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ALTITUDE-DRIVEN VARIATION IN MORPHOLOGY, PHYTOCHEMICALS, AND ANTI-NUTRITIONAL FACTORS IN BUCKWHEAT (*Fagopyrum esculentum)*

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ABSTRACT

Buckwheat, a naturally gluten-free pseudocereal belonging to the genus *Fagopyrum* (family *Polygonaceae*), is increasingly recognized for its exceptional nutritional and nutraceutical value. It is a rich source of high-quality proteins, essential amino acids, dietary fiber, vitamins, and minerals, along with a diverse array of bioactive compounds such as flavonoids and phenolic acids that contribute to its health-promoting properties. The present study was conducted to assess the variation in morphological traits and phytochemical composition of buckwheat cultivars collected from different altitudinal regions of Uttarakhand. The findings demonstrated a clear and significant trend: as altitude increased, there was a corresponding enhancement in key nutritional and biochemical parameters, including total phenolic content, total flavonoids, β-carotene, protein levels, and essential amino acids such as lysine, arginine, and tryptophan. This suggests that high-altitude environments may induce favorable metabolic adaptations that enrich the crop's nutraceutical profile. However, the study also observed a simultaneous increase in anti-nutritional factors, including tannins, phytic acid, and oxalic acid, indicating a complex balance between beneficial and limiting components influenced by altitude. Among the studied sites, Nijmola emerged as the most promising location, exhibiting the highest concentrations of bioactive compounds, whereas Kotdwar recorded comparatively lower values. Overall, the results highlight that higher altitudinal conditions are conducive to improving the nutritional quality and functional attributes of buckwheat, underscoring its potential as a valuable crop for the development of nutritionally superior and health-beneficial food products.

Keywords **:** Buckwheat, phytochemicals, anti-nutrients, amino acids, β-carotene.

Introduction

Buckwheat is a pseudocereal belonging to the genus *Fagopyrum* of the family Polygonaceae. It is a grain-like seed that is used in the same way as cereals such as wheat or rice but it does not belong to the grass family (Ferreira *et al*., 2022). Buckwheat is a dicot plant that produces irregular and triangular-shaped seeds. The seed is covered in brown-to-black colored hulls and has a white to light green kernel. The color and hardness of the buckwheat hulls may vary depending on the cultivar of the plant (Roy *et al*., 2019). Buckwheat is a low-maintenance crop that can be grown on marginal soils, making it a good cover or rotational crop. It has a quick maturity rate and can mature in as little as 30 days. The growth of buckwheat is so rapid that it can crowd out weed growth and is used to prepare soil for organic crops. The two most widely cultivated species are the common buckwheat *(F. esculentum)* and Tartary buckwheat *(F. tataricum)*. These species are rich in micro and macro nutrients, including proteins, amino acids, fatty acids, dietary fibres, vitamins, minerals, and bioactive compounds. These nutrients make buckwheat an exceptional superfood with various health benefits such as promoting heart health, regulating blood sugar levels and aiding digestion (Jin *et al*., 2022). Additionally, buckwheat contains bioactive polyphenolic compounds such as flavonoids and phenolic acids that can increase its nutraceutical potential. Flavonoids like rutin, isoorientin, quercetin, isovitexin, vitexin, and orientin are particularly abundant in buckwheat (Raguindin *et al*., 2021). Among all pseudocereals, rutin is

