



## Phytochemical screening and antioxidant evaluation of millet varieties of Pakistan

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### ABSTRACT

The current research was planned to measure the comparative phytochemical and anti-oxidative potential of aqueous and methanolic extracts of two indigenously grown millet varieties of Pakistan. The locally available millet varieties, *i.e.* MB-87 and AF-POP flours were chemically characterized through the proximate and mineral analysis. The antioxidant extract was prepared in water and methanol. The extracts were screened for saponins, terpenoids, flavonoids and tannins; methanolic extract of MB-87 and AF-POP showed flavonoids in an average quantity (++)<sup>+</sup>, whilst aqueous extract had only trace (+) values. The phytochemical screening showed the presence of saponins only in the aqueous extract of both varieties. However, terpenoids and tannins were present in both methanolic and aqueous extracts. During antioxidant evaluation, millet methanolic extracts showed higher level of TPC and DPPH of MB-87 and AF-POP as  $0.30 \pm 0.001$  &  $0.23 \pm 0.0012$  mg GAE/g and  $48 \pm 0.96$  &  $46 \pm 1.09\%$ , respectively. However, the  $\beta$ -carotene activity of the aqueous extract of both varieties (MB-87 and AF-POP) was recorded higher. In a nutshell, the methanolic extract of MB-87 has an appreciable antioxidant profile. Further research should be planned to screen the important constituents of Pakistani millet varieties.

## Introduction

Millet is used as staple food in many regions of Pakistan. It is known for its rich polyphenol profile. The major millet varieties are pearl millet, foxtail millet, barnyard millet, little millet and kodo millet (Saleh et al., 2013). Numerous studies have reported millet as a cheap source of protein and energy. Millets are considered exclusive among the cereals due to high polyphenol, mineral, dietary fibre and carbohydrate contents. Several health benefits are also associated with the polyphenol contents of millet. Millet is comprised of both water and fat soluble vitamins like niacin, ascorbic acid, riboflavin, thiamine and tocopherol. The water soluble B complex vitamins are concentrated in an aleurone layer and germ of millet, and the fat soluble vitamins are present in the germ portion (Obilana and

Manyasa, 2002). It is a good source of phenolic acids with high antioxidant and anti-proliferative potential.

Previous research has documented the whole millet grain as an efficient source of phenolics (Jideani et al., 2014). Millet phenolics are present either in free or conjugated form. These phenolics can be classified into two types, *i.e.* hydroxycinnamic and hydrobenzoic acids. The hydroxybenzoic acids are derivatives of benzoic acid, *i.e.* vanillic, p-hydroxybenzoic, gallic and syringic acid, whereas hydroxycinnamic acids include caffeic, coumaric and ferulic acid with a structure of C6-C3 (Manach et al., 2004). Previous researches have shown that the administration of polyphenol rich food has potential to raise the blood antioxidant capacity (Pandey and Rizvi, 2009). The polyphenols are absorbed in the intestinal tract depending on their structure and the binding ability with the cell structure of a living system

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(Yang et al., 2001). The phenolic compound bioavailability is different in various cell structures of intestine. They are metabolized in tissues as absorbed from the gut with the help of intestinal enzymes or are metabolized by the microflora. The remaining unabsorbed fraction is excreted out via bile and eliminated from the body (Scalbert et al., 2002). Numerous analyses are in practice to explore the antioxidant potential as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, total polyphenol content (TPC) and  $\beta$ -carotene assay. In this manner, the current research was designed to screen the phytochemical, as well as the anti-oxidative potential of two millet varieties available at Pakistan for further recommendation as a dietary source of polyphenol.

## Materials and methods

Two locally available millet varieties, *i.e.* AF-POP and MB-87 were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan. The chemicals were purchased from Lab scan Sigma Aldrich. The millet varieties were ground into flour using China Chakki. Flours obtained from millet were used for analysis and product development.

### Chemical characterization of millet samples

The proximate composition including moisture, crude fat, crude fibre, crude protein, ash and nitrogen free extract (NFE) of millet varieties was estimated using the respective procedures of AACC. The moisture percentage of millet flour was calculated following the Method No. 44-15A discussed in AACC (2006). The total crude fat was determined using Soxhlet apparatus along with petroleum ether as a chemical following the Method No. 30-25 (AACC, 2006). The percentage of ash of flour samples was estimated by Method No. 08-01 of AACC (2006). The percentage of crude protein and crude fibre was calculated following the Method No. 46-10 and 32-10, respectively as mentioned in AACC (2006). The nitrogen free extract (NFE) was measured using the following equation:

$$\text{NFE\%} = 100 - (\text{moisture} + \text{crude protein} + \text{crude fibre} + \text{crude fat} + \text{ash}) \%$$

### Mineral assay

The mineral profile of millet flours was calculated considering the instruction of AOAC (2006). Magnesium (Mg) and Phosphorus (P) were estimated using the atomic absorption spectrophotometer.

