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**Research Article****Biochemical variations among native apple (*Malus domestica*) varieties; Saspolo, Kachura Ambari and exotic varieties from Skardu Baltistan, Pakistan**Samreen Fatima¹, Ishtiaq Hussain¹, Saiqa Ishtiaq², Shazia Kousar³, Muhammad Muneeb⁴, Muhammad Khalid Saeed⁵, Konain Zahra Maqbool⁶, Tajgul¹¹Department of Botany, University of Baltistan, Skardu 16100, Gilgit Baltistan, Pakistan.²Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan.³Centre for Plant Conservation, University of Karachi, Pakistan.⁴Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore Campus, Pakistan.⁵Pakistan Council of Scientific Industrial Research (PCSIR) Lahore, Pakistan.⁶Department of Plant Production Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro-080-0834, Japan.**ABSTRACT**

The present study investigates the biochemical composition and antioxidant activity of indigenous and exotic apple varieties (*Malus domestica* Borkh.) grown in the Baltistan region of Pakistan. Two local varieties, Saspolo and Kachura Ambari, along with two exotic varieties, Five Star and Golden Delicious, were analyzed for primary and secondary metabolites, including proteins, carbohydrates, phenols, flavonoids, tannins, and alkaloids. Quantitative analysis revealed significant differences ($P < 0.001$) in total phenolic content (TPC) and total flavonoid content (TFC) among the studied varieties. The highest TPC was recorded in the peel of Saspolo apple (41.59 ± 0.11 mg GAE/100 g), while the flesh of Five Star showed the highest TPC (43.71 ± 0.05 mg GAE/100 g). Antioxidant activity, assessed using the DPPH radical scavenging assay, demonstrated maximum activity in Saspolo variety ($82.42\% \pm SD$) at the tested concentration (100 $\mu\text{g/mL}$). The results indicated substantial biochemical diversity among apple varieties from Baltistan, with native cultivars exhibiting superior antioxidant potential, particularly in the peel. These findings highlight the nutritional and functional importance of native apple germplasm adapted to high-altitude agro-climatic conditions in mountain areas of Gilgit-Baltistan, Pakistan.

Keywords: Native germplasm; Saspolo & Kachura apple; phytochemistry; secondary metabolites; apple peel; high-altitude horticulture; nutritional profiling; Gilgit-Baltistan.

INTRODUCTION

The apple (*Malus domestica* Borkh.), which belongs to the Rosaceae family, is one of the most prevalent fruits in the world and is mostly cultivated in temperate climates because of its flexibility, prolonged shelf life, and economic significance. Apples are used fresh and processed into a range of products such as juice, jam, vinegar, cider, and dried slices, contributing significantly to human nutrition and worldwide food systems (Violeta et al., 2010; Hussain et al., 2014; Nayik and Gull, 2020; Patocka et al., 2020). Among commonly consumed fruits, apples are one of the primary sources of dietary phenolic compounds. A wide range of bioactive components, such as flavonoids, dietary fiber, phenolic acids, vitamins and tannins, which exhibit strong antioxidant capacity, are primarily linked to their health-promoting benefits. Consistent consumption of apples has been linked with lower risks of chronic

**Correspondence**Ishtiaq Hussain
ishtiaq.mondq@uobs.edu.pk**Article History**

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diseases such as type-2 diabetes, cardiovascular disorders, and some types of cancer because they can neutralize reactive oxygen species and moderate oxidative stress (Kroon & Williamson, 2005; Hagen et al., 2007). Climate, altitude, growth conditions, genetic background, and fruit tissue type (peel versus flesh) all have a major impact on the biochemical makeup of apples. Apple peel has considerably larger amounts of phenolic compounds and flavonoids than apple flesh, according to various studies (Wolfe et al., 2003; Kalinowska et al., 2014). As a result, unpeeled fruits have stronger antioxidant activity. The accretion of secondary metabolites and overall fruit quality are further controlled by environmental factors as temperature, sun radiation, moisture availability, and physiological conditions (Chun et al., 2005; Melke, 2015; Lee et al., 2023).

Apples rank in the top four in terms of production and consumption, making it one of Pakistan's most significant fruit crops. Punjab, Khyber Pakhtunkhwa, and Gilgit-Baltistan are central apple-growing regions (Mehdi et al., 2020; Wang et al., 2025). Gilgit-Baltistan, especially Baltistan region, offers unique high-altitude agro-climatic conditions described by little precipitation, extreme sun radiation, and significant daylight temperature variations. These conditions support the production of fruit and other nutraceutical crops, and biochemical profiles (Hussain et al., 2016; Baig et al., 2022). In this zone, apples are utilized as an important cash crop that is vital to mountain people's capability to produce income and ensure their food security (Hussain et al., 2014).

