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# Phenolic contents, antioxidant and $\alpha$ -glucosidase inhibition properties of Nepalese strain buckwheat vegetables

Pankaja Sharma<sup>1\*</sup>, Amal Kumar Ghimeray<sup>1</sup>, Anup Gurung<sup>2</sup>, Cheng Wu Jin<sup>1</sup>, Ho Sik Rho<sup>3</sup> and Dong Ha Cho<sup>1</sup>

<sup>1</sup>College of Biomedical Science, Dept. of Bio-Health Technology Kangwon National University, Chuncheon 200-701, Republic of Korea.

<sup>2</sup>Department of Biological Environment, Kangwon National University, Chuncheon 200-701, Republic of Korea.

<sup>3</sup>R&D Center, Amore Pacific Corporation, Yongin, 446-729. S. Korea.

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The sprouts, microgreens and leafy greens of common and tartary buckwheat of Nepalese strain were compared for the phenolic contents and biological activity. The tartary buckwheat samples expressed higher total phenolic and flavonoid contents compared to the common buckwheat. The sprouts had the highest total phenolic contents (9333.48  $\pm$  150.23 and 6976.21  $\pm$  213.65 mgGAE/100g dw in tartary and common buckwheat, respectively) whereas, the highest total flavonoid content was present in the leafy greens (7635.39  $\pm$  141.40 and 4414.61  $\pm$  70.85 mgRE/100g dw in tartary and common buckwheat respectively). The high performance liquid chromatography (HPLC) results revealed that the tartary buckwheat vegetables had higher rutin, (3800.28  $\pm$  434.41 mg/100g in leafy greens), quercetin (159.75  $\pm$  9.04 mg/100g in sprouts) and chlorogenic acid (293.47  $\pm$  65.06 mg/100g in microgreens) contents than those of common buckwheat. However, other phenolics like vitexin, isovitexin, orientin and isoorientin contents were more abundant in common buckwheat. In biochemical assay, all three types of vegetable of common and tartary buckwheat showed higher antioxidant and  $\alpha$ -glucosidase inhibition effect in dose dependent manner. Based on these results, it can be conformed that all the vegetables (microgreens, sprouts and leafy greens) of both varieties of buckwheat of Nepalese strains can be regarded as a potent source of functional food.

**Key words:** Antioxidant,  $\alpha$ -glucosidase, buckwheat vegetables, Nepalese strain buckwheat, phenolics.

## INTRODUCTION

Reactive oxygen species (ROS), such as superoxide radical, hydroxyl radical, hydrogen peroxide, and singlet oxygen are associated with different diseases like cancer, aging, cardiovascular diseases, inflammation and neurodegenerative diseases (Ames, 1983; Stadtman, 1992; Sun, 1990). Likewise, diabetes is a metabolic disorder disease that occurs worldwide and its incidence

is increasing rapidly in most parts of the world. In modern medicine no satisfactory effective therapy is still available to cure the diabetes mellitus. Hence, there has been increasing interest in using medicinal plants to control diabetes and ROS mediated diseases.

Buckwheat is a plant that possesses both antioxidant and antidiabetic properties which is attributed to its phenolic contents like rutin and quercetin (Aliaga and Lissi, 2004). This plant is considered as a functional food as it is rich in phenolic compounds including rutin, quercetin, orientin, vitexin, isovitexin and isoorientin (Li and Zhang, 2001). Among these compounds rutin, a flavonol glycoside has been recognized as a major antioxidant component that accounts for about 85-90% of the total antioxidant activity (Morishita et al., 2007). Rutin is also known to have anti-inflammatory, anti-carcinogenic

\*Corresponding author. E-mail: [chodh@kangwon.ac.kr](mailto:chodh@kangwon.ac.kr); [sharmapankaja@gmail.com](mailto:sharmapankaja@gmail.com). Tel: 82-33-250-6475. Fax: 82-33-243-6475.

**Abbreviations:** HPLC, High performance liquid chromatography; ROS, reactive oxygen species; LDL, low-density lipoprotein; GAE, gallic acid equivalents; TF, total flavonoid.

effects (Liu et al., 2008) and is effective for preventing hemorrhagic disease and arteriosclerosis (Fabjan et al., 2003). Likewise, quercetin (aglycone), a major bioflavonoid of human diet, present in buckwheat, has been identified as a strong antioxidant, anti-angiogenesis and anticancer (Jackson and Venema, 2006). It is also known to reduce the risk of hypertension (Edwards et al., 2007). Besides rutin and quercetin, the other flavonoids like vitexin, isovitexin, orientin and isoorientin are also considered as good antioxidant compounds present in buckwheat (Kim et al., 2008) and have been reported to exhibit 4-40% of antioxidant activities (Szostak, 2004). Vitexin has been found to be effective in the prevention of skin cell damage caused by UV- radiation (Kim et al., 2005). Likewise, potential antimicrobial and antifungal activity have been shown by isovitexin (Morris, 2003) and chlorogenic acid (Bowels and Miller, 1994). Orientin has the ability to protect radiation-induced lipid peroxidation in mouse liver (Devi et al., 2000) and isoorientin is known to scavenge free radicals and prevent human LDL (low-density lipoprotein) against oxidation (Ko et al., 1998).

