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RESEARCH ARTICLE

Comparative Analysis of Nutritional Values, Bioactive Compounds and Antinutrients in Tartary and Common Buckwheat Leaves

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Abstract

Among the millets grown in the Himalayan region of India, common and tartary buckwheat are the most cultivated species. Due to its extensive nutritional and antioxidant characteristics, buckwheat is attracting much interest, especially in producing nutrient-rich healthy foods. The objective of this study was to investigate nutritional values (protein, lysine, arginine and tryptophan content), bioactive compounds (total phenolic and flavonoid contents, antioxidant and rutin content) and antinutrients (phytate, tannin and oxalate) in tartary and common buckwheat leaves. Till now, very little information is available on the phytochemical constituents of buckwheat leaves as compared to the seeds. Common buckwheat leaves contain significantly ($p < 0.05$) more protein (22.21 ± 0.23 gm/100 g), lysine (12.41 ± 0.41 gm/ 100 g protein), arginine (6.61 ± 0.29 gm/100 g protein) and tryptophan (3.58 ± 0.36 gm/100 g protein) content then tartary buckwheat. There were relatively minor differences in the contents of amino acids. The results showed that the phenol (1768.16 ± 0.78 mg GAE/100 g), flavonoid (678.31 ± 0.27 mg QE/100 g), total antioxidant activity (52.89 ± 0.77 mg/100 g), rutin (11.32 ± 0.10 gm/100 gm), phytate (2.84 ± 0.07 gm/100 gm), tannin (2.76 ± 0.08 gm/100 gm), and oxalate (4.27 ± 0.03 gm/100 gm) content of the tartary buckwheat samples were significantly ($p < 0.05$) higher as compared to that of the common buckwheat leaves. On the basis of this study, it can be concluded that tartary buckwheat leaves are superior as green vegetables compared to common buckwheat for preventative nutrition.

Keywords: Buckwheat, Phenol, Flavonoid, Lysine, Antinutrients, Rutin

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Introduction

Buckwheat is an ancient worldwide accepted, nutrient dense and healthy crop of Polygonaceae family and is cultivated in diverse ecological regions mostly to produce grains for human sustenance. Buckwheat, in India, is grown chiefly in hilly areas of Jammu and Kashmir, Uttarakhand, Himachal Pradesh, Uttar Pradesh, Darjeeling, upper Assam region, Nilgiris, Sikkim and Tamil Nadu and Kerala hills (Phull and Gupta 2023). *Fagopyrum* is a small genus consisting of two cultivated species and over 25 wild buckwheat species, the majority of which are endemic to southwest China, which is regarded the origin, distribution, and diversity centre. Common buckwheat (*F. esculentum* Moench) and tartary buckwheat (*F. tataricum* Gaertn) are two species that are widely cultivated and spread all over the world. Both cultivated species have health benefits, are gluten-free, and have one of the most balanced amino acid composition of any cereal product (Luthar *et al*., 2021). Buckwheat is an annual or perennial herb that grows 0.5 to 2.5 m tall and has hollow, branching or unbranched reddish stems with white or pink blossoms. Plants grow rapidly, with little heart-shaped leaves and slender hollow stems (Ahmed *et al*., 2014). Buckwheat is an underutilized food crop that is gaining popularity,

especially in the development of nutrient-rich health foods due to its extensive nutritional, therapeutic, and antioxidant characteristics (Kumari & Chaudhary 2020). Buckwheat is an annual plant that is grown for both its seeds and its leaves. Buckwheat leaves and flowers are utilised in traditional medicine in India. Buckwheat leaves are used in the hills of Uttarakhand as a green leafy vegetable (hara saag).

