± 2.4 ^{c,2}	11.3 ± 1.6 ^{c,1}	11.1 ± 1.3 ^{b,1}	11.4 ± 1.1 ^{b,1}	1.3
ii) Caffeic acid	29.2 ± 2.6 ^{d,3}	20.4 ± 2.1 ^{d,2}	17.6 ± 1.6 ^{c,1}	Nd	2.1
iii) p-Coumaric acid	1.9 ± 0.2 ^{a,1}	1.9 ± 0.4 ^{a,1}	1.4 ± 0.3 ^{a,1}	Nd	0.7
iv) m-Coumaric acid	50.2 ± 4.6 ^{e,4}	41.3 ± 3.2 ^{e,3}	31.5 ± 2.4 ^{d,2}	25.6 ± 1.6 ^{c,1}	3.1
v) o-Coumaric acid	6.5 ± 2.1 ^{b,2}	4.6 ± 1.1 ^{b,1}	2.1 ± 0.5 ^{a,1}	12.4 ± 1.4 ^{b,3}	2.5
vi) Ferulic acid	65.6 ± 5.1 ^{f,4}	53.2 ± 3.5 ^{f,3}	35.2 ± 2.1 ^{e,2}	2.8 ± 0.6 ^{a,1}	5.3
vii) Sinapic acid	3.3 ± 1.1 ^{a,1}	2.7 ± 1.1 ^{a,1}	Nd	Nd	1.1
LSD	2.1	1.8	1.5	1.6	
Total HCA	175.5 ± 2.6	135.4 ± 1.8	98.9 ± 1.4	52.2 ± 1.2	
% of HCA	32.9	36.8	36.4	20.8	
<i>c) Flavonoids</i>					
i) Gallocatechin	Nd	Nd	Nd	3.5 ± 1.3 ^a	–
ii) Catechin	Nd	2.1 ± 0.56 ^a	Nd	Nd	–
iii) Epicatechin gallate	Nd	Nd	Nd	3.4 ± 1.1 ^a	–
iv) Epicatechin	6.1 ± 1.2 ^{b,2}	Nd	3.4 ± 1.1 ^{a,1}	Nd	1.3
v) Apigenin-7-O-rutinoside	7.5 ± 1.4 ^{b,2}	4.2 ± 1.1 ^{a,1}	3.8 ± 1.6 ^{a,1}	Nd	1.2
vi) Naringenin-7-O-glucoside	1.3 ± 0.3 ^a	Nd	Nd	Nd	–
vii) Apigenin-7-O-glucoside	1.5 ± 0.3 ^a	Nd	Nd	Nd	–
viii) Quercetin	59.3 ± 3.2 ^{e,4}	48.6 ± 3.6 ^{d,3}	41.4 ± 2.7 ^{c,2}	36.2 ± 1.6 ^{c,1}	3.3
ix) Isoquercetin	30.4 ± 2.1 ^{d,4}	22.6 ± 1.7 ^{c,3}	16.2 ± 1.4 ^{b,2}	15.4 ± 1.3 ^{b,1}	2.2
x) Myricetin	Nd	6.6 ± 1.2 ^b	Nd	Nd	–
xi) Kaempferol	10.4 ± 1.3 ^c	Nd	Nd	Nd	–
LSD	2.2	2.4	1.8	1.3	
Total flavonoids	116.5 ± 1.4	84.1 ± 1.2	64.8 ± 1.7	58.5 ± 1.3	
% of flavonoids	50.1	46.9	44.1	53.1	
<i>d) Anthocyanins</i>					
i) Cyanidin-3, 5-diglucoside	7.3 ± 1.1 ^a	Nd	Nd	Nd	–
ii) Pelargonidin-3,5-diglucoside	5.4 ± 1.1 ^a	Nd	Nd	Nd	–
iii) Cyanidin-3, glucoside	16.4 ± 2.1 ^b	Nd	Nd	Nd	–
iv) Cyanidin-3, rutinoside	25.6 ± 2.3 ^c	Nd	Nd	Nd	–
LSD	2.5				

Values are mean ± SD (n = 3); LSD least significant difference (p ≤ 0.05), nd not detected

Values with different superscript lowercase letters (a–f) and numerical (1–4) differ significantly (p ≤ 0.05)

4.6 Content of flavonoids

Table 2 and Fig. 1. HPLC analysis demonstrated the identification of eleven flavonoids in various sections of the mulberry plant. Quercetin and isoquercetin emerged as the prominent flavonoids present across all parts of the mulberry plant. The content of quercetin ranged from 36.2 ± 1.6 to 59.3 ± 3.2 mg/100 g, while the content of isoquercetin ranged from 15.4 ± 1.3 to 30.4 ± 2.1 mg/100 g. Among the assessed parts, the quantities of quercetin and