Even though apples are vital for Baltistan's economy, there is still a lack of comparative biochemical data on native apple varieties, especially when it comes to the distribution of metabolites of peel and flesh and the potential for antioxidants. Native apple varieties, conventionally farmed and well adapted to the severe climatic conditions of Baltistan, especially Paari village and adjoining areas in Kharhong, whereas Kachura Ambari grown and in Kachura village near Shangilla, Sakrdu. These varieties have not been scientifically studied in detail. For the first time, Agriculture Department established two private Saspolo fruit nurseries in Karmong as pilot to promote its orchard under One Village One Product (OVOP) project. To the best of our knowledge, this study is the first biochemical and antioxidant analysis of Gilgit-Baltistan region. Thus, the aim of the current study was to examine and evaluate the biochemical, mineral profile, and antioxidant activity of local apple varieties (Saspolo and Kachura Ambari) and the exotic varieties (Five Star and Golden Delicious) cultivated in Baltistan. In addition to emphasizing the nutritional and functional value of local apple germplasm adapted to high-altitude conditions, this study intention to illuminate varietal and tissue-specific changes in bioactive chemicals by examining both peel and flesh tissues.

MATERIALS AND METHODS

Study area

This research was carried out in the Baltistan region of northern Pakistan's Gilgit-Baltistan in 2023 (Figure 1). High-altitude topography, little yearly precipitation (less than 125 mm), and chilly weather, especially in the winter, are characteristics of the area with great diversity of vegetation (Hussain et al., 2011), and these environmental conditions have a significant impact on their chemical properties and constituents (Naseer et al., 2022).



Figure 1. Map of study area, Skardu and Kharhong, Gilgit-Baltistan.

Sampling, drying and grinding

Apple samples were gathered from orchards in the Baltistan region in the fall of 2023 (September–October). Two local (Saspolo and Kachura Ambari) and two exotic (Five Star and Golden Delicious) types were chosen. Following Ajila et

al. (2010), samples were cleaned with distilled water, physically peeled, shade-dried for a week at room temperature, and crushed into a fine powder at Botany Lab, University of Baltistan Skardu and stored for further analysis.

Laboratory analysis

Antioxidant activity tests were conducted at the PCSIR Laboratories Complex Lahore, Pakistan, while all biochemical and physicochemical studies were completed at the University of the Punjab, Department of Pharmacy, Lahore. Figures (2 & 3) show the flow of work done for both qualitative and quantitative biochemical analysis.

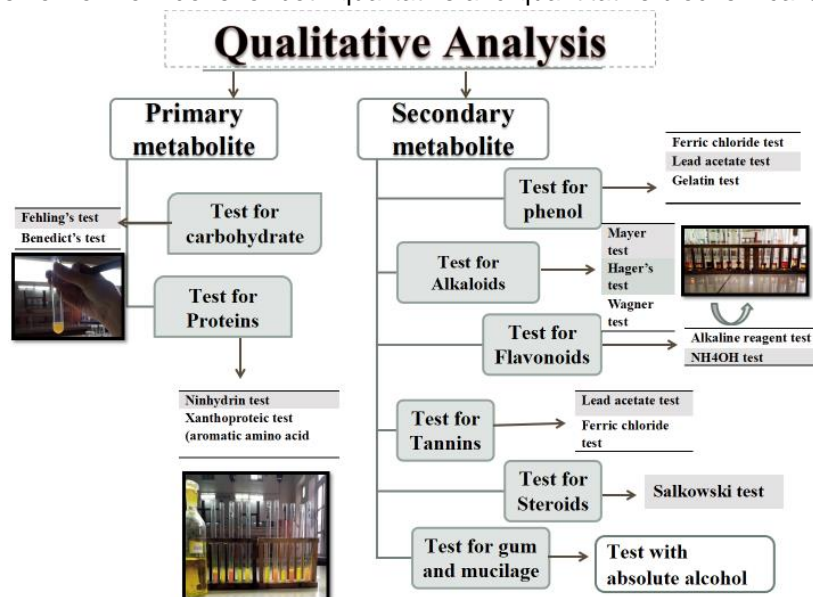


Figure 2. Flowchart of qualitative biochemical analysis of primary and secondary metabolites in apple varieties.