abundantly present in buckwheat, and it has higher antioxidant, anti-inflammatory, and anticancer properties than other flavonoids (Zhu, 2016). The flavonoid content in tartary buckwheat (40 mg/g) is higher than that in common buckwheat (10 mg/g) (Li and Zhang 2001). The accumulation of rutin is predominant in distinct regions of the buckwheat flora. Greater levels of rutin content are specifically present in the leaves with concentrations ranging from 0.08-0.10 mg/g. The seeds of the buckwheat plant exhibit a more variable range of rutin content which spans from 0.12-0.36 mg/g as indicated by Brunori *et al*. (2010) and Park *et al.* (2008). Phenolic acid content of buckwheat is 275.5-532.0 mg gallic acid equivalents/ 100 g dw (Liu *et al.*, 2019; Rochetti *et al*., 2019). Buckwheat is known to possess superior nutritional value compared to traditional cereals due to their significantly higher protein levels. In buckwheat, the protein content typically ranges from 5.7% to 14.2% (Shukla *et al*., 2018; Thanh-Tien *et al*., 2018). Buckwheat is also rich in vitamin A, B1, B2, and E. Carotenoids, a type of terpenoid, are known for their natural antioxidant properties and their ability to protect against UV radiation. Additionally, carotenoids like α- and β-carotene serve as precursors of vitamin A (Fiedor *et al*., 2014). The carotenoid content in buckwheat seeds has been reported to be around 3.6-3.7 mg/kg dw (Tang *et al.*, 2016; Tuan *et al.*, 2013).

Buckwheat is considered as nutritious food but like many other plant-based foods it contains certain compounds called antinutrients. Antinutrients such as oxalate, phytic acids, tannins, saponins, and enzyme inhibitors which are known to decrease the bioavailability of minerals like iron, calcium, and zinc. Antinutrients are well known for their ability to impair or interfere with certain biochemical reactions in our body, and decrease the bioavailability of nutrients in foods (Kaspchak *et al*., 2018). Antinutrients can be reduced through various technological and processing methods. The effectiveness of these methods depends on the type, structure, and chemical properties of the antinutrients present in the food (Samtiya *et al*., 2020).

Materials and Methods

Collection of seed samples

The seeds were collected from five different geographically hilly regions of Uttarakhand namely Nijmola, Joshimath, Lohaghat, Petshal and Kotdwar. The sources of seed samples of Buckwheat (*Fagopyrum esculentum*) with their locations are summarized in the (Table 1).

Table 1: Buckwheat seed samples with their locations

S. No.	Sample's locations	Latitude (N)	Longitude (E)	Altitude (Meters)
1.	Nijmola (Chamoli)	30.3680	79.4563	2590
2.	Joshimath (Chamoli)	30.5561	79.5617	1875
3.	Lohaghat (Champawat)	29.42	80.10	1706
4.	Sariyatal (Nainital)	29.3793	79.4310	1220
5.	Petshal (Almora)	29.5973	79.6609	520
6.	Kotdwar (Pauri Garhwal)	29.7453	78.5148	454

Source: www.uk.gov.in

Preparation of Methanolic Extract

The seeds (15 gm) were weighed using an electronic balance and then crushed into a fine powder with a grinder. The resulting powder was wrapped in filter paper and placed in the thimble of the Soxhlet apparatus. Methanol was used as the solvent, with the Soxhlet temperature set at 50°C. After eight hours, a mixture of the solvent and extract was obtained in the round-bottom flask of the assembly. The distillation unit was then used to vaporize the solvent, leaving behind the required methanolic extract in the flask.

Morphological characters of different buckwheat samples

Seed coat colour

The buckwheat seed coat ranged from blackish brown to light brown in colour, as visualized in the study by Ratan and Kothiyal (2011). Table 2 shows the various colours observed in different buckwheat samples.

Seed shape

The shape of buckwheat seeds is pyramidal (Table 2). These findings are consistent with those reported by Misra *et al.* (2019).

Seed coat texture

Upon observation, the texture of buckwheat seeds was found to vary from smooth to rough in nature (Table 2). These findings align with those reported by Bhavsar *et al.* (2013).

Table 2: Morphological characters of different buckwheat seeds samples

S.No.	Sample location	100 seeds weight (gm)	Seed coat colour	Seed texture	Extract Yield (g /100g)
1.	Nijmola	2.106	Light brown	Smooth	1.458
2.	Joshimath	2.006	Light brown	Rough	1.472
3.	Lohaghat	2.185	Blackish brown	Smooth	1.430
4.	Sariyatal	2.176	Light brown	Rough	1.447
5.	Petshal	2.160	Dark brown	Smooth	1.442
6.	Kotdwar	2.114	Light brown	Smooth	1.469

*The shape of the seeds in all the collected samples was pyramidal.