Nowadays, buckwheat sprout and microgreens have gained popularity due to the functional compounds present in them and are considered as a new vegetable (Kim et al., 2001). So far, several researches have been reported regarding the phenolic content and antioxidant activities of the buckwheat sprouts (Kim et al., 2008, 2006; Liu et al., 2008). However, the results were varied in different researches. This variation could be due to the fact that the buckwheat phenolics can be influenced by geographic origin of seed as well as environmental conditions (Kitabayashi et al., 1995). Likewise, phenolics or flavonoids (rutin) content in buckwheat can also be influenced by solar radiation (Ohara et al., 1989), photoperiods (Ohsawa and Tsutsumi, 1995) and cultivation time (Oomah and Mazza, 1996).

In context to Nepal, buckwheat is an important crop of the hilly area, and is a staple food crop in the remote hills. Apart from the grains, the young shoots (3 to 4 weeks old) of the buckwheat plant are also generally consumed as leafy vegetable by the people of Himalayan region. In a previous study, high rutin content in different parts of buckwheat that originate from Nepal has been reported (Park et al., 2004). However, there are no reports regarding the phenolic compositions and bioactivity of the Nepalese strain of buckwheat sprout, micro greens and leafy greens. Hence, the objective of this study was to compare the three kinds of vegetables viz. sprouts, microgreens and leafy greens of common and tartary buckwheat, originating from the eastern hills of Nepal for their phenolic contents and other biological (antioxidant and  $\alpha$ -glucosidase inhibition) properties.

## MATERIALS AND METHODS

### Plant materials and sprout production

Buckwheat sample of two varieties *Fagopyrum esculentum*

(common buckwheat) and *Fagopyrum tartaricum* (tartary buckwheat) were collected from local market of Illam hills of Eastern Nepal. The sprouts of common and tartary buckwheat were grown in a dark chamber at a temperature of 25°C, watered from time to time and were harvested at 7 days. The sprouts were dried at a temperature of 50°C, crushed and stored in the refrigerator for further experiments.

### Cultivation of buckwheat microgreens and leafy greens

The seeds of *F. tartaricum* and *F. esculentum* were sown in an open field in natural environment and watered from time to time (one time in a day till 7 days and alternate day till 3 weeks). On the 7 days and 21 days of germination, the young plants were harvested as microgreens and leafy greens, respectively. The harvested samples were washed and the roots were removed before drying at a temperature of 50°C.

### Preparation of plant extracts

The dried powdered samples (2 g) of both varieties of buckwheat were taken and 100 ml of 80% ethanol was added to each and incubated overnight in a shaker followed by filtration using Advantech 5B Tokyo Roshi Kaisha, Japan. The extract was dried using a rotatory evaporator (Eyela N-1000, Japan) at a temperature of 40°C. The extracts were vacuum freeze dried to remove the remaining moisture. The yield was measured and stored in the refrigerator for further experiment.

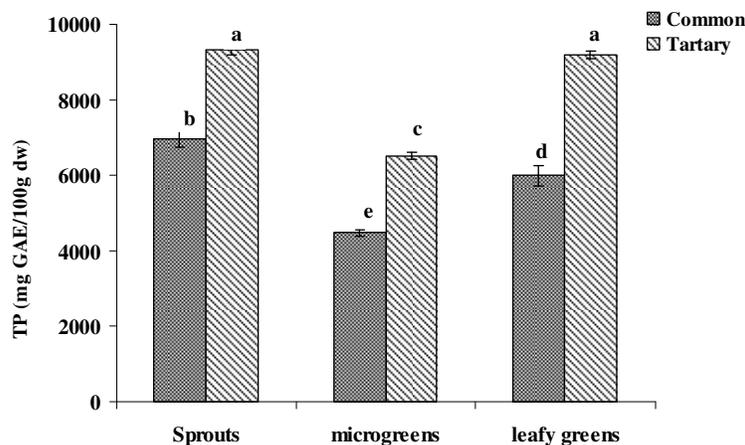
### Estimation of total polyphenol and flavonoid content

The total phenolic (TP) content was determined by the Folin-Ciocalteu assay (Eom et al., 2008). A sample aliquot of 200  $\mu$ l was added to a test tube containing 200  $\mu$ l of phenol reagent (1 M). The volume was increased by adding 1.8 ml of distilled deionized water and the solution was allowed to stand for 3 min for reaction after vortex. Further to continue reaction, 400  $\mu$ l of Na<sub>2</sub>CO<sub>3</sub> (10%, v/v) was added and vortexed and the final volume (4 ml) was adjusted by adding 1.4 ml of distilled deionized water. A reagent blank was prepared using distilled deionized water. The absorbance was measured at 725 nm after incubation for 1 h at room temperature. The TP content was expressed as Gallic acid equivalents (GAE) in mg/100g (dw) of sample.

