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ORIGINAL ARTICLE

# Evaluation of nutraceutical and antinutritional properties in barnyard and finger millet varieties grown in Himalayan region

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**Abstract** Five elite varieties of barnyard (*Echinochloa frumentacea*) and finger (*Eleusine coracana)* growing at northwestern Himalaya were investigated for nutraceutical and antinutritional properties. Barnyard millet contained higher amount of crude fiber, total dietary fiber, tryptophan content, total carotenoids, α-tocopherol compared to the finger millet whereas the finger millet contains higher amount of methionine and ascorbic acid as compared to the barnyard millet. The secondary metabolites of biological functions were analyzed and found that barnyard millet contained the higher amount of polyphenols, tannins and ortho-dihydroxy phenol content compared to finger millet. Among antinutitional compounds barnyard millet contained lower phytic acid content compare to finger millet whereas no significant difference in trypsin inhibition activity of barnyard millet and finger millet varieties were found. Barnyard millet contained higher acid phosphatase, α-galactosidase and α-amylase inhibitor activity compared to finger millet. Finger millet seeds contained about 10–13 folds higher calcium content and double amount of manganese content in comparison to barnyard millet seeds. Present study suggests that barnyard millet varieties studied under present investigation were found nutritionally superior compared to finger millet varieties.

**Keywords** Barnyard millet · Finger millet · Nutritional · Nutraceutical · Antinutritional

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## Introduction

Millets are a group of grassy plants with short slender culm and small grains possessing remarkable ability to survive under severe drought. Millets are one of the oldest foods known to humans and possibly the first cereal grain to be used for domestic purposes. Finger millet is a very good source of micronutrients, vitamins, dietary fibers, polyphenols, pigments and phytates (Antony et al. 1996). In case the millet is processed to separate out the seed coat matter as is normally done in millet malting and milling (Malleshi 2003). Consumption of whole grain of finger millet is associated with health benefits, such as its hypoglycemic (Lakshmi Kumari and Sumathi 2002), hypocholestrolemic (Hegde et al. 2005) characteristics and anti-ulcerative properties (Tovey 1994). It is a naked caryopsis with brick red-colored seed coat and is generally used in the form of the whole meal for preparation of traditional foods, such as roti (unleavened breads or pancake), mudde (dumpling) and ambali (thin porridge). Epidemiological studies have demonstrated that regular consumption of whole grain cereals and their products can protect against the risk of cardiovascular diseases, type II diabetes, gastrointestinal cancers and a range of other disorders (McKeown 2002). Barnyard millet (*Echinocola frumentacea*) is important millet grown in the arid and semiarid region of the world. Barnyard millet is comparable with other cereals, such as rice and wheat as a source of protein, fat, carbohydrates and crude fiber (Kumar and Parameshwaran 1998; Veena et al. 2005; Devi et al. 2014) apart from mineral and vitamins (Veena et al. 2005). It also contains phytochemicals, such as phenolic acids, flavonoids and tannins (Kulkarni et al. 1992) which serve as good source of natural antioxidants. Although barnyard millet like any

other minor millet is nutritionally superior to cereals, yet it is under utilization. Finger millet and barnyard millet are also well known for their antinutrient constituents such as α-amylase inhibitor, trypsin inhibitors, phytate and tannins. Antinutritional factors in plant-based foods are in focus to understand their potential health benefits (Yabuno 2001). Phytates protects against oxidative stress by chelating iron involved in Fenton's reaction, and some phenolics and tannins act as antioxidants (Yabuno 2001). In Uttarakhand small millets are grown on a fairly large area of about 223–278 thousand hectare producing about 159–237 thousand tones grain annually (Singh 1997). Among them, finger millet is the staple food, which stands second after the rice in kharif season. The striking features of finger millet are its ability to adjust to different agro-climatic conditions and high resistance to stored insect pests and moulds. Uttarakhand occupies about 230,000 ha, with barnyard millet almost equally divided between hills and plains. Minor millets have been well characterized for their nutritional and nutraceutical properties, however the characterization of finger millet and barnyard millet grown in Himalayan region is lacking and also their comparative study for nutritive and antinutritive compounds is very scarce.

## Materials and methods

### Plant materials

Seeds of different varieties of barnyard millet (VL21, VL29, VL172, VL207 and PRJ1) and finger millet (VL146, VL149, VL204, VL315 and VL324) were procured from ICAR- Vivekananda Parvatiya Krishi Anushandhan Sansthan, Almora and Uttarakhand University of Horticulture and Forestry, Bharsar, Uttarakhand, India.