Estimation of nutritional parameters

Total Phenol Content

The total phenolic content was estimated using the Folin-Ciocalteu reagent. The method followed was given by Bray and Thorpe, (1954). 0.5 mL of methanolic extract was combined with 3 mL of distilled water in test tubes. Then, 0.5 mL of 2.0 M FCR was added, followed by 2 ml of 20% sodium carbonate solution. After 3 minutes, the mixture was thoroughly mixed and incubated at room temperature for 60 minutes. The absorbance was measured at 650 nm to calculate the total phenol content in milligrams per 100 grams of the sample using the standard graph of gallic acid.

Total Flavonoid Content

The total flavonoid content in sample extracts was analysed using the $AlCl_3$ colorimetric method (Jagadish *et al*. 2009). A 0.5 mL sample of methanol was pipetted into test tubes and made up to 1 mL with the solvent. Then, 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ were added. After 5 minutes, 1.5 mL of 2% methanolic $AlCl_3$ solution was added, followed by 2 mL of 1 M NaOH and 10 mL of distilled water after another 5 minutes. The mixture was vigorously stirred, and after 10 minutes of incubation, the absorbance was measured at 367 nm. The total flavonoid content was calculated from a standard graph of quercetin and expressed in milligrams per 100 grams of the sample.

β-carotene Estimation

β-carotene was estimated using the method suggested by Srivastava & Kumar, (2002). Sample (5 g) was ground and homogenized with 10 ml acetone in the presence of a few crystals of anhydrous sodium sulfate. The supernatant was collected after decantation. The same process was repeated three times and the resulting supernatants were mixed and put in a separating funnel. It was mixed thoroughly by adding petroleum ether (10 ml). On standing, two layers were formed. The bottom layer was discarded and the top layer was collected in a 100 ml volumetric flask. Petroleum ether was added to fill up the flask to a volume of 100 ml and using petroleum ether as a reference. The optical density was measured at 452nm. The β-carotene was calculated using the following formula:

$$\beta - \text{carotene } (\mu g/g) = \frac{\text{Optical density X 13.9 104 X 100}}{\text{Weight of sample X 560 X 1000}}$$

Extraction and Determination of Protein

before extraction. Approximately 1 gm of seeds were then mixed with 0.2 M phosphate buffer (pH 7.2) and were grinded using mortar and pestle over an ice bucket. Then centrifuge it at 10,000 rpm for 20 minutes at 4ºC. The collected supernatant was then quantified according to the method given by Lowry *et al*. (1951). The standard curve was prepared using BSA and the protein content in the sample was expressed in g/100g of dry wt powder.

Estimation of Lysine Content

The estimation of Lysine was carried out by the method given by Sadasivam and Manickam, (1992). 100 mg of defatted sample treated with 5 ml papain solution, incubated at 65°C overnight. After centrifugation, collect the supernatant. Various required reagents were added to measure the lysine content. A standard curve was prepared using standard lysine solution and carbonate buffer. Papain was added to each tube and then an amino acid mixture and copper phosphate suspension were added. These steps were repeated. The standard curve was plotted for lysine concentrations of 40, 80, 120, 160 and 200 µg/ml. Then, the lysine content was calculated from the standard graph.

Estimation of Arginine Content

The arginine content was determined by Sakaguchi method as described by Tomlinson and Viswanatha, (1974). The sample was hydrolyzed in 6N HCl at 120°C for 8 hours, neutralized with NaOH and diluted to 40ml with D.W. Then, α- naphthol, KOH,

urea and potassium hypobromite were added successively. The absorbance at 520 nm was measured after 20 minutes and the arginine content was determined using a standard curve.

Estimation of Tryptophan Content

Tryptophan content was determined using spectrophotometric method given by Spies and Chambers, (1949). In a conical flask, 50 mg powdered defatted sample was mixed with 30 mg of p-dimethyl amino-benzaldehyde and 10 mL of 19N H_2SO_4. The mixture was kept in the dark for 12 hours at room temperature then centrifuged for 15 min at 5000 rpm. After adding 0.1 mL of 0.045% $NaNO_2$ solution, absorbance was measured at 454 nm. Tryptophan content was calculated from a standard curve and expressed as g/100 g of protein.

Estimation of Antinutrients

Total Tannin content

The total tannin content was determined according to the spectrophotometric method given by Chandran and Indira, (2016). A 10 ml volumetric flask was filled with 0.5 ml of methanolic extract, 0.5 ml of ddH_2O, 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and 10 ml of ddH_2O. The mixture was kept at room temperature for half an hour. Tannic acid standard solutions (20, 40, 60, 80, 100g/ml) were prepared using the same method. A UV/Visible spectrophotometer was used to measure the absorbance of the solutions at 700 nm. The tannin content was calculated from the standard graph of tannic acid and measured in mg of tannic acid equivalents per 100 grams of dry weight sample.

Total Phytic Content

The phytic acid concentration in a 0.5 g powdered sample was estimated using the spectrophotometric method described by Haug and Lantzsch (1983). The sample was extracted with 25 mL of 0.2 N HCl, filtered and then mixed with ferric ammonium sulfate. After boiling and adding bipyridine solution, the absorbance was measured at 519 nm, using distilled water as the blank.

Total Oxalate Content

Total oxalate was analyzed by extraction with hydrochloric acid as described by Baker, (1952). In a volumetric flask, 2 g of powdered seed sample was digested in 10 mL of 6 M HCl for one hour. The pH of the filtrate was adjusted with NH_4OH until the color changed to pale yellow. Insoluble oxalate was precipitated with 10 mL of 5% $CaCl_2$, then centrifuged. The precipitate was dissolved in 10 mL of 20% H_2SO_4, resulting in a total volume of 300 mL. A 125 mL aliquot was titrated against 0.05 M $KMnO_4$ solution to calculate the oxalate content.

Result and Discussion

Nutritional Parameters of untreated buckwheat seeds

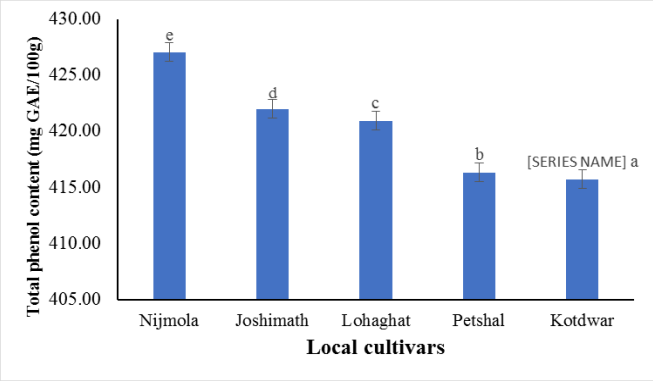
Total Phenols, total flavonoid and β-carotene content in buckwheat seeds

Buckwheat seeds were collected from different locations Nijmola, Joshimath, Lohaghat, Petshal and Kotdwar. The measurements of total phenol content were taken for the samples from each location ranged from 427.05±0.83-415.72±0.84 mg GAE/100 g DW, respectively. The findings showed that the Nijmola seeds had the highest total phenol content of 427.05 ±0.83 mg GAE/100 g DW while the Kotdwar seeds had the lowest at 415.72±0.84 mg GAE/100g DW (Fig 1, Table 3). These findings were consistent with previous findings by Liu *et al*. (2019); Rochetti *et al*. (2019). Further analysis for total flavonoids content showed that the Nijmola seeds had the highest amount at 329.66±1.23 mg QE/100 g DW sample whereas the seeds from Kotdwar had the lowest level at 321.20±0.81 mg QE/100 g DW sample (Fig 2, Table 3). These findings were in agreement with the results of Beitane *et al*. (2018). Additionally, the Nijmola seeds exhibited the highest level of β-carotene, measuring at 2.89±0.12 mg/100 g, while the seeds from Kotdwar had the lowest amount at 2.82±0.15 mg/100 g (Fig 3, Table 3). The results of present study were consistent with the results obtained by Wijngaard and Arendt (2006).