Tea is made from the leaves of plants, which lowers cholesterol and glucose levels in the blood. The high amount of phenolic compounds and dietary fibre in buckwheat leaves contributes to these qualities. Buckwheat seeds are also recognised as an important dietary source due to their high nutritional value proteins and other bioactive compounds. Buckwheat seeds are processed into groats, flour, pasta, and bread (Dziadek *et al*., 2018). Buckwheat has grown in popularity as a health food in several countries in recent years, due to its capacity to grow in unfavourable climatic conditions and low cultivation cost. Buckwheat holds a lot of promise as a substitute staple crop because it meets all of the requirements present in commonly cultivated crops (Rana *et al*., 2012). Rutin, a valuable antioxidant found in abundance in buckwheat leaves, is used extensively in the cosmetic and pharmaceutical industries. It is a common functional component of buckwheat, having biological properties such as anti-hypertension activity, lowering cholesterol levels, reducing headaches and fatigue, and lowering body mass index, as well as anti-inflammatory, antithrombotic, and cytoprotective activities. It can also aid in the prevention of cancer and cardiac disease (Saraswat & Kumar, 2019**).** Most of the studies are focus on chemical composition of buckwheat seeds as well as health properties. There are little data concerning the study of other plant parts of buckwheat. Therefore, this study aimed to determine the nutritional composition, bioactive compounds, antioxidants and ant-nutrients in leaves between most cultivated buckwheat species in Uttarakhand.

Material and Methods

Collection and cultivation of buckwheat species

Seed samples of both the species (*F. esculentum* and *F. tataricum*) were collected from Magroli and Jageshwar regions of Uttarakhand. Seeds were dried and cleaned to remove all the foreign particles and damaged seeds. The dried and cleaned seed samples were packed in glass jars and stored at room temperature in dark condition. Seeds were grown at Norman E. Borlaug Crop Research Centre, Pantnagar in November 2022 and the plant leaves were used in the study.

Preparation of extract

Leaves were dried in natural condition [at ambient temperature (20–25°C) in a shaded, well-ventilated area]. Natural drying is the most popular method for preservation of herbs in various types of plants. The methanolic extract were prepared as per A.O.A.C., 1965. In this method, powdered samples (100 g) of buckwheat were wrapped in muslin cloth and placed in the thimble of the soxhlet apparatus. Methanol (80%) was used as solvents for extract preparation (0.5 mg/mL). Soxhlet was run at 50°C temperature for 8 hours. Solvents were separated using distillation unit, followed by further concentrating the extracts on water bath.

Estimation of total phenol content

The total phenolic content (TPC) was ascertained using the Folin-Ciocalteu method (Bray and Thorpe, 1954). A 0.5 mL (1 mg/10 mL) aliquot of methanol was taken in the test tubes. A volume of 3 mL of distilled water and 0.5 mL of 2.0 M Folin-Ciocalteu reagent were added. The tube was slowly filled with 2 mL of a 20% (w/v) Na_2CO_3 solution after 3 minutes of incubation. The absorbance at 650 nm was measured following 60 minutes of incubation at room temperature. Using a calibration curve for 98% gallic acid, the total phenol content was determined and expressed as mg/100 g of dry wt. sample.

Estimation of total flavonoid content

The $AlCl_3$ method was used to calculate the total flavonoid content (TFC) (Jagadish *et al*., 2009). The test tubes were filled with a 0.5 mL aliquot of the methanolic extract, and the volume was kept constant at 1-mL using methanol 0.3 mL of 5% $NaNO_2$ and 4 mL of dH_2O were used to prepare the blank. Then, 1.5 mL of a 2% $AlCl_3$ solution was added after 5 minutes. After adding 2 mL of 1 M NaOH to each tube, 10 mL of distilled water was then added. The mixture was vortexed 10 minutes after it had been incubated. Different quercetin concentrations (20, 40, 60, 80, and 100 g/mL) were used to make the standard curve, and the absorbance was measured at 367 nm. Total flavonoid content was expressed in 98% quercitin milligrams per 100 gm of dry wt. sample.