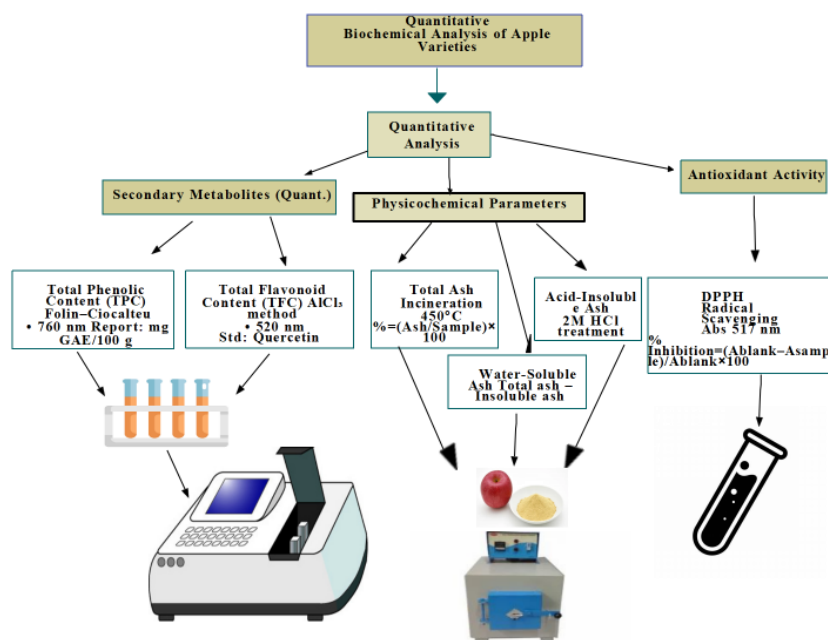


Figure 3. Quantitative biochemical analysis of Native and Exotic apple varieties.

Methanolic extract preparation

Dried powdered (20.0 g) apple (peel and flesh) and 40 mL of methanol were combined to create the methanolic extract. The mixture was filtered through Whatman No. 1 filter paper after being left at room temperature for a day. The solvent was evaporated to reduce the final volume to half of the initial amount, as stated by El-Desoukey (2015).

Biochemical analysis

To determine whether the apple samples included primary and secondary metabolites, such as proteins, carbohydrates, alkaloids, tannins, steroids, flavonoids, phenols, gums, and mucilage, a number of biochemical assays

were carried out. The overview of each test utilized in preliminary phytochemical screening is shown in (Table 1 & figure 2-3). The phytochemical screening techniques were modified from Godlewska et al. (2022), Ahmad et al. (2013). Table (1) shows qualitative biochemical analysis of primary and secondary metabolites in apple varieties, including tests for proteins, carbohydrates, alkaloids, tannins, steroids, flavonoids, gums and mucilage, and phenols.

Table1. Qualitative biochemical analysis of primary and secondary metabolites in apple varieties, including tests for proteins, carbohydrates, alkaloids, tannins, steroids, flavonoids, gums and mucilage, and phenols.

Qualitative Biochemical analysis		Biochemical test
Primary Metabolites Analysis		
1	Protein	Ninhydrin test Xanthoproteic test (aromatic amino acid)
2	Carbohydrate	Fehling's test Benedict's test
Secondary Metabolites Analysis		Biochemical test
1	Alkaloid	Mayer test Hager's test Wagner test
2	Tannin	Lead acetate test Ferric chloride test
3	Steroid	Salkowski test
4	Flavonoid	Alkaline reagent test NH ₄ OH test
5	Gum and Mucilage	Test with absolute alcohol
6	Phenol	Ferric chloride test Lead acetate test Gelatin test

Total Phenolic and flavonoid content

A quantitative analysis was performed on the secondary metabolites. The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent and a gallic acid standard curve (Hinneburg et al., 2006). 2.5 mL of a 7.5% Na₂CO₃ solution and 2.5 mL of a 10x diluted Folin-Ciocalteu reagent were combined with 1 mL of the sample extract. A spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the absorbance at 760 nm after the mixture had been incubated for 30 minutes at room temperature. A standard gallic acid calibration curve was used to determine the phenolic content, which was then reported as mg GAE/100 g.

The aluminum chloride (AlCl₃) colorimetric method was used to calculate the total flavonoid content (TFC) (Kumar et al., 2008). Standard solutions of quercetin (10–100 µg/mL) were prepared in methanol. A 400 µg/mL methanolic apple extract was diluted to 3 mL using methanol. 0.3 mL of 5% sodium nitrite (NaNO₂) was mixed with 1 mL of each standard and apple extract in a 10 mL flask. After five minutes, 0.3 mL of AlCl₃ solution was added, and after six minutes, 2 mL of 1 M NaOH. After diluting the combination with 10 milliliters of distilled water, the absorbance at 520 nanometers was measured. The absorbance of the quercetin standards (12.5-100 µg/mL) was used to create a calibration curve.

Physicochemical analysis

Total ash content

Three grams of the sample were burned at 450°C until all organic material was removed in order to calculate the overall ash content. After the ash was weighed, the following formula was used to determine the percentage of total ash according to the formula by Rajashree et al., (2012):

The weight of ash divided by the sample weight * 100 yields the total ash percentage (%)

Acid-insoluble ash content

To find the percentage of acid-insoluble ash, the sample's ash was heated and treated with 25 milliliters of 2M hydrochloric acid. It was further ashed after filtration. The weight of the air-dried sample (medication) was used to determine the percentage of acid-insoluble ash (Sandeep et al., 2014).