Table 3: Total phenol, flavonoid and β-carotene content of raw buckwheat grains

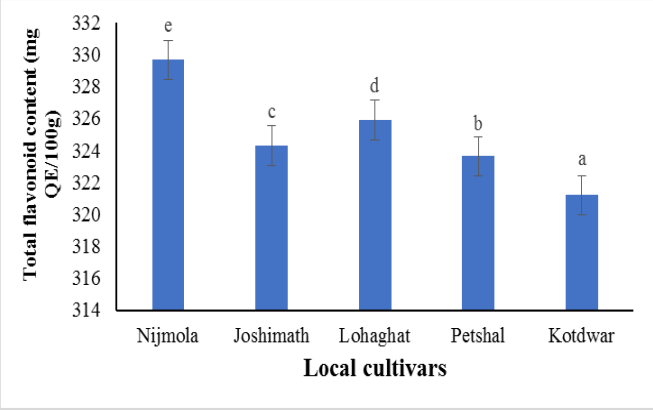
Location	Total phenol content (mg GAE/100gm)	Total flavonoid content (mg QE/100gm)	β-carotene content (mg/100gm)
Joshimath	427.05 ±0.83	324.30±0.80	2.85±0.08
Kotdwar	420.95±0.84	323.64±0.82	2.82±0.15
Lohaghat	422.01±0.83	325.89±0.81	2.84±0.08
Nijmola	416.33±0.83	329.66±1.23	2.89±0.12
Petshal	415.72±0.84	321.20±0.81	2.84±0.06

Note: Values are mean of triplicate ± SD



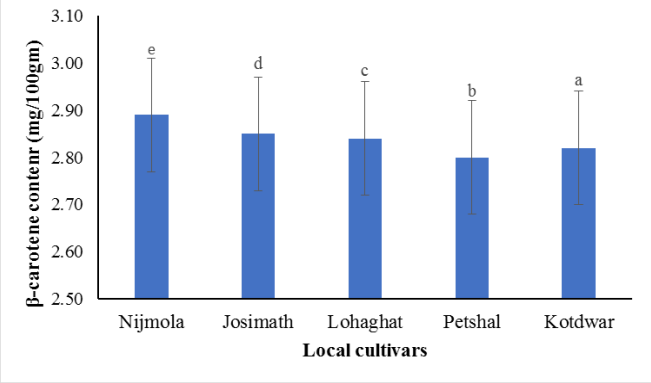
Note: Error bars represent mean of triplicate ± SD. Different letters denote statistically different values ($p<0.05$)

Fig. 1: Variability in total phenol content of different buckwheat cultivars



Note: Error bars represent mean of triplicate ± SD. Different letters denote statistically different values ($p<0.05$)

Fig. 2: Variability in total flavonoid content of different buckwheat cultivars



Note: Error bars represent mean of triplicate ± SD. Different letters denote statistically different values ($p<0.05$)

Fig. 3: Variability in β-carotene content of different buckwheat cultivars

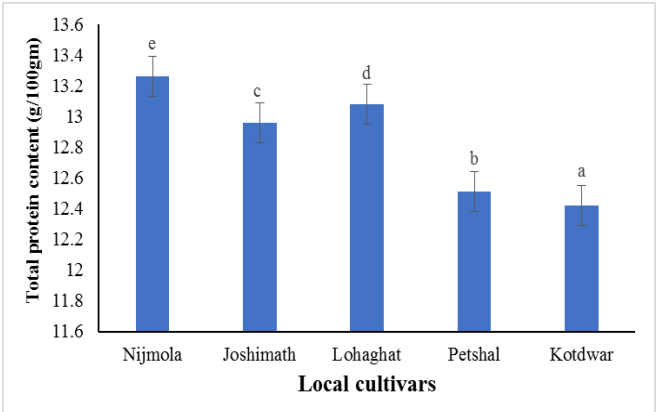
Protein, Lysine, Arginine and Tryptophan content of untreated buckwheat seeds

