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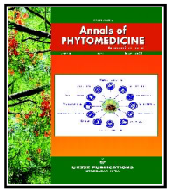

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Impact of germination on the nutritional, antioxidant and antinutrient characteristics of selected minor millet flours

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Abstract

The present study was conducted to evaluate the effect of germination on nutritional, antinutritional, total phenolic content, total flavonoid content and antioxidant activity (DPPH radical scavenging activity) in barnyard, foxtail and little millet flours. Grains were germinated for 24 h after soaking for 12 h, dried, milled and used for the analysis. Nutritional composition of millet flours was analyzed using AOAC methods. Antinutritional and antioxidant properties were measured in terms of oxalates, tannins, total phenolic contents and total flavonoid contents. Antioxidant activity was done using DPPH radical scavenging activity at six different concentrations and 50% inhibitory concentration was drawn from the linear regression. Germination of millets has decreased energy value and oxalate content, and increased crude fiber, tannins, total phenolic compounds and total flavonoid compounds. It also increased DPPH radical scavenging activity of millet flours. As germinated millet flours are rich source of antioxidant properties with good radical scavenging activity could be recommended to develop functional foods for the treatment of various degenerative diseases.

1. Introduction

There are increasing evidences worldwide that free radicals produce molecular alterations in human cells that are correlated with various degenerative diseases such as atherosclerosis, cancers, parkinson's disease, diabetes, asthma, alzheimer's disease, arthritis, immune deficiency diseases and ageing (Okoh *et al*., 2014; Sen *et al*., 2010) which can be reduced by health promoting diets including millets, fruits and vegetables. Millets are the most commonly consumed food items in India, Africa, China and elsewhere. They contain wide range of high-quality protein with good amino acid balance, fibre, magnesium, manganese, niacin, phosphorus, iron, potassium, B-complex vitamins, vitamin A and E (Shashi *et al*., 2007; Saleh *et al*., 2013). Millets are also natural source of many bioactive compounds including phenolic acids, athocyanins and flavonols which possess antioxidant properties and can act as nutraceutical and functional food ingredient in health promotion and risk reduction in degenerative diseases (Kayode *et al*., 2007; Rao *et al*., 2011; Sreeramulu *et al*., 2009). Millets are least allergenic, non-glutinous, non-acid forming and easy to digest. All these make millets an important nutritional biosource and, hence termed as nutracereal (Devi *et al*., 2014; Nazni and Shobana, 2016).

The main drawback of millet nutrition is its bioavailability owing to high antinutritional factors which can be reduced by various traditional processing techniques (Sheela *et al*., 2018). Soaking followed by germination improves nutrient levels of millets by activating hydrolytic enzymes, endo and exopeptidases. Germinated grains contain low unsaturated fatty acids, high protein, vitamins, minerals such as calcium, phosphorus, copper and zinc than ungerminated grains (Dicko *et al*., 2006; Inyang and Zakari, 2008). Several studies on germination of pearl and sorghum millets have shown lessened antinutritional factors (Elmaki *et al*., 1999; Nwasike, 1989; Correia *et al*., 2010); increased nutritive value (Correia *et al*., 2008; Nkama *et al*., 2015) and minerals (Badau *et al*., 2005; Arora *et al*., 2003; Suma and Urooj, 2014). Very less research works has been done on the nutritional changes occurring in minor millets after germination, so the aim of the present study is to investigate the effect of germination on proximate composition, antinutrients, total phenolic content, total flavonoid content and antioxidant activity including 50% inhibitory concentrations in barnyard, foxtail and little millets.

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2. Materials and Methods

2.1 Procurement and germination

Barnyard, foxtail and little millet were procured directly from the farmers. Barnyard and little millet grains were germinated at 35°C and foxtail millet at 30°C for 24 h in BOD incubator after overnight steeping in distilled water. Germination time and temperature were optimized according to Al-Mudaris (1998). Then, the germinated grains were dried in tray dryer at 60°C for 6 h. Dried germinated grains and raw grains were milled and stored in an air tight bag for further analysis.