Estimation of total antioxidant capacity

The total antioxidant capacity was determined by Prieto *et al*., (1999) using a phosphomolybdenum-based spectrophotometric technique. Methanolic extract (0.5 mL) and 80% methanol were combined in a test tube, and then 3 mL of phosphophomolybdenum reagent was added. The test tube was then incubated for 90 minutes at 95°C in a water bath. At 37°C, samples were allowed to cool. The standard was ascorbic acid (1-mg/mL), and the absorbance was determined at 695 nm. In terms of ascorbic acid equivalent, the total antioxidant activity was measured in gram per 100 gram of dw sample.

Estimation of crude protein

The Kjeldahl method was used to determine crude protein (Lynch and Barbano, 1999). Digestion, distillation, and titration are the three main steps. Strong acids, such as

sulfuric acid, were used to digest the leaf samples, and bases were used for distillation and an indicator dye for titration. After that, nitrogen content was calculated using the formula below:

$$\%\text{Nitrogen} = \frac{\text{Titre value} \times 0.00014}{\text{Weight of the sample}} \times \frac{\text{Volume made up}}{\text{aliquot of distilled}} \times 100$$

Crude protein content (g/100 g of dry wt. sample) was calculated by multiplying nitrogen content by conversion factor (6.25).

Estimation of lysine content

An established given by Sadasivam and Manickam (1992) was used to ascertain the lysine content. Defatted leaf sample 100 mg was taken, 5 mL papain solution was added and incubated at 65°C whole night. At room temperature, it was centrifuged at 3000 rpm for 10 minutes. In a centrifuge tube, supernatant (1-mL) was combined with copper phosphate reagent (0.5 mL) and carbonate buffer (0.5 mL) followed by centrifugation. Supernatant (1-mL) was mixed with 0.1 mL of 2-chloro-3, 5-dinitrophyridine solution. After 2 hours 5 mL of 1.2 N HCl was added and in a separatory flask 5 mL of ethyl acetate was added in previously obtained, top layer was removed. At 390 nm, the absorbance was calculated against a reagent blank (papain). For lysine concentrations of 40 to 200 g/mL, the standard curve was created and lysine content measured as mg/100 g of protein through standard graph.

Estimation of arginine content

The Sakaguchi method was used to calculate the arginine content (Tomlinson and Viswanatha, 1974). Defatted buckwheat leaf powder (100 mg) were hydrolyzed at 120°C for 8 hours with 2 mL of HCL (6 N). Sodium hydroxide was used for neutralization, and distilled water was used to dilute it to 40 mL. α -naphthol (0.1%) and KOH (10%) both 1-mL were added to 0.5 mL of the neutralized mixture and thoroughly blended. Following the addition of a drop of urea (5%) and 2 mL of potassium hypobromite, the mixture was continuously shaken. The arginine solution was substituted for distilled water to prepared a reagent blank, which was then incubated for 20 minutes at room temperature. A standard curve was created using various 20 to 100 mg/mL of 99% pure arginine concentrations by taken absorbance at 520 nm. The arginine content was expressed as g/100 g of dry wt. sample.

Estimation of tryptophan content

Spectrophotometric method was used to determine the tryptophan content of leaf samples (Spies and Chambers, 1949). A conical flask containing defatted 50 mg leaf powder, 30 mg p-dimethyl amino-benzaldehyde, and 10 mL of H_2SO_4 (19N) was thoroughly mixed, and the mixture was then left at 37°C in the dark for 12 hours. After incubation, 0.1 mL of $NaNO_2$ (0.045%) was added, the mixture was centrifuged for 15 minutes at 5000 rpm. Tryptophan was used to draw the standard graph, absorbance was measured at 454 nm, and finally, the amount of tryptophan was expressed in mg/100 g of protein.