The protein quantities of buckwheat seed samples collected from different locations varied between 13.26±0.13 g/100g dw to 12.42±0.11 g/100g dw. Nijmola had the highest protein content while Kotdwar had the lowest (Fig. 4, Table 4). Results are in consistent with findings by Steadman *et al*. (2001). Also, the lysine concentrations of seeds from Joshimath, Kotdwar, Lohaghat, Nijmola and Petshal were ranged from 5.24±0.07-5.17±0.06, respectively with Lohaghat having the highest lysine content and Kotdwar having the lowest. Moreover, arginine content among the same seed samples ranged from 7.23±0.06 to 7.11±0.06 with Nijmola having the highest and Kotdwar having the lowest amounts of arginine. Finally, tryptophan levels in the seeds of Nijmola, Joshimath, Lohaghat, Petshal and Kotdwar were ranged from 2.44±0.01-2.39±0.02, respectively, with Nijmola seeds containing the highest and Kotdwar seeds containing the least amount of tryptophan (Fig 5, Table 4). These results are in accordance with the findings by Aubert *et al*. (2021).

Table 4: Total protein, lysine, arginine and tryptophan content of raw buckwheat grains of different geographical regions

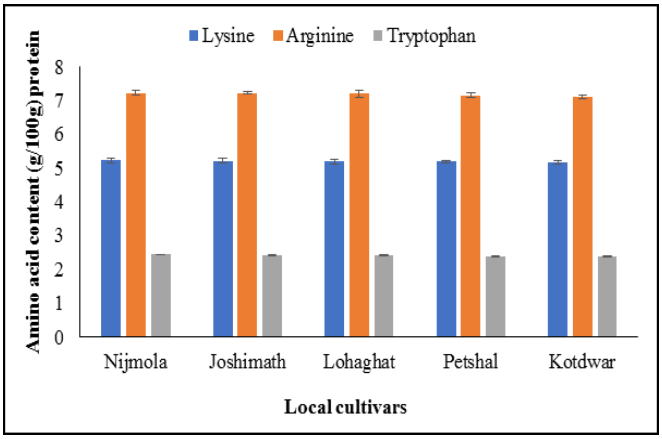
Location	Protein (g/100gm)	Lysine (g/100gm protein)	Arginine (g/100gm protein)	Tryptophan (g/100gm protein)
Nijmola	13.26±0.13	5.24±0.07	7.23±0.06	2.44±0.01
Joshimath	12.96±0.15	5.22±0.08	7.22±0.04	2.42±0.02
Lohaghat	13.08±0.15	5.20±0.07	7.20±0.11	2.43±0.01
Petshal	12.51±0.12	5.19±0.04	7.16±0.06	2.40±0.02
Kotdwar	12.42±0.11	5.17±0.06	7.11±0.06	2.39±0.02
CD at 1%	0.4626515	0.4576657	0.4815504	0.7184774
CD at 5%	0.3255004	0.3219927	0.3387968	0.5054878
SEM	0.1033291	0.1022156	0.1075500	0.1604656

Note: Values are expressed as mean of triplicate ± SD



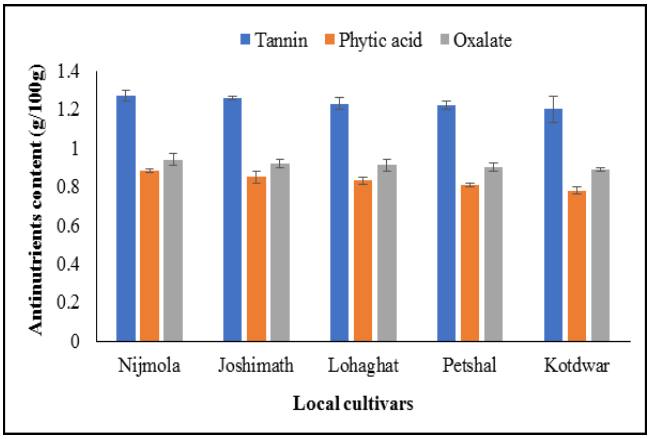
Note: Error bars represent mean of triplicate ± SD. Different letters denote statistically different values ($p<0.05$)