The total flavonoid (TF) content was determined according to Eom et al. (2008) with slight modifications. Briefly, an aliquot of 0.5 ml of sample (1 mg/ml) was mixed with 0.1 ml of 10% aluminum nitrate and 0.1 ml of potassium acetate (1 M). In the mixture, 3.3 ml of 80% methanol was added to make the total volume 4 ml. The mixture was vortexed and the absorbance was measured after 40 min at 415 nm in spectrophotometer and calculated. Rutin was used as a standard and the values of TF content were expressed in rutin equivalent (RE) mg/100g (dw).

### HPLC analysis

The quantitative estimation of different compounds (rutin, quercetin, vitexin, isovitexin, orientin, isoorientin and chlorogenic acid) were performed by HPLC. The HPLC system (CBM-20A, Shimadzu Co, Ltd., Japan) with two gradient pumps (LC-20AT, Shimadzu), an auto sample injector (SIL-20A, Shimadzu), a UV-detector (SPD-10A, Shimadzu) and a column oven (35°C CTO-20A, Shimadzu) were used for analysis. The separation was performed on a C18 column (Synergi 4  $\mu$  MAX-RY, 150  $\times$  4.6 mm, 4 micron Phenomenex). Flow rate of mobile phase solution was 1.0 ml/min, and detection was at 355 nm. 10  $\mu$ l of each sample was injected in to the HPLC machine.



**Figure 1.** Total polyphenol (TP) contents in the 80% ethanolic extract of sprouts, microgreens and leafy greens of common and tartary buckwheat. TP contents are expressed in Gallic acid equivalent (GAE) in mg/100 g dw of sample. Each value is expressed as the mean  $\pm$  SD (n=3). Different letters indicate that the values are significantly different ( $P \leq 0.05$ ).

HPLC conditions were as follows: Solvent A (water in 0.1% Trifluoroacetic acid) and solvent B (acetonitrile). Gradient elution used was 0-10 min, 5-6% B; 10-15 min, 6-10% B; 15-45 min, 10-19% B; 45-65 min, 19-20% B.

#### 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The antioxidant activity of the samples (sprout, microgreens and leafy greens) was determined on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Bracca et al. (2003) with slight modifications. Briefly, 1 ml of each of the extracts at different concentrations (0.125, 0.25, 0.5 and 1 mg/ml) was added to 4 ml (0.15 mM DPPH solution) of DPPH. The mixtures were shaken vigorously and left to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm in a spectrophotometer (Hitachi U-2001, Japan) and the percent inhibition activities of the extracts were calculated against a blank using the following expression:

Inhibition (%) =  $(1-B/A) \times 100$ , where, A is the absorbance of the mixture without extract and B is the absorbance of the mixture containing the extract of buckwheat vegetables.

#### Metal chelating power

The samples were analyzed for the metal chelating activity according to the procedure of Dinis et al. (1994) with slight modification. Briefly, 0.5 ml of the sample extracts at different concentrations (0.25, 0.5, 1 and 2 mg/ml) were mixed with 0.1 ml of 1 mM  $FeCl_2$  followed by the addition of 0.2 ml of 5 mM ferrozine, vortexed and kept for 10 min. For blank, the sample extracts was replaced by 80% ethanol. The absorbance of the mixtures was measured against the blank at 562 nm after the addition of 3.2 ml 80% ethanol.

#### $\alpha$ -Glucosidase inhibitory activity

$\alpha$ -Glucosidase inhibitory assay was performed according to Kim et

al. (2004). 100  $\mu$ l of 5 mM pNPG (p-nitrophenyl  $\alpha$ -D-glucoside) in 0.2 M sodium phosphate buffer (pH 6.8) was added as a substrate to the mixture of 50  $\mu$ l of  $\alpha$ -glucosidase (0.15 unit/ml) and 50  $\mu$ l of sample to start the reaction. The reaction was conducted at 37°C for 15 min and stopped by the addition of 300  $\mu$ l of 0.1 M  $Na_2CO_3$ .  $\alpha$ -glucosidase activity was assessed by measuring the release of p-nitrophenol from pNPG at 405 nm. All tests were performed in independent triplicate (n=3) and data were expressed as mean  $\pm$  SD.