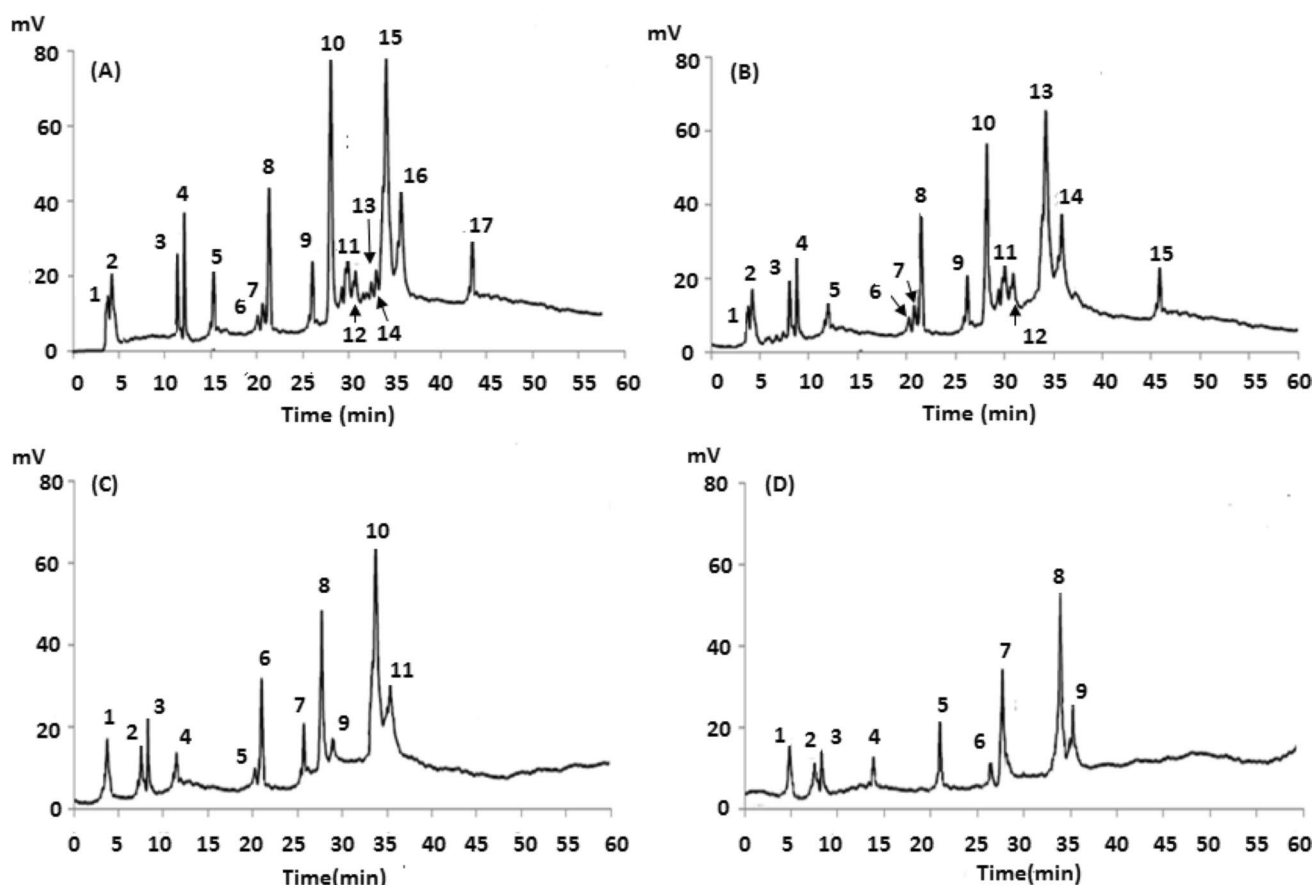


Fig. 1 HPLC chromatogram of phenolic acids and flavonoids of fruit (A), root (B), shoot (C) and leaf (D) of Mulberry Cv. Ichinose. Peak identification. Fruit (A): 1=gallic acid, 2=chlorogenic acid, 3=caffeic acid, 4=syringic acid, 5=epicatechin, 6=gentisic acid, 7=p-coumaric acid, 8=m-coumaric acid, 9=apigenin-7-o-rutinoside, 10=ferulic acid, 11=o-coumaric acid, 12=sinapic acid, 13=naringenin,7-o-glucoside, 14=apigenin,7-o-glucoside, 15=quercetin, 16=isoquercetin, 17=kaempferol. Root (B): 1=gallic acid, 2=chlorogenic acid, 3=caffeic acid, 4=syringic acid, 5=catechin, 6=gentisic acid, 7=p-coumaric acid, 8=m-coumaric acid, 9=apigenin-7-o-rutinoside, 10=ferulic acid, 11=o-coumaric acid, 12=sinapic acid, 13=quercetin, 14=isoquercetin, 15=myricetin. Shoot (C): 1=chlorogenic acid, 2=caffeic acid, 3=syringic acid, 4=epicatechin, 5=p-coumaric acid, 6=m-coumaric acid, 7=apigenin-7-o-rutinoside, 8=ferulic acid, 9=o-coumaric acid, 10=quercetin, 11=isoquercetin. Leaf (D): 1=chlorogenic acid, 2=protocatechuic acid, 3=gallo-catechin, 4=epicatechingallate, 5=m-coumaric acid, 6=ferulic acid, 7=o-coumaric acid, 8=quercetin, 9=isoquercetin