Estimation of rutin

The amount of rutin was calculated based on spectrophotometric method (AOAC, 1995). Extract was prepared by 100 mg of leaf powder using methanol: aceticacid: water (100:2:100 V/V) for 60 minutes at room temperature. The absorbance of the diluted sample was measured at 352 nm. Rutin content (%) in samples was calculated using standard curve of rutin.

Estimation of tannin content

The total tannin content was determined using the spectrophotometric method (Chandran and Indira, 2016). A volumetric flask containing 0.5 mL of methanolic extract, 0.5 mL of distilled water, 1-mL of Na_2CO_3, and 0.5 mL of Folin-Ciocalteu reagent (FCR) was properly mixed followed by incubation for 30 minutes at room temperature. The same procedures were used to create a tannic acid standard calibration curve for a range of concentrations (20–100 µg/mL). At 700 nm, the absorbance was calculated. A standard graph was used to calculate the tannin acid content (measured in mg of tannic acid equivalents per 100 g of dry wt. sample).

Estimation of phytic acid content

To calculate the amount of phytic acid, the spectrophotometric method described by Haug and Lantzsch, 1983. The 0.5 g of powdered leaf sample was extracted using 0.2 N, 25 mL HCl for 3 hours on a shaker and then filtered through No. 1 Whatman filter paper. Filtrate (0.5 mL) was taken from the mixture, added to 0.9 mL of distilled water, 1-mL of ferric ammonium sulphate, and 30 minutes of incubation was done in a boiling water bath. After mixing 1.5 mL of freshly made bipyridine solution and 1-mL of the supernatant in a blank tube, absorbance was measured at 519 nm. Utilising a standard sodium phytate graph, the amount of phytic acid in the sample was calculated and expressed as g of phytic acid per 100 g of dry wt. sample.

Estimation of oxalate content

The method was described by Baker, 1952 in a volumetric flask, 2 gm of powdered buckwheat leaf sample was digested for one hour with 10 mL of 6M HCl before being filtered through Whatman No. 1 filter paper. Using a concentrated NH_4OH solution, the pH of the filtrate was raised until a pale yellow color appeared in place of the salmon pink color. Following this, the insoluble oxalate was precipitated from the filtrate using 10 mL of $CaCl_2$ solution (5%) and centrifuged for 10 minutes at 2500 rpm. The precipitate obtained was redissolved in 10 mL of 20% H_2SO_4. The filtrate's volume was increased to 200 mL, and then it was heated till boiling. This filtrate was titrated for 30 seconds with a 125 mL aliquot of 0.05 M standard potassium permanganate solution, and the

volume of the standard solution used was recorded. The titre value was used to calculate the oxalate content in mg/100 gof dry wt. Sample.

Statistical Analysis

All the experiments were being performed three times and one-way analysis of variance (ANOVA) was done on a program developed by Computer, Mathematics and Statistics Department of Pantnagar University, to determine variations in the estimated phytoconstituents with respect to the species and with other biochemical parameters. All readings were presented as mean ± SD (standard deviation).

Results and Discussion

Total phenol content (TPC) in leaves of common buckwheat was 1477.47 ± 0.98 and 1768.16 ± 0.78 mg GAE/100 gm of dry wt. in tartary buckwheat leaves. The TFC of the common and tartary buckwheat leaves was 561.79 ± 0.65 and 678.31 ± 0.27 mgQE/100 gm of dry wt. sample respectively. Obtained results were in agreement with Jiang *et al*., 2007, who stated that tartary buckwheat seeds has been shown to contain a higher content of flavonoids in comparison with common buckwheat. It was also reported that tartary buckwheat exhibited a remarkably higher TPC and TFC compared to common buckwheat (Liu *et al*., 2019). Total antioxidant activity in common and tartary buckwheat was 41.63 ± 0.62 and 52.89 ± 0.77 mg/ 100 gm of dry wt. sample. It is reported that antioxidant capacity of the common buckwheat was significantly lower than that of tartary buckwheat (Tien *et al*., 2018). Rutin content was 8.75 ± 0.07 and 11.32 ± 0.10 g/100 gm of dry wt. in common and tartary buckwheat, respectively (Table 1). Tartary buckwheat seeds contained more rutin than common buckwheat seeds (Fabjan *et al*., 2003).