### Determination of nutritional parameters

Total dietary fibre was determined calorimetrically by following the alkali treatment method (Englyst and Hudson 1987). Tryptophan content was determined by following the method of Dalby and Tsai (1975). Standard curve was prepared for tryptophan by taking different concentration 20–200 µg. Amount of methionine was determined by the method of Horn et al. (1946). The standard curve was prepared using methionine at different concentration from 100 to 1000 µg. Total flavonoids was determined by the method of Kim et al. 2003. A calibration curve was prepared with quercetin and the results were expressed as mg quercetin equivalent/g (QE/g) sample. Ascorbic acid was determined calorimetrically by following the method of Law et al. 1983. In this assay ascorbic acid reduce the $Fe^{3+}$ to $Fe^{2+}$ in acidic solution and $Fe^{2+}$ then forms complexes with bipyridyl, to gives a pink colour that has maximum absorbance at $\lambda_{525\ nm}$. A standard curve of ascorbic acid ranged 0–40 µg was prepared. The total carotenoids were extracted in organic solvents (acetone/methanol) on the basis of their solubility. The amount of carotenoids presents in samples was estimated calorimetrically by following the method of Johnson (2002). A standard curve for carotenoids was prepared using β-carotene as a standard. Vitamin-E reduces ferric ions to ferrous ions which combine with bathophenanthroline to form an orange coloured complex which gives absorbance at $\lambda_{536\ nm}$. α-Tocopherols was estimated calorimetrically by following the method of Kayden et al. (1973).

### Micronutrient analysis

The oven-dried grinded samples were passed through a 0.2 mm sieve for estimation of micronutrients content. Sieved samples were digested with a mixture of nitric acid and perchloric acid in the ratio of 10:4 (v/v) on hot plates sand bath. After complete digestion, samples were cooled at room temperature and appropriately diluted and filtered with a Whatmann No. 1 filter paper and transferred to storage vials. These digested samples were analyzed for zinc (Zn), iron (Fe), copper (Cu), calcium (Ca) and manganese (Mn) by the help of double beam atomic absorption spectrophotometer (ECIL, Hyderabad).

### Extraction of phenolic compounds

Powdered sample (500 mg) was mixed with 5 ml of 80 % methanol and ground thoroughly in pestle and mortar. The ground material was centrifuged at 600 rpm for 15 min. The volume was made to 50 ml by washing with 80 % methanol. The extract thus prepared was used for the estimation of total phenol, orthodihydroxy phenol, tannins and flavonoids.

### Estimation of phenolic compounds

The total polyphenols and total tannins content were determined by Folin Ciocalteu reagent (Swain and Hillis 1959) and calculated from a standard calibration curve based on gallic acid (0–0.1 mg/ml) and tannic acid (0–0.1 mg/ml) respectively. The results were expressed as gallic acid equivalents in mg GAE/g DW for total polyphenols and as tannic acid equivalents in mg TAE/g DW for total tannin content. Saponin content was determined calorimetrically by following the method of Fenwick and Oakenfull (1983). A standard curve of diosgenin was used for calculation of saponin (10–80 µg). For the

estimation of Ortho dihydroxy phenol, methanolic extract 1 ml was taken in a test tube and evaporated to dry. The residue was dissolved in 1 ml of distilled water by vortex. The Orthodihydroxyl phenol content was estimated in dissolved residue by the method of Nair and Vaidyanathan (1964). Standard curve was prepared using catechol (10–100 µg).

### Determination of antinutritional properties

Phytic acid (PA) contents in defatted flour samples were determined by the method of Davies and Reid (1979). The phytic acid content was calculated from a standard curve using phytate phosphorus salt in the range of 10–50 µg. Saponin content was determined by the method of Fenwick and Oakenfull (1983). A standard curve of diosgenin was used for calculation of saponin in range of 10–80 µg. Trypsin inhibitor activity (TI) was determined by the method of Kakade et al. (1974). Trypsin inhibitory activity of the extract was determining its ability to inhibit caesinolytic activity of trypsin and results were expressed as percent trypsin inhibition.

### Determination of enzymes activities

*Acid phosphatase*

Seeds (100 mg) were ground in a chilled mortar, with 3 ml of chilled 100 mM sodium acetate buffer (PH 5.0). The homogenate was centrifuged at 10,000*g* for 30 min at 4 °C. All procedure of extraction was carried at 4 °C. The supernatant was used as the crude extract for the acid phosphatase assay. The enzyme was assayed by using the procedure of Agoreyo (2010). The assay was performed in triplicate and acid phosphatase activity was expressed as µmol *ρ*–nitrophenol released min/g/fresh wt.