isoquercetin followed the trend fruit > root > shoot > leaf as shown in Table 2. It is noteworthy that kaempferol at 10.4 ± 1.3 mg/100 g was exclusively detected in fruit, while myricetin at 6.6 ± 1.2 mg/100 g was solely found in the root. Apigenin-7-O-rutinoside, in the range of 3.8 ± 1.6 – 7.5 ± 1.5 mg/100 g, was present in all parts except the leaf. Epicatechin, at 6.1 ± 1.2 mg/100 g, and 3.4 ± 1.1 mg/100 g, was identified solely in the fruit and shoot of the mulberry plant. Catechin and gallo catechin, at concentrations of 2.1 ± 0.56 mg/100 g and 3.5 ± 1.3 mg/100 g, were detected only in the root and leaf of the mulberry plant. Additionally, epicatechin gallate, at 3.4 ± 1.1 mg/100 g, was exclusively found in the mulberry leaf. Two flavonoid glycosides, naringenin-7-O-glucoside and apigenin-7-O-glucoside, present at lower levels (1.3 ± 0.3 and 1.5 ± 0.5 mg/100 g), were identified solely in the mulberry fruit. The comparison of data also underscored that the leaf (53.1%) and fruit (50.1%) of the mulberry plant significantly contributed to the flavonoid content compared to the root and shoot.

4.7 Content of anthocyanins

The study exclusively detected anthocyanins in mulberry fruit as depicted in Fig. 2A. The HPLC analysis unveiled the existence of four anthocyanins in different parts of the mulberry plant, including two monoglucoside anthocyanins and two diglucoside anthocyanins. Significantly, the levels of monoglucoside anthocyanins surpassed those of diglucoside

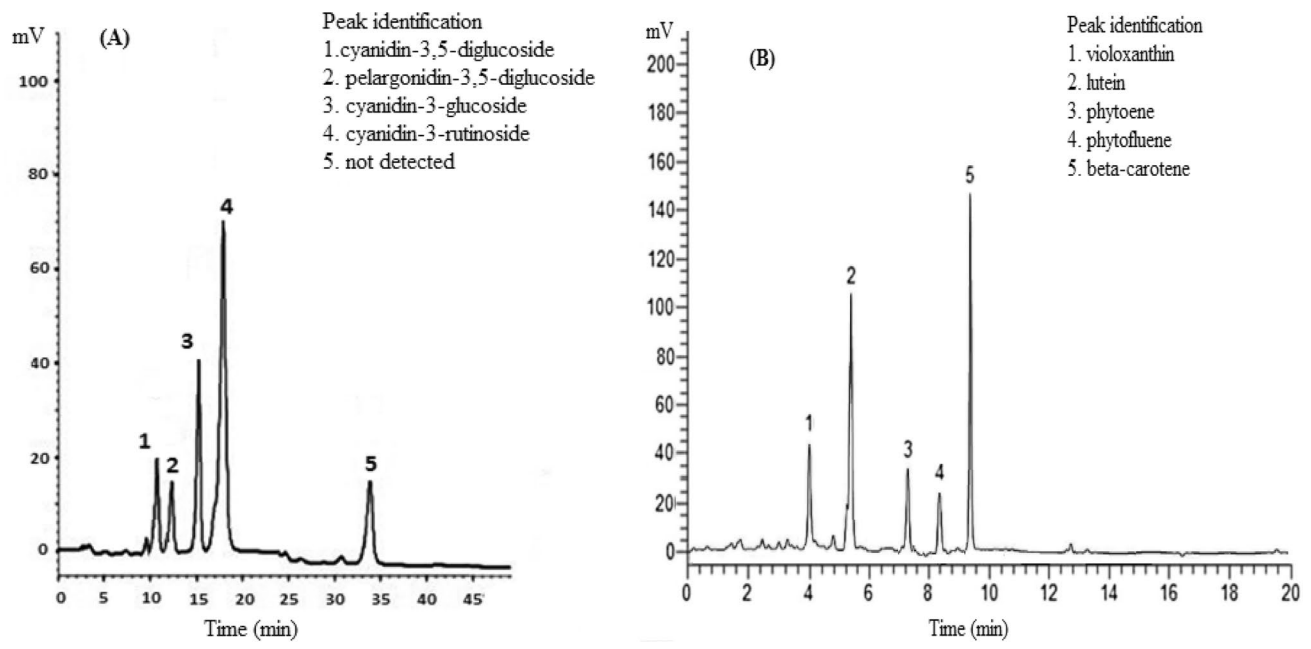


Fig. 2 HPLC chromatogram of fruit anthocyanins (A) and leaf carotenoids (B) of Mulberry Cv. Ichinose

anthocyanins as detailed in Table 2. Among the monoglucoside anthocyanins, cyanidin-3-rutinoside emerged as the major anthocyanin in mulberry fruit with a content of 25.6 ± 2.3 mg/100 g. Conversely, the contents of the two diglucoside anthocyanins exhibited a slight variance in the mulberry fruits. Previous studies have indicated that cyanidin derivatives were the primary anthocyanins in mulberry fruits. Furthermore, the content of cyanidin-3-rutinoside in Chinese mulberry fruits was reported to range from 31.9 ± 1.4 to 43.2 ± 3.8 mg/100 g fw, while Cyanidin-3-glucoside ranged from 65.6 ± 1.6 to 436.7 ± 24.3 mg/100 g fw [30, 32]. The disparities in anthocyanin content among mulberries can be attributed to factors such as cultivars and the stage of maturity.

4.8 Content of carotenoids

Figure 2B HPLC analysis revealed the presence of 5 carotenoids in various parts of mulberry. The content of individual carotenoids identified in different parts of mulberry is reported in Table 3. Among the parts, leaf had significantly higher content of carotenoids followed by shoot. Among the identified carotenoids, beta carotene emerged as the primary carotenoid in leaf followed by lutein. Conversely, lutein was predominant carotenoid in the shoot, followed by violaxanthin. In the leaf, beta-carotene and lutein were quantified at levels of 2.1 ± 0.3 and 0.92 ± 0.03 mg/100 g, respectively. In the shoot, the quantities of lutein and violaxanthin were 0.32 ± 0.01 and 0.16 ± 0.03 mg/100 g. Beta-carotene contributed approximately 47.7% to the carotenoid content in the leaf, while lutein contributed 37.6% in the shoot. Analysis

Table 3 Content of individual carotenoids (mg/100 g) in different parts of mulberry (*Morus alba* L.) Cv. Ichinose

Carotenoid compound	Fruit	Root	Shoot	Leaf	LSD
Violaxanthin	$0.05 \pm 0.01^{a,1}$	$0.05 \pm 0.01^{a,1}$	$0.16 \pm 0.03^{a,1}$	$0.75 \pm 0.02^{b,2}$	0.3
Lutein	$0.07 \pm 0.02^{a,1}$	$0.07 \pm 0.02^{a,1}$	$0.32 \pm 0.01^{b,2}$	$0.92 \pm 0.03^{b,3}$	0.4
Phytoene	$0.02 \pm 0.01^{a,1}$	$0.02 \pm 0.01^{a,1}$	$0.12 \pm 0.01^{a,1}$	$0.35 \pm 0.02^{a,2}$	0.2
Phytofluene	$0.03 \pm 0.01^{a,1}$	$0.04 \pm 0.03^{a,1}$	$0.11 \pm 0.02^{a,1}$	$0.21 \pm 0.01^{a,2}$	0.1
Beta carotene	$0.09 \pm 0.01^{a,1}$	$0.03 \pm 0.01^{a,1}$	$0.14 \pm 0.01^{a,1}$	$2.1 \pm 0.3^{c,2}$	0.2
LSD	0.1	0.1	0.1	0.2	
Total carotenoids	0.26 ± 0.4	0.21 ± 0.03	0.85 ± 0.03	4.4 ± 0.2	

Values are mean $\pm$ SD (n = 3); LSD least significant difference ($p \leq 0.05$)

Values with different superscript lowercase letters (a–c) and numerical (1–3) differ significantly ($p \leq 0.05$)

of the data also revealed marginal differences in carotenoid contents between fruit and root. It has been reported that beta-carotene and lutein are the primary carotenoids in mulberry leaves [33]. Variations in carotenoid composition and concentration among different parts of the mulberry could be attributed to genetic diversity across various varieties and organs. [1, 32].

4.9 Antioxidant activity of mulberry parts

Antioxidant activity of mulberry parts was assessed using four different assays. Ascorbic acid, gallic acid, and butylated-hydroxy toluene (BHT) were employed as positive controls in the assays. Results depicted in Table 4 exhibited significant variations in antioxidant activity across different mulberry parts. Fruit displayed the highest DPPH radical scavenging, ferric reducing ability power, hydroxyl radical scavenging activity, and inhibition in beta-carotene bleaching activity, followed by root, shoot, and leaf. DPPH radical scavenging activity of mulberry parts ranged from 71.5 ± 1.4 – $89.3 \pm 1.8\%$, while hydroxyl radical scavenging activity varied from 36.7 ± 1.8 – $78.3 \pm 1.3\%$. Inhibition of beta-carotene bleaching activity ranged from 18.1 ± 1.9 – $29.4 \pm 2.2\%$. Our findings on antioxidant activity align with previous reports on mulberry. Evaluation of 25 mulberry fruit varieties showed that DPPH radical scavenging activity ranged from 39.0 ± 1.7 – $72.8 \pm 0.8\%$, while hydroxyl radical scavenging activity was between 21.0 ± 1.2 – $64.5 \pm 1.1\%$. Assessment of antioxidant activity in mulberry leaves from various varieties indicated DPPH radical scavenging activity in the range of 31.2 – $56.4 \mu\text{mol TE/g}$. Furthermore, evaluation of DPPH radical scavenging activity in white and black mulberry fruits revealed scavenging activity ranging from 3.62 – 12.9 mg TE/g [31, 34]. Based on the literature comparison, it can be inferred that the Ichinose mulberry variety assessed in this study exhibits high antioxidant potential, particularly in DPPH radical scavenging and hydroxyl radical scavenging activity. Variation in antioxidant activity among mulberry parts may be attributed to varying polyphenol contents, which were notably higher in fruit and root. In the present study, positive correlations existed between the total phenols and the antioxidant activity measured by DPPH radical scavenging assay ($r=0.96$) and hydroxyl radical scavenging assay ($r=0.91$).

4.10 EC_{50} values of mulberry parts for antioxidant assays

For better comparison of antioxidant activity, results obtained from the four antioxidant assays were expressed as EC_{50} values. The EC_{50} values of extracts of different mulberry parts were compared with standard ascorbic acid, gallic acid and BHT and are presented in Table 4. EC_{50} values of the fruit were significantly lower compared to other parts for all the antioxidant assays, thus confirming that fruit had the highest antioxidant potential. For the antioxidant assays studied in the present work, EC_{50} values ranged from 152.5 ± 2.1 – $181.3 \pm 3.5 \mu\text{g/mL}$ for DPPH radical scavenging, 201.2 ± 2.5 – $325.6 \pm 3.2 \mu\text{g/mL}$ for FRAP, 174.2 ± 2.2 – $236.2 \pm 2.6 \mu\text{g/mL}$ for hydroxyl radical scavenging and 472.2 ± 4.5 – $535.2 \pm 3.5 \mu\text{g/mL}$ for beta-carotene bleaching activity respectively. Based on the EC_{50} data, it can be inferred that extracts of different mulberry parts do possess significant antioxidant potential especially the fruit extract. But, close comparison of EC_{50} values with the standard antioxidants indicated that DPPH radical scavenging, FRAP and inhibition in beta-carotene bleaching activity of the studied mulberry parts was significantly lower than standard antioxidants. However, for hydroxyl radical scavenging assay; EC_{50} values of mulberry fruit, root and shoot were significantly lower than standard antioxidants, thus indicating that these parts of mulberry can serve as potential antioxidants and could exert higher antioxidant effect than the standard antioxidants. In a study examining two mulberry fruit varieties of Kashmir, north India reported an EC_{50} value of $107.9 \pm 3.8 \mu\text{g/mL}$ in *Zagtul* variety and $307.2 \pm 4.2 \mu\text{g/mL}$ in *Chattatual* variety for DPPH radical scavenging activity [3].

