Antimicrobial and antioxidant activity of minor millet extracts

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**Abstract**

*In vitro* antimicrobial assay and antioxidant property of the three genders of minor millets (*Echinochloa crus-galli*, *Panicum miliaceum* L., *Panicum sumatrense*) were evaluated. Antimicrobial assay of six extracts of three species were evaluated against bacterial strains of *Staphylococcus aureus* (MTCC 96), *Bacillus megaterium* (MTCC-428), *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC1688) and fungal pathogens *Aspergillus niger* (MTCC 282) and *Fusarium oxysporum* (MTCC-1755). It is concluded from the results that phytochemicals are responsible for such inhibition of multi resistance microorganisms and could be a source of new antibacterial drugs. IC\textsubscript{50} for various extracts were determined for DPPH scavenging, bleaching of β-carotene and percentage inhibition of \( \cdot \Theta \) and compared with standard positive controls viz. butylated hydroxytoluene (BHT) for DPPH, propyl gallate for β-carotene and ascorbic acid for \( \cdot \Theta \) assay.

**Citation:**


**Keywords:**

Antimicrobial assay, Antioxidant activity; Phenolic content; Minor millet; flavonoid

1. Introduction

*Echinochloa crus-galli*, *Panicum sumatrense*, and *Panicum miliaceum* L. are considered richest sources of phenolics and flavonoids, which have high biological activity (Bhagirath, 2013). Many synthetic antioxidants are available but these compounds must be used under strict regulation due to their potential hazards (Kim et al., 2012; Husrev et al., 2012). Therefore, new interest has been developed as purifying and characterizing safe antioxidants from natural sources. The predominant flavonoids and phenolic acids are almost exclusively present in glycosylated forms in *Echinochloa crus-galli*, *Panicum sumatrense*, and *Panicum miliaceum* (Reddy et al., 2012; Arunachalam et al., 2012). There have been many attempts made to determine the contents and physiological activity of phenolic compound in *Echinochloa crus-galli*, *Panicum sumatrense*, and *Panicum miliaceum* due to the apparent relationship of phenolics in minor millet with prevention of chronic diseases (Barik, 2003). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis, cancer and type-2 diabetes (Solomakos et al., 2003). Due to environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods as well as physical stress, free radicals cause depletion of the immune system, the change in gene expression and induce abnormal proteins. The oxidation process is one of the most important routes for producing free radicals in food, drugs, and even living systems. Antioxidants are important species, which possess the ability of protecting organisms from damage caused by free radical-
induced oxidative stress (Zheng et al., 2003). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Chen et al., 2006). A number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively added to foodstuffs, although their use has begun to be questioned because of their toxicity. Therefore, there is considerable interest in preventive medicine and in the food industry in the development of natural antioxidants obtained from botanical sources, especially herbal plants (Cheung et al., 2003). It is believed that antioxidant activity might be correlated with antimicrobial activity and therefore, it is interesting to see the potential of extracted active ingredients against bacterial strains of Staphylococcus aureus (MTCC 96), Bacillus megaterium (MTCC-428), Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC1688) and fungal pathogens used in the study Aspergillus niger (MTCC 282) and Fusarium oxysporum (MTCC-1755). The medicinal value of these plants lies in some chemical substances that produce a definite physiological effect on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, phenolic compounds and flavonoids (Desmarchelier et al., 2000). Among these, flavonoids are the ubiquitous group of plant secondary metabolites demonstrating a wide range of biochemical and pharmacological effects, including anti-diabetic, antioxidant, anti-inflammatory, antibacterial, antifungal (Madamombe et al., 2003; Kobus et al., 2009). In the present study, we focused on antioxidant and antimicrobial assays using six crude extracts from three genders of minor millets (Repetto et al., 2002; Pradeep et al., 2003; Huang et al., 2011).

2. Experimental

2.1 Plant material

Seeds of Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum were collected from local marker at Bhavnagar, Gujarat-India. The collected samples were preserved in dark and dry place at ambient temperature with passive ventilation prior to extraction.

2.2 Preparations of extracts

The aerial parts of Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum were cleaned with deionized water, oven dried at 40°C for 60 h and powdered in a grinder. The plant material (100 g) was extracted with three different solvents 1% acidified methanolic, 95% ethanolic, water (1500 ml) using Soxhlet apparatus for 48 h at temperature not exceeding the boiling point of the respective solvents. The obtained extracts were filtered using Whatmann filter paper No.1 and concentrated under vacuum at 40°C using a rotary vacuum evaporator (Buchi Laboratoriums, Switzerland) to dryness. The extractive values of the extracts were calculated. The extraction conditions were optimized based on different percentage recovery of extracts in the different experimental conditions. The solvent was evaporated under reduced pressure to give the maximum percentage yield from Eleusine coracana (L.). An aliquot of 1% acidified methanol was chromatographed by open column chromatography on silica gel, using chloroform: methanol mixtures of increasing polarity.

2.3 Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH), b-carotene, linoleic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ferrous chloride and Folin–Ciocalteu reagent were purchased from Hi-Media Lab. Pvt. Ltd., Mumbai, India. Sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate dehydrate and sodium acetate was purchased from E-Merck India Ltd. Other chemicals and solvents were procured from SD Fine- Chem Ltd., Mumbai, India and were of analytical grade, while Hydrogen peroxide (H₂O₂) was purchased from RFCL Limited (Rankem), Mumbai, India. Reference standard diclofenac sodium was obtained as gift sample from Unique Pharmaceuticals, Ankleshwer, India. For biological assay we used Nutrient Agar (Himedia, g/L) and nutrient broth (Himedia, g/L) while Fungus were assayed on Potato Dextrose Agar (Himedia, g/L).

2.4 Antibacterial assay

2.4.1 Microbial stain

The studied bacterial strains include Staphylococcus aureus (MTCC 96), Bacillus megaterium (MTCC-428), Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC1688), were procured from IMTECH, Chandigarh and screening were carried out at Junagadh Agricultural University, Junagadh (India).

2.4.2 Culture media

Bacteria were assayed on Nutrient Agar (Himedia, g/L) and nutrient broth (Himedia, g/L) while Fungus were assayed on Potato Dextrose Agar (Himedia, g/L) were used in this study.

2.4.3 Agar disc diffusion method

Echinochloa crus-galli, Panicum miliaceum L. and Panicum sumatrense extracts (50,100 & 200 µg/ml) were prepared in six different solvents (Water, Ethyle Acetate, Acetone, 95% Ethanol, Chloroform and 1% acidified methanol).
The dishes were left for 30 min at room temperature to allow the diffusion of extracts, which were incubated at 37°C for 12 h to 48 h for bacteria (Nostro et al., 2000).

### 2.5 Extraction process

The yields of the extracts obtained per 100 g of dry plant material with the different solvents are given in Table.

The highest yield of the extract was obtained from extraction with 1%-acidified methanol.

### 2.6 Determination of total phenols and flavonoids

Total phenolic content was determined according to Folin–ciocalteu method. The results are expressed as grams of gallic acid equivalents per 100 g of dry extract.

The concentration of phenolic compounds was calculated according to the following Eq. (1) obtained from the standard Gallic acid (5–50 µg) curve.

\[
\text{Absorbance} = 0.016 \times \text{GAE in } \mu\text{g} + 0.029 \quad (R^2 = 0.996)
\]  

(1)

Flavonoid content in the various extracts was determined by a colorimetric method. The results are expressed as grams of quercetin equivalents per 100 g of dry extract. The concentration of flavonoid compounds was calculated according to the following Eq. (2) obtained from the standard quercetin (20–100 µg) curve (Lila et al., 2013).

\[
\text{Absorbance} = 0.00001 \times \text{Quercetin in } \mu\text{g} + 0.011 \\
(R^2 = 0.994)
\]  

(2)

### 2.7 DPPH radical scavenging assay

In this assay, free radical scavenging activity of crude extract was determined by measuring the bleaching of purple-colored methanol solution of DPPH. The radical scavenging activity was determined as described elsewhere. One milliliter from a 0.5 mM methanol solution of the DPPH radical was mixed to 2.0 ml of different concentrations of 95% ethanol; methanol and water extract and were added 2.0 ml of 0.1 M sodium acetate buffer (pH 5.5). The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a UV Spectrophotometer. BHT was used as positive control, whereas methanol was used as negative one. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation-3.

\[
\% \text{RSA} = \frac{[A_0-A_1]}{A_0} \times 100
\]  

(3)

Where A₀ and A₁ are the absorbance of the control (containing all reagents, except the test compound) and test compound respectively (Veenashri et al., 2011).

### 2.8 Reducing sugar power

The reducing power of crude extract was determined using the method as described previously. Different concentrations of extracts (20 to 100 µg/ml) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide [K₃Fe(CN)₆] (1%). The mixture was incubated at 50°C for 20 min. Aliquots (2.5 ml) of 10% trichloroacetic acid were added to the mixture. The above mixture was then centrifuged at 1036 x g for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 2.5 ml of 1% ferric chloride solution. The absorbance was measured at 700 nm in a UV spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power and compared with those of standard antioxidant BHA and BHT (Geronikaki et al., 2006).

### 2.9 H₂O₂ assay

The ability of all seeds extracts to scavenge hydrogen peroxide was determined according to the. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts samples (10 to 50 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both extracts and standard compounds are calculated by using following equation 5 (Lin et al., 2002).

\[
\% \text{Scavenged H}_2\text{O}_2 = \left(\frac{[A_0-A_1]}{A_0}\right) \times 100
\]  

(5)

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### Table 1: The yields of the extracts obtained from three different minor millets in three different solvents

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Echinochloa crus-galli</th>
<th>Panicum miliaceum</th>
<th>Panicum sumatrense</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Methanol</td>
<td>3.87 gm ± 1.25</td>
<td>3.049 gm ± 0.85</td>
<td>3.02 gm ± 1.13</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>2.45 gm ± 1.20</td>
<td>1.004 gm ± 1.52</td>
<td>1.49 gm ± 1.03</td>
</tr>
<tr>
<td>Water</td>
<td>2.39 gm ± 1.10</td>
<td>1.83 gm ± 1.05</td>
<td>2.315 gm ± 0.86</td>
</tr>
</tbody>
</table>
Where $A_0$ was the absorbance of the control, and $A_t$ was the absorbance in the Presence of the extract and standards.

### 2.10 β-carotene bleaching

Antioxidant activity was determined using β-carotene bleaching test. 1 ml of β-carotene solution (0.2 mg/ml in chloroform) was added to 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. (Shon et al., 2003) The mixture was evaporated at 40°C for 10 min using rotary evaporator to remove chloroform. The resultant mixture was immediately diluted with 100 ml of distilled water to form emulsion. 5 ml of the emulsion was transferred into different test tubes containing 0.2 ml of samples in 70% ethanol at different concentrations (500, 250, 100, and 50 μg/ml). 0.2 ml of 70% ethanol in 5ml of the above emulsion was used as control. Standard (propyl gallate) at the same concentration as samples was used for comparison. The tubes were gently shaken and placed at 45°C in a water bath for 60 min. The absorbance of the samples, standard and control was measured at 470 nm using a UV Spectrophotometer against a blank, standard and control was measured at 470 nm using a UV Spectrophotometer against a blank, the absorbance of the negative control at 60 min. The absorbance of the control, and the absorbance of the negative control at 60 min. The extract concentration providing 50% antioxidant activity (IC50) was calculated from the graph of antioxidant activity percentage against extract concentration.

### 3. Results and Discussion

#### 3.1 Antibacterial assay of the extracts

Antibacterial assay of extracts of three minor millets were carried out and zone of inhibition for each extract was measured at three concentrations (Ncube at al., 2000).

Table-2 represents the data for zone of inhibition at 200 μg/ml concentration of each extract.

Antimicrobial activity was manifested by the 1% acidified methanol extract of seed of Echinochloa crus-galli E. coli with maximum zone of inhibition of 16 mm, while seed extract of Panicum miliaceum L in acetone showed good activity against Staphylococcus aureus with 16.4 mm zone of inhibition. Acetone extract of Panicum sumatrense showed good activity against S. aureus with 17.7 mm zone of inhibition (Mahesh et al., 2008). Moreover, it was observed that acetone extract of Panicum sumatrense showed good activity against both gram-positive and gram-negative bacterial strains. The results of antifungal activity for both fungal pathogens were evaluated and as shown in the table 2, zone of inhibition for F. oxysporum species found predominant with maximum zone of inhibition was achieved for acetone extract of Panicum sumatrense. If we compare the results, the extracts of Panicum sumatrense were showed the highest activities for bacterial strains and fungal pathogens.

This result suggests that the acetone extract of Panicum sumatrense may be exerting a metabolic interference for gram-negative bacteria and may possibly inhibit gram-negative bacterial growth. This finding also suggests that the potential bioactive compound(s) in Panicum sumatrense extract have distinct influence on gram-negative bacteria cell growth and function by interfering with any of the steps involved in the growth of gram-negative bacteria. Similarly, it can be attributed for F. oxysporum species.

The results obtained in this study indicate differences in antimicrobial assay between extracts depending on the species of minor millets as a function of the type of extracting solvents. Other authors (Cho et al., 1999) have observed similar differences.

These results were in agreement with the suggestion of Oloke et al, (2008) those phytochemicals of any medical plant have different solubility in different extracting solvents.

The reason for weak activity of aqueous extracts was that phytochemicals present in the species of minor millets were insoluble or poorly soluble in water. The results also indicate that the efficacious interaction effect may be dependent on the precise concentrations of certain phytochemicals in an extract. In spite of the fact that most independent plant-derived extracts have shown weak potency against pathogenic bacteria compared to antibiotics, plants usually, fight infections successfully in their natural environment.

Antimicrobial activity was manifested by the 1% acidified methanol extract of seed of Echinochloa crus-galli E. coli with maximum zone of inhibition of 16 mm, while seed extract of Panicum miliaceum L in acetone showed good activity against S. aureus with 16.4 mm zone of inhibition.
Table 2: Average zone of inhibition (mm) from six extracts of three minor millets species at 200 µg/ml concentration of each extract

<table>
<thead>
<tr>
<th>Solvents</th>
<th>gram positive</th>
<th>gram negative</th>
<th>fungal pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echinochloa crus-galli</strong></td>
<td><strong>S. aureus</strong></td>
<td><strong>E. Coli</strong></td>
<td><strong>P. Aeruginosa</strong></td>
</tr>
<tr>
<td>Water</td>
<td>8.33±0.9</td>
<td>11±2.1602</td>
<td>8.66±2.8674</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>11±6.815</td>
<td>13.66±3.258</td>
<td>11±3.5900</td>
</tr>
<tr>
<td>Acetone</td>
<td>4.33±1.24</td>
<td>7.33±2.422</td>
<td>9.33±2.6246</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>6.00±1.885</td>
<td>11.33±2.799</td>
<td>10.33±2.624</td>
</tr>
<tr>
<td>1% Methanol</td>
<td>11.66±7.5</td>
<td>10.66±7.85</td>
<td>10±2.690</td>
</tr>
<tr>
<td><strong>Panicum miliaceum</strong></td>
<td><strong>S. aureus</strong></td>
<td><strong>E. Coli</strong></td>
<td><strong>P. Aeruginosa</strong></td>
</tr>
<tr>
<td>Water</td>
<td>10±1.922</td>
<td>13.0±2.150</td>
<td>9.0±2.414</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>9.33±2.05</td>
<td>10.66±7.69</td>
<td>10±2.160</td>
</tr>
<tr>
<td>Acetone</td>
<td>12.0±1.41</td>
<td>16.33±4.13</td>
<td>12.66±5.311</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>9.00±2.1602</td>
<td>11.0±5.665</td>
<td>8.33±4.0428</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8.66±7.45</td>
<td>9.66±5.099</td>
<td>7.33±2.427</td>
</tr>
<tr>
<td>1% Methanol</td>
<td>12.33±3.3</td>
<td>16.0±2.439</td>
<td>13.66±5.299</td>
</tr>
<tr>
<td><strong>Panicum sumatrense</strong></td>
<td><strong>S. aureus</strong></td>
<td><strong>E. Coli</strong></td>
<td><strong>P. Aeruginosa</strong></td>
</tr>
<tr>
<td>Water</td>
<td>8.33±0.9</td>
<td>12.0±2.859</td>
<td>8.66±2.6246</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>10.0±1.41</td>
<td>14.0±2.160</td>
<td>9.66±3.298</td>
</tr>
<tr>
<td>Acetone</td>
<td>12.34±0.94</td>
<td>17.66±7.247</td>
<td>16.66±7.140</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>11.34±3.29</td>
<td>11.33±2.624</td>
<td>10.67±6.246</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8.66±7.45</td>
<td>9.66±5.099</td>
<td>7.33±2.427</td>
</tr>
<tr>
<td>1% Methanol</td>
<td>13.0±2.160</td>
<td>12.33±4.085</td>
<td>13.66±3.681</td>
</tr>
</tbody>
</table>

Table 3: Total phenolic and total flavonoid contents in seeds of *Minor millets*

<table>
<thead>
<tr>
<th>Millets</th>
<th>Total phenolic contents</th>
<th>Total flavonoids contents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 % Methanol (30 µg/ml)</strong></td>
<td><strong>Echinochloa crus-galli</strong></td>
<td>245.33±2.49</td>
</tr>
<tr>
<td></td>
<td><strong>Panicum miliaceum</strong></td>
<td>155.81±8.55</td>
</tr>
<tr>
<td></td>
<td><strong>Panicum sumatrense</strong></td>
<td>157.61±4.13</td>
</tr>
<tr>
<td><strong>95% Ethanol (30 µg/ml)</strong></td>
<td><strong>Echinochloa crus-galli</strong></td>
<td>845.33±2.49</td>
</tr>
<tr>
<td></td>
<td><strong>Panicum miliaceum</strong></td>
<td>164.50±3.52</td>
</tr>
<tr>
<td></td>
<td><strong>Panicum sumatrense</strong></td>
<td>171.70±4.13</td>
</tr>
<tr>
<td><strong>Water (30 µg/ml)</strong></td>
<td><strong>Echinochloa crus-galli</strong></td>
<td>345.33±7.45</td>
</tr>
<tr>
<td></td>
<td><strong>Panicum miliaceum</strong></td>
<td>145.49±6.28</td>
</tr>
<tr>
<td></td>
<td><strong>Panicum sumatrense</strong></td>
<td>108.65±7.16</td>
</tr>
</tbody>
</table>

Table 4: IC₅₀ for DPPH scavenging, β-Carotene scavenