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Exploring The Nutritional Profile of Wild Mulberry Landraces Grown in Northern Pakistan: A Promising Dietary Option

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Summary: The rising demand for nutritious and functional foods has spurred interest in underutilized natural resources to enhance consumer nutritional security. This study investigates the nutritional and functional properties of fruits from five wild Wild mulberry *landraces* (WM1–WM5) collected from Gilgit, Pakistan. The fruits were analyazed for pH, soluble solids titratable acidity, moisture, protein, fibre, ash, ascorbic acid, total sugar, anthocyanins, flavonoids, carotenoids, total phenolics and antioxidants activity. Results indicated pH values ranging from 3.62- 6.79, total soluble solids at 14.14 –17.06 °Brix, titratable acidity between 0.17–0.28%, moisture content of 62.65–81.7%. Protein, fiber and ash content ranged from 0.85–1.66%, 6.83–10.88%, and 0.351–0.672%, respectively. Ascorbic acid levels varied between 12.41–21.09 mg/100 g, total sugars 5.7–9.86 mg/100 g, anthocyanins 9.72–12.27 mg/100 g, flavonoids 71.64–81.9 mg/100 g, carotenoids 0.65–1.04 mg/100 g, and total phenolics 16.35–31.29 mg/100 g. Antioxidant activity, measured as DPPH scavenging, ranged from 70.99–87%. These results are comparable with reported values of common edible mulberry. These findings underscore the nutritional and functional richness of wild mulberry fruits, presenting them as a promising resource for food and feed applications. The study provides critical baseline data to support the development of value-added products and establish a wild mulberry value chain, fostering socioeconomic growth in mountain communities.

Keywords: Wild mulberry (*Morus*), Nutritional composition, Antioxidant activity, Functional foods, Value-added products.

Introduction

Mulberry (*Morus. L*) of the Moraceae family is an out-breeding, heterozygous and commercially significant perennial tree known for its high biomass yield and protein rich leaves, widely utilized in sericulture, agroforestry, and horticultural practices [1]. Mehmood *et al* (2012) states that the genus *Morus* is includes 68 recognized species with at least 100 identified varieties also includes 24 species and one subspecies [2]. In Pakistan, India and Iran the mulberries of various colors are extensively cultivated which includes black (*Morus nigra L*) white (*Morus alba L*) and red (*Morus rubra L*). In Gilgit-Baltistan of Pakistan mulberries are grown in extensive quantity and the cultivation is extending in high altitude in the Himalaya-Hindu Kush region [3].

The edible mulberry landraces species black (*Morus nigra L*) white (*Morus alba L*) and red (*Morus rubra L*). are rich in essential nutrients such as nutrients, minerals, and phytochemicals with significant health attributes [4-5]. Various carotenoids such as cyanidin-3-glucoside and cyanidin 3-rutinoside) and flavonoids (e.g cynadin 3-glcoside, isoquercitrin, rutin) are also found in mulberry fruit [4, 6] which play an essential role in maintenance of good health In addition mulberry is also a source of alkaloid deoxynojirimycin (DNJ), which is effective againts HIV/AIDS [7, 8]. Khalid *et al*. (2011) reported that the anti-HIV activity of mulberry is over four times greater than that of blueberries, potentially due to the lignified components in mulberry juice [9]. Mulberries are consumed as dried and fresh fruits, and processed into molasses, juices, and alcoholic beverages [10-11].

Mulberry holds significant ecological and economic importance in the agroecology of Gilgit-Baltistan. It is the most prominent plant cultivated under social forestry in both irrigated and water-stressed areas, serving as an excellent feed source for livestock. Its wood is utilized for fuel and construction purposes. The region is home to various wild mulberry landraces. While some improved varieties are consumed as fresh and dried fruits, wild species

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remain underutilized and are often wasted on farms [12].

In Gilgit-Baltistan mulberry (*Morus* spp.) is recognized as one of the common traditional fruit plants along with other fruits such as apricot, apple, almond, and walnut. Locally called Edible mulberry (Shahtoot) comprises white mulberry (*Morus alba*), brown mulberry (*Morus laevigata*) and black mulberry *(Morus nigra)*, which categorized by relatively large fruit size. These types are generally grafted or improved cultivars, widely cultivated and commonly consumed in both fresh and dried forms. In contrast, wild mulberry locally known as Shatoo) has a wide array of races in the region with common fruiting landraces and some non-fruiting species. Wild mulberry remains mainly underutilized, with its fruits often left unharvested, the principal investigation of this study is to investigate the nutritional profile of wild mulberries, aiming to enhance their value as a food source and a potential revenue stream. This research seeks to explore innovative business and food processing opportunities to ensure food security and promote the economic well-being of the farming community. By encouraging cottage industries and establishing a robust mulberry value chain, the study aims to contribute to the sustainable development of the region.

Experimental

*Collection of fruit sample***:** The fruits of five wild mulberry landraces (WM1, WM2, WM3, WM4, and WM5) were collected from different localities of district Gilgit, Gilgit-Baltistan, Pakistan in 2023. Samples were obtained according to the established botanical field collecting methods [13]. For each sample, several fruits were gathered, mixed and enclosed in opaque plastic bags to protect them from sunlight. Soon after they were collected, samples were moved to the lab. The fruits were cleaned of obvious dirt and insect remains before analysis. Fruits were dehydrated, compressed, and put into dark glass bottles before being placed in a deep freezer until more studies could be done. Fresh fruit samples that had just arrived in the lab were used to evaluate pH and titratable acidity as followed by Imran *et al*. (2010) [4].

*Sample preparation***:** An electric blender was used to remove pulp for analytical samples. To remove solid particles, the pulp was sieved through a muslin cloth. The chemicals and reagents utilized in the experiment were of the highest quality.

*Physico-chemical Characteristics***:** Physicochemical analysis (moisture, T SS, pH, titratable acidity, ash, sugars, protein, and ascorbic acid) of mulberry samples. A digital pH meter that had been calibrated with pH 4 and pH 7 was used to monitor pH The titrimetric technique was used to test titratable acidity [4,14].

Functional composition

Total phenolic Content (TPC): The technique used to determine the total phenolic content was Folin-Ciocalteu following by the methodology adopted by Keskin *et al*. (2014) with modifications slightly [15]. Gallic acid of various concentration was used to construct a standard curve. The total phenolic content was calculated in milligram of Galic acid equivalent per gram of dry weight based on absorbance value obtained.

Anthocyanin: The methodology adopted by Viskelis *et al*. (2009) [16] was used to determine the anthocyanin content in wild mulberry. Cynaidin-3-O glucoside chloride as slandered was used for the calibration curve for quantification. The anthocyanin content was expressed as cyaidin-3-O-glucoside (CyE) based on calibration curve.

Flavonoids: The total flavonoid content was determined by following the method of Chandra *et al.* (2014) [17]. A calibration curve was constructed for the quantification of total flavonoid using different concentration of quercetin as internal standard.

Carotenoids: Carotenoid content were evaluated by adopting the methodology as outlined by Cerón-García *et al.* (2018), with modifications slightly [18]. Then finally expressed the results as carotene equivalents in milligram per 100 grams of wet weight.

Antioxidant capacity: The anti-oxidant activity of the samples of wild mulberry were determined by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) protocol followed by Elsharkawy *et al*. (2019) and Merculieff *et al*. (2014) [19-20]. During this method 100 µl of extract (at a concentration oof 1mg/ml) was mixed with 1.9 ml of methanolic DPPH solution (150 µM) in a reaction flask. The mixture was shaken thoroughly to ensure proper mixing. Afterward, the solution was incubated in the dark at 37°C for 35 minutes. UV spectrophotometer was used to measure the absorbance of the resulting solution at 517 nm (Thermo Spectronic GENESYS 10UV). The antioxidant activity was calculated as the percentage of DPPH scavenging capacity using the formula as follows.

$$\text{Antioxident capacity}(\%) = \frac{\text{Absorbance at blank state} - \text{sample}'\text{sabsorbance}}{\text{Absorbance at blank state}} x100$$

Statistical analysis: The results were reported as the mean ± standard deviation (SD) based on three independent assessments. Statistical analysis was performed using the software Statistix 8.1. One-way analysis of variance (ANOVA) were employed to determines significance difference among the means, with the level of significance set at $p < 0.05$.

Results and Discussion

Physico-chemical profile of the wild mulberry landrace

The analytical results for pH, total soluble solids, titratable acidity and moisture content of the wild mulberry landraces are summarized in Table 1. The pH with highest value was observed in WM5 (6.79), while the lowest was recorded in WM2 (3.62). Similarly, the total soluble solids ranged from a maximum of 17.06 °Brix in WM5 to a minimum of 14.14 °Brix in WM3. Titratable acidity showed the highest value in WM5 (0.282%) and the lowest in WM3 (0.17%). In terms of moisture content, WM1 exhibited the highest percentage (81.7%), whereas WM5 recorded the lowest (62.65%).