2.2 Proximate analysis

Proximate composition of raw and germinated millet flours was done using standard AOAC methods such as moisture-oven drying method (AOAC, 2005), ash-charring method (AOAC, 2005), protein-kjeldhal method (AOAC 992.23 - 2005), crude fiber-acid-base extraction method (AOAC 962.09 - 2016) and fat-soxhlet extraction method (AOAC 922.06 - 2016). Carbohydrate content was calculated by subtracting the total of moisture, fat, protein, and ash from 100. Energy values were obtained by the formula:

Energy value= Protein × 4 + Carbohydrate × 4+ Fat × 9.

2.3 Antinutrient analysis

Tannins were analyzed spectrophotometrically at 700 nm by using tannic acid as standard. Titration method was used to determine oxalate content. One gram of the sample was weighed into 100 ml conical flask where 75 ml of 3M H_2SO_4 was added and stirred intermittently with a magnetic stirrer for 1 h. It was then filtered using Whatman No.1 filter paper. From the filtrate, 25 ml was taken and titrated against hot (80-90°C) 0.1N $KMnO_4$ solution up to faint pink color persisted for at least 30 s (Nissar *et al*., 2017).

2.4 Antioxidant properties and antioxidant activity

Samples were extracted using cold maceration technique in methanol (1 g/100 ml) for 24 h then centrifuged at 3000 rpm for 30 min, filtered through Whattman number 41 filter paper and extracts were preserved at 4°C to analyze TFC (Total Flavonoid Content), TPC (Total Phenolic Content) and antioxidant activity. TPC and TFC were done spectrophotometrically at 750 and 415 nm and expressed in GAE (Gallic Acid Equivalents) and RE (Rutin Equivalents), respectively (Slinkard and Slingleton, 1997; Meda *et al*., 2005). Antioxidant activity was determined using DPPH radical scavenging activity at different concentrations such as 1, 2, 4, 6, 8, 10 mg and absorbance was read at 517 nm (Domain *et al*., 2004). Inhibitory concentration of 50% (IC_{50}) was obtained by interpolation of linear regression analysis from the data obtained at various concentrations.

2.5 Statistical analysis

Experiments were carried out in triplicates. Data was subjected to CRD-ANOVA to determine significant difference between group means at 5% level of significance using Windostat version 9.1.

3. Results

3.1 Effect of germination on proximate composition

Data pertaining to proximate composition and per cent change of raw and germinated millet flours have been given in Table 1, and Figure 1 on dry weight basis. From the Table 1 it can be seen that moisture content ranged from 8.52 to 7.41% with the highest content in WBF (Whole Barnyard millet Flour), followed by WLF (Whole Little millet Flour), WFF (Whole Foxtail millet Flour), GBF (Germinated Barnyard millet Flour), GFF (Germinated Foxtail millet Flour) and GLF (Germinated Little millet Flour). Germination has shown non-significant effect of moisture levels in barnyard and foxtail millet flour, but it significantly decreased in little millet flour by 11.07%. Protein content of the millet flours ranged from 12.05 to 10.19%. The crude protein content was highest in little millet (12.05 and 11.14%), followed by foxtail (11.14 and 10.75%) and barnyard millet (10.19 and 10.9%) of raw and germinated millet flours. Ash content was ranged between 4.99 to 3.25%. Significant difference was found among the three germinated millet flours, even though there was no difference in raw foxtail and little millet flours. It might be due to increase in ash content of WLF than the WFF (Figure 1). Crude fiber content of samples ranged from 11.21 to 7.96%. Germination has significantly increased crude fiber content in barnyard and foxtail millet, while no significant increase was found in little millet. Crude fiber content was increased by 12.1, 8.66 and 4.29 % in GBF, GFF and GLF, respectively. It was highest in GBF (Figure 1) as crude fiber is high in raw barnyard millet flour. Fat content of all flour samples was in the range of 5.29 to 3.5%; it was highest in GLF, followed by WBF, GBF, WFF, WLF and GFF (Table 1). Carbohydrates of raw and germinated millet flours ranged from 66.44 to 60.55%. Carbohydrate content was highest in foxtail millet (65.59 and 66.44%) followed by little (63.3 and 63.58%), and barnyard millet (65.59 and 66.44%) among raw as well as germinated millet flours. Energy values ranged from 346 to 327 Kcal with GLF and WFF having the highest value, followed by GFF, WLF, WBF and GBF.