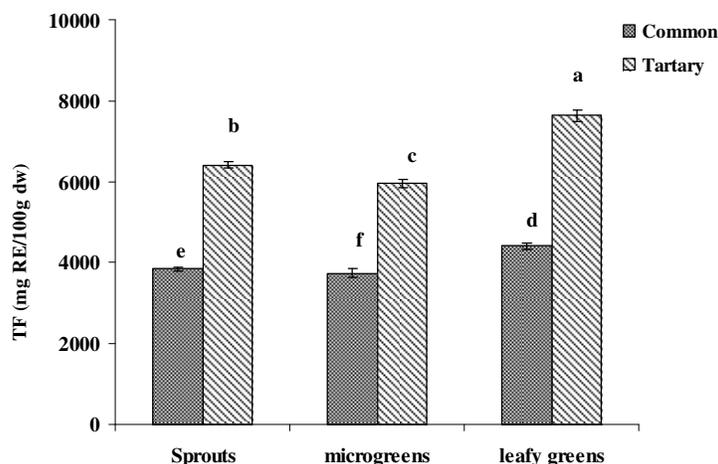
#### Statistical analysis

All data were expressed as mean value  $\pm$  standard deviation of the number of experiments (n=3) using Microsoft EXCEL program. Differences between the mean values of the multiple groups were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple tests using the SPSS 16.0 Inc., USA package. Statistical significance was considered at  $P \leq 0.05$ .

## RESULTS

### Total polyphenol (TP) and total flavonoid (TF) content of sprouts, microgreens and leafy greens

The TP contents in the ethanolic extracts of buckwheat sprouts, microgreens and leafy greens were determined from regression equation of calibration curve and expressed in gallic acid equivalent (mg GAE /100g) of dry plant material (Figure 1). The results reveal that the TP content of tartary buckwheat was comparatively higher than the common buckwheat in all the three types of vegetables studied in the research. In both types of buckwheat, the content of TP was in the order: sprouts > leafy greens > microgreens. Among all, the highest content of TP was found in the sprouts and leafy greens of tartary buckwheat ( $9333.48 \pm 150.23$  and  $9194.19 \pm$



**Figure 2.** Total flavonoid (TF) contents in the 80% ethanolic extract of sprouts, microgreens and leafy greens of common and tartary buckwheat. Flavonoid content was expressed in rutin equivalent (RE) in mg/100 g dw of sample. Each value is expressed as the mean  $\pm$  SD (n=3). Different letters indicate that the values are significantly different ( $P \leq 0.05$ ).

**Table 1.** Different phenolic contents in 80% ethanolic extract of sprouts, microgreens and leafy greens of common buckwheat, determined by HPLC.

Compound (mg/100g dw)	Common buckwheat		
	Sprouts	Microgreens	Leafy greens
Rutin	384.42 $\pm$ 36.43	595.81 $\pm$ 65.10	1440.92 $\pm$ 354.14
Vitexin	581.27 $\pm$ 21.92	394.15 $\pm$ 43.32	201.51 $\pm$ 96.04
Isovitexin	370.14 $\pm$ 17.22	247.14 $\pm$ 21.05	122.56 $\pm$ 5.03
Orientin	353.25 $\pm$ 28.47	208.56 $\pm$ 8.02	98.02 $\pm$ 0.39
Isoorientin	751.53 $\pm$ 31.72	431.24 $\pm$ 53.29	233.14 $\pm$ 13.07
Quercetin	8.52 $\pm$ 3.14	4.74 $\pm$ 0.22	17.12 $\pm$ 2.25
Chlorogenic acid	27.21 $\pm$ 6.70	156.23 $\pm$ 2.04	3.04 $\pm$ 0.17

Each value is expressed as the mean  $\pm$  SD (n=3).

113.97 mg/100g, respectively).

The TF content of the sprouts of tartary and common buckwheat were expressed in rutin equivalent (mg RE /100g dw) of dry plant material (Figure 2). The TF content was also higher in tartary buckwheat vegetables compared to the common type. The TF in the leafy greens of both types of buckwheat were higher (7635.39  $\pm$  141.40 mg/100g in tartary and 4414.61  $\pm$  70.85 mg/100g in common buckwheat) compared to the sprouts and microgreens. The TF content in both types of buckwheat was in the order, leafy greens > sprouts > microgreens.

#### Quantification of different phenolic contents in sprouts, microgreens and leafy greens by HPLC

The quantitative estimation of phenolic compounds (rutin,

vitexin, isovitexin, orientin, isoorientin, quercetin and chlorogenic acid) in common and tartary buckwheat vegetables is shown in Tables 1 and 2. The results show that the rutin was present in highest amount in all the tartary buckwheat vegetables (3100.98  $\pm$  202.80, 3000.12  $\pm$  343.12 and 3800.28  $\pm$  434.41 mg/100g dw in sprouts, micro greens and leafy greens, respectively). Besides rutin, quercetin and chlorogenic acid were also higher in tartary buckwheat vegetables, the former being highest in the leafy greens (171.43  $\pm$  2.02 mg/100g dw) followed by sprouts (159.75  $\pm$  9.04 mg/100g dw), while the later was highest in the microgreens (293.47  $\pm$  65.06 mg/100g dw). Similar trend was also observed in common buckwheat vegetables for quercetin and chlorogenic acid contents; however, their contents were lower than in tartary buckwheat (Table 1). In common buckwheat, the highest rutin content was found in the

**Table 2.** Different phenolic contents in 80% ethanolic extract of sprouts, microgreens and leafy greens of tartary buckwheat, determined by HPLC.

Compound (mg/100g dw)	Tartary buckwheat		
	Sprouts	Microgreens	Leafy greens
Rutin	3100.98 ± 202.80	3000.12 ± 343.12	3800.28±434.41
Vitexin	40.12 ± 7.07	26.01 ± 8.04	4.64 ± 1.87
Isovitexin	18.44 ± 8.11	11.73 ± 6.75	3.01 ± 0.02
Orientin	85.15 ± 4.27	ND	ND
Isorientin	152.65 ± 11.43	85.10 ± 5.42	ND
Quercetin	159.75 ± 9.04	7.13 ± 2.02	171.43 ± 2.02
Chlorogenic acid	41.26 ± 6.31	293.47 ± 65.06	51.55 ± 6.32

Each value is expressed as the mean ± SD (n=3). ND: Not detected.

**Table 3.** IC<sub>50</sub> of DPPH free radical scavenging activity, metal chelating activity and α-glucosidase inhibitory activity expressed in ppm.

Analysis	Sprouts		Microgreens		Leafy greens	
	Common	Tartary	Common	Tartary	Common	Tartary
DPPH free radical scavenging activity	219.08	150.11	239.68	152.51	187.23	127.44
Metal chelating activity	839.05	572.88	291.82	150.29	290.79	226.06
α-glucosidase inhibitory activity	78.36	44.56	6086.65	1532.33	874.93	323.22

leafy greens (1440.92 ± 354.14 mg/100g dw) followed by the microgreens (595.81 ± 65.10 mg/100g dw) and then the sprouts (384.42 ± 36.43 mg/100g dw). The other compounds viz, vitexin, isovitexin, orientin and isoorientin were more abundant in the vegetables of common than in the tartary buckwheat. These compounds were present in highest amounts (581.27 ± 21.92, 370.14 ± 17.22, 353.25 ± 28.47 and 751.53 ± 31.72 mg/100g dw respectively) in the sprouts of common buckwheat, followed by the microgreens (394.15 ± 43.32, 247.14 ± 21.05, 208.56 ± 8.02 and 431.24 ± 53.29 mg/100g dw, respectively) and leafy greens (201.51 ± 96.04, 122.56 ± 5.03, 98.02 ± 0.39 and 233.14 ± 13.07 mg/100g dw, respectively). These four compounds (vitexin, isovitexin, orientin and isoorientin) were also present in significant amount (40.12 ± 7.07, 18.44 ± 8.11, 85.15 ± 4.27 and 152.65 ± 11.43 mg/100g dw, respectively) in the sprout of tartary buckwheat. A good amount (85.10 ± 5.42) of isoorientin was detected in the microgreens of tartary buckwheat but not observed in the leafy greens. Similarly, orientin was not detected in microgreens and leafy greens of tartary buckwheat.

### DPPH free radical scavenging activity

The hydrogen donating ability of the ethanolic extracts of the sprouts, microgreens and leafy greens of common and tartary buckwheat measured by using DPPH free radicals showed that vegetables of both types of buckwheat have high free radical scavenging activity.

The results are presented in IC<sub>50</sub> value and are given in Table 3. The results reveal that all the three types of vegetables viz. sprouts, microgreens and leafy greens of tartary buckwheat were superior to those of common buckwheat in DPPH free radical scavenging activity. The leafy greens of tartary buckwheat showed the highest IC<sub>50</sub> value with an IC<sub>50</sub> of 127.44 ppm. The sprouts and microgreens of tartary buckwheat also showed good inhibition with IC<sub>50</sub> values of 150.11 and 152.50 ppm, respectively. Among the vegetables of common buckwheat, the leafy greens showed the highest free radical scavenging activity with an IC<sub>50</sub> value of 187.23 ppm. The IC<sub>50</sub> values of the sprouts and microgreens were 219.08 and 239.68 ppm, respectively being less potent than the other vegetables. Overall, the tartary vegetables showed higher free radicals scavenging activity than the common buckwheat and the leafy greens of both types of buckwheat were more potent than the sprouts and microgreens.