5 Conclusions

The findings from this study indicate that the Ichinose cultivar of mulberry fruit is significantly enriched with bioactive compounds and demonstrates superior antioxidant properties, particularly notable in its hydroxyl radical scavenging capacity when contrasted with the root, shoot, and leaf tissues. Given the substantial presence of antioxidants and bioactive molecules in the Ichinose variety, it is proposed that its cultivation be expanded on a commercial scale not only to benefit sericulture at the foundational level of silkworm diet but also to enhance value addition in the silk industry. Moreover, due to the high concentration of bioactive compounds, the Ichinose mulberry fruit holds considerable potential for further application in the formulation of nutraceuticals aimed at health promotion. Additionally, owing to

Table 4 Antioxidant activity and corresponding EC₅₀ values for the used assays in different parts of mulberry (*Morus alba* L.) Cv. *Ichinose*

Antioxidant activity	Mulberry part					EC ₅₀ (µg/mL)						
	Fruit	Root	Shoot	Leaf	LSD	Fruit	Root	Shoot	Leaf	GA	AA	BHT
DPPHSA(%P)	89.3±1.8	82.2±1.3	73.5±2.5	71.5±1.4	2.6	152.5±2.1	166.3±2.4	178.5±3.1	181.3±3.5	16.1±0.36	19.6±0.45	18.9±0.41
FRAP (O.D)	0.77±0.04	0.7±0.11	0.32±0.08	0.12±0.04	0.31	201.2±2.5	209.4±2.1	288.9±3.5	325.6±3.2	9.7±0.16	30.6±0.55	57.7±1.1
HRSA (%)	78.3±1.3	73.3±1.5	61.9±1.6	36.7±1.8	3.2	174.2±2.2	179.6±2.3	189.4±2.1	236.2±2.6	216.6±1.6	237.2±2.1	–
BCBA(%P)	29.4±2.2	24.1±2.6	21.4±3.9	18.1±1.9	2.8	472.2±4.5	493.4±3.6	518.6±3.7	535.2±3.5	–	–	345.2±4.4

Values are mean ± SD (n = 3); LSD = least significant difference (p ≤ 0.05)

DPPHSA DPPH scavenging activity, FRAP ferric reducing ability power, HRSA hydroxyl radical scavenging activity, BCBA beta-carotene bleaching activity, IP inhibition power, o. d optical density, GA gallic acid, AA ascorbic acid, BHT butylatedhydroxy toluene

its potent antioxidant capabilities and considerable anthocyanin content, it offers promising utility as a natural colouring additive within both the food and pharmaceutical sectors. This would not only benefit the sericulture industry but also open avenues for its application in nutraceutical formulations aimed at health promotion. It thus stands as a multifunctional crop with diverse benefits for human health and industry.

Author contributions Lubna Altaf performed the main extraction, analysis, and data tabulation of the bioactive compounds, and wrote the main manuscript text; Shabir Ahmad Wani designed and supervised the experimental studies; Peerzada Rashid Hussain supervised the study, analyzed the data and co-authored the manuscript; Prashant Suradkar performed the HPLC analysis of bioactive compounds; Mohammad Farooq Baqual facilitated the financial support and helped in designing the experiment and Aabid Ahmad Bhat helped in data analysis and tabulation. All authors reviewed the manuscript.

Data availability The data used in this study is available from Krishikosh, the institutional repository of the Indian National Agricultural Research System, and can be accessed at <https://krishikosh.egranth.ac.in/handle/1/5810197942>.

Declarations

Ethics approval and consent to participate The plant material used in this study was obtained from the germplasm collection maintained by College of Temperate Sericulture, Mirgund, Sher-e- Kashmir University of Agricultural Sciences and Technology. All plant material was cultivated within the university's facilities. The collection and usage of plant material strictly adhered to the guidelines provided by the university's ethical committee and complied with local regulations regarding plant research. Ethical approval for the collection and use of plant material was obtained from the relevant authorities at College of Temperate Sericulture, Mirgund, Sher-e- Kashmir University of Agricultural Sciences and Technology.