Water-soluble ash content

The entire ash of the sample was heated for five minutes in 25 milliliters of water. After gathering the insoluble material on ashless filter paper and cleaning it with hot water, it was burned for 15 minutes at a maximum temperature of 450°C. By subtracting the weight of the insoluble material from the overall weight of the ash, the weight of the water-soluble ash was calculated. The weight of the air-dried medicine was used to calculate the percentage of water-soluble ash (Gami & Parabia, 2010).

Antioxidant activity

A modified DPPH test was used to assess the antioxidant activity (Saeed et al., 2022). Various quantities (25, 50, 75, 100 μ L) of a 1 mM DPPH solution were added to 1000 μ L of the apple sample stock solution. The reaction mixture was incubated at room temperature for 30 minutes before the absorbance at 517 nm was measured. The following formula was used to determine the percentage of free radical inhibition:

$$\text{(Percentage Inhibition)} = \frac{(\text{Ablank} - \text{Asample})}{\text{Ablank}} * 100$$

where is the absorbance of the test compound and Ablank is the absorbance of the control reaction, which contains all of the reagents but the test compound.

Statistical analysis

The data for various biochemical parameters and antioxidant properties of each apple cultivar were recorded and kept in MS Excel 2010. Three independent replicates were used to calculate the mean, standard deviation (SD), minimum, maximum, and standard error of the mean (SEM). Statistix software (USA, version 8.1) was used to conduct descriptive statistics, correlation analysis, analysis of variance (ANOVA), and the Least significant difference (LSD) test.

RESULTS AND DISCUSSION

The present study provides a comprehensive evaluation of the biochemical composition, mineral profile, and antioxidant activity of four apple varieties, including two local and two exotic varieties grown in Baltistan region. The findings revealed pronounced varietal and tissue-specific (peel vs. flesh) variability in both primary and secondary metabolites, highlighting the strong influence of genetic makeup and high-altitude agro-climatic conditions on apple nutritional quality and phytochemical compositions.

Qualitative phytochemical composition

Qualitative phytochemical screening demonstrated the presence of a wide range of bioactive constituents in plants with medicinal and aromatic properties (Ismail et al., 2022). Such bioactive components include carbohydrates, flavonoids, phenols, tannins, alkaloids, sterols, gums, and mucilage, with marked differences between peel and flesh tissues. Apple peels were particularly rich in secondary metabolites such as flavonoids, phenols, and tannins, whereas alkaloids were absent in the flesh. Proteins, gums, and mucilage were predominantly detected in the flesh rather than the peel (Figure 04). These observations are consistent with earlier reports indicating that apple peel acts as a protective tissue, and also accumulating higher concentrations of secondary metabolites with antioxidant potential (Khan et al., 2022; Saeed et al., 2023).

Total phenolic and flavonoid contents

This study demonstrated significant variation in Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) among four apple varieties cultivated in Gilgit Baltistan. Local cultivars such as Saspolo and Kachura Ambari consistently exhibited higher concentrations of bioactive compounds, particularly in the peel, compared to exotic varieties. Specifically, Saspolo recorded the highest TPC in the peel (41.59 mg GAE /100g), whereas the exotic cultivar Five Star exhibited the highest TPC in the flesh (43.71 mg GAE /100g) (Table 02 & 03). In terms of flavonoids, Kachura Ambari had the greatest levels both in peel (44.71 mg QE/100g) and flesh (19.75 mg QE/100g) . (Table 2 & 3). These results align with previous research suggesting that phytochemicals are predominantly localized in the fruit peel, contributing substantially to such activities (Boyer & Liu, 2004). The observed variations in phytochemical profiles among cultivars are consistent with multiple comparative assessments of apple germplasm. In a comprehensive analysis of six apple varieties from Nagar and Hunza; Mehdi et al. (2020) reported that total phenolic contents ranged widely from 151.27 to 203.03 mg GAE/100 mg, reinforcing cultivar dependent variation in phenolic accumulation.