### Metal chelating power

The result of metal chelating assay of the three types of buckwheat vegetables (sprouts, microgreens and leafy greens) was obtained in a dose dependent manner and the IC<sub>50</sub> values are presented in Table 3. According to the data, the tartary buckwheat vegetables possess higher metal chelating property compared to the common buckwheat. Microgreens of tartary buckwheat showed the highest IC<sub>50</sub> value of 150.29 ppm followed by the leafy

greens with IC<sub>50</sub> value of 226.06 ppm. The chelating property of microgreens and leafy greens of common buckwheat were almost similar with the IC<sub>50</sub> values of 291.82 and 290.78 ppm, respectively. The sprouts of both types of buckwheat expressed the lowest metal chelating properties with IC<sub>50</sub> values >500 ppm.

### **α-Glucosidase inhibition activity**

In order to determine if buckwheat vegetables possess anti-diabetic properties, the inhibitory activity of the 80% ethanolic extracts on α-glucosidase activity was studied and compared between two species. The results (IC<sub>50</sub> values) of α-glucosidase inhibitory activity of sprouts, microgreens and leafy greens are given in Table 3. The data reveal that the tartary buckwheat vegetables were more potent than those of the common buckwheat in α-glucosidase inhibition. Among the three types of vegetables, the sprouts of tartary and common buckwheat had the highest inhibitory property with IC<sub>50</sub> values of 44.56 and 78.36 ppm, respectively followed by leafy greens of tartary and common buckwheat with IC<sub>50</sub> value of 323.22 and 874.93 ppm, respectively. The microgreens of both tartary and common buckwheat were comparatively weaker inhibitors of α-glucosidase showing the IC<sub>50</sub> value of 1532.33 and 6086.65 ppm, respectively.

### **DISCUSSION**

The variability in the flavonoid content and the antioxidative activity of buckwheat due to cultivar, cultivation time and location was reported by Oomah and Mazza, (1996). Park et al. (2004) also reported the significant variation in the rutin content in the tartary buckwheat strains collected from different regions. In our study, we observed that the TP and TF content of the sprouts, microgreens and leafy greens of tartary buckwheat were varied and comparatively higher than those of the common variety. This variation in the TP and TF content among the vegetables could be due to the fact that exposure to natural light and the age of the buckwheat sprouts affects the phenolic and flavonoid composition of buckwheat (Kim et al., 2008). This result is in accordance with the previous reports of Park et al. (2005) in which tartary buckwheat contained higher TP and TF than the common buckwheat.

In case of tartary buckwheat, the contents of some compounds like rutin, orientin, isoorientin and quercetin were much higher in our study, whereas, vitexin, isovitexin were lower compared to the sprouts reported by Kim et al. (2008). It was also noticeable in our research that the chlorogenic acid content in the microgreens was much higher (293.47 ± 65.06) compared to the sprouts (41.26 ± 6.31 mg/100g dw). This is in accordance with previous research by Kim et al., (2008),

where they also found similar trend showing the microgreens having twice the amount of chlorogenic acid compared to the sprouts. According to the data (Tables 1 and 2), vitexin, isovitexin, orientin, and isoorientin seemed to decrease gradually in the microgreens and the leafy greens, suggesting that growth in the light or soil (as in microgreens) or longer growth (from microgreens to leafy greens) might have decreased the level of these compounds. While the higher level of rutin accumulated in the leafy greens compared to the sprouts and microgreens implies that longer growth in the presence of light might increase the rutin contents in buckwheat vegetables (Yao et al., 2004)