Competing interests All the authors declare that they have no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Jan B, Parveen R, Zahiruddin S, Khan MU, Mohapatra S, Ahmad S. Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals. A review. Saudi J Biol Sci. 2021;28:3909–21. <https://doi.org/10.1016/j.sjbs.2021.03.056>.
2. Kadam RA, Dhupal ND, Khyade VB. The mulberry, *Morus alba* (L.): the medicinal herbal source for human health. Int J Curr Microbiol App Sci. 2019;8:2941–64. <https://doi.org/10.20546/ijcmas.2019.804.341>.
3. Jan B, Zahiruddin S, Basist B, Irfan M, Abass S, Ahmad S. Metabolomic profiling and identification of antioxidant and antidiabetic compounds from leaves of different varieties of *Morus alba* Linn grown in Kashmir. ACS Omega. 2022;7:24317–28.
4. Hao J, Gao Y, Xue J, Yang Y, Yin J, Wu T, Zhang M. Phytochemicals, pharmacological effects and molecular mechanisms of mulberry. Foods. 2022;11:1170. <https://doi.org/10.3390/foods11081170>.
5. Li H, Wang L, Feng J, Jiang L, Wu J. Effects of oral intake fruit or fruit extract on skin aging in healthy adults: a systematic review and meta-analysis of randomized controlled trials. Front Nutr. 2023;10:1232229. <https://doi.org/10.3389/fnut.2023.1232229>.
6. Polumackanycz M, Wesolowski M, Viapiana A. *Morus alba* L. and *Morus nigra* L. leaves as promising food source of phenolic compounds with antioxidant activity. Plant Foods Human Nutr. 2021;76:458–65. <https://doi.org/10.1007/s11130-021-00922-7>.
7. Yu Y, Li H, Zhang B, Wang J, Shi X, Huang J, Yang J, Zhang Y, Deng Z. Nutritional and functional components of mulberry leaves from different varieties: evaluation of their potential as food materials. Int J Food Prop. 2018;21:1495–507. <https://doi.org/10.1080/10942812.2018.1489833>.
8. Hussain PR, Suradkar P, Sadiya J, Heena A, Sadaf P. Influence of postharvest gamma irradiation treatment on the content of bioactive compounds and antioxidant activity of fenugreek (*Trigonella foenum-graceum* L.) and spinach (*Spinacia oleracea* L.) leaves. Innov Food Sci Emerg Technol. 2016;33:268–81. <https://doi.org/10.1016/j.ifset.2015.11.017>.
9. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. Food Chem. 2005;91:571–7. <https://doi.org/10.1016/j.foodchem.2004.10.006>.
10. Guisti MM, Wrolstad RE. Characterization and measurement of anthocyanins by UV–visible spectroscopy. In: Wrolstad RE, editor. Current protocols in food analytical chemistry. New York: John Wiley & Sons Inc.; 2001. p. F1.2.1–F1.2.13.
11. Kimura M, Rodriguez-Amaya DB. Harvest plus handbook for carotenoids analysis. Washington DC: International food policy research Institute; 2004. p. 2-11–2 & #x02212;54.
12. Kuppusamy P, Lee KD, Song CE, Ilavenil S, Srigopalram S, Arasu MV, Choi KC. Quantification of major phenolic and flavonoid markers in forage crop *Lolium multiflorum* using HPLC-DAD. Braz J Pharmacog. 2018;28:282–8. <https://doi.org/10.1016/j.bjp.2018.03.006>.

13. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft Technol.* 1995;28:25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
14. Oyaizu M. Studies on products of browning reactions: antioxidant activity of products of browning reaction prepared from glucosamine. *Jap J Nutr.* 1986;44:307–15. <https://doi.org/10.5264/eiyogakuzashi.44.307>.
15. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. *Int J Pharmacol.* 1990;58:237–40. [https://doi.org/10.1016/S0378-8741\(03\)00043-6](https://doi.org/10.1016/S0378-8741(03)00043-6).
16. Sarkar A, Bishayee A, Chatterjee M. Beta-carotene prevents lipid peroxidation and red blood cell membraneprotein damage in experimental hepato carcinogenesis. *Cancer Biochem Biophys.* 1995;15:111–25.
17. Cochran WG, Cox GM. *Experimental designs*. 2nd ed. New York: John Wiley & Sons Inc.; 1992. p. 95–100.
18. Danica SD, Kostic DA, Stojanovic GS, Mitic SS, Mitic MN, Dordevic AS. Phenolic composition, antioxidant activity, mineral content and antimicrobial activity of fresh fruit extracts of *Morus alba* L. *J Food Nutr Res.* 2014;53:22–30.
19. Muhammad AK, Aziz AR, Shafiqul I, Proma K, Shahnaj P, Md BI, Mosharrof H, Mamunur R, Golam S, Shamima N, Nurul M, H. M. and AHM, K. A. A comparative study on the antioxidant activity of methanolic extracts from different parts of *Morus alba* L. (Moraceae). *BMC Res Notes.* 2013;6:24.
20. Denise S, Maykelis D, Solare Y, Enrique MR, Inelvis CC, Leydi F, Carballo NP, Yudit LM, Gecernir C, Jacqueline A. Roots from mulberries (*Morus alba*) natural and hybrids varieties, phenolic content and nutraceutical potential as antioxidant. *J Appl Pharm Sci.* 2016;6:063–9.
21. Ines T, Walid E, Nizar T, Manel Z, Maria GC, Ali F. Phenols, flavonoids and antioxidant and antibacterial activity of leaves and stem bark of *Morus* Species. *Int J Food Prop.* 2014;17(4):842–54.
22. Srivastava S, Kapoor R, Thathola A, Srivastava RP. Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). *Int J Food Sci Nutr.* 2006;57:305–13.
23. Alidee T, Jales M, Hjaïj ND, Almouna A. Estimation of some phytochemical compounds and antioxidant properties of leaves from different mulberry varieties grown in Syria. *Evid-Based Complement Alter Med.* 2023;9929276:1–6. <https://doi.org/10.1155/2023/9929276>.
24. Kalaivani M, Jebanesan A, Maragathavalli S, Annadurai B, Gangwar SK. Studies on chlorophyll content, soluble protein, carbohydrate and moisture content of *Morus alba* Linn. *Int J Sci Nat.* 2013;4:131–7.
25. Monika P, Flaczyk E, Kmiecik D, Buchowski MS, Staniek H, Tomczak-Graczyk A, et al. Functional properties and antioxidant activity of *Morus alba* L. leaves var. Zolwinska Wielkolistna (WML-P)- The effect of controlled conditioning process. *Antioxi.* 2020;9:9080668. <https://doi.org/10.3390/antiox9080668>.
26. Nguyen NHK, Nguyen TDA, Anh PK, Truc TT. Microwave assisted extraction of chlorophyll and polyphenol with antioxidant activity from *Pandanus amaryllifolius* Roxb. Vietnam. *IOP conf Ser Mat Sci Engg.* 2021;1166:012039. <https://doi.org/10.1088/1757-899X/1166/1/012039>.
27. Venkatesh KR, Chauhan S. Biochemical constituents of different parts of mulberry genotypes. *Int J Agric Sci.* 2011;3:90–6.
28. Kim I, Lee J. Variations in anthocyanins profiles and antioxidant activity of 12 genotypes of mulberry (*Morus* Spp.) fruits and their changes during processing. *Antioxi.* 2020;9:242–53. <https://doi.org/10.3390/antiox9030242>.
29. Butkhup L, Samappito W, Samappito S. Phenolic composition and antioxidant activity of white mulberry (*Morus alba* L.) fruits. *Int J Food Sci Technol.* 2013;48:934–40. <https://doi.org/10.1111/ijfs.12044>.
30. Sanchez-Salcedo EM, Mena P, Garcia-Viguera G, Martinez JJ, Hernandez F. Phytochemical evaluation of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry fruits, a starting point for the assessment of their beneficial properties. *J Funct Foods.* 2015;12:399–408. <https://doi.org/10.1016/j.jff.2014.12.010>.
31. Gundogdu M, Muradoglu F, Sensoy RG, Yilmaz HJSH. Determination of fruit chemical properties of *Morus nigra* L., *Morus alba* L. and *Morus rubra* L. by HPLC. *Scien Hort.* 2011;132:37–41. <https://doi.org/10.1016/j.scienta.2011.09.035>.
32. Chen T, Fei-Fan S, Qing-Yue F, Yu-Xiong J, Chen-Man Z, Wei-Guo Z, Dong-Yang Z, Xiao-Hui Y, Fu-Liang C. Evaluation of the chemical composition and antioxidant activity of mulberry (*Morus alba* L.) fruits from different varieties in China. *Mole.* 2022;27:27092688. <https://doi.org/10.3390/molecules27092688>.
33. Zhu L, Yu-Qing Z. Identification and analysis of the pigment composition and sources in the colored cocoon of the silkworm, *Bombyx mori* by HPLC-DAD. *J Insect Sci.* 2014;14:1–10. <https://doi.org/10.1673/031.014.31>.
34. Imran M, Khan H, Shah M, Khan F. Chemical composition and antioxidant activity of certain *Morus* species. *J Zhejiang Uni Sci-B, Biomed Biotechnol.* 2010;11:973–80. <https://doi.org/10.1631/jzus.B1000173>.