Zoo Botanica
ISSN: 3007-2050 (E), 3007-2042 (P)

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Research Article

Evaluation of phytochemical properties in leaves and tubers of Beta vulgaris varieties grown at Skardu

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ABSTRACT

Sugar beet (*Beta vulgaris*) is a biennial herbaceous crop with important agricultural and therapeutic uses. The present study aims to evaluate phytochemical constituents in two varieties (V1 = local variety, V2 = red sugar beet, obtained from Agriculture Department Skardu) grown under two conditions at Skardu, including the farmer field (FF) and experimental field (EF). Plant samples (n=03) including leaves and tuber were collected from each location and analyzed for total phenolic content (TPC), total flavonoid content (TFC), total anti-oxidant capacity (TAC), total reducing power (TRP) and free radical scavenging assay (DPPH). Results obtained were tested for significance (ANOVA and LSD) by using statistical software package (Statistix 8.1, USA). The results revealed that highest phenolic content was found in leaves of V2 experimental field (48.94 ± 0.7 µg GAE/mg), whereas higher TFC was found in tubers of V1 at experimental field (47.98 ± 0.99 µg QE/mg), and TRP was maximum in leaves of V2 at experimental field (209.64 ± 0.89 µg AAE/mg). The highest antioxidant capacity was recorded in the leaves of V1 under experimental field conditions (103.63 ± 1.73 µg AAE/mg), while the maximum DPPH radical scavenging activity was observed in the leaves of V2 at farmer field conditions (44.16 ± 0.88%). The ANOVA test revealed significant differences ($p < 0.05$) in the levels of TAC, TFC, TPC, TRP and DPPH activities in tested samples. This study provides novel insights into the phytochemical composition of *Beta vulgaris* grown under two contrasting conditions, demonstrating that agronomic practices in the experimental field significantly influenced the phytochemical profiles across different varieties. Such variations in chemical constituents not only elaborate the potential role of sugar beet as functional food grown at highlands of Skardu, but also elucidate its ability to cope under the stressful conditions. Both varieties of sugar beet grown under experimental field conditions exhibited enhanced productivity and bioactive potential, underscoring its value as a functional food crop for sustainable mountain agriculture.

Keywords: Sugar beet; phytochemical; antioxidant capacity; agronomic practices; mountain farming, Gilgit-Baltistan.

INTRODUCTION

Plants have been essential to human survival and health treatment from all of recorded history. Medicinal plants have a multitude of natural compounds having traditional uses that can greatly promote pharmaceutical development (Parekh and Chanda 2007; Hussain et al., 2011). Plants grown in mountain areas have great diversity with respect to biochemical constituents in various parts (Hussain et al., 2016; Ishtiaq et al., 2024), their uses with respect to diversity in farming systems (Öztürk et al., 2022; Ismail et al., 2022) and phytochemical properties (Khan et al., 2011; Zahra et al., 2017; Naseer et al., 2022). Free radicals, which are by products of metabolic activities, are one of the major health issues that humans face today.

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Article History
Received: August 01, 2025
Accepted: August 22, 2025
Published: August 31, 2025

Numerous illnesses, such as cancer, arthritis, ischemia, gastritis, and atherosclerosis, are linked to these reactive oxygen species (Mazandarani et al., 2012). According to recent studies, phytochemicals having antioxidants properties can prevent disease development and improve health (Parveen et al., 2025).

The therapeutic efficacy of this species is due to its bioactive components, which include tannins, alkaloids, flavonoids, phenolic acids, betalains and reported to for cure of many diseases including traditional therapies (Pitalua et al., 2010; Vasconcellos et al., 2016). Farming methods, environmental factors, and genotype all affect sugar beet's chemical makeup. Compared to crops grown conventionally, organically grown varieties have higher amounts of advantageous metabolites (Ventrano et al., 2020). Research on sugar beet juice supplementation emphasizes its potential to improve health by improving oxygen efficiency during physical activity and lowering altitude sickness symptoms (Masschelein et al., 2012). However, external environmental cues like temperature and light, which affect the root's storage of sugar, have a major impact on its output (Ebrahimian et al., 2009).

The sugar beet is an essential crop for the production of sugar since it contains between 15% and 21% sugar by weight. The plant is a mainstay of the world's sugar industry because of its great yielding potential and ability to adapt variety of climatic conditions. Sugar is essential for food texture, stability, and volume in addition to its ability to sweeten and provide energy. With a 30% global contribution to sugar production, sugar beet ranks as the second most important sugar crop after sugarcane. With more than 60% of the world's production, sugar beet is the most prevalent in Europe (Kumar and Pathak, 2013). In many European and Asian nations, it is an essential part of the agricultural economy because to its versatility and high sucrose yield (European Commission, 2021) and contributes approximately 20% of the world’s total sugar production (Wimmer and Sauer, 2020). In Pakistan Sugar beet is primarily cultivated in KPK, specifically in Peshawar, Mardan Charsadda areas, and cultivation of this crop supporting local economies and sugar factories (Mubarak et al., 2016).

Cultivation of the crop at higher altitudes of Gilgit-Baltistan (G.B) may increase its output with respect to food security, and phytochemical profiling would bring better understanding about its health benefit and importance to the local economy in GB. Studies related to phytochemical constituents of sugar beet varieties were not investigated so far in mountain farming system of GB. Hence present study evaluates phytochemical properties in different organs of two varieties grown under farmer-managed and experimental field conditions in Skardu.

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MATERIALS AND METHODS

Study Area

The study was carried out at Skardu, the administrative center of Baltistan, which is situated in Pakistan's north. At a height from 2,200 to 2,500 meters above sea level, Skardu is an area known for its stunning scenery. The climatic conditions of Skardu are highly variable. Winter temperatures can plunge to -21°C, while summer temperatures may soar to 35°C, and fertility evaluation of mountainous soils shows optimum nutrients with no pollutants (Hussain et al., 2019), hence providing a challenging yet distinct environment for agricultural research. This unique climate made Skardu an ideal location to study the growth and development of *Beta vulgaris* varieties.

Plant Materials and Sample Preparation

Plant sample of both varieties of *Beta vulgaris* (V1 and V2) were collected from two different areas of Skardu the V1 (local variety of sugar beet) and V2 (red sugar beet obtained from Agriculture Department Skardu). The seed were tested for germination and grown under two conditions at Skardu, that is farmer field (FF) and experimental field (EF). At the experimental field, both varieties were grown at Agriculture Department Skardu with proper spacing (20cm between plants) including timely irrigation (6-8 times) (Tamiru et al., 2017) and other management practices as compared to the traditionally farmer field (FF), where no standard agronomic practice were followed like proper spacing, irrigation and fertilizer treatments.

Leaves and tubers samples (n=03) were harvested from each locations with proper coding, shade-dried, powdered, and extracted in methanol (1:3 solvent ratio w/v). Extracts were concentrated on a hot plate, stored, and dissolved in DMSO (20 mg/ml), followed by sonication to ensure dissolution. A stock solution (04 mg/ml) was prepared for all assays mentioned below.

Phytochemical Tests

Total phenolic content (TPC)

Extract (20.0 ul) was taken from 4mg/ml stock solution in 96 well plates (micro plate) then 90ul of folin-ciocalteu reagent (FCR) was added with the micro-pipette. At room temperature, the mixture was allowed for incubation (5 minutes).

Then of sodium carbonate (90 µL) was added. For negative control, the DMSO was used whereas gallic acid was used for positive control (Ul Haq et al., 2012). Reading was taken at 630nm wavelength on a micro-plate reader.

Total flavonoid content (TFC)

Extracts (20µl) was transferred to each well and added 10µl each of potassium acetate, 10ul aluminum chloride. At the end 160µl of distilled water was added using micro pipette the respective wells. Quercetin was used at various concentrations (2.5, 5.0, 10.0, 20.0, and 40.0 µg/ml) as the positive control for plotting a calibration curve for TFC, while DMSO served as the negative control. The plate was incubated at room temperature (30 minutes), and the absorbance of the test extracts was measured at 415 nm. The results were expressed as (µg QE)/mg of the extract (Kamal et al., 2022; Zahra et al., 2017).

Total reducing power (TRP)

The total reducing power of the samples was determined by using the potassium ferricyanide assay based on colorimetric method. From sample extract (4 mg/ml DMSO) 100µl was added in eppendorf tube with 200µl of phosphate buffer and 250µl potassium ferricyanide (1% w/v in distilled water) then placed in water bath for incubation at 50°C for 20 minutes. The mixture was then centrifuged at 3000 rpm at room temperature for 10 min after pouring 200µl of trichloroacetic acid (10% w/v in distilled water) to each eppendorf. Then the supernatant of 150 µl was taken and mixed with 50µl of FeCl3 (0.1% w/v in distilled water) in 96-well plate. Ascorbic acid (1 mg/ml in DMSO) was used as the positive control, whereas for the negative control, simple DMSO was used. The absorbance was measured at 630 nm, and the reducing power of the samples was expressed as micrograms of ascorbic acid equivalent per milligram of sample (µg AAE/mg) (Brewer et al., 2011; Zahra et al., 2017).

Total antioxidant capacity (TAC)

A phosphomolybdenum-based technique was used for the TAC assay. Out of total 1000 µL mixture, an aliquot of sample (100 µL) and the reagent (900 µL) was used where the reagent contains (sulfuric acid, 0.6 M, sodium phosphate, 28 mM, and ammonium molybdate, 4 mM). For positive control an ascorbic acid was used, whereas for the negative control, the DMSO was used in the assay. The reaction mixture was then allowed to incubate at 95 °C in water bath for more than hour time (90 minutes). The absorbance was measured at 630 nm for both standard and test samples and antioxidant activity was calculated according the method described by Ul Haq et al., (2012). The results were expressed as (µg AAE/mg DW) for TAC.

Free radical scavenging assay (FRSA based on DPPH Assay)

The free radical scavenging assay (FRSA) was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) for all samples. An aliquot of 10 µL of sample extract and the DPPH solution (190 µL) was mixed yielding a final concentration of 200 µg/mL in the reaction mixture. After incubation at 37 °C for 30 minutes, the absorbance was measured at a wavelength of 517 nm on a microplate reader. Formula for %free radical scavenging activity used is as under:

$$\%\mathrm{FRSA} = (1 - \mathrm{Ab_s}/\mathrm{Ab_c}) \times 100$$

Where Ab_c represents the absorbance of the control and Ab_s represents absorbance of sample. Whereas, for the positive control the ascorbic acid was used (Zahra et al., 2017).

Analysis of Data

The experiments were carried out in triplicate. Recorded data were analyzed for ANOVA by using software package (Statistix 8.1,) followed by Least Significant Difference (LSD) test at $p < 0.05$.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

The leaves of V2 at EF had the highest phenolic content (48.94 ± 0.7 µg GAE/mg) than the tubers of V1 at EF (9.57 ± 0.9 µg GAE/mg), while V2 leaves of FF had a similar amount (47.98 ± 0.87 µg GAE/mg). Significant variations were found between the two types and conditions in terms of phenolic content, according to the phytochemical evaluation (Fig 1 & 2). The results revealed that the phytochemical profiles of V2 and V1 differed significantly. The maximum phenolic concentration was found in the V2 under both growing conditions which is consistent with Ediziri et al. (2019), who found that methanolic extracts were very effective in extracting phenol.

Total flavonoid content (TFC)

Total flavonoid content recorded lowest in leaves of V2 at FF (4.49 ± 0.62 µg QE/mg) and greatest in tubers of V1 at EF (47.98 ± 0.99 µg QE/mg) (Figure 3 & 4). Because of their bioactive potential, which includes anti-cancer and antioxidant properties, phenolic chemicals are essential. In a similar vein, the V1 under EF had showed maximum flavonoid content as compare to V1 grown under FF and such findings are contrary to the recent work on varieties of sugar beet (Almeida et al., 2025), who have reported maximum chemical composition and other activities including antioxidant as well as antimicrobial activity in leaves than that of tubers. This variation emphasizes how local growing circumstances affect the build-up of phytochemicals, probably due to growing of such crops at higher elevations and effect of radiations and colder climate supports more phenolic compounds in their organs (Karimi et al., 2025).

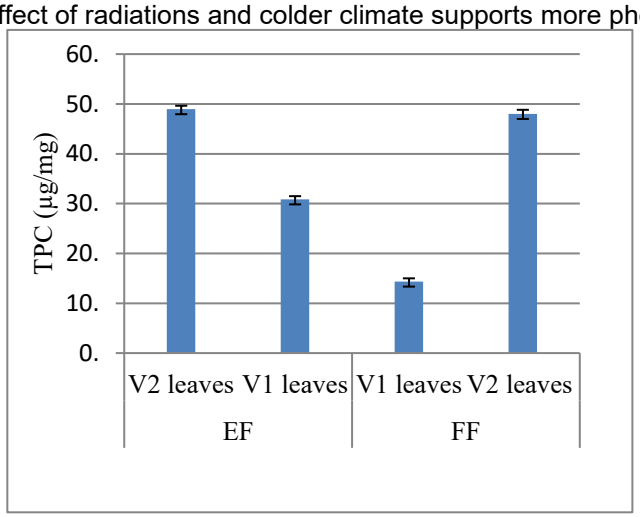

Figure 1. TPC in Leaves of *Beta vulgaris.*

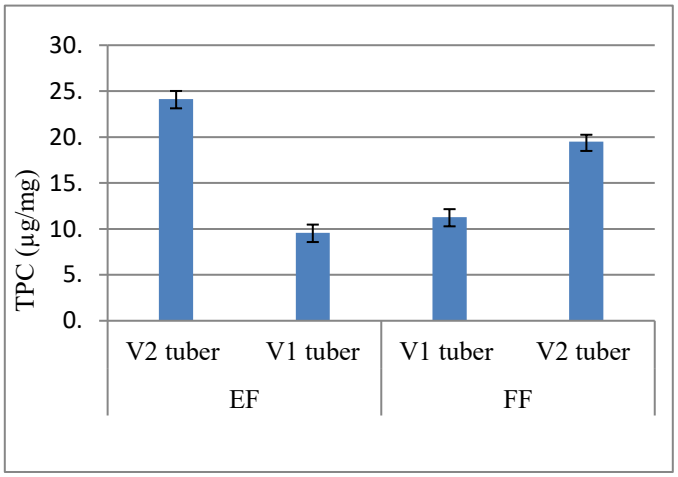

Figure 2. TPC in tubers of *Beta vulgaris.*

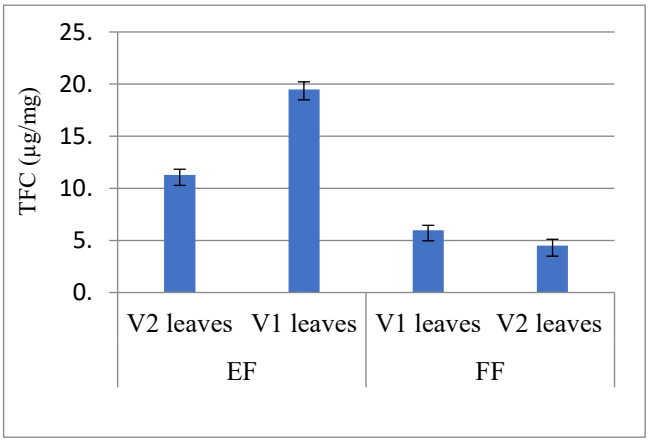

Figure 3. TFC in Leaves of *Beta vulgaris*.

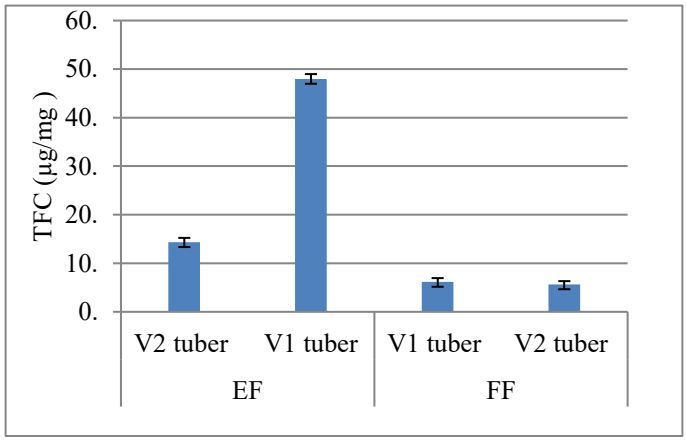

Figure 4. TFC in tubers of *Beta vulgaris*.

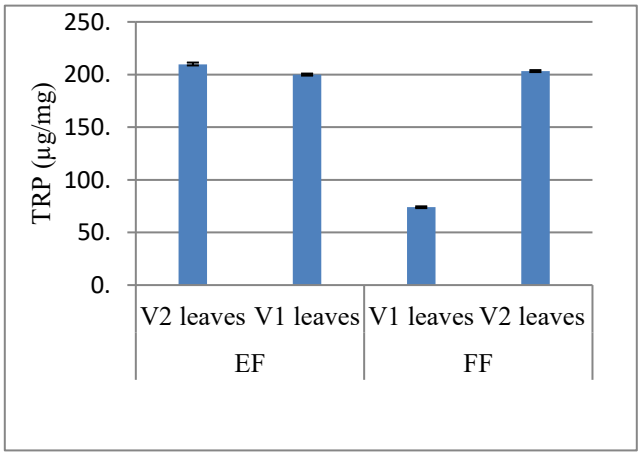

Figure 5. TRP in Leaves of *Beta vulgaris.*

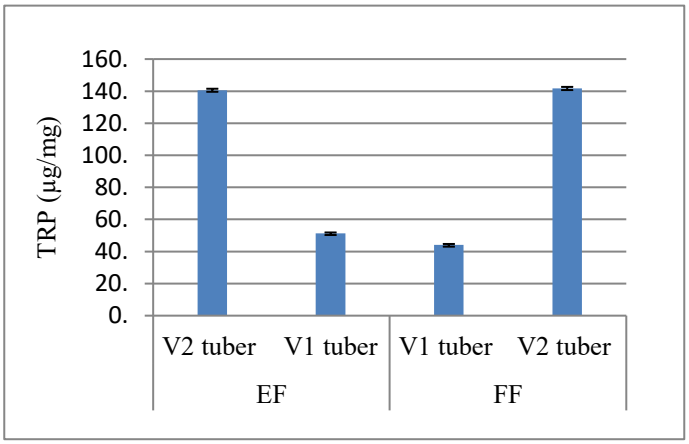

Figure 6. TRP in tubers of *Beta vulgaris.*

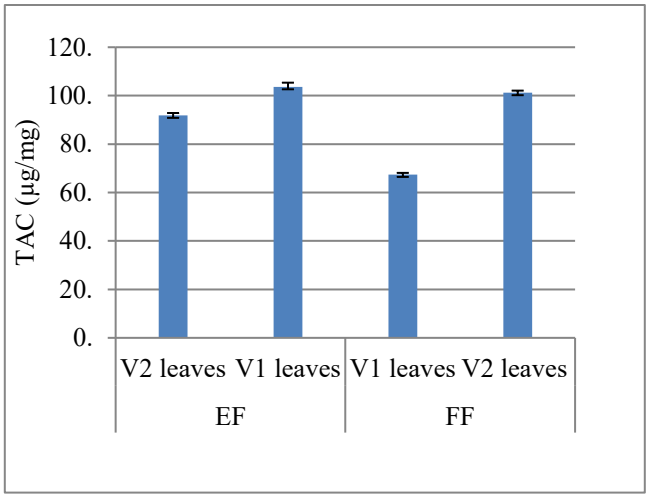

Figure 7. TAC in Leaves of *Beta vulgaris.*

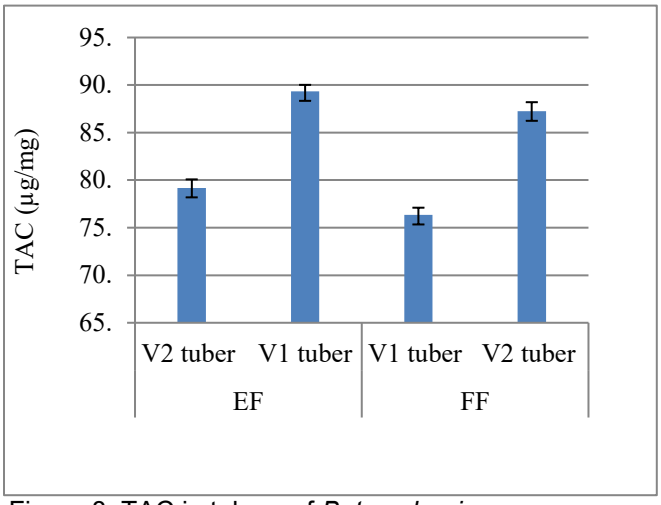

Figure 8. TAC in tubers of *Beta vulgaris.*

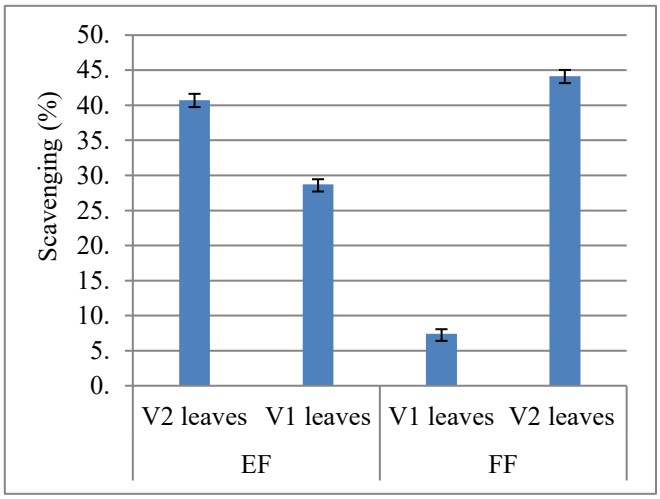

Figure 9. DPPH in Leaves of *Beta vulgaris.*

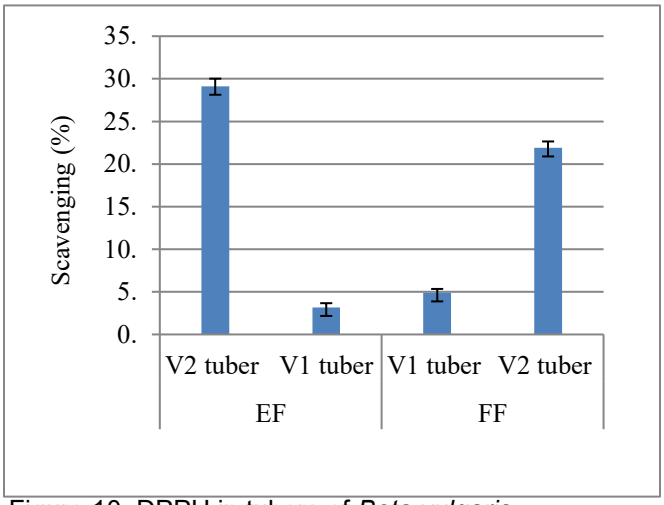

Figure 10. DPPH in tubers of *Beta vulgaris.*

Total Reducing Power (TRP)

The statistical findings presented in figure (5 & 6) showed that tubers of V1 at FF had the lower reducing power (44.04 -140.00 µg AAE/mg), than that of samples from EF (209.64 ± 0.89 µg AAE/mg). Such variations not only supports recent studies carried out on sugar beet varieties, but also suggest a great potential for health beneficial properties exhibited by leaves, having maximum chemical composition and leading environmental sustainability (Almeida et al., 2025). On the other hand the TRP was recorded higher in V2 tubers as compared to V1 under both growing conditions, such varietal differences were already reported by Hussain et al., (2025).

Total Antioxidant Capacity (TAC)

The total antioxidant capacity for tested samples was highest in V1 leaves at EF (103.63 ± 1.73 µg AAE/mg) than that of V2 leaves at FF (101.20± 0.02 µg AAE/mg). The decreasing patterns given by TAC for various extracts were observed in local variety V1 leaves at (FF) as compared to EF conditions and V1 showed least TAC at FF conditions (Figure 7 & 8), indicating potential as a rich antioxidant source grown at EF with better agronomic practices. These findings are in agreed with Wootton et al. (2011), who reported variations in antioxidant potential and bio-accessibility between juice fractions and beet juices. The variation in antioxidant capacity not only provides health beneficial effects (Hussain et al., 2025), but also have positive effect in crop management and breeding potential. Mahmoud et al. (2024) concluded that ascorbic acid plays critical role in protection against the drought stresses faced by sugar beet, suggesting it ability to cope extreme climatic conditions.

Free radical scavenging assay

The highest free radical scavenging activity was exhibited by V2 under both growing conditions (EF and FF) as compared to V1. The antioxidant properties of *Beta vulgaris* are influenced by environmental conditions and cultivation

practices, as seen by the considerable variation in DPPH scavenging activity in both conditions.
The antioxidant properties of sugar beet are influenced by environmental conditions and cultivation practices, as seen by the considerable variation in DPPH scavenging activity amongst the samples. The LSD test exhibited significant differences ($p < 0.05$) in the levels of antioxidant activities performed while comparing its variation in organs (leaves and tuners), locations, and among varieties (Figure 9 & 10). The soil enzymes may also influence the edaphic factor favouring growth of crops (Nosheen et al., 2018) and not only the growing conditions favour the variation but also the genotypes or varietal differences influences such changes in crops (Javid et al., 2024; Hussain et al., 2025). The statistical results for such attributes by *Beta vulgaris* with respect to phytochemical characteristics were significantly different under experimental field (EF) practices as compared to conventional farmer field (FF). The V1 performed better than V2, showing greater adaptability to optimized cultivation in Skardu.
The enhancement under EF practices may be mainly due to the optimized spacing, balanced nutrient management, and improved irrigation, which created favorable conditions for secondary metabolite synthesis. Skardu's unique environment like high altitude, low pressure, and large temperature variations would be another possible stimulant for accumulation phytochemical accumulation. Such variations are also in agreement with Čeryová et al., (2025), who has concluded that variability in chemical composition influenced by plant parts, varieties and the growing conditions.
Overall, sugar beet grown under EF conditions tailored to Skardu's environment can significantly enhance crop quality, yield and crop protection against oxidative stresses supporting higher phytochemical constituents by local sugar beet variety of Skardu (V1) showing strong potential for sustainable cultivation in mountain areas.

CONCLUSION

The results of this investigation offer important new information about the phytochemical of two varieties of *Beta vulgaris*. Especially in controlled experimental settings, the V1 proved to be a superior choice in terms of productivity and bioactive chemicals. Because of this, it is a better option for growing in mountain regions of Pakistan (Gilgit-Baltistan) as healthy food. Such variation in chemical composition exhibited under contrasting growing conditions at higher attitude not only elaborate the potential role of sugar beet as functional food, but also elucidates its ability to cope under the stressful condition thereby Identifying a better solution to upturn the tolerance faced by sugar beet varieties. Furthermore, sugar beet's high phenolic and antioxidant content highlights its potential as a functional food and a source of bioactive chemicals for use in medicine.
The genetic and molecular causes of the observed differences in agronomic and phytochemical features require further investigation. Further investigation can aid in the formation of dietary supplements for mountain farmers and functional foods that can enhance health and wellbeing.

AUTHOR'S CONTRIBUTION

All authors agreed for publication for the present work and confirm that all materials, data, and results reported in this study are based on field and lab work followed by manuscript preparation and submission, where all authors made their due contributions.

FUNDING

No funding was received for the present study.

AVAILABILITY OF DATA AND MATERIAL

The datasets supporting this study are included in the article. Extended methodological details and raw data can be accessed by contacting the corresponding author, subject to ethical and institutional guidelines.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors acknowledge field work performed at Agriculture Complex, Department of Agriculture Skardu based on joint collaboration with the University of Baltistan Skardu UoBS and the lab work was performed at Dr. Ihsan-ul-Haq's Laboratory, QAU Islamabad, Pakistan.

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