Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol.6 (2), April-June, 2018

VARIATION IN TOTAL PHENOLS AND RUTIN CONTENT IN THE LEAVES, GRAINS, AND HULLS OF PROMISING COMMON AND TARTARY BUCKWHEAT GENOTYPES

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ABSTRACT

The dry mature grains, hulls and leaves of four promising common buckwheat and one tartary buckwheat varieties/ genotypes procured from MAREC, Sangla were analysed for total phenols and rutin content. The total phenols content in different plant parts of the buckwheat i.e., leaves, grains and hulls of the versatile buckwheat genotypes ranged from 2.1 (PRB-1) to 3.2 (VL-7) per cent in the leaves, 0.5 (PRB-1) to 0.7(OC-2) per cent in grains and 0.35 (Hassoska) to 0.59 (OC-2) per cent in hull, whereas, values for this parameter in tartary buckwheat var. Shimla-B-1 grains were observed as 1.81, 2.0 and 0.58 per cent in the leaves, grains and hulls. Variation in the rutin content was found from 1.70 to 2.86 per cent in leaves, 0.03 to 0.09 in grains and 0.02 to 0.10 per cent in hulls of common buckwheat genotypes. However, values for rutin content in tartary buckwheat var. Shimla-B-1 was observed as 1.63, 1.46 and 0.08 per cent in the leaves, grain and hulls, indicating the adequacy of this nutraceutically important constituent relatively in the leaves for further use in food and pharmaceuticals industries.

Keywords: Buckwheat. Phenols . Rutin . Hull . Grain . Leaves

INTRODUCTION

During the present era of scientific and agricultural development, nutraceuticals are reported to play a crucial role in the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions, beyond adequate nutritional effects. In recent years, cereals and its ingredients are accepted as functional foods and nutraceuticals because of providing dietary fibre, proteins, minerals, vitamins and exhibiting antioxidant properties required for human health. Search for such plant species/ food imparting nutraceutical properties is provide medical or health benefits including the prevention and treatment of disease. [1, 2]

Chemically, cereals include dietary fibre such as β - glucan and arabinoxylan, carbohydrates such as resistant starch and oligosaccharides (galacto- and fructo- oligosaccharides). Wheat, buckwheat, oat, barley, flaxseed, psyllium, brown rice, soy and products are notified the most common cereal and pseudo cereal based functional foods and nutraceuticals. Preventing cancer and cardiovascular disease, reducing tumor incidence,

lowering, lowering blood pressure, risk of heart disease, cholesterol and rate of fat absorption, delaying gastric emptying and supplying gastrointestinal health are the protective effects of the cereals.

In view of the increasing importance of nutraceuticals in human nutrition considerable efforts have been made for the evaluation of pseudocereals and cereals like catechins in strawberries and green and black tea; Kaempferol from brussel sprouts and apples; quercetin from beans, onions and apples and lignans from nuts, seeds and whole grain cereals which exhibiting antioxidant / nutraceutical properties. Undoubtedly, cereal and legume form the major food of nutraceutical importance consumed by humans and contribute significantly towards food technological innovations. In this context, interest has also been aroused in the nutraceuticals other than cereals and legumes. Some efforts seems to have been made in the past to investigate the profile of pseudocereals.

In Himachal Pradesh, buckwheat crop is important because their grains are used in various forms of hill food and products. The hilly terrains of Himachal Pradesh represent several diverse eco-geographic cultivation pockets which are more suitable for cultivation of hardy crops. In the state of Himachal Pradesh, buckwheat is grown in Kinnaur, Lahul Spiti and Sirmour districts. Since, the crop is adapted to temperate climate; hence, Himachal Pradesh can play an important role in production of this crop.

Buckwheat contain nutraceutically important biomolecules of vital significance. Buckwheat is reported to contain various phenols and bioflavonoids including rutin, which are considered to be effective in prevention or cure of cardiovascular disorders. [3] The status of rutin content in different plant parts of buckwheat grown in Himalayan terrain in the state is still scanty. However, apparently systematic information on the status of rutin as antioxidant in general and pharmaceutical in particular is still limited.

It would be, therefore, worthwhile to evaluate promising genotypes for total phenols and rutin content in different plant parts of buckwheat.