*Alpha-galactosidase*

Seeds (100 mg) were ground in a chilled pestle and mortar, in 2 ml of chilled 100 mM sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 10,000*g* for 30 min at 4 °C. All procedure of extraction was done at 4 °C to minimize the denaturation. The supernatant was used as the crude extract for the enzyme assay. The enzyme was assayed by using the procedure of McCleary and Matheson (1974). A standard curve of *ρ*-nitrophenol (100–1000 µM) was prepared to calculate concentration of *ρ*–nitrophenol formed during enzymatic reaction. One Alpha-galactosidase Unit (α-gal U) is that amount of enzyme which liberates 1 µmol of *ρ*–nitrophenol in 1 min under the assay conditions.

*Alpha-amylase inhibitor activity*

Seeds (1 g) were finely ground in pestle mortar and mixed with 10 ml 1 % NaCl (w/v) by shaking for 1 h and kept overnight at 4 °C. The mixture was centrifuged and final volume of supernatant was made to 25 ml with 1 % NaCl solution. Alpha-amylase inhibitor activity was measured in supernatant. The enzyme was assayed by using the procedure of Bernfeld (1955). One unit of amylase was defined as the amount of enzyme which yields 1 µmol of glucose at 37 °C for 1 min at pH 6.7. One unit of inhibitory activity is that which reduce the activity of amylase by one unit under the assay conditions.

## Results and discussion

### Nutritional properties

Crude fiber content in barnyard millet varieties ranged from 10.17 (VL207) to 11.91 % (VL29), while in finger millet varieties crude fiber content ranged from 3.10 (VL204) to 3.90 % (VL146) (Table 1). Crude fiber content in barnyard millet seeds was three times higher than finger millet (Bisoi et al. 2012; Khouloud et al. 2013). Total dietary fiber content in barnyard millet varieties seeds ranged from 23.25 (VL207) to 31.70 % (VL29). In finger millet varieties total dietary fiber content ranged from 16.28 (VL324) to 18.06 % (VL315) (Table 1). It showed that barnyard millet had higher amount of total dietary fiber compared to the finger millet. Cavallo et al. (1989) stated that increased intake on total dietary fiber appears to be useful for the treatment of both obesity and diabetes mellitus, since barnyard millet variety VL29 contained the highest amount of crude fiber and dietary fiber, hence incorporation of barnyard millet in diets may improve the human health. Chethan and Malleshi (2007) reported the total dietary fiber content (15–19 %) in finger millet which was similar to the finger millet varieties. The dietary fiber in oat bran is 15–22 % which is comparatively high then barnyard millet variety VL29 as both are important for their hypoglycemic activity (Chatuevedi et al. 2011). The seed coat of the millet is an edible component of the kernel and is a rich source of phytochemicals, such as dietary fiber and polyphenols (0.2–3.0 %) (Hadimani and Malleshi 1993).

Tryptophan content in seeds of barnyard millet varieties ranged from 58.35 (VL29) to 78.89 µg/g (VL172) whereas in seeds of finger millet varieties, tryptophan content was found from 53.57 (VL149) to 59.44 µg/g (VL315) (Table 1). Hence barnyard millet contained the higher amount of tryptophan as compared to the finger millet. Comai et al. (2007) reported the tryptophan content

**Table 1** Crude fiber content, total dietary fiber content, tryptophan, methionine, flavonoids, ascorbic acid, total carotinoids and α-tocopherol content in grain of barnyard millet and finger millet varieties

| Millets | Varieties | Crude fiber (%) | Total dietary fiber (%) | Tryptophan (μg/g) | Methionine (μg/g) | Flavonoids (mg/g) | Ascorbic acid (μg/g) | Total carotinoids (μg/g) | α-Tocopherol (μg/g) |
|---|---|---|---|---|---|---|---|---|---|
| Barnyard millet | VL21 | $11.30^{b}$ | $29.80^{b}$ | $60.55^{cd}$ | $139.09^{c}$ | $5.26^{a}$ | $51.0^{bcd}$ | $40.17^{c}$ | $26.75^{ab}$ |
| | VL29 | $11.91^{a}$ | $31.70^{a}$ | $58.35^{cd}$ | $204.00^{b}$ | $3.56^{d}$ | $47.53^{cd}$ | $43.65^{b}$ | $30.94^{a}$ |
| | VL172 | $10.33^{d}$ | $28.29^{c}$ | $78.89^{a}$ | $129.82^{c}$ | $3.12^{e}$ | $53.05^{bcd}$ | $36.72^{d}$ | $30.57^{a}$ |
| | VL207 | $10.17^{d}$ | $23.25^{e}$ | $73.39^{b}$ | $194.73^{b}$ | $4.70^{b}$ | $44.05^{d}$ | $50.79^{a}$ | $24.72^{bc}$ |
| | PRJ1 | $10.74^{c}$ | $24.37^{d}$ | $62.38^{c}$ | $185.45^{b}$ | $4.65^{b}$ | $54.15^{bcd}$ | $42.22^{bc}$ | $23.69^{bcd}$ |
| Finger millet | VL146 | $3.90^{e}$ | $17.26^{f}$ | $58.53^{cd}$ | $294.29^{a}$ | $3.98^{c}$ | $60.28^{ab}$ | $21.47^{fg}$ | $18.27^{de}$ |
| | VL149 | $3.70^{e}$ | $17.31^{f}$ | $53.57^{e}$ | $196.73^{b}$ | $3.50^{d}$ | $55.65^{abc}$ | $23.21^{efg}$ | $21.47^{bcde}$ |
| | VL204 | $3.10^{g}$ | $17.55^{f}$ | $56.88^{de}$ | $213.27^{b}$ | $3.34^{d}$ | $64.92^{a}$ | $25.95^{e}$ | $18.27^{de}$ |
| | VL315 | $3.60^{f}$ | $18.06^{f}$ | $59.44^{cd}$ | $296.73^{a}$ | $4.10^{c}$ | $59.13^{ab}$ | $24.31^{ef}$ | $17.52^{e}$ |
| | VL324 | $3.50^{f}$ | $16.28^{g}$ | $56.15^{de}$ | $176.18^{b}$ | $4.16^{c}$ | $54.49^{abcd}$ | $20.03^{g}$ | $19.72^{cde}$ |

Different letters in the same column indicate significant difference using LSD ($P \leq 0.05$); Values are the mean of three determinations

(7.4 ± 0.9 mg/100 g dry wt.) in millets which was found similar with barnyard millet variety VL207 (73.39 ± 1.46 μg/g).

Methionine content in seeds of barnyard millet varied from 129.82 (VL172) to 204.00 μg/g (VL29) whereas in seeds of finger millet, methionine content ranged from 176.18 (VL324) to 296.73 μg/g (VL315) (Table 1). Overall the methionine content found less in barnyard millet as compared to finger millet. Yeoh and Watson (1981) reported higher methionine content (356 μg/g) in finger millet. Mbithi-Mwikya et al. (2000) reported 290 and 298 μg/g methionine content in finger millet that were found approximately similar with finger millet varieties VL146 and VL315. Flavonoids content in seeds of barnyard millet varieties ranged from 3.12 (VL172) to 5.26 (VL21) mg QE/g, whereas in seeds of finger millet varieties it ranged from 3.34 (VL204) to 4.16 (VL324) mg QE/g (Table 1). Kim et al. (2010) also reported total flavonoid in proso millet (3.4 mg QE/g), foxtail millet (4.0 mg QE/g), which was found lesser in comparison to barnyard millet variety VL21 (5.26 ± 0.24 mg QE/g). Ascorbic acid concentration in varieties of barnyard millet ranged from 44.05 (VL207) to 54.15 μg/g (PRJ1) whereas in varieties of finger millet, ascorbic acid concentration ranged from 54.49 (VL324) to 64.92 μg/g (VL204) (Table 1). Results sowed that finger millet contained the higher amount of ascorbic acid as compared to the barnyard millet. Ascorbic acid is required for cardiovascular function, immune cell development, iron utilization and antioxidant properties (Chen et al. 2003). Total carotenoids content in seeds of barnyard millet varieties ranged from 36.72 (VL172) to 50.79 μg/g (VL207) whereas in finger millet varieties, total carotenoids ranged from 20.03 (VL324) to 25.95 μg/g (VL204) of seeds (Table 1). Results showed that barnyard millet contained twofold higher amount total carotenoids compared to finger millet. Asharani et al. (2010) also reported total carotenoids in finger millet (29 ± 7.7 μg/g), little millet (7.8 ± 1.9 μg/g), foxtail millet (17.3 ± 2.5 μg/g) and proso millet (36.6 ± 10.4 μg/g). α-Tocopherol content in barnyard millet varieties ranged from 23.69 (PRJ1) to 30.94 μg/g (VL29) whereas in finger millet varieties, α-Tocopherol ranged from 17.52 (VL315) to 21.47 μg/g (VL149) (Table 1). It showed that barnyard millet contained 1.5 fold higher α-tocopherol content compared to finger millet. Results are in agreement of previous study by Asharani et al. (2010), reported α-Tocopherol analyzed by reverse phase HPLC in finger millet (37 ± 0.2 μg/g), little millet (13 ± 0.2 μg/g), foxtail millet (1.2 ± 0.008 μg/g) and proso millet (36 ± 0.1 μg/g). The vitamin is a peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and in plasma lipoproteins (Levine et al. 1999).

