Evaluation of nutritional and antioxidant potential of Indian Buckwheat grains

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In this study, grains of two buckwheat species: *Fagopyrum esculentum* (IC37309) and *Fagopyrum tataricum* (var. *himpriya*) were phytochemically investigated for their nutritional, and antioxidant potential for their use as functional foods. Methanolic extracts of grains of two *Fagopyrum* species showed higher phenolic content and antioxidant activity. Inductively coupled plasma optical emission spectrometry (ICP-OES) analysis presented these grains as a good source of minerals. This work demonstrated that grains of *Fagopyrum* species under study possess high protein, carbohydrate, phenolic content and antioxidative potential thus can be a good source of nutrition.

**Keywords:** *Fagopyrum esculentum, Fagopyrum tataricum, Antioxidant activity, Polyphenols, ICP-OES*

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Buckwheat (*Fagopyrum species*) (*Kuttu* in Hindi) is an old traditional underutilized crop plant belonging to the family Polygonaceae that had been used as staple food for local communities in India, Nepal, Bhutan and China, predominantly in the hilly and temperate areas of Europe and East Asia., curative herb or feed for animals long back until the expansion and industrialization of agriculture masked this crop and left behind all its significance, advantages and potential in the race towards the maximum amount producing crops. Buckwheat is often used in Japan to make traditional buckwheat soba noodles. It was also used as a protective crop for poor farmers below poverty line in China as it grows well in areas with low fertile soil, limited rainfall, used as green manure and plant for erosion control thus, source of income for farmers. Buckwheat is eaten during fasting in India considering it to be a fruit not as grain.

Genus *Fagopyrum* includes many species but only two are mainly cultivated and are commonly known as sweet buckwheat and bitter buckwheat, respectively. Buckwheat has been reported as a hypotensive, antihaemorrhagic, vasculoprotector drug, and can be used against circulatory disorders. Buckwheat proteins are proved to lower blood cholesterol, delay consenescence, prevent fat accumulation, and constipation. Buckwheat is known to contain the flavonoid rutin which helps in normalization of increased vascular permeability and fragility, hyaluronidase inhibition and oedema protection.

Research studies show a strong correlation between the antioxidant properties of plant derived polyphenolic compounds and their health-promoting and disease-preventing effects. Polyphenols are secondary plant metabolites which constitute a large group of structurally diverse compounds including flavonoids, lignins and tannins, and have demonstrated antibacterial, anti-carcinogenic, antiviral and vasodilator activity. Studies show that flavonoids exhibit antioxidant activities in addition to inhibition of enzymes related to tumorigenesis and induction of detoxifying enzymes. Flavonol intake is inversely proportional to the risk of heart disease, stroke, and lung cancer. Phenolic compounds are bound to cell wall components of hull in most cereal grains but buckwheat contains a majority of phenolics in free form, evenly distributed in the entire grain. All parts of grain (flour, hull and whole buckwheat) have been reported to exhibit high antioxidant activity.

In view of the growing interest towards health, there is a need to identify and quantify important compounds in underutilized grains to evaluate their nutritional potential and health benefits. The relationship between the intake of foods containing antioxidants and the illnesses caused by oxidative damage has become an important research area for...
food and nutrition. Recently many researchers have focused on the development of buckwheat as functional food material. In Japan, China and Italy buckwheat noodles are very popular made from buckwheat flour. Buckwheat is rich in nutrients and the limiting amino acids Thr and Met thus can be used as protein supplement additive. As buckwheat is eaten during fasting in India and has not been explored in terms of dietary usage, the aim of the present study was to determine various nutritional phytochemicals present in underutilized Indian buckwheat grains and their use as a potential source of natural antioxidants in order to understand their nutritional and other health benefits.

Methodology

Grain material
Grains of *F. esculentum* (accession no. IC37309) and *F. tataricum* variety himpriya were obtained from NBPGR, Shimla, India. The samples were crushed partially including hulls and flour, and were then analyzed for various parameters.

Solvent extraction
Partially crushed grains of the two buckwheat species were extracted with methanol using Soxhlet apparatus and the extracts were dried under vacuum and stored at 4°C.