The nutritional profile of wild mulberry

The nutritional profile of the wild mulberry landraces is detailed in Table 2. The protein content ranged from the highest value of 1.66 g/100 g FW in WM5 to the lowest of 0.85 g/100 g FW in WM1. Fiber content was notably highest in WM1 (10.88 g/100 g FW) and lowest in WM4 (6.83 g/100 g FW). The ash content peaked at 0.672 g/100 g FW in both WM3 and WM4, while the lowest value of 0.351 g/100 g FW was observed in WM1.The content of ascorbic acid was highest in WM2 (21.09 mg/100g) and lowest in WM4 (12.41 mg/100 g). Similarly, total sugar content was recorded at a maximum of 9.86 mg/100 g in WM4 and a minimum of 5.7 mg/100 g in WM2. These variations in nutritional parameters were statistically significant ($p < 0.05$), reflecting distinct patterns among the wild mulberry landraces.

Functional attributes of wild mulberry

The functional attributes of the wild mulberry landraces at different locations are presented in Table 3. The anthocyanin content ranged from a minimum of 9.72 mg CGE/100 g in WM3 to a maximum of 12.27 mg CGE/100 g in WM5. Total flavonoid content varied from 71.64 mg CGE/100 g in WM4 to 81.9 mg CGE/100 g in WM3. Similarly, total carotenoid content ranged between 0.65 mg CGE/100 g in WM5 (lowest) and 1.04 mg CGE/100 g in WM3 (highest). The antioxidant activity was highest in WM3 (87%) and lowest in WM4 (70.99%). Likewise, total phenolic content reached its maximum in WM3 at 31.29 mg/100b, while the lowest was observed WM4 at 16.35 mg/100 g. These findings demonstrate significant variations in the functional properties of the wild mulberry landraces.

Table-1: Comparative analysis of physicochemical characteristics of wild mulberry landrace.

Landraces	pH	Soluble solids (ºBrix)	Titratable acidity (%)	Moisture contents (%)
WM_1	5.79 ± 0.03^{a}	15.04± 0.12^{d}	0.24± 0.12e^{d}	82.70± 0.22^{c}
W_{M2}	3.62 ± 0.02$^{a\,f}$	15.37± 0.20^{d}	0.197± 0.04^{h}	78.18± 0.04^{d}
W_{M3}	3.87± 0.02^{f}	14.14± 0.11^{f}	0.177± 0.13^{h}	74.84± 0.33^{h}
W_{M4}	4.24± 0.04^{e}	14.74± 0.04^{ef}	0.28± 0.11^{ab}	67.32± 0.42^{l}
W_{M5}	6.79± 0.12^{c}	17.06± 0.12^{a}	0.28± 0.32^{ab}	62.65± 0.04^{m}
P value	0.05<	0.05<	0.05<	0.05<

Note: Wild Mulberry landraces (WM_1= wild mulberry 1, WM_2= wild mulberry 2, WM_3= wild mulberry 3, WM_4= wild mulberry 4 and WM_5= wild mulberry 5:
Values are means ± SD, Values having same letter on superscript within column do not differ significantly among five landraces varieties at $p \leq 0.05$.

Table-2: The nutritional characteristics of wild mulberry landrace.

Landraces	Protein content (g/100 g FW)	Fiber (g/100 g FW)	Ash content (g/100 g FW)	Ascorbic Acid (mg/100 FW)	Total Sugar (g/100 g FW
WM_1	0.80 ± 0.42^{c}	10.88± 0.04^{a}	0.35± 0.12^{a}	18.19± 0.52^{f}	7.47± 0.04^{fg}
WM_2	1.53± 0.23^{a}	9.27± 0.13^{cd}	0.43± 0.13^{a}	21.09± 0.02^{c}	5.70± 0.41^{j}
W_{M3}	1.54± 0.04^{a}	8.11± 0.12^{ef}	0.67± 0.41^{c}	19.54± 0.24^{d}	7.24± 0.12^{f}
W_{M4}	1.35± 0.12^{a}	6.83± 0.03^{e}	0.67± 0.15^{a}	12.41± 0.32^{j}	9.86± 0.05^{a}
W_{M5}	1.66± 0.03^{d}	7.47± 0.13^{e}	0.36± 0.25^{c}	13.00± 0.04^{k}	7.96± 0.12^{a}
P value	0.05<	0.05<	0.05<	0.05<	0.05<

Note: Wild Mulberry landraces (WM_1= wild mulberry 1, WM_2= wild mulberry 2, WM_3= wild mulberry 3, WM_4= wild mulberry 4 and WM_5= wild mulberry 5:
Values are means ± SD , Values having same letter on superscript within column do not differ significantly among five landraces varieties at $p \leq 0.05$.

Table-3: Comparative analysis of functional attributes of wild mulberry landrace.

Landraces	Anthocyanin content mg/100g	Total flavonoid mg/100g	Total carotenoid mg/100g	Antioxidant Activity %	Total phenolics mg/100g
WM_1	10.41 ± 0.04[a]	76.18± 0.02[a]	0.65± 0.02[g]	77.90± 0.04[a]	20.71± 0.12[a]
WM_2	10.48± 0.03[a]	78.28± 0.04[b]	0.91± 0.12[d]	81.30± 0.14[b]	30.42± 0.22[b]
WM_3	9.72± 0.02[b]	81.90± 0.12[c]	1.04± 0.03[a]	87.00± 0.32[c]	31.29± 0.02[c]
WM_4	10.48± 0.04[a]	71.64± 0.32[d]	0.84± 0.04[j]	70.99± 0.02[d]	16.35± 0.04[d]
WM_5	12.27± 0.12[c]	73.38± 0.04[f]	0.94± 0.02[j]	73.31± 0.03[e]	18.02± 0.32[e]
P value	0.05<	0.05<	0.05<	0.05<	0.05<

Note: Wild Mulberry landraces (WM_1= wild mulberry 1, WM_2= wild mulberry 2, WM_3= wild mulberry 3, WM_4= wild mulberry 4 and WM_5= wild mulberry 5:

Values are means ± SD , Values having same letter on superscript within column do not differ significantly among five landraces varieties at $p \leq 0.05$.

This study was conducted to explore the nutritional profile of five underutilized wild mulberry landraces grown in Gilgit-Baltistan. The fruits of these landraces are not used and wasted on the ground. This study explored nutritional potentials of these underutilized wild mulberry landraces and compared with improved /edible mulberry landraces varieties known as common edible mulberry such as *M. Alba, (white), M. Nigra, (black),* and *M. Laevigata* (brown) and fruits commonly used as fresh and dried) form. Different parameters such as pH, soluble solids titratable acidity, moisture, protein, fibre, ash, ascorbic acid, total sugar, anthocyanins, flavonoids, carotenoids, total phenolics and antioxidants activity were carried out. The pH values recorded in the current study ranged from 3.62–6.79. The lowest pH values were observed in landraces WM2, WM3 and WM4, which are comparable to those reported by Imran *et al*. (2010), In contrast, WM1 and WM5 exhibited relatively higher pH values. Soluble solids content (TSS) serves as an essential sensory characteristic indicating fruit sweetness [24]. In this study TSS ranged from 14.14 - 17.06 °Brix, which is slightly lower than reported ranged 17.60-21.97 °Brix in edible mulberry [5]. Similarly, Ercisli and Orhan (2007), Yilmaz *et al* (2012), and Oktan *et al* (2016) reported parallel levels of TSS and pH in mulberries while level of total solids is lower [21-23]. The variation in pH values among mulberry landraces varieties may due to organic acid concentration [25]. These variations could be attributed primarily to differences in genotypic among the landraces. Like-wise the moisture content in five Wild Mulberry landraces were ranged from 62.32 - 82.70 %. The moisture content in landraces WM1 and WM2 are comparable to reported values [5]. They reported moisture content ranged from 78.03 – 82.40 % in various species of *Morus* species [5]. In contrast, the landraces WM3, WM4 and WM5 exhibited lowers values than reported range. The variation in moisture level may be due to different harvesting time and ecological conditions (Koyuncu *et al*. 2014) [26].

Protein contents ranged from 0.85–1.66 g/100 g DW. Among the landraces, WM5 exhibited the highest protein content (1.66%), while WM1 recorded the lowest (0.85%). These values are comparable to those reported by Imran *et al*. (2010) and Wang *et al*. (2022) [5, 27]. Imaran *et al*., (2010) observed protein contents ranging from 0.96 - 1.73 g/100 g DW in edible mulbery. Dietary fibre content ranged from 6.83–10.88 g/100 g DW . This range overlaps well with the wide variation reported by Imran *et al*. (2010), who recorded fibre contents from 0.57 to 11.75 g/100 g DW, with *M. nigra* identified as particularly fibre-rich. Similarly, Uzumucu *et al* (2021) [28] also reported similar values of fiber content in mulberry. The high fibre content observed in WM1 highlights its potential role in promoting digestive health and positions it as a nutritionally valuable landrace.

The ash content indicates the concentration of minerals in fruit. In this study the ash content in wild mulberry landraces ranged from 0.35- 0.67 g/100 g DW. This ranged is comparable to reported ranged (0.46-0.87 g/100 g DW) from edible mulbery [5] and a similar range of ash content reported by Koyuncu *et al*. (2014) [26].

In current study, the content of ascorbic acid ranged from 12.41 - 21.09 mg/100 g, with WM2 showing the highest content and WM4 the lowest. These values are in close agreement with those reported by Imran *et al*. (2010), who reported ascorbic acid contents ranged from 15.20 - 17.03 mg/100 g FW. Similarly, Eyduran *et al*. (2015) and Ercisli *et al*. (2010), also determined ascorbic acid concentration ranges from 18.87 to 20.79 mg/100 g in mulberries [29-30]. Total sugar content ranged from 5.7 to 9.86 mg/100 g, with WM4 having the greatest whereas WM2 the smallest values. This trend is consistent with Imran *et al*. (2010), who has reported sugar contents ranging from 6.64 - 10.89 g/100 g FW in edible Morus species. Like-wise, Mahmood *et al*. (2012) also reported the slight variations in sugar content (7.93–8.19 mg/100 g) in mulberry fruits from Pakistan. Genotypic diversity, environmental and geological factors are responsible for impacting wild mulberry landraces. [31].

The antioxidant reported in mulberries have the properties of preventing chronic diseases such as cancer as reported by Aljane *et al*, (2016) and Liu *et al* (2004) [32-33]. The total flavonoid content observed during aur study is closely align with the investigation by He *et al*. (2020, 2022), who analyzed flavonoid levels in various *Morus* fruits [34-35]. On the other hand, Mehmmod *et al*., (2012) reported a higher content of total flavonoids (110–1021 mg CE/100 g DW) in mulberry fruit (M. alba, M. nigra, M. macroura, M. laevigata collected from Faisalabad and Lahore, Pakistan.

Carotenoid content was closely related with the findings of Manzoor *et al*. (2022) and Sun *et al*. (2020) [36-37]. Antioxidant activity is also agreed with those of Alijane *et al*. (2016), who observed the similar results of antioxidant attributes in mulberry cultivars [38].

Our findings of total phenolic content are closely related with Ozgen *et al* (2009) and Ercili and Orhan (2008), who reported the phenolic content ranging from 19.43 to 23.37 mg GAE/g FW in mulberries [39-40]. Memon *et al*. (2010) and Isabelle *et al*. (2008) also studied the comparable phenolic content from Pakistan and south of China [41-42]. Mehmmod *et al*., (2012) reported higher total phenolics (201–2287 mg GAE/100 g DW) in mulberry fruits from Faisalabad and Lahore. Variation in total phenolic composition of mulberry can be occurred due to genetic diversity, environmental factors and maturity stages (Lima *et al*., 2005) [43]

Conclusion

This study was conducted to explore the chemical composition, nutritional value, and antioxidant potential of five underutilized wild mulberry landraces grown in Gilgit-Baltistan**.** The fruits of these varities are not used and wasted on the ground. According to the findings these mulberry fruits are nutrient rich and have a significant part in balance diet. The high phenolic content and antioxidant activity further increase their nutritional and phyto-therapeutic properties. Different parameters such as pH, soluble solids, moisture, protein, fibre, ash, ascorbic acid, total sugar, anthocyanins, flavonoids, carotenoids, total phenolics and antioxidants activity are comparable with improved /grafted mulberry landraces varieties commonly known as common edible mulberry (*M. Alba, (white), M. Nigra, (black),* and *M. Laevigata* (brown) and their fruits are commonly used as fresh and dried form. Being the first study of its kind, it provides reference data for further research along with highlighting the nutritional advantages of these unique fruits. Moreover, it lays the groundwork for promoting these fruits as valuable resources for the food and pharmaceutical industries.

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