## Compound of bioactive properties

The results indicated that barnyard millet contained the higher polyphenolic content compared to finger millet, which is approximately twofold higher in barnyard millet (Table 2). Barnyard millet contained total polyphenols ranged from 20.30 (VL172) to 27.80 (VL21) mg GAE/g. Kim et al. (2010) reported the total phenolic content ranged from 18.0 to 26.5 mg GAE/g in proso millet and from 12.0 to 26.7 mg GAE/g in foxtail millet. Tannin content in seeds of barnyard millet varieties ranged from 3.25 (VL172) to 3.96 mg/g (VL21) whereas in finger millet varieties, tannin content ranged from 2.05 (VL204) to 2.62 (VL149) mg

**Table 2** Bioactive constituents and antinutritional compounds of grains of barnyard millet and finger millet varieties

| Millets | Bioactive properties | | | | | Antinutitional compounds | |
|---|---|---|---|---|---|---|---|
| | Varieties | Total phenol (mg/g) | Tannins (mg/g) | Saponins (mg/g) | Ortho dihydroxy phenol (μg/g) | Phytic acid (mg/g) | Trypsin inhibition (%) |
| Barnyard millet | VL21 | $27.80^{a}$ | $3.96^{a}$ | $10.26^{a}$ | $107.14^{c}$ | $3.62^{e}$ | $8.07^{b}$ |
| | VL29 | $20.35^{b}$ | $3.42^{c}$ | $8.53^{b}$ | $122.85^{ab}$ | $3.30^{g}$ | $8.37^{a}$ |
| | VL172 | $20.30^{b}$ | $3.25^{d}$ | $8.38^{b}$ | $117.86^{b}$ | $3.43^{f}$ | $7.85^{c}$ |
| | VL207 | $27.55^{a}$ | $3.62^{b}$ | $10.11^{a}$ | $130.71^{a}$ | $3.70^{d}$ | $7.87^{c}$ |
| | PRJ1 | $27.70^{a}$ | $3.34^{d}$ | $8.42^{b}$ | $127.14^{ab}$ | $3.37^{g}$ | $8.24^{ab}$ |
| Finger millet | VL146 | $12.55^{c}$ | $2.57^{e}$ | $2.13^{c}$ | $59.28^{def}$ | $5.56^{b}$ | $8.06^{abc}$ |
| | VL149 | $11.35^{cd}$ | $2.62^{e}$ | $2.63^{c}$ | $64.28^{d}$ | $5.58^{a}$ | $8.02^{abc}$ |
| | VL204 | $12.10^{c}$ | $2.05^{g}$ | $2.32^{c}$ | $53.57^{f}$ | $5.54^{c}$ | $8.36^{a}$ |
| | VL315 | $10.35^{d}$ | $2.18^{fg}$ | $2.23^{c}$ | $62.85^{ef}$ | $5.57^{ab}$ | $8.24^{b}$ |
| | VL324 | $11.45^{cd}$ | $2.33^{f}$ | $2.52^{c}$ | $59.28^{ef}$ | $5.58^{a}$ | $8.34^{a}$ |

Different letters in the same column indicate significant difference using LSD ($P \leq 0.05$); Values are the mean of three determinations

TAE/g (Table 2). Results showed that barnyard millet contained high amount of tannins compared to finger millet, which is about 1.5-fold higher. Previous study showed that in finger millet tannin content ranged from 0.04 to 3.47 % by Ramachandra et al. (1977). Tannin content was also estimated in hilly region varieties and found to be less compared to base region varieties (Wadikar et al. 2006). Saponins content in seeds of barnyard millet varieties ranged from 8.38 (VL172) to 10.26 (VL21) mg diosgenin eq/g, whereas in seeds of finger millet varieties it ranged from 2.13 (VL146) to 2.63 (VL149) mg diosgenin eq/g (Table 2). It indicated that barnyard millet contain the higher phenolic content compared to finger millet, which is approximately fivefold higher in barnyard millet. Results are in agreement of previous study by Banno et al. (2004), reported that saponins exert various biological benefits, such as anti-inflammatory, anti-diabetic, anti-HIV, anti-atherosclerotic and serve as protective functions like gastro-protective, hepatoprotective and hypolipidemic, thus saponin content is a desirable trait from functional food. Ortho-dihydroxy phenol content in seeds of barnyard millet varieties ranged from 107.14 (VL21) to 130.71 (VL207) μg catechol/g, whereas in finger millet varieties ranged from 53.57 (VL204) to 64.28 (VL149) μg catechol/g (Table 2). It indicated that barnyard millet contain higher Ortho-dihydroxy phenol content compared to finger millet, which is approximately twofold higher in barnyard millet. Ortho-dihydroxy phenols are an important group of plant polyphenolics and responsible for a range of bioactive properties of difference plant extracts. The presence of 3, 4-dihydroxyphenyl ethanol glucoside and 3, 4-dihydroxy-6-(*N*-ethylamino) benzamide in green pepper has been shown to be responsible for its antimicrobial properties (Pradhan et al. 1991).

## Antinutitional compounds

Phytic acid content in seeds of barnyard millet varieties ranged from 3.30 (VL29) to 3.70 mg/g (VL207), whereas in finger millet varieties, ranged from 5.54 (VL204) to 5.58 mg/g (VL149 and VL324) (Table 2). Results showed that barnyard millet contain lower phytic acid content compare to finger millet. Results of present study are in agreement of Lorenz (1983). Phytic acid content in legumes, cereals, oil seeds, pollens, and nuts is 1–5 % which is comparatively high barnyard and finger millet (Petry et al. 2015). Phytic acid has long been recognized as an antinutritional factor, for its ability to bind, precipitate and decrease the availability of di-and trivalent cationic minerals (Phillipy 2003). Phytic acid was known to inhibit number of digestive enzymes such as pepsin, α-amylase and trypsin (Ravindran 1992). Thus, a lower level of phytic acid is a desirable trait which is shown by barnyard millet.

The inhibitory potential of trypsin inhibitor of seeds of barnyard millet and finger millet varieties were estimated and expressed in % inhibition of trypsin enzyme activity. In barnyard millet varieties, percent inhibition varied from 7.85 (VL172) to 8.37 (VL29), while in finger millet varieties it ranged from 8.02 (VL149) to 8.36 (VL204) (Table 2). There was not any significant difference in trypsin inhibition activity of barnyard millet and finger millet varieties.

## Acid phosphatase enzyme activity

Acid phosphatase enzyme activity in seeds of barnyard millet varieties was ranged from 216.28 (VL29) to 331.21 (PRJ1) U/g seed and in seeds of finger millet varieties, was found from 192.02 (VL204) to 215.63 (VL315) U/g

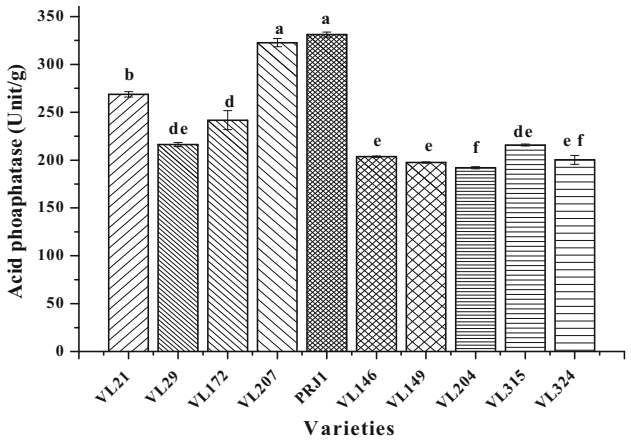


**Fig. 1** Acid phosphatase in different varieties of barnyard millet (VL21, VL29, VL172, VL207, PRJ1) and finger millet (VL146, VL149, VL204, VL315, VL324). Values are expressed as mean ± standard deviation (n = 3). Means with *different letters* (*a*, *b*, *c*, *d*, *e* and *g*) were significantly different at the level of $P < 0.05$

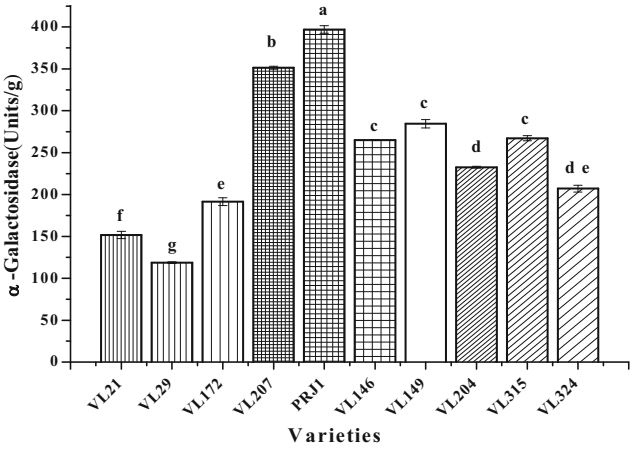


**Fig. 2** α-Galactosidase in different varieties of barnyard millet (VL21, VL29, VL172, VL207, PRJ1) and finger millet (VL146, VL149, VL204, VL315, VL324). Values are expressed as mean ± standard deviation (n = 3). Means with *different letters* (*a*, *b*, *c*, *d*, *e* and *g*) were significantly different at the level of $P < 0.05$

(Fig. 1). It showed that barnyard millet contain higher acid phosphatase enzyme activity compared to finger millet.