Chemical analysis
Macro Kjeldhal method Ref. 988.05 was used for estimation of total nitrogen content and crude proteins. Nitrogen content was multiplied by a factor of 6.25 to get total protein. Moisture content was also determined. The ash content was analyzed by AOAC method Ref. 942.05. The fat content of the samples was determined by AOAC method Ref. 920.39. Total carbohydrate and Energy calorific value were also calculated. Total reducing sugar was calculated using AOAC method Ref. 920.183. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

Phytochemical analysis
Concentration of crude alkaloids, saponins and tannins in the dried powdered samples were determined. Total phenolic content of extracts of *F. esculentum* and *F. tataricum* was determined by Folin Ciocalteu reagent method and expressed in terms of mg gallic acid equivalents/g of dry extract (GAE). Aluminum chloride colorimetric method was used for flavonoids determination. Total flavonoid values were expressed in terms of mg rutin equivalents/g of dry extract (RE). Total flavonol content of the two extracts was determined and expressed as mg of RE per g of dry extract.

Antioxidant activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity
Extract (0.1 ml) was mixed with 1 ml of 0.1 mM DPPH. After 30 min of incubation at room temperature, absorbance was measured at 517 nm. Ascorbic acid and trolox (6-hydroxy-2,5,7,8-tetramethylecholramone-2-carboxylic acid) were used as standards.

ABTS (2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) assay
Different concentrations of extracts (10 µl) was mixed with 990 µl ABTS reagent and incubated for 10 min. Absorbance was measured at 734 nm. Ascorbic acid and trolox were used as standards.

FRAP (Ferric reducing antioxidant power) assay
Various concentrations of extracts (100 µl) was mixed with 900 µl of FRAP reagent. After 4 min of incubation at room temperature absorbance was measured at 593 nm. FRAP values were calculated as mg of trolox equivalents/g extract (TE).

Reducing power assay
The reducing power of the extracts of *F. esculentum* and *F. tataricum* was also determined. Increase in absorbance (700 nm) of the reaction mixture indicated increasing reducing power. Trolox was used as standard.

Results and discussion

Nutrition analysis
Nutritional studies have demonstrated potential benefits of buckwheat grains. Previous studies have shown almost similar content of proteins, fat and ash in *F. esculentum* and *F. tataricum*. In present studies, *F. tataricum* was found to be rich in proteins, carbohydrates and reducing sugars as compared to *F. esculentum*. However, crude fat was found to be higher in *F. esculentum* than *F. tataricum*. The two species were found to contain almost similar levels of ash and moisture on dry weight basis (Table 1). Energy calorific value of *F. esculentum* and *F. tataricum* was found to be 405.23±1.45 Kcal and 372.43±0.5 Kcal, respectively (p<0.0001). Basic
Phenolics were found to be more abundant in F. esculentum than F. tataricum (Table 3). High quantity of alkaloids was found in F. tataricum. The methanolic extracts of both buckwheat species were found to be nearly similar in both the species. The value of total phenolics, flavonoids and flavonols showed almost similar scavenging as standards ascorbic acid (IC$_{50}$ = 0.007±0.001 mg/ml) and trolox (IC$_{50}$ = 0.005±0.002 mg/ml) showed higher radical scavenging than buckwheat extracts. The extract of the two species were significantly not different with each other (p>0.05) but showed significant difference with two standards (p<0.0001).

**ABTS scavenging activity**

ABTS scavenging assay is applicable for screening both lipophilic and hydrophilic antioxidants. As shown in Fig. 2, methanolic extracts of F. esculentum (IC$_{50}$ = 0.06±0.001 mg/ml) and F. tataricum (IC$_{50}$ = 0.05±0.005 mg/ml) showed almost similar scavenging as standards ascorbic acid (IC$_{50}$= 0.003±0.003 mg/ml) and trolox (IC$_{50}$= 0.002±0.001 mg/ml) at concentrations above 0.2 mg/ml. There was significant difference (p<0.005) in ABTS scavenging activity of the extracts of the two buckwheat species as well as that of standards (p<0.0001).