WBF: Whole barnyard millet flour; WFF: Whole foxtail millet flour; WLF: Whole little millet flour; GBF: Germinated barnyard millet flour; GFF: Germinated foxtail millet flour; GLF: Germinated little millet flour. The results are given as Means ± SD of three measurements. Means within a column with the same superscript alphabet are not significantly different (p<0.05).

Table 1: Effect of germination on proximate composition of minor millets

	Moisture (%)	Ash (%)	Protein (%)	Crude fiber (%)	Fat (%)	CHO (%)	Energy (Kcal)
WBF	8.52 ± 0.04^{a}	4.97 ± 0.03^{a}	10.19 ± 0.27^{c}	10 ± 0.24^{b}	4.898 ± 0.11^{b}	61.42 ± 0.19^{d}	331 ± 0.84^{c}
WFF	7.81 ± 0.06^{ab}	3.24 ± 0.03^{c}	11.14 ± 0.15^{b}	7.96 ± 0.2^{d}	4.26 ± 0.06^{d}	65.59 ± 0.3^{b}	345 ± 1.13^{a}
WLF	8.49 ± 0.66^{a}	3.36 ± 0.01^{bc}	12.05 ± 0.43^{a}	8.61 ± 0.26^{c}	4.2 ± 0.04^{d}	63.3 ± 0.76^{c}	339 ± 1.64^{b}
GBF	7.81 ± 0.04^{ab}	4.99 ± 0.03^{a}	10.9 ± 0.05^{b}	11.21 ± 0.1^{a}	4.54 ± 0.07^{c}	60.55 ± 0.13^{d}	327 ± 0.34^{d}
GFF	7.41 ± 0.04^{b}	3.25 ± 0.003^{c}	10.75 ± 0.1^{b}	8.65 ± 0.18^{c}	3.50 ± 0.09^{e}	66.44 ± 0.15^{a}	340 ± 0.17^{b}
GLF	7.55 ± 0.09^{b}	3.48 ± 0.03^{b}	11.14 ± 0.15^{b}	8.98 ± 0.21^{c}	5.29 ± 0.12^{a}	63.58 ± 0.08^{c}	346 ± 0.8^{a}
CD at 5%	0.75	0.127	0.586	0.57	0.222	0.982	2.646
CV %	3.68	1.27	2.07	2.4	1.94	0.6	0.3

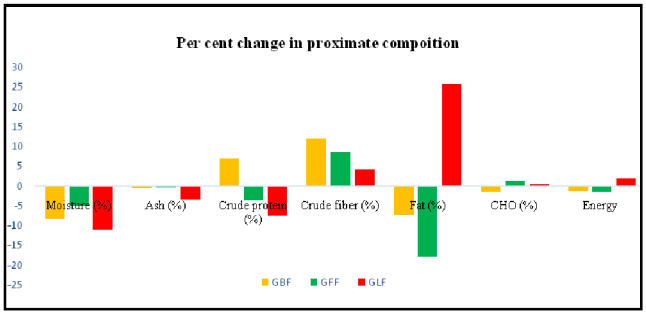

Figure 1: Per cent change in proximate composition of millet flours on germination.

GBF: Germinated barnyard millet flour; GFF: Germinated foxtail millet flour; GLF: Germinated little millet flour.

3.2 Effect of germination on antinutritional and antioxidant properties

Antinutritional and antioxidant properties of millet flour obtained from raw millets and germinated millets was presented in Table 2. Oxalate content has nutritional importance due to interference with mineral (calcium) bioavailability. Khokhar and Apenten (2003) opined the removal of oxalates to a safe level *via* the germination process. Oxalate content of millet flours was ranged from 0.56 to 0.37 g/100 g with the highest content in WFF, followed by WLF, GBF, WBF, GFF and GLF. From Table 2, it can be seen that tannin content of millet flours was ranged from 9.1 to 4.72%. Tannins were high in little millet (6.14 and 9.1%), followed by foxtail (4.94 and 6.27%) and barnyard millet (4.72 and 5.78%)) both in raw and germinated millet flours.

Total phenolic compounds of millet flours ranged from 55.98 to 132.98 mg GAE/100 g with the highest content in WLF, followed by GLF, GBF, GFF, WBF and WFF 123.3, 105.83, 69.28, 66.78 and 55.98 mg GAE/100 g, respectively. Total flavonoid content of millets ranged from 699.76 to 1343 mg RE/100 g with the highest content in GFF, followed by WFF, GLF, GBF, WLF and WBF (Table 2). Total flavonoid content of 27 RE mg/g in pearl millet and 391 RE mg/100 g in roasted proso millet were reported by Pushparaj and Urooj (2014) and Kalam Azad *et al.* (2019), respectively per cent increase in total flavonoid content after germination was 22.81, 11.9 and 34.81 in barnyard, foxtail and little millet, respectively.

Table 2: Effect of germination on antinutrient and antioxidant properties of minor millets

	Oxalates (g/100 g)	Tannins (%)	TPC (mg GAE/100 g)	TFC (mg RE/100 g)
WBF	0.44 ± 0.004^{b}	4.72 ± 0.09^{e}	66.78 ± 3.85^{d}	699.76 ± 20.51^{e}
WFF	0.56 ± 0.004^{a}	4.94 ± 0.12^{e}	55.98 ± 0.39^{e}	1200.08 ± 65.76^{b}
WLF	0.44 ± 0.004^{b}	6.14 ± 0.06^{c}	132.98 ± 7.74^{a}	743.7 ± 29.87^{e}
GBF	0.44 ± 0.001^{b}	5.78 ± 0.09^{d}	105.83 ± 0.88^{c}	859.44 ± 30.32^{d}
GFF	0.37 ± 0.004^{c}	6.27 ± 0.04^{b}	69.28 ± 1.24^{d}	1343 ± 38.18^{a}
GLF	0.37 ± 0.004^{c}	9.1 ± 0.06^{a}	123.3 ± 1.84^{b}	1002.65 ± 26.38^{c}
CD at 5%	0.003	0.225	10.28	80.439
CV %	0.25	1.43	4.33	3.21

WBF: Whole barnyard millet flour; WFF: Whole foxtail millet flour; WLF: Whole little millet flour; GBF: Germinated barnyard millet flour; GFF: Germinated foxtail millet flour; GLF: Germinated little millet flour. TPC: Total phenolic content, TFC: Total flavonoid content, GAE: Gallic acid equivalent, RE: Rutin equivalent. The results are given as Means ± SD of three measurements. Means within a column with the same superscript alphabet are not significantly different (p<0.05)

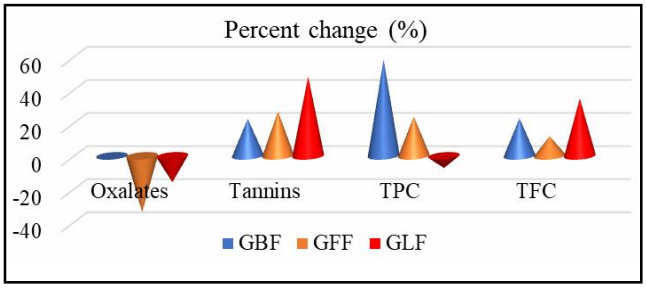

Figure 2: Percent change in antinutrient and antioxidant properties on germination.

GBF: Germinated barnyard millet flour; GFF: Germinated foxtail millet flour; GLF: Germinated little millet flour.

3.3 Germination effect: Antioxidant activity by DPPH radical scavenging activity and IC_{50}

Figures 3-5 summarizes data on DPPH radical scavenging activity of raw and germinated millet flours. IC_{50} values were presented in Figure 6. In the DPPH scavenging assay, the color stable DPPH radical is brought down in the presence of an antioxidant which donates hydrogen to non-radical DPPH–H (Ragaee *et al.*, 2006). IC_{50} values of barnyard millet was increased on germination from 2.72 to 3.31 mg/ml (Figure 4) which means WBF has higher scavenging activity than GBF. Inhibition per cent was increased with an increase in the concentration in barnyard millet, it is high in GBF up to 6 mg and later it raised but not more than WBF (Figure 3). Decreased scavenging activity was reported after germination in two pearl millet varieties (Pushparaj and Urooj, 2014). Radical scavenging activity has significantly increased in GFF than WFF at all the concentrations (Figure 4). IC_{50} value in GFF (3.61 mg/ml) was slightly higher than raw foxtail millet flour (3.51 mg/ml). Germinated little millet also has shown a significant increase in free radical scavenging activity at all the concentrations than WLF (Figure 5). The same was observed in 50% inhibitory concentration of free radicals (1.92 and 1.99 mg/ml) (Figure 6). Effect of germination on DPPH radical scavenging activity in the barnyard, foxtail and little millet (Figures 3-5).