In the present study, it was observed that the antioxidant activities such as, DPPH free radical scavenging activity and the metal chelating ability of the tartary buckwheat vegetables (sprouts, microgreens and leafy greens) were higher than the vegetables of common buckwheat. This higher antioxidant activity was due to the higher amount of rutin, quercetin and chlorogenic acid content in the tartary buckwheat (Yang et al., 2008; Kumar et al., 2009; Lamson and Brignall, 2000). Our result support previous finding of Liu et al. (2008) where they also reported that the sprouts of tartary buckwheat scavenged higher percent of free radicals compared to those of the common buckwheat. However, the concentration required for scavenging approximately 88 % of DPPH radicals in their experiment was 5 mg/mL. Whereas, according to our data, the IC<sub>50</sub> value for free radical scavenging activity of tartary sprout was 150.11 ppm (Table 3), which was approximately 90 % at 1 mg/ml (percent data not shown). All this dissimilarity in the research could be due to the difference in phenolic compositions in different cultivar, growing season and location, soil types, harvesting times and other environmental conditions (Oomah and Mazza, 1996; Kitabayashi et al., 1995; Hagels et al., 1995). In the metal chelating assay, the microgreens of tartary buckwheat expressed the highest metal chelating activity with an IC<sub>50</sub> value of 150.29 ppm. This may be due to the presence of high level of chlorogenic acid (Table 2), as it has been previously reported that a melandion-like polymer derived from chlorogenic acid was the main metal chelating substance in coffee (Takenaka et al., 2005). In the α-glucosidase inhibition assay, the sprouts were the best inhibitors of the enzyme with an IC<sub>50</sub> of 44.56 and 78.36 ppm for tartary and common buckwheat respectively.

In conclusion, all the vegetables (microgreens, sprouts and leafy greens) of both varieties of buckwheat can be regarded as a potent source of phenolics (rutin, quercetin, vitexin, isovitexin, orientin isoorientin and chlorogenic acids) and has high antioxidant activities. In an *in vitro* antidiabetic assay using the enzyme α-glucosidase conformed that both the mentioned species of buckwheat vegetables can be esteemed as a potent inhibitor of α-glucosidase activity which can contribute in the treatment of diabetes. Overall, through this research, it is suggested

that Nepalese strain buckwheat vegetables contain high phenolics with higher biological (antioxidant and  $\alpha$ -glucosidase inhibition) activity and can be used as an alternative food. Therefore, mass production of more and more buckwheat food products should be encouraged and included in the daily diet, which would help the people to prevent diabetes and many other diseases caused by the free radicals.