### Antioxidant extract preparation

The millet flour was subjected to an antioxidant extraction using methanol and water. Purposely, the sample (15 g) was added in respective solvents, *i.e.* methanol and water (200 mL) and placed in a mechanical shaker for 24 h. The resultant extracts were evaporated through the rotary evaporator. Afterwards, the extracts were re-dissolved to a 4 mg/mL concentration in respective solvents for further analysis (Suma and Urooj, 2012).

### Phytochemical screening of the prepared extracts

The prepared samples were screened for phytochemicals of millet grain using the protocol of Mojab et al. (2003). In this context, tannins, terpenoids, saponins and phenolics were assessed by the colourimetric method and change in colour was noticed.

### Antioxidant potential

The estimation of the total phenolic compounds was done by the Folin-Ciocalteu method (FCM) as described by Sun et al. (2007). The spectrophotometer (IRMECO Germany) was used to estimate the absorbance of samples at the wavelength of 760 nm. The antioxidant activity, *i.e.* the radical scavenging ability via DPPH method of methanolic extract of millet flour samples was estimated following the procedures done by Afify et al. (2012). Moreover, the absorbance level of the extract was calculated at 515 nm. The antioxidant activity ( $\beta$ -carotene) of the flour samples was calculated following the techniques of Kim et al. (2006).

### Statistical analysis

The whole experimentation was carried out in triplicate and the resultant data were reported in term of means with respective standard deviation, *i.e.* means  $\pm$  S.D. The analysis of variance was determined to check the level of significance.

## Results and discussion

### Chemical characterization of millet varieties

Moisture content in MB-87 was reported  $9.4 \pm 0.07$  % followed by  $8.9 \pm 0.08$  % in the AF-POP during the study. The mean values of millet moisture content are presented in Table 1. The total ash content in millet was reported  $1.47 \pm 0.1$  % (MB-87) and  $1.83 \pm 0.25$  % (AF-POP). The percentage of crude protein of both examined millet varieties ranged from  $12 \pm 1.7$  to  $15 \pm 1.3$  %. The percentage of crude fibre ranged between

3.5±0.15 % (MB-87) to 3.73±0.2 % (AF-POP) in millet flour. The percentage of crude fat for AF-POP and MB-87 was reported to be 2.97±0.33 % and 2.26±0.16 %, respectively. The values of nitrogen free extract NFE in both varieties of millet ranged from 66.1±0.59 % (AF-POP) to 64.3±0.51 % (MB-87).

The values of phosphorus (P) in millet varieties were 346±2.4 mg/100 g in AF-POP and 357±1.5 mg/100 g in MB-87. The MB-87 has comparative higher amount of phosphorus. Whilst in case of magnesium, MB-87 has about 134±1.5 mg/100 g of magnesium (Mg), whereas about 133±1.57 mg/100 g recorded in AF-POP.

**Table 1.** Chemical composition

	<b>MB-87</b>	<b>AF-POP</b>
<b>Moisture(%)</b>	9.4±0.07	8.9±0.08
<b>Ash(%)</b>	1.47±0.1	1.83±0.25
<b>Protein(%)</b>	12±1.7	15±1.3
<b>Fiber(%)</b>	3.5±0.15	3.73±0.2
<b>Fat(%)</b>	2.26±0.16	2.97±0.33
<b>NFE(%)</b>	64.3±0.51	66.1±0.59
<b>P(mg/100 g)</b>	357±1.5	346±2.4
<b>Mg(mg/100 g)</b>	134±1.5	133±1.57

#### Phytochemical screening

The results pertaining to phytochemical screening of aqueous and methanolic extracts of millet varieties are presented in Table 2. The methanolic extract of MB-87 and AF-POP showed flavonoids in an average quantity (++) , whilst aqueous extract had only trace (+) values. The tannins were in traces (+) in the methanolic extract, but absent in the aqueous extract. In case of terpenoids, MB-87 showed average and AF-POP as traces in the methanolic extract. However, only traces were found in the MB-87 aqueous extract. Lastly, the saponins were absent in methanolic solution and were present as traces in the aqueous extract. The phytochemicals within MB-87 and AF-