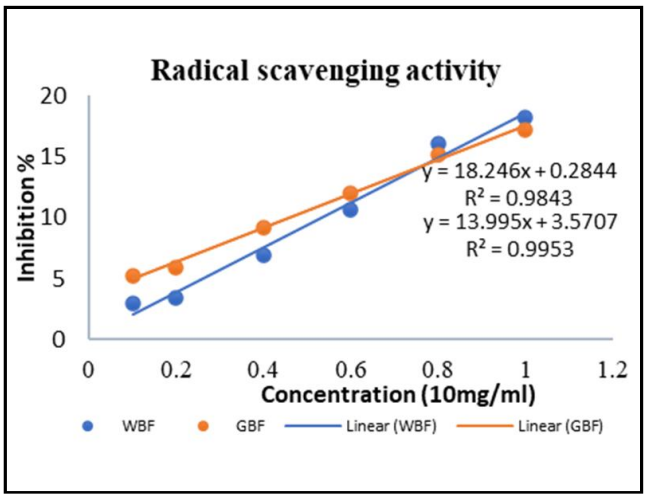

Figure 3: Barnyard millet.

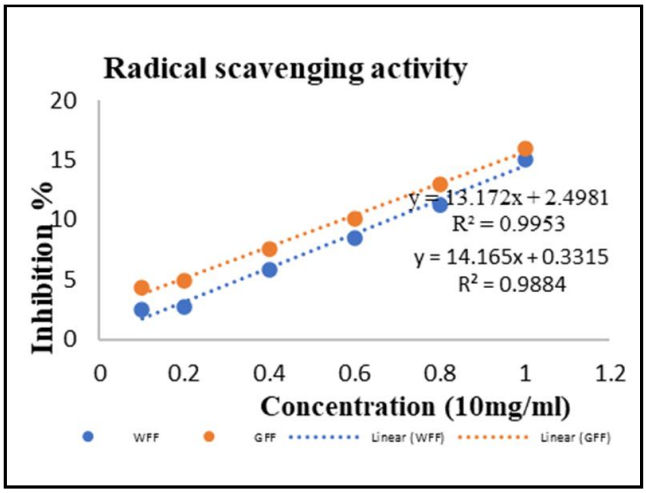

Figure 4: Foxtail millet.

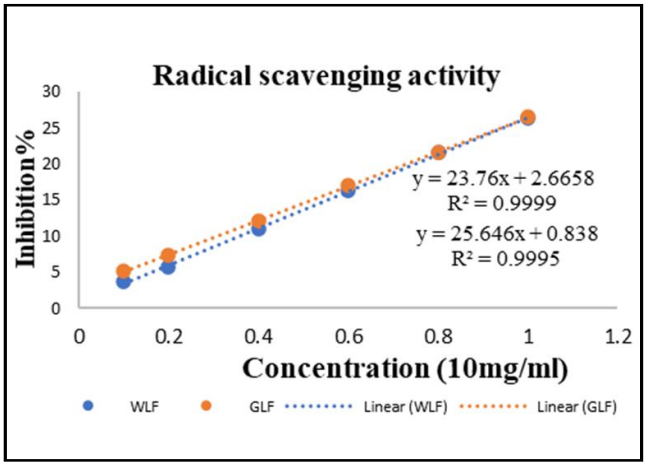

Figure 5: Little millet.

WBF: Whole barnyard millet flour; WFF: Whole foxtail millet flour; WLF: Whole little millet flour; GBF: Germinated barnyard millet flour; GFF: Germinated foxtail millet flour; GLF: Germinated little millet flour.