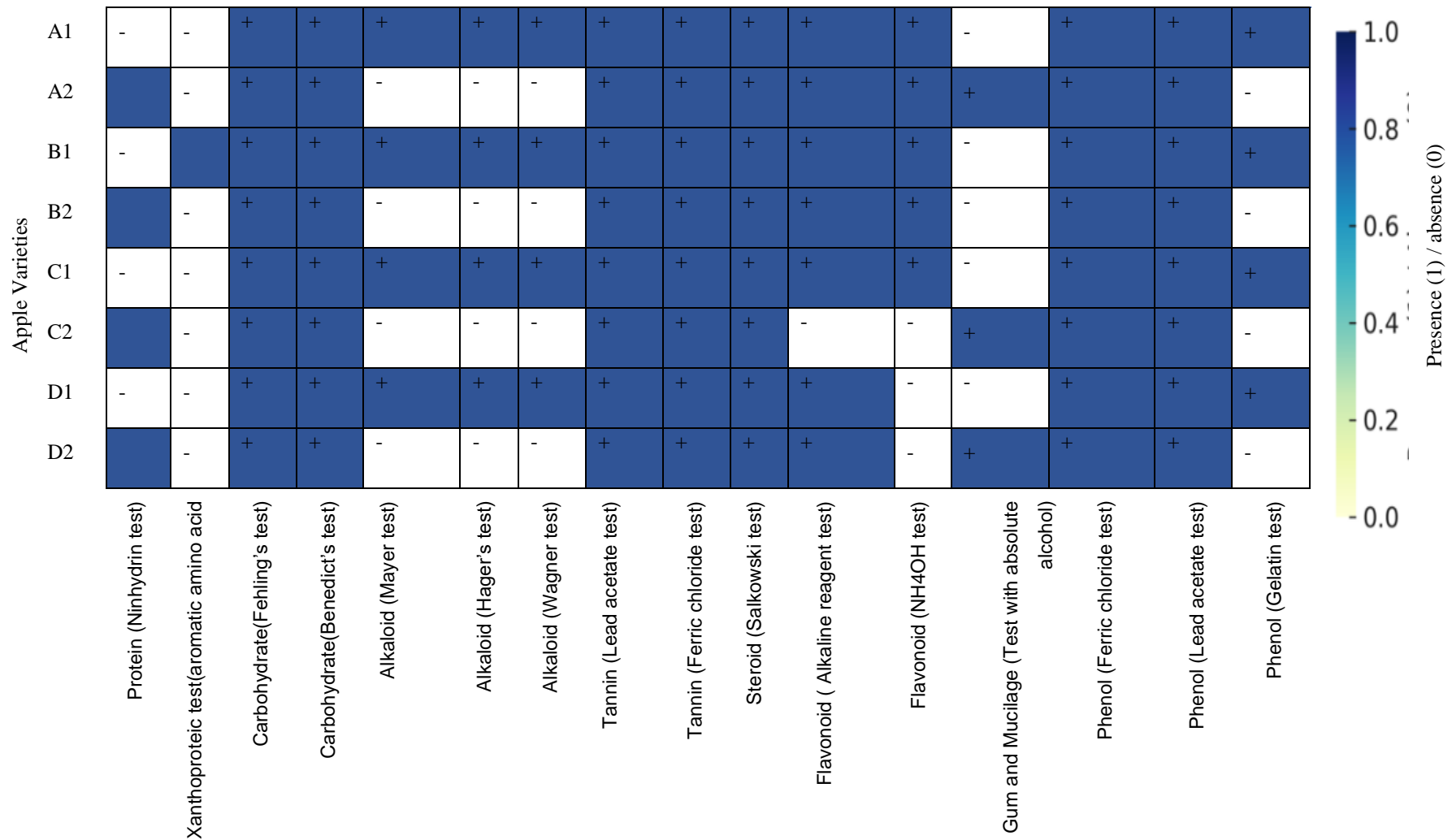


Figure 4. Qualitative biochemical analysis of primary and secondary metabolites of local and exotic apple (peel and flesh) varieties from Baltistan region, Gilgit Baltistan, Pakistan. Keys: +ve Positive (present), -ve negative (absent) Keys: A1= Saspolo (Methanolic peel extract), A2= SaspoloKharman (Methanolic flesh extract), B1= Kachura Ambari (Methanolic peel extract), B2= Kachura Ambari (Methanolic flesh extract), C1= Golden Delicious (Methanolic peel extract), C2= Golden Delicious (Methanolic flesh extract), D1= Five Star (Methanolic peel extract), D2= Five Star (Methanolic flesh extract)

Similarly, Saeed et al. (2023) documented markedly high TPC values in apple peel, with methanolic extracts yielding 320 ± 5.4 mg GAE/100 g and aqueous extracts 201 ± 4.20 mg/100 g, further confirming the peel as a rich source of phenolic antioxidants. These findings corroborate the present study's observation of higher phenolic concentrations in peel tissues relative to flesh. Significant diversity in bioactive compound distribution among apple genotypes grown in Gilgit Baltistan reported that the cultivar Red Delicious exhibited the highest flavonoid content (105.75 mg/100 g FW), and Red Full Star demonstrated the greatest TPC (4.00 mg GAE/g FW), highlighting the influence of genotype on phytochemical expression (Tipu et al., 2025). The unique phytochemical profiles and quality attributes of indigenous apple may be valuable for conservation and breeding programs.

Table 2. TPC, TFC, TAC, WSA and AIA of apple peel samples.

Varieties	TPC	TFC	TAC	WSA	AIA	AA
S	41.590 ^A	28.827 ^C	2.4500 ^B	16.390 ^C	54.200 ^C	74.143 ^A
KA	23.080 ^D	44.113 ^A	2.2967 ^B	13.540 ^D	22.830 ^D	69.843 ^{AB}
GD	35.797 ^C	33.913 ^B	1.8100 ^C	32.560 ^A	62.463 ^A	64.084 ^{AB}
FS	39.830 ^B	21.550 ^D	4.4700 ^A	24.600 ^B	55.510 ^B	51.390 ^B
Mean	35.074	32.101	2.7567	21.772	48.751	64.866
C.V	21.55	27.35	38.622	35.679	32.761	19.041
LSD	0.1907	2.7355	0.1914	0.4756	0.161	20.194
p values	0.000	0.000	0.000	0.000	0.000	0.000

All such means sharing a common English letter in the same column are non-significant $p < 0.0$.

Keys: S (Saspolo), KA (Kachura Ambari), GD (Golden Delicious), FS (Five Star), TPC (Total Phenolic Content), TFC (Total Flavonoid Content), WSA (Water soluble ash), AIA (Acid Insoluble Ash), AA (Antioxidant activity).

Table 3. TPC, TFC, TAC, WSA and AIA of apple flesh samples.

Varieties	TPC	TFC	TAC	WSA	AIA
S	21.59 ^C	17.35 ^D	1.51 ^B	52.38 ^A	31.820 ^A
KA	2.97 ^D	19.74 ^A	1.65 ^{AB}	28.57 ^B	15.417 ^D
GD	26.52 ^B	17.73 ^C	1.79 ^A	20.83 ^D	31.580 ^B
FS	43.71 ^A	19.50 ^B	1.43 ^B	23.06 ^C	28.570 ^C
Mean	23.7	18.584	1.5967	31.211	26.847
C.V	63.947	6.3244	10.61	41.974	26.154
LSD	0.1527	0.1448	0.2448	0.096	0.1099
p values	0.001	0.001	0.001	0.001	0.001

All such means sharing a common English letter in the same column are non-significant.

Keys: S (Saspolo), KA (Kachura Ambari), GD (Golden Delicious), FS (Five Star), TPC (Total phenolic content), TFC (Total flavonoid content), WSA (Water soluble ash), AIA (Acid insoluble Ash).

Collectively, these studies emphasize the importance of cultivar selection in determining the health promoting potential of apples. The preferential accumulation of phenolics and flavonoids in peel underscores the nutritional value of this often discarded tissue. From a horticultural and industrial perspective, local varieties such as Saspolo and Kachura Ambari represent promising genetic resources for functional food development and bioactive compound extraction. These results advance our understanding of phytochemical diversity in local apple germplasm from high altitude agroecosystems and support targeted utilization in breeding strategies aimed at enhancing nutritional quality.

Mineral composition

Significant differences were observed in the mineral composition of apple varieties, particularly in total ash content (TAC), Water-soluble ash (WSA), and Acid-insoluble ash (AIA) (Table 02 & 03), indicating varietal and tissue-specific variability. In the present study, Five Star exhibited the highest TAC in the peel (4.47%), while Golden Delicious showed the highest TAC in the flesh (1.79%). Similarly, WSA was highest in the peel of Golden Delicious (35.56%) and in the flesh of Saspolo (54.38%), suggesting a greater proportion of readily bioavailable minerals, especially in peel tissues. These findings are consistent with Mehdi et al., (2020), who reported notable variation in ash content among apple cultivars from Nagar and Hunza, with Golden Delicious showing the highest ash content (2.92%). The influence of genotype as well as adequate soil fertility and growing environment on mineral accumulation and natural fertilizers may

also influence the better growth (Nosheen et al., 2018; Hussain et al., 2019). The comparatively higher ash values observed in the present study, particularly in peel samples, may be attributed to differences in cultivar selection and agro-climatic conditions of Baltistan. Overall, elevated TAC and WSA values confirm that apple peels are mineral-rich components that may contribute to essential physiological functions and physicochemical properties as suggested by Gowman et al. (2019).

Antioxidant activity

The antioxidant activity of apple varieties differed significantly in the present study, with the local cultivar Saspolo showing the highest DPPH radical scavenging activity (82.42% at 100 $\mu\text{g}/\text{mL}$), whereas Kachura Ambari exhibited comparatively lower activity (64.59%) (Figure 5). These values are substantially higher than those reported by Mehdi et al. (2020) for apple cultivars from Nagar and Hunza (15.12–18.61%), indicating strong varietal and methodological influences on antioxidant capacity.