Tartary buckwheat grains possessed three to four times more antioxidative activity then common buckwheat grains, and their rutin content was more than ten times that of common buckwheat (Lee *et al*., 2016). Polyphenols contribution to antioxidative activity differs between common and tartary buckwheat. Not only rutin content was lower in common buckwheat than tartary buckwheat, but so were other polyphenol components (Morishita *et al*., 2007). Tartary buckwheat has higher concentrations (at least two fold) of 61 flavonoids and 94 non-flavonoid secondary metabolites than common buckwheat. It is proposed that tartary and common buckwheat grains are both rich in secondary metabolites that are beneficial to human health. Non-flavonoid metabolites, especially, may have contributed to tartary buckwheat's better health-promoting value as compared to common buckwheat. Plants of common and tartary buckwheat survived at high altitudes by gradually accumulating genes for secondary metabolite synthesis, which allowed the plants to survive and reproduce in the less favorable environment. The richness and diversity of tartary buckwheat compounds protect plants from UV radiation, pathogens, and grazing (Kreft *et al*., 2022).

High protein content was observed in the common buckwheat leaves 22.21 ± 0.23 gm/100 g dry wt. as in tartary buckwheat *i.e.* 19.27 ± 0.52 g/100 gm of dry wt. Sample (Table 2). Qin *et al*., 2010 reported that there were no significant differences in protein content between tartary buckwheat flour and common buckwheat flour on average. The protein content of the tartary buckwheat groats was higher than that of the common or tartary buckwheat groats (Tien *et al*., 2018). The lysine content was 12.41 ± 0.41 g min the leaves of common buckwheat, while 10.91 ± 0.36 g/100 gm in dry wt. of protein in tartary buckwheat leaves. More arginine content was observed in the leaf of common buckwheat *i.e.* 6.61 ± 0.29 g and the slightly lower was found in the tartary buckwheat *i.e.,* 5.64 ± 0.32 g/100 gm dry wt.

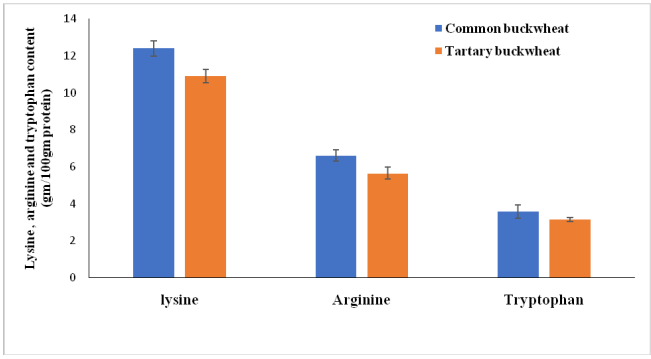

Figure 1: Lysine, arginine and tryptophan content (g/100 gm protein) in common and tartary buckwheat

Table 1: Phenol, flavanoid, antioxidant and rutin content of common and tartary buckwheat leaves

Parameters / *Species*	*Total phenol content (mg GAE/100 g of dry wt.)*	*Total flavonoid content (mg QE/100g of dry wt.)*	*Antioxidant (mg/ 100g of dry wt.)*	*Rutin (g/100g of dry wt.)*
Common Buckwheat	1477.47 ± 0.98	561.79 ± 0.65	41.63 ± 0.62	8.75 ± 0.07
Tartary Buckwheat	1768.16 ± 0.78	678.31 ± 0.27	52.89 ± 0.77	11.32 ± 0.10
CD at 1%	40.42295	32.14022	1.930120	.5736279
CD at 5%	28.84563	22.93511	1.377325	.4093383
SEM	9.363048	7.444543	.4470680	.1328677