Material and methods

The leaves, grains and hulls (grain coat) of four common buckwheat varieties and one variety of tartary buckwheat collected from Mountain Agriculture Research and Extension Centre (MAREC), Sangla of CSK HPKV, Palampur. Samples were analysed in triplicate for total phenols and rutin content by following standard procedures.

Extraction of phenolics was carried out by the method followed by Makkar et al.,1993. Rutin was extracted from plantparts/grains after modification of the protocol already reported by Kreft *et al.,* 2006.

100 mg dried powdered samples each of different plant parts i.e., leaves, grain and hull (grain coat) were extracted with ethanol-water 50:50 (v/v), with sonication (2 x 30 min., 45^0C). The extracts were combined, filtered and the solvent was removed under vacuum. The extracts (100mg/ml) obtained from each sample of plant material were prepared in HPLC-grade methanol for quantitative analysis.

Standard rutin was purchased from Chromadex, Life Technologies, India. All HPLC grade solvents (acetonitrile, water) were purchased from J.T.Baker (USA). Trifluoroacetic acid was purchased from E.Merck, India. Standard stock solution (1mg/ml) of rutin was prepared in HPLC - grade methanol.

Samples (100 mg) were extracted with 10 ml methanol/water (50:50) kept in sonnicator at 40^0C for half an hour. The extracted material was filtered and solvent was distilled off completely. After lyopholiztion, the sample was dissolved in 5 ml of methanol (HPLC grade) and filtered through milipore (0.45 nm) membrane filter.

Chromatograhic conditions and identification:

HPLC analysis was performed with a Waters HPLC system equipped with 600 quaternary gradient pump, (7725i Rheodyne injector) Waters 717 plus autosampler, 996 PDA detector and empower 2 software (version- 4.01). All the samples were filtered through 0.45 µm (Millipore) filters. Extracts of plant samples were separated on a LiChrosphere 100-RP-18e column (250mm x 4mm x 5µm particles) from E. Merck (Germany). The temperature of the column was set at 25^0C. Elution of standards and samples (20µl) was performed. The mobile phase was a gradient prepared from 0.05 per cent Trifluroacetic acid (TFA) in water(A) and acetonitrile (B) with a linear gradient elution: 15-60 per cent B in 0-30 min and 15 per cent B in 35 min. The flow rate was 1ml/min, the run time 30 min. and the detection wavelength 270 nm. Identification of compounds was done on the basis of the retention time, co-injections, and spectral matching with standards.

Result and discussion

Keeping in view the significance of phenols as an the anti-oxidant factor, total phenol status in different plant parts of the common buckwheat i.e., leaves, grains and hulls of the versatile genotypes presented in Table 1 showed variation from 2.1 (PRB-1) to 3.2 (VL-7) per cent in the leaves, 0.5 (PRB-1) to 0.7 (OC-2) per cent in grains and 0.35 (Hassoska) to 0.59 (OC-2) per cent in hulls. Therefore, the values obtained in the present investigation reported that in common buckwheat total phenol content was found higher in leaves followed by grains and hulls. However, the values for total phenols content was observed as 1.81, 2.0 and 0.58 per cent in the leaves, grains and hulls, accordingly of tartary buckwheat variety Shimla-B-1. It was found that in tartary buckwheat, grains contained more total phenol content followed by leaves and hulls. Leaves also contained good amount of total phenol content . So, it is concluded that leaves in common buckwheat and grains in tartary buckwheat contained higher amount of total phenol content than hulls (grain coat) of both the varieties.

Variety VL-7 followed by Hassoska in leaves; Shimla-B-1 (tartary buckwheat) followed by OC-2 and VL-7, Hassoska in grains and OC-2 followed by Shimla-B-1 (taratry buckwheat) were emerged out to be overall superior varieties/ genotypes containing total phenol content.

Studies conducted earlier by Tahir and Farooq (1985) revealed phenolics content as 0.73, 0.79 and 0.77 per cent (on dry weight basis) in hull, groat and whole grain in that order in buckwheat. [6] In another study, buckwheat grains contained total phenols content as 726 mg/100g as ferulic acid equivalents. [7] Oomah *et al*. (1996) determined phenolic acid content in grains from five buckwheat cultivars grown at three locations in western Canada for four years and reported 12-16 g/kg of total phenolic acids, about 3 g/kg of esterified phenolic acids, and 8-13 g/kg of etherified phenolic acids. [8] Variation in phenolic acids attributes was mainly due to cultivar, seasonal effects and their interaction.