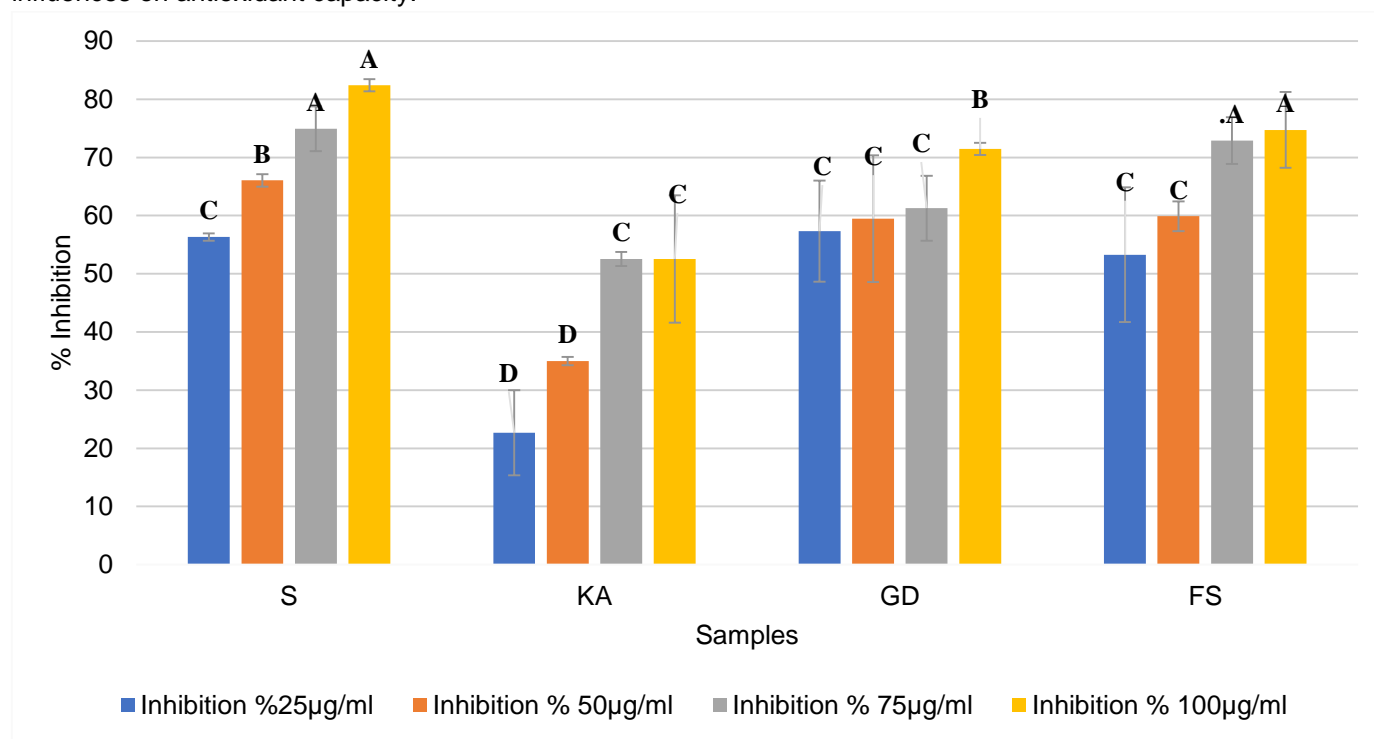


Figure 5. Antioxidant activity of apple peel at 25, 50, 75, 100 $\mu\text{g}/\text{m}$; Keys: S (Saspolo), KA (Kachura Ambari), GD (Golden Delicious), FS (Five Star).

Comparable trends were reported by Saeed et al. (2023), who observed concentration-dependent antioxidant activity in methanolic extracts of apple peel powder (25.40–69.2%), exceeding that of aqueous extracts and the synthetic antioxidant BHT. The variation in different tissues including pulp and peels samples of apple are in agreement with previous findings with diversity in antioxidant capacities (Manzoor et al., 2012). In the present study, the use of apple peel powder and methanolic extraction likely enhanced radical scavenging efficiency due to the higher solubility of phenolic and flavonoid compounds in methanol. Additionally, the high-altitude agro-climatic conditions of Baltistan, where these local apple varieties are adopted may have favored greater accumulation of antioxidant metabolites. The strong positive correlation between total phenolic content and antioxidant activity ($r = 0.99$) further supports the role of phenolic compounds as major contributors to the antioxidant potential of apples, in agreement with earlier findings (Guerrero et al., 2014).

Correlation analysis

Correlation analysis revealed significant relationships among various biochemical parameters, underscoring the functional interplay between apple metabolites. TPC in the peel showed a very strong positive correlation with antioxidant activity ($r = 0.99$), highlighting the central role of phenolics in oxidative stress mitigation. A strong correlation was observed between alkaloid and phenol as well as steroid ($r=0.99$) and alkaloid and protein ($r=0.98$) (Table 4 and 5). Such correlations between phytochemical and nutritional attributes among genotypes are in agreement with Overall, the present study, in agreement with earlier regional investigations, confirms that apples grown in Gilgit-Baltistan

Table 4. Correlation between qualitative and quantitative biochemical traits of apple peel.