Table 2: Protein, lysine, arginine tryptophan, phytate, tannin and oxalate content of common and tartary buckwheat leaves

Parameters / Species	Protein	Lysine	Arginine	Tryptophan	Phytic acid	Tannin	Oxalate
Common Buckwheat	22.21 ± 0.23	12.41 ± 0.41	6.61 ± 0.29	3.58 ± 0.36	2.23 ± 0.19	2.11 ± 0.07	4.40 ± 0.20
Tartary Buckwheat	19.27 ± 0.52	10.91 ± 0.36	5.64 ± 0.32	3.14 ± 0.12	2.84 ± 0.07	2.76 ± 0.08	4.27 ± 0.03
CD at 1%	.8936064	.7803922	.4605601	.4047715	.6021335	.1659603	.2958711
CD at 5%	.6376734	.5568843	.3286535	.2888431	.4296797	.1184285	.2111322
SEM	.2069834	.1807599	.1066782	.09375602	.1394704	.03844089	.06853174

*Unit of all the parameters in the above table is g/100 gm dry wt. of sample

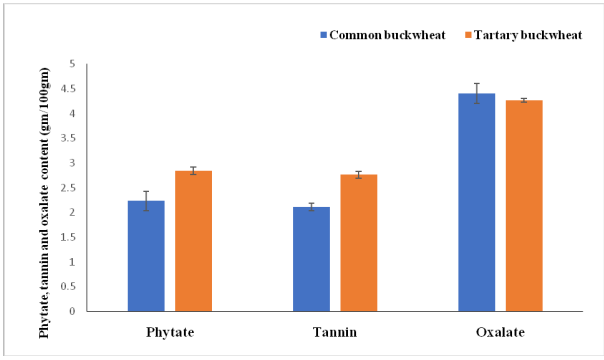

Figure 2: Phytate, tannin and oxalate content in common and tartary buckwheat

of protein. Tryptophan content in the leaves of common buckwheat was 3.58 ± 0.36 g, while in tartary buckwheat 3.14 ± 0.12 g/100 gm dry wt. of protein (Table 2, Figure 1). Amino acid profile is comparatively similar in both the buckwheat species (Bonafaccia *et al*., 2003)

Phytic acid content was 2.23 ± 0.19 and 2.84 ± 0.07 g/100 gm dry wt. sample in common and tartary buckwheat (Table 2, Figure 2).It is reported that tartary buckwheat having considerable higher content of phytic acid (Bonafaccia and Fabjan 2003). The tannin content in common and tartary buckwheat was 2.11 ± 0.07 and 2.76 ± 0.08 g/100 gm dry wt. Similar findings were also reported by Gadžo *et al*., 2010. The oxalate content in common and tartary buckwheat samples was 4.40 ± 0.20 and 4.27 ± 0.03 gm/100 gm of dry wt. Buckwheat genotypes showed a significant ($p \leq 0.05$) variation in oxalate content present in seeds (Dogra and Awasthi 2015).

Conclusion

There were significant (≤5%) phytochemical differences among cultivated buckwheat species in Himalayan regions. It may be due to the geographical locations, environmental conditions, harvesting time and genetic factors. The leaves of tartary buckwheat had significantly higher TPC, TFC, rutin, and antioxidant capacity than those of the common buckwheat. Tartary buckwheat was found to be a good source of nutrients and functional components and could be used for food processing. Higher amount of rutin content in tartary buckwheat will pave the way to fulfil the demand of rutin in cosmetic and pharmaceutical industries. Common buckwheat is also an important food, as it contains proteins with high biological value and balanced amino acids (lysine, arginine and tryptophan). This study will be helpful in improving various traditional buckwheat food products as per nutritional point of view. High value of rutin in tartary buckwheat will also boost the food, cosmetic and pharmaceutical industries.

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