Acid phosphatase is the key enzyme involved in acquisition, transport and recycling of phosphorus (Yan et al. 2001). Ungerminated seeds have low level of acid phosphatase activity which gets enhanced during germination and early seedling growth (Biwas and Cundiff 1991). Higher acid phosphatase enzyme in barnyard millet may provide protection to plants against various abiotic stresses, like salt, osmotic and water, by maintaining a certain level of inorganic phosphate.

## Alpha-galactosidase enzyme activity

Alpha-galactosidase enzyme activity in seeds of barnyard millet varieties ranged from 118.62 (VL29) to 396.99 (PRJ1) U/g and in seeds of finger millet varieties ranged from 207.14 (VL324) to 284.51 (VL149) U/g (Fig. 2), indicated that barnyard millet contained higher α-galactosidase enzyme activity compare to finger millet.

Acid and alkaline alpha-galactosidase plays a significant role during seed germination (Dey and Pridham 1972). It was reported that down regulation of alpha galactosidase enhances freeze tolerance (Pennycooke et al. 2003). Thus α-galactosidase may be an important enzyme responsible for mobilization of stored carbohydrates during germination.

## Alpha-amylase inhibition

Alpha-Amylase inhibition in seeds of barnyard millet varieties varied from 41.26 % ((VL21) to 60.32 % (PRJ1), while in seeds of finger millet varieties it ranged from

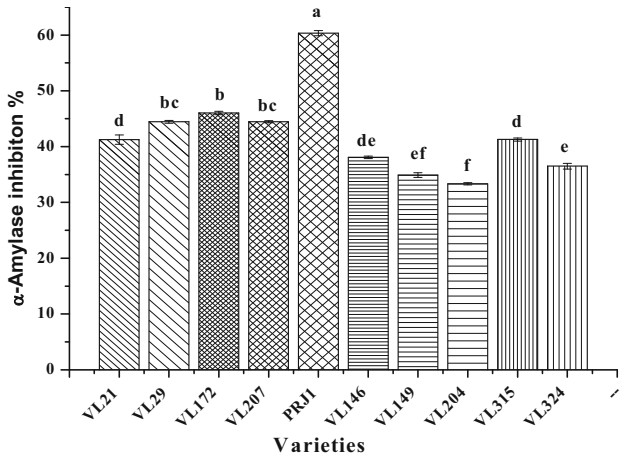


**Fig. 3** Percent α-amylase inhibition in seeds of different varieties of barnyard millet (VL21, VL29, VL172, VL207, PRJ1) and finger millet (VL146, VL149, VL204, VL315, VL324). Values are expressed as mean ± standard deviation (n = 3). Means with *different letters* (*a*, *b*, *c*, *d*, *e* and *g*) were significantly different at the level of $P < 0.05$

33.34 % (VL204) to 41.25 % (VL315) (Fig. 3). Results showed that barnyard millet seeds contain higher α-amylase inhibition activity compared to finger mille seeds.

Kokiladevi et al. (2005) studied the α-amylase inhibitory activity of *Vigna sublobata* on insect (*Callosobruchus analis*). Result showed that crude protein extracts from the seeds of ten *Vigna* genotypes were assayed for inhibitory activity against the larval α-amylase of *C. analis*. *Vigna umbellate* (110.01 %) *V. sublobata* (80.50 %) and *V. glabracens* (70.03) showed higher levels of inhibitory activity.

**Table 3** Micronutrients content of grains of barnyard millet and finger millet varieties

| Millets | Varieties | Calcium (mg/100 g) | Iron (mg/100 g) | Zinc (mg/100 g) | Cooper (μg/100 g) | Mn (mg/100 g) |
|---|---|---|---|---|---|---|
| Barnyard millet | VL21 | $27.10^{d}$ | $19.53^{b}$ | $4.75^{b}$ | $0.013^{d}$ | $3.13^{d}$ |
| | VL29 | $22.27^{d}$ | $22.98^{a}$ | $4.95^{b}$ | $0.018^{a}$ | $3.43^{cd}$ |
| | VL172 | $23.34^{d}$ | $19.27^{b}$ | $4.21^{b}$ | $0.016^{b}$ | $3.13^{d}$ |
| | VL207 | $26.72^{d}$ | $20.25^{b}$ | $5.92^{a}$ | $0.014^{c}$ | $3.31^{cd}$ |
| | PRJ1 | $22.56^{d}$ | $19.93^{b}$ | $4.44^{b}$ | $0.016^{b}$ | $3.63^{d}$ |
| Finger millet | VL146 | $302.4^{b}$ | $4.82^{cd}$ | $2.96^{c}$ | $0.012^{e}$ | $7.11^{b}$ |
| | VL149 | $309.79^{b}$ | $4.08^{d}$ | $2.82^{c}$ | ND | $7.62^{a}$ |
| | VL204 | $331.92^{a}$ | $5.56^{c}$ | $2.72^{cd}$ | $0.012^{e}$ | $7.12^{b}$ |
| | VL315 | $298.72^{b}$ | $5.30^{c}$ | $2.24^{cd}$ | ND | $7.01^{b}$ |
| | VL324 | $276.60^{c}$ | $5.27^{c}$ | $2.00^{d}$ | $0.012^{e}$ | $7.21^{ab}$ |