Genotype/ Varieties	Total phenols (%)			Rutin (%)		
	Leaves	Grain	Hull	Leaves	Grain	Hull
VL-7	3.2±0.1	0.6±0.01	0.53±0.01	2.86 ± 0.02	0.03 ± 0.01	0.02 ± 0.001
PRB-1	2.1±0.25	0.5±0.01	0.48±0.01	1.70± 0.02	0.05 ± 0.01	0.04 ± 0.001
OC-2	2.4±0.2	0.7±0.10	0.59±0.02	2.24± 0.02	0.09 ± 0.01	0.10 ± 0.01
Hassoska	3.1±0.1	0.6±0.10	0.35±0.03	2.82 ± 0.02	0.05± 0.26	0.04 ± 0.01
Shimla -B-1	1.81±0.09	2.0 ±0.1	0.58±0.01	1.63 ± 0.03	1.46± 0.02	0.08 ± 0.01

Table 1: Variation in total phenols and rutin in leaves, grain and hull (grain coat) of promising buckwheat genotypes (Values on dry wt. basis)

The data pertaining to rutin content in the leaves, grains and hulls of promising buckwheat genotypes are presented in Table 1. It is evident that the rutin content ranged from 1.70 to 2.86 per cent in leaves, 0.03 to 0.09 in grains and 0.02 to 0.10 per cent in hulls of promising common buckwheat genotypes. The lowest and highest value of rutin content was exhibited by varieties PRB-1 and VL-7 in the leaves.The next higher value was exhibited by genotype Hassoska followed by variety OC-2. The minimum and maximum value of rutin was observed VL-7 and OC-2 in grains and VL-7 and OC-2 in hulls, accordingly. It was observed that rutin content was found higher in the leaves followed by grains and hulls in common buckwheat. The rutin content was observed as 1.63,1.46 and 0.08 per cent in leaves, grains and hulls of tartary buckwheat i.e., released variety Shimla-B-1. It was noticed that leaves contained more rutin content than grains and hulls. However, grains of tartary buckwheat contained appreciable amount of rutin.

Variety VL-7 followed by Hassoska and OC-2 was found overall superior varieties/genotypes in leaves. However, Shimla-B-1 contained highest amount of rutin content than other varieties/genotypes in grains and variety OC-2 followed by Shimla-B-1 was observed higher rutin content in hulls.

Kitabayashi *et al*. (1995) estimated rutin content in the buckwheat leaves to range from 1,880 to 3600 mg/100g. [9] The rutin content in grains showed wide range of variation from 12.6 to 35.9 mg/100g on dry matter basis among 27 cultivars of common buckwheat grown in Japan, China, Nepal and Europe. Similar trend of variation in rutin content in grain, leaves and hulls have been observed by other investigators. [10, 11] It is notable that rutin content in the grain of tartary buckwheat was higher than common buckwheat; whereas its value was noticed to be higher in common buckwheat leaves as compared to tartary buckwheat. In all, buckwheat leaves exhibited higher values for rutin content than grains and hulls.

Zhanrong and Xiulian (2006) and Suzuki *et al*. (2005) also reported that rutin concentration was high in leaves and flowers. [12,13] Studies conducted by Krkoskova and Mrazova (2005) showed that flavonoids

composition in buckwheat grains was different in different buckwheat species during diverse growing phases and had slight differences owing to difference among growing circumstances. [14]

Conclusion

The study thus, indicates that on average total phenols content was found higher in the leaves (2.1 to 3.2 per cent) followed by grain (0.5 to 0.7 per cent) and hull (0.35 to 0.59 per cent) in the common buckwheat, whereas in tartary buckwheat total phenols content was observed in grain (2.0 %) followed by leaves (1.81%) and hull (0.58%), in that order. Relatively higher value for the bioflavonoid 'rutin' was observed in the leaves accompanied by grains and hull in common buckwheat. However, tartary buckwheat leaves contained much more concentration of rutin as compared to leaves and hull. It is therefore, concluded that buckwheat leaves and grains exhibited appreciable amounts of phenolic compounds and rutin content both in common buckwheat and tartary buckwheat to be beneficial in imparting anti-oxidant properties on human consumption.

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