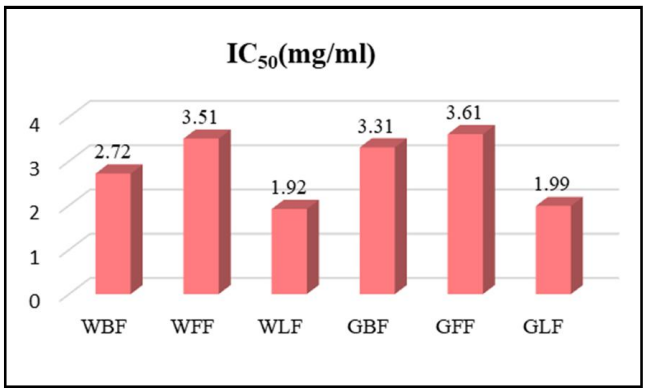

Figure 6: Effect germination on 50% inhibitory concentration in minor millets.

WBF: Whole barnyard millet flour; WFF: Whole foxtail millet flour; WLF: Whole little millet flour; GBF: Germinated barnyard millet flour; GFF: Germinated foxtail millet flour; GLF: Germinated little millet flour.

4. Discussion

Moisture content has significantly decreased in little millet and no difference was observed in barnyard and foxtail millet on germination. Similar results were reported in pearl millet, finger millet, sorghum (Singh *et al*., 2017), maize (Anaemene and Fadupin, 2020) and green gram (Dattatray *et al*., 2019) after germination. Due to the drying process in flour development, lower moisture levels help in longer shelf life of flours. Germination has shown an effect on crude protein content; it significantly increased in barnyard millet (6.96%) and decreased in little millet (7.55%). Germination of pearl millet has increased protein content; it might be due to enzymatic synthesis (Sade, 2009). In contrast, no difference was found in foxtail millet. Similar observations were reported by Xu *et al*. (2012) in germinated brown rice. Germination has shown a non-significant decrease in ash content of millet flours. Other authors observed similar results in pearl millet after germination (Suma and Urooj, 2014; Sade, 2009). The reduction could be due to losses of total soluble solids during the soaking of grains prior to germination (Wang *et al*., 1997). Crude fiber content has increased in millet flours after processing; similar results were observed by Shreeja *et al*. (2021), Gunashree *et al*. (2014) and Owheruo *et al*. (2019) in buckwheat, ragi and wheat, respectively. During the germination process, starch in the grain is broken down, and sugar is generally used up, leaving only the fibrous grain and this might cause an increase in fiber content (Ikenebomah *et al*., 1986; Shreeja *et al*., 2021).

Germination has significantly reduced fat content of barnyard and foxtail millet, it reduced by 7.3 and 17.84%, respectively. At the same time, the germination has increased fat content of little millet flour by 25.95% (Figure 1). Reduced fat content was observed after germination in pearl millet, finger millet, sorghum (Singh *et al*., 2017), maize (Anaemene and Fadupin, 2020), wheat and ragi (Pandhre *et al*., 2011). Crude fat content increased after germination in brown rice (Xu *et al*., 2012), millet dakuwa and sorghum dakuwa (Nkama *et al*., 2015). Germination has shown non-significant decrease of carbohydrate content in barnyard and foxtail millet, while it significantly increased in foxtail millet (1.29%). An increase in carbohydrate content was reported in germinated maize (Anaemene

and Fadupin, 2020). Reduced carbohydrate content in germinated green gram was observed by Dattatray *et al.* (2019). Bello *et al.* (2017) reported non-significant increase in carbohydrate content at 24 and 48 h of germination and a significant decrease at 72 h of germination in pearl millet flour. Carbohydrate levels depend on moisture, ash, protein, fiber and fat content of the grains. Reduction in moisture, ash, crude protein and fat may be attributed to rise carbohydrates in foxtail millet on germination. Germination has decreased energy content of barnyard and foxtail millet by 1.2 and 1.44%, while it increased in little millet by 2.06%. Significant rise of fat content in little millet and decrease in barnyard and foxtail millet on germination might be the reason for changes in energy value, as it contains about twice the energy values of protein and carbohydrate (Osborne and Voogt, 1978). A significant decrease in energy value was observed at 24, 48 and 72 h of germination time in a brown and yellow variety of tiger nut flour; which was attributed to an increase in alpha-amylase activity, which breaks down complex carbohydrates into simpler and more absorbable sugars which are utilized by the growing seedlings during the early stage of germination (Chinma *et al*., 2009).

Germination of barnyard, foxtail and little millet has significantly reduced oxalate content in foxtail and little millet by 33.9 and 15.9% (Figure 2). Suma and Urooj (2014) reported significant reduction in oxalate content by 24 and 48% in pearl millet varieties after germination. Germination has reduced large portions of oxalate content in brown rice (Nissar *et al*., 2017), maize (Anaemene and Fadupin, 2020) and horsegram (Pal *et al*., 2016; Handa *et al*., 2017). Reduction in oxalate content might be due to the activation of oxalate oxidase during the germination process, which breaks down oxalic acid into carbon dioxide and hydrogen peroxide, consequently releasing calcium (Murugkar et al, 2013). Germination has increased tannin content of all three millet flours, per cent increase in tannin content was 22.45, 26.93 and 48.2 in GB, GF and GL, respectively. Tannin content of two germinated sorghum and pearl millet varieties were increased (Elkhier and Hamid, 2008; Pushparaj and Urooj, 2014). Tannin is concentrated in seed coat, which is not affected by the germination process, since the major food consumption was taken from the cotyledon. The remained cotyledon parts are less in weight compared with seed coat of germinated grains. Therefore, overall tannin content was raised (Elkhier and Hamid, 2008). Germination has shown significant changes in total phenolic content of millet flours, it increased in barnyard and foxtail millet flour by 58.47 and 23.75%, whereas it decreased in little millet flour by 7.27%. Germination has increased total phenols in brown rice (Nissar *et al*., 2017), corn (Sokrab *et al*., 2012), finger and pearl millet (Chauhan, 2017). It could be due to enzymatic release of bound phenols and an increase in the free forms due to the breakdown of the cell wall during germination (Nissar *et al*., 2017). Reduced total phenols in germinated pearl millet, finger millet, sorghum (Singh *et al*., 2017) and horse gram were observed (Pal *et al*., 2016; Handa *et al.*, 2017). In contrast, Bello *et al*. (2017) observed increased polyphenol content at 24 h of germination and decreased at 48 and 72 h of germination in pearl millet flour. Total flavonoid content has significantly increased in barnyard, foxtail and little millet on germination. Increased flavonoids were observed in germinated horsegram flour (Ojha *et al*., 2020). Phenols and flavonoids have potential applications in food, pharmaceutical and medical aspects, especially for antioxidant effects, antibacterial effect, anticancer effect, cardioprotective effects, immune system promoting and anti-inflammatory effects (Landete, 2012; Tungmunnithum *et al*., 2018).

DPPH free radical scavenging activity was studied at six different concentrations (1, 2, 4, 6, 8 and 10 mg/ml). Free radical scavenging activity varied with the treatment and was concentration dependent in millet flours. IC_{50} values of germinated millet flours were increased, where low IC_{50} value indicates high antioxidant activity because it requires lower amount of antioxidants to scavenge 50% of free radicals. Zhang *et al*. (2015) observed similar results in germinated buckwheat. Little millet has the lowest IC_{50} value followed by barnyard and millet among raw and germinated millets. Germination has significantly increased radical scavenging activity of millet flours. This might be due to the decomposition of high molecular weight polymers during germination leads to the generation of biofunctional substances. Radical scavenging activity was highest in little millet followed by barnyard and foxtail millet flours among raw and germinated millet flours. Increased DPPH inhibition was observed after germination in brown rice (Nissar *et al*., 2017) and horsegram flour (Ojha *et al*., 2020).

5. Conclusion

The present research work results reveal that germination has shown significant changes in barnyard, foxtail, and little millet; however, the nutrient content is grain dependent. Germination has increased protein, fiber and tannins in barnyard millet; crude fiber, carbohydrate and tannins in foxtail millet and fat, energy value and tannins in little millet. In addition, germination has increased phenolic, flavonoid contents and radical scavenging activity in all millet flours. This improved bioactive compounds and antioxidant activity suggests developing a nutrient rich product with germinated millet flours for therapeutic diets. However, future studies are needed to carry out human health outcomes.

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Conflict of interest

The authors declare that there are no conflicts of interest relevant to this article.

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