Different letters in the same column indicate significant difference using LSD ($P \leq 0.05$); *ND* not detected. Values are the mean of three determinations

## Micronutrients

In seeds of barnyard millet varieties the calcium content was ranged between 22.27 (VL29) to 27.10 (VL21) mg/100 g whereas in seeds of finger millet the calcium content ranged between 276.60 (VL324) to 331.92 (VL204) mg/100 g (Table 3). The results showed that, finger millet seeds contains about 10–13 folds higher calcium content in comparison to barnyard millet seeds. The results are in agreement of previous study by Rao et al. (1973), reported that the calcium content in finger millet in range from 203 to 690 mg/100 g.

Iron content in seeds of barnyard millet varieties ranged from 19.27 (VL172) to 22.98 (VL29) mg/100 g whereas, in seeds of finger millet varieties, iron content ranged from 4.08 (VL149) to 5.56 (VL204) mg/100 g (Table 3). Results of present study are in agreement with previous study in finger millet by Admassu et al. (2009). Rao et al. (1973) reported higher iron content (20.9 to 93.5 mg/100 g) in 15 varieties of finger millet. The average iron concentration 55 μg/g in beans (*Phaseolus vulgaris*) is quite low compared to millet (Petry et al. 2015). Zinc content in seeds of barnyard millet varieties ranged from 4.21 (VL172) to 5.92 (VL207) mg/100 g whereas, in seeds of finger millet varieties, zinc content ranged between 2.00 (VL324) to 2.96 (VL146) mg/100 g (Table 3). Results of present study are in agreement with previous study in white and brown variety of finger millet by Antony and Chandra (1998) reported the zinc content. The varieties of barnyard millet and finger millet under study showed a significant ($P \leq 0.05$) difference in their zinc content. Copper content in seeds of barnyard millet varieties ranged from 0.013 (VL21) to 0.018 (VL29) μg/100 g whereas in seeds of finger millet varieties, copper content was found 0.012 μg/100 g in VL146, VL324 and VL204 (Table 3). The Copper content was not detected in some varieties of finger millets. In several previous studies, the copper content was not reported in barnyard millet and finger millet seeds, however copper content (4–6 μg/kg) in pearl millet have been reported by (Buerkert et al. 2001). This indicated that seeds of all millets contained very less amount of copper content.

Manganese content in seeds of barnyard millet varieties ranged between 3.13 (VL21) to 3.63 (PRJ1) mg/100 g and in seeds of finger millet varieties, manganese content ranged from 7.01 (VL315) to 7.62 (VL149) mg/100 g (Table 3). The results of present study are in agreement with previous study in finger millet by Antony and Chandra (1998). Manganese (Mn) is an essential trace metal that is found in all tissues and is required for normal amino acid, lipid, protein, and carbohydrate metabolism. Manganese metalloenzymes include arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and Mn superoxide dismutase (Mn-SOD) (Aschner and Aschner 2005).

## Conclusion

Present study showed that barnyard millet contained higher amount of crude fiber, total dietary fiber, tryptophan content, total carotenoids, α-tocopherol compared to the finger millet whereas the finger millet contains higher amount of methionine and ascorbic acid as compared to the barnyard millet. The bioactive constituents in barnyard millet contain the higher amount of polyphenols, tannins and ortho-dihydroxy phenol content compared to finger millet. Among antinutitional compound barnyard millet contain lower phytic acid content compare to finger millet whereas no significant difference in trypsin inhibition activity of barnyard millet and finger millet varieties were found. Barnyard millet contained higher acid phosphatase, α-

galactosidase and α-amylase inhibitor activity compared to finger millet. Finger millet seeds contain about 10–13 folds higher calcium content and double amount of manganese content in comparison to barnyard millet seeds. Hence incorporation of flour of barnyard millet and finger millet in different ratio may enhance the nutraceutical value of the processed food.

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