POP showed wide variations in the aqueous and methanolic extract. Such difference was reported due to the polarity difference. Methanol is an organic solvent polar in nature when compared to other extracting media. Thus, most of the organic phytochemicals are detected in methanol (Adom and Liu, 2002; Suma and Urooj, 2012). Flavonoids are one of the phenolics found in the millet. Depending on the variety of millet and phenolics, the quantity of flavanoids, hydrobenzoic acids and hydroxycinnamic acids may change (Rao and Muralikrishna, 2004). Moreover, millet grains have tannins, which help the plant to protect from mold and their deterioration (Waniska, 2000). The tannins and phytic acid are the antinutritional factors that are present in the millet irrespective of the varieties. Tannins can be considered as inhibitors that affect the carbohydrates, protein and mineral digestion, but can also play a role in anti-carcinogenic, anti-ulcerogenic and cholesterol lowering entity (Siwela et al., 2007).

#### Antioxidant potential of MB-87 and AF-POP

The total phenolic contents (TPC) of the methanolic extract were observed to be higher, i.e. 0.30±0.001 mg GAE/g (MB-87) and 0.23±0.0012 mg GAE/g (AF-POP). The aqueous extract showed relatively lower values as 0.26±0.001 mg GAE/g (MB-87) and 0.19±0.01 mg GAE/g (AF-POP). Likewise, methanolic extract presented peak values 48±0.96 mg GAE/g and 46±1.09 mg GAE/g for MB-87 and AF-POP, whilst lesser for aqueous extract (Table 3). The DPPH values were maximum in case of the methanolic extract. Contrarily,  $\beta$ -carotene was reported higher in the aqueous extract (27±12.05 and 19.33±1.74 g/100 g; MB-87 and AF-POP) than the methanolic (24±15.07 and 16±0.98 g/100 g; MB-87 and AF-POP). Among both varieties, MB-87 had greater TPC, DPPH and  $\beta$ -carotene values.

**Table 2.** Phytochemical screening of millet flour samples

	<b>Saponins</b>		<b>Terpenoids</b>		<b>Tannins</b>		<b>Flavonoids</b>	
	MB-87	AF-POP	MB-87	AF-POP	MB-87	AF-POP	MB-87	AF-POP
<b>Methanolic extract</b>	-	-	++	+	+	+	++	++
<b>Aqueous extract</b>	+	+	+	-	-	-	+	+

+traces, ++average, -absent

**Table 3.** Means of TPC (mg GAE/g),  $\beta$ -carotene (g/100 g), DPPH (g/100 g) of millet varieties

	<b>TPC (mg GAE/g)</b>		<b>DPPH (g/100g)</b>		<b><math>\beta</math>-carotene (g/100g)</b>	
	MB-87	AF-POP	MB-87	AF-POP	MB-87	AF-POP
<b>Methanolic extract</b>	0.30±0.001	0.23±0.0012	48±0.96	46±1.09	24±15.07	16±0.98
<b>Aqueous extract</b>	0.26±0.001	0.19±0.01	36±0.83	29±0.79	27±12.05	19.33±1.74

Millet grains are a good source of phenolic compounds. The heat treatment can cause the reduction in the antioxidant activity and phenolic content of cereals and it depends on the time, type and severity of the heat exposure. Fermentation, malting and germination can increase the amount of phenolic content and thus antioxidant activity to some extent (Gorinstein et al., 2007). Free radicals initiate chain reaction in food items rich in unsaturated fats. Contrarily, the antioxidants also break down the free radical, donate the hydrogen, thus stabilize the products. The antioxidants from MB-87 and AF-POP were extracted using water and methanol as the extracting medium. The antioxidants react with DPPH that is a steady free radical and converts it to 1,1-diphenyl-2-picryl hydrazine. The free radical chelating activity was assessed through DPPH assay as mentioned in Table 3. The discolouration of the testing component showed the scavenging power of the antioxidant present in the methanolic extract (Singh et al., 2002). Numerous reports have presented higher antioxidant potential of methanolic extract as compared to aqueous, ethanolic and acetone solvent. Thus, the results indicated higher free radical scavenging potential of methanolic extract compared to the aqueous one.

## Conclusions

The methanolic extract of millet varieties MB-87 and AF-POP had higher residues of antioxidants as compared to the aqueous extracts. The inter comparison of these two varieties presented relatively higher contents of polyphenols and phytochemicals in MB-87 and proved it as a good source of antioxidants. Although millet is used in numerous domestic edible products and birds feed in Pakistan, it still has not attained researchers' attention. Future research should be planned to explore its beneficial chemical constituents.

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