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## REFERENCES

- Aliaga C, Lissi EA (2004). Comparison of the free radical scavenger activities of quercetin and rutin-an experimental and theoretical study. *Can. J. Chem.* 82(12): 1668-1673.
- Ames BN (1983). Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*, 221: 1256-1264.
- Bowels BL, Miller AJ (1994). Caffeic acid activity against *Clostridium botulinum* spores. *J. Food Sci.* 59: 905-908.
- Bracca A, Fico G, Morelli I, Simone FD, Tome F, De TN (2003). Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *J. Ethnopharmacol.* 86: 63-67.
- Devi PU, Ganasaundari A, Vrinda B, Srinivasan KK, Unnikrishnan MK (2000). Radiation protection by the Ocimum flavonoids orientin and vicenin: Mechanism of action. *Radiat. Res.* 154: 455-460.
- Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* 315: 161-169.
- Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T (2007). Quercetin reduces blood pressure in hypertensive subjects. *J. Nutr.* 137: 2405-2411.
- Eom SH, Park HJ, Jin CW, Kim DO, Seo DW, Jeong YH, Cho DH (2008). Changes in antioxidant activity with temperature and time in *Chrysanthemum indicum* L (Ganguk) teas during elution processes in hot water. *Food Sci. Biotech.* 17: 408-412.
- Fabjan N, Rode J, Kosir IJ, Zang Z, Kreft I (2003). Tartary buckwheat (*Fagopyrum tartaricum* Gaertn.) as a source of dietary rutin and quercetin. *J. Agric. Food Chem.* 51: 6452-6455.
- Hagels HD, Wagenbreth, Schilcher H (1995). Phenolic compounds of buckwheat herb and influence of plant and agricultural factors (*Fagopyrum esculentum* Moench and *Fagopyrum tartaricum* Gaertn.). In: Matano T and Ujihara A Editors, *Current Advances in Buckwheat Research*, Vol. I, Shinshu University Press: Matsumoto, Japan. pp. 801-809.
- Jackson SJ, Venema RC (2006). Quercetin inhibits eNOS, microtubule polymerization, and mitotic progression in bovine aortic endothelial cells. *J. Nutr.* 136: 1178-1184.
- Kim JH, Lee BC, Kim JH, Sim GS, Lee DH, Lee KE, Yun YP, Pyo HB (2005). The isolation and antioxidative effects of vitexin from *Acer palmatum*. *Arch. Pharm. Res.* 28: 195-202.
- Kim SJ, Zaidul ISM, Suzuki T, Mukasa Y, Hashimoto N, Takigawa S, Noda T, Chie ME, Yamauchi H (2008). Comparison of phenolic compositions between common and Tartary buckwheat (*Fagopyrum*) sprouts. *Food Chem.* 110: 814-820.
- Kim SJ, Kawaharada C, Suzuki T, Saito K, Hashimoto N, Takigawa S (2006). Effect of natural light periods on rutin, free amino acid and vitamin C contents in the sprouts of common (*Fagopyrum esculentum* M) and Tartary (*F. tartaricum* G) buckwheats. *Food Sci. Technol. Res.* 12: 199-205.
- Kim SL, Young KS, Hwang JJ, Kim SK, Hur HS, Park CH (2001). Development and Utilization of Buckwheat Sprouts as Functional Vegetables. *Fagopyrum*, 18: 49-54.
- Kim YM, Wang MH, Rhee I (2004). Novel  $\alpha$ -glucosidase inhibitor from pine bark. *Car. Res.* 339: 715-717.
- Kitabayashi H, Ujihara A, Hirose T, Minami M (1995). Varietal differences and heritability for rutin content in common buckwheat, *Fagopyrum esculentum* Moench. *Jpn. J. Breed.* 45: 75-79.
- Kumar V, Rani A, Dixit AK, Bhatnagar D, Chauhan GS (2009). Relative changes in tocopherols, isoflavones, total phenolic content and antioxidative activity in soybean seeds at different reproductive stages. *J. Agric. Food Chem.* 57: 2705-2710.
- Ko FN, Chu CC, Lin CN, Chang CC, Teng CM (1998). Isoorientin-6''-O-glucoside, a water-soluble antioxidant isolated from *Gentiana arisanensis*. *Biochimica et Biophysica Acta.* 1389: 81-90.
- Lamson DW, MS Brignall (2000). Antioxidant and cancer III: Quercetin. *Altern. Med. Rev.* 5 (3): 196-208.
- Li SQ, Zhang QH (2001). Advances in the development of functional foods from buckwheat. *Crit. Rev. Food Sci. Nutr.* 41: 451-464.
- Liu CL, Chen YS, Yang JH, Chiang BH (2008). Antioxidant Activity of Tartary (*Fagopyrum tartaricum* (L.) Gaertn.) and Common (*Fagopyrum tataricum* Gaertn.) Buckwheat Sprouts. *J. Agric. Food Chem.* 56 (1): 173-178.
- Morishita T, Yamaguchi H, Degi K (2007). The contribution of polyphenols to antioxidative activity in common buckwheat and tartary buckwheat grain. *Plant Prod. Sci.* 10: 99-104.
- Morris B (2003). Bio-functional legumes with nutraceutical, pharmaceutical, and industrial uses. *Economic Bot.* 57: 254-261.
- Ohara T, Ohinata H, Muramatsu N, Matsuhashi T (1989). Determination of rutin in buckwheat foods by high performance liquid chromatography. *Nippon Shokuhin Kogyo Gakkaishi.* 36: 114-120.
- Ohsawa R, Tsutsumi T (1995). Improvement of rutin content in buckwheat flour. In: Matano T and Ujihara A Editors, *Current Advances in Buckwheat Research*, Vol. I, Shinshu University Press: Matsumoto, Japan. pp. 365-372.
- Oomah BD, Mazza G (1996). Flavonoids and antioxidative activities in buckwheat. *J. Agric. Food Chem.* 44: 1746-1750.
- Park BJ, Park JI, Chang KJ, Park CH (2004). Comparison in Rutin content in seed and plant of tartary buckwheat (*Fagopyrum tartaricum*). *Proceedings of the 9th International Symposium on Buckwheat*. Prague. pp. 626-629.
- Park BJ, Kwon SM, Park JI, Chang KJ, Park CH (2005). Phenolic compounds in common and tartary buckwheat. *Kor. J. Crop Sci.* 50: 175-180.
- Stadtman ER (1992). Protein oxidation and aging. *Science*, pp. 257-1220-1224.
- Sun Y (1990). Free radicals, antioxidant enzymes and carcinogenesis. *Free Radic. Biol. Med.* 8: 583-599.
- Szostak DD (2004). Flavonoids in hulls of different varieties of buckwheat and their antioxidant activity. In: *Proceedings of the 9th International Symposium on Buckwheat*. Prague, pp. 621-625.
- Takenaka M, Sato N, Asakawa H, Wen X, Murata M, Homma S (2005). Characterization of a metal chelating substance in coffee. *Biosci. Biotechnol. Biochem.* 69 (1): 26-30.
- Yang J, J Guo, J Yuan (2008). In vitro antioxidant properties of rutin. *LWT - Food Sci. Technol.* 41(6): 1060-1066.
- Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R (2004). Flavonoids in food and their health benefits. *Plant Foods Human Nutr.* 59: 113-122.
- Zou Y, Lu Y, Wei D (2004). Antioxidant activity of a flavonoid rich extract of *Hypericum perforatum* L. *in vitro*. *J. Agric. Food Chem.* 52: 5032-5039.