	TPC	TFC	% Inhibition	Protein	Carbohydrate	Alkaloid	Tannin	Steroid	Flavonoid	Gum and Mucilage	Phenol
TPC	1										
TFC	-0.853	1									
% Inhibition	0.99	-0.853	1								
Protein	-0.816	0.870	-0.816	1							
Carbohydrate	0.001	0.174	0.004	-0.333	1						
Alkaloid	-0.816	0.870	-0.816	0.980	-0.333	1					
Tannin	0.002	0.174	0.003	-0.333	0.990	-0.333	1				
Steroid	-0.816	0.870	0.816	0.970	-0.333	0.999	-0.333	1			
Flavonoid	0.003	0.522	0.002	0.333	0.333	0.333	0.333	0.333	1		
Gum and Mucilage	0.004	0.174	0.006	-0.333	0.980	-0.333	0.989	-0.333	0.333	1	
Phenol	-0.816	0.870	-0.816	0.980	-0.333	0.990	-0.333	0.989	0.333	-0.333	1

In bold, significant values (except diagonal) at the level of significance alpha = 0.050 (two-tailed test).;Keys: TPC (Total Phenolic Content), TFC (Total Flavonoid content), inhi % (inhibition percentage).

Table 5. Correlation between qualitative and quantitative biochemical traits of apple flesh

	TPC	TFC	Protein	Carbohydrate	Alkaloid	Tannin	Steroid	Flavonoid	Gum and Mucilage	Phenol
TPC	1									
TFC	0.000	1								
Protein	-0.816	0.577	1							
Carbohydrate	0.000	-0.577	-0.333	1						
Alkaloid	-0.816	0.577	0.999	-0.333	1					
Tannin	0.000	-0.577	-0.333	0.986	-0.333	1				
Steroid	-0.816	0.577	0.988	-0.333	0.999	-0.333	1			
Flavonoid	-0.816	-0.577	0.333	0.333	0.333	0.333	0.333	1		
Gum and Mucilage	0.000	-0.577	-0.333	0.988	-0.333	0.988	-0.333	0.333	1	
Phenol	-0.816	0.577	0.996	-0.333	0.998	-0.333	0.995	0.333	-0.333	1

In bold, significant values (except diagonal) at the level of significance alpha = 0.050 (two-tailed test); Keys: TPC (Total Phenolic Content), TFC (Total Flavonoid Content).

possess substantial biochemical diversity. The superior phenolic content and antioxidant activity of local apple varieties such as Saspolo and Kachura Ambari, particularly in the peel—underscore their nutritional, functional, and therapeutic potential. These findings strongly support the conservation, promotion of high value crop, and value-added utilization of indigenous apple germplasm and for functional foods, nutraceutical development, and sustainable horticultural practices in high-altitude regions.

CONCLUSION

This study concludes biochemical diversity within apple varieties from Baltistan region, with indigenous varieties such as Saspolo Apple from Kharmonj and Kachura Ambari from Skardu district exhibiting higher levels of phenolic compounds, other activity, particularly in the peel tissue. The promotion of local apple varieties in remote valleys of Gilgit-Baltistan is promising for local farmers and biochemical profiling of such varieties revealed great diversity in metabolites at tissue level. The strong correlation between total phenolic content and antioxidant activity highlights the functional importance of phenolic compounds in apples. These findings signify the nutritional value and also the functional potential of local apple germplasm acclimatized to the high-altitude environments and utilization of nutraceutical applications.

There is great potential as functional food and product development at commercial level. Further studies are recommended to discover detailed pharmacological properties, metabolomic studies, explore antimicrobial potential for these native apple varieties, and value-added products. The conservation of native species in national gene bank is recommended to delineate its function at molecular level.

AUTHOR'S CONTRIBUTION

Samreen Fatima conducted the experimental work and data acquisition. Ishtiaq Hussain supervised the overall experimental design and manuscript preparation. Shazia Kousar contributed to statistical analysis and manuscript preparation. Saiqa Ishtiaq, Muhammad Muneeb, and Muhammad Khalid Saeed provided continuous technical support and guidance throughout the experimental work under different lab. Konain Zahra Maqbool and Tajgul assisted in sample handling and data collection. All authors duly contributed this final manuscript.

FUNDING

No funding was received for this study.

AVAILABILITY OF DATA AND MATERIAL

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with ethical guidelines for plant research. No ethical approval or consent to participate was required as the research did not involve human or animal subjects.

CONSENT FOR PUBLICATION

All authors have reviewed the manuscript and approved it for publication.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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