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**Table 1—Nutritional content of F. esculentum and F. tataricum on dry weight basis (gm/100gm)**

<table>
<thead>
<tr>
<th>Buckwheat species</th>
<th>Ash</th>
<th>Moisture</th>
<th>Crude fat</th>
<th>Total protein</th>
<th>Total carbohydrate</th>
<th>Total reducing sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. esculentum</td>
<td>2.23±0.02$^a$</td>
<td>8.51±0.05$^a$</td>
<td>8.95±0.42$^a$</td>
<td>10.12±0.21$^a$</td>
<td>70.4±0.32$^a$</td>
<td>7.19±0.08$^a$</td>
</tr>
<tr>
<td>F. tataricum</td>
<td>2.4±0.03$^b$</td>
<td>8.42±0.06$^b$</td>
<td>3.11±0.36$^b$</td>
<td>14.34±0.30$^b$</td>
<td>71.8±0.12$^b$</td>
<td>8.38±0.14$^b$</td>
</tr>
</tbody>
</table>

Mean values within each column followed by different letter are significantly different at $p<0.05$.

**Table 2—Minerals, trace elements and heavy metals contents of F. esculentum and F. tataricum on concentration basis (ppm)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>F. esculentum</th>
<th>F. tataricum</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>2409.7±1.23$^a$</td>
<td>3132.91±0.40$^b$</td>
</tr>
<tr>
<td>P</td>
<td>2307±1.04$^a$</td>
<td>1541±0.48$^b$</td>
</tr>
<tr>
<td>Mg</td>
<td>1817.1±1.66$^a$</td>
<td>1230±0.43$^b$</td>
</tr>
<tr>
<td>Ca</td>
<td>748.3±0.51$^a$</td>
<td>505.4±0.40$^b$</td>
</tr>
<tr>
<td>Na</td>
<td>126±0.36$^a$</td>
<td>314.6±0.25$^b$</td>
</tr>
<tr>
<td>Mn</td>
<td>12.79±0.45$^a$</td>
<td>10.19±0.42$^b$</td>
</tr>
<tr>
<td>Fe</td>
<td>2.208±0.01$^a$</td>
<td>15.92±0.45$^b$</td>
</tr>
<tr>
<td>Cu</td>
<td>3.779±0.38$^a$</td>
<td>1.332±0.34$^b$</td>
</tr>
<tr>
<td>Ti</td>
<td>0.932±0.03$^a$</td>
<td>0.23±0.02$^b$</td>
</tr>
<tr>
<td>Sr</td>
<td>0.778±0.01$^a$</td>
<td>0.51±0.03$^b$</td>
</tr>
<tr>
<td>Ni</td>
<td>0.698±0.01$^a$</td>
<td>0.65±0.04$^b$</td>
</tr>
<tr>
<td>Cr</td>
<td>0.534±0.02$^a$</td>
<td>0.24±0.02$^b$</td>
</tr>
<tr>
<td>Mo</td>
<td>0.496±0.01$^a$</td>
<td>0.16±0.04$^b$</td>
</tr>
<tr>
<td>Li</td>
<td>0.181±0.001$^a$</td>
<td>N.D.$^b$</td>
</tr>
<tr>
<td>Co</td>
<td>0.126±0.001$^a$</td>
<td>N.D.$^b$</td>
</tr>
<tr>
<td>Pb</td>
<td>0.100±0.05$^a$</td>
<td>N.D.$^b$</td>
</tr>
</tbody>
</table>

N.D. Not defined. Mean values within each row followed by different letter are significantly different at $p<0.05$. 

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**chemical composition of the two buckwheat species demonstrated that F. tataricum is better source of nutrition than F. esculentum.**

Buckwheat grains have been reported to be rich in K, Mg, Ca and Na and an important nutritional source of microelements Fe, Mn and Zn. In present study also, both species of buckwheat were found to be rich in minerals Mg, P, K, Ca and Na (Table 2). Heavy metals and trace elements were under acceptable limits.

**Phytochemical analysis**

The phytochemical content of F. tataricum and F. esculentum were analyzed and the values of tannins, saponins and crude alkaloids were determined on dry weight basis (gm/100gm) (Table 3). High quantity of alkaloids was found in both buckwheat species. Tannin content was found to be nearly similar in both the species. The value of saponins was found to be higher in F. tataricum than F. esculentum.

The antioxidant activity of phenolics can be attributed to their potential to act as reducing agents, hydrogen donors, free radical scavengers, and metal chelators which are generally based on the number and location of hydroxyl groups present. Colorimetry of total phenolics, flavonoids and flavonols showed some differences between the two species (Table 3). Phenolics were found to be more abundant in F. tataricum than in F. esculentum. Flavonoid content of F. tataricum extract was about five times higher than that of F. esculentum. The content of flavonols in F. tataricum extract was about three times to that of F. esculentum.

**Antioxidant activity**

**DPPH scavenging activity**

DPPH is a free-radical generating compound and has been widely used to evaluate the free-radical scavenging ability of various antioxidant compounds. The methanolic extracts of F. esculentum (IC$_{50}$ = 0.2±0.02 mg/ml) and F. tataricum (IC$_{50}$ = 0.19±0.04 mg/ml) showed comparable scavenging of DPPH free radicals (Fig.1). However, standards ascorbic acid (IC$_{50}$= 0.007±0.001 mg/ml) and trolox (IC$_{50}$= 0.005±0.002 mg/ml) showed higher radical scavenging than buckwheat extracts. The extract of the two species were significantly not different with each other (p>0.05) but showed significant difference with two standards (p<0.0001).
FRAP assay
In this assay, reduction of the ferric-tripryridyltriazine to the ferrous complex forms an intense blue color which can be measured at a wavelength of 593 nm. The intensity of the color is related to the amount of antioxidant reductant in the samples. The ferric reducing activity of the methanolic extracts of *F. esculentum* and *F. tataricum* was 1.026±0.005 and 0.968±0.018 mg TE/gm respectively. The extract of both species were significantly different (*p*<0.05), in their FRAP values.

Reducing power activity
In the reducing power assay, the antioxidant compounds convert the oxidation form of iron (Fe^{2+}) to ferrous (Fe^{2+}). As depicted in Fig. 3, maximum activity was shown by methanolic extract of *F. tataricum* which was even greater than standard gallic acid at 200 µg/ml. The reducing activities of extracts were found to be dose dependent. The reducing power activity was significantly different (*p*<0.05) in both species.

Conclusion
Buckwheat species were found to be a very good source of proteins, carbohydrates, minerals. Its low fat content contributes it to be a very interesting constituent for several diets. In our study, the methanolic extract of both *Fagopyrum* species showed the high total phenolics, total flavonoids, total flavonol and radical scavenging activity. *F. tataricum* has proved to be better among two species of *Fagopyrum* which were investigated in terms of nutrition and antioxidant potential. Therefore, buckwheat species could be used in balanced diets and functional foods which can be consumed safely without any concern of health risk. In countries with high population where the food requirements are not being fulfilled by seasonal crops due to less availability of fertile land and optimum conditions for growth, buckwheat can be a good substitute. Thus, delving deep into the composition and bioactive compounds present in grains of buckwheat can lead to a better understanding and appreciation of the

<table>
<thead>
<tr>
<th>Buckwheat species</th>
<th>Crude alkaloids (g/100g)</th>
<th>Tannins (g/100g)</th>
<th>Saponins (g/100g)</th>
<th>Total phenolics (mg GAE/g)</th>
<th>Total flavonoids (mg RE/g)</th>
<th>Total flavonols (mg RE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. esculentum</em></td>
<td>2.51±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.21±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>317.47±1.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>993.13±56.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>252.449±14.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>F. tataricum</em></td>
<td>2.04±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>524.66±5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5821.46±60.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>760.233±17.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values within each column followed by different letter are significantly different at *p* < 0.05.

Fig.s (1-3)—DPPH scavenging activities of methanolic extracts of the two *Fagopyrum* species and standards; 2—ABTS scavenging activities of methanolic extracts of the two *Fagopyrum* species and standards; 3—Reducing power activities of methanolic extracts of the two *Fagopyrum* species and gallic acid.
nutraceutical and therapeutic value that these grains offer and an increased consumption of the buckwheat by the general public.

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