Fig. 4: Variability in total protein content of different buckwheat cultivars



Note: Error bars represent mean of triplicate ± SD. Different letters denote statistically different values ($p<0.05$)

Fig. 5: Variability in lysine, arginine and tryptophan content of different buckwheat cultivars



Note: Error bars represent mean of triplicate ± SD. Different letters denote statistically different values ($p<0.05$)

Fig. 6: Variability in tannin, phytic acid and oxalate content of different buckwheat cultivars

Anti-nutritional Parameters of untreated buckwheat seeds

Tannin, Phytic acid and Oxalate content

The tannin levels present in buckwheat seeds collected from regions Nijmola, Joshimath, Lohaghat, Petshal and Kotdwar were ranged from 1.27±0.01 to 1.20±0.02, respectively. The samples from Nijmola and Kotdwar exhibited the highest and lowest levels of tannins, respectively. Meanwhile, the phytic acid concentration was ranged from 0.88±0.01 to 0.78±0.02. It was observed that the samples taken from Nijmola had the highest phytic acid content whereas those taken from Kotdwar had the lowest. Additionally, the oxalate levels in samples from these regions was ranged from 0.94±0.03-0.89±0.01, respectively, with the highest oxalate levels being found in the samples from Nijmola and the lowest in those from Kotdwar (Fig 6, Table 5). The results are in confirmation with the study of Kasar *et al*. (2021).

Table 5: Tannin, Phytic acid and Oxalate content of raw buckwheat grains of different geographical regions

Location	Tannin (gm TAE/100gm)	Phytic acid (g/100gm dw)	Oxalate (gm /100gm dw)
Nijmola	1.27±0.01	0.88 ±0.01	0.94±0.03
Joshimath	1.26±0.07	0.85 ±0.03	0.92±0.02
Lohaghat	1.23±0.03	0.83 ±0.02	0.91±0.03
Petshal	1.22±0.03	0.81 ±0.01	0.90±0.02
Kotdwar	1.20±0.02	0.78 ±0.02	0.89±0.01
CD at 1%	0.1084583	0.4639068	0.1895070
CD at 5%	0.7630631	0.3263836	0.1333285
SEM	0.2422321	0.1036095	0.4232472

Note: Values are expressed as mean of triplicate ± SD

Conclusion

The present investigation clearly revealed a pronounced and consistent elevation in the biochemical composition of buckwheat samples with increasing altitude, underscoring the strong influence of agro-ecological conditions on nutritional quality. A marked and progressive enhancement was observed in total phenols, total flavonoids, β-carotene, total protein, and essential amino acids such as lysine, arginine, and tryptophan. Notably, this enrichment was accompanied by a simultaneous rise in anti-nutritional factors including tannins, phytic acid, and oxalic acid, indicating a complex altitude-driven metabolic response. Among all the studied locations, Nijmola distinctly emerged as the most superior site, exhibiting exceptionally high concentrations of bioactive compounds and amino acids in unprocessed buckwheat, thereby highlighting its potential as a nutritionally rich source. In stark contrast, samples from Kotdwar consistently recorded the lowest levels of these constituents, emphasizing the comparatively limited nutritional accumulation at lower altitudes.

Overall, these findings strongly suggest that higher altitudinal environments significantly enhance both the nutraceutical and biochemical profile of buckwheat, making such regions highly favourable for cultivating nutritionally enriched crops.

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Author Contribution

P.C: Conceptualization, Data curation, Formal analysis, Methodology, Writing- original draft. **K.C:**

Conceptualization, Overall Supervision Formal analysis, Writing- review and editing. **P.T:** Sample collection and data analysis. All authors contributed to and approved the final draft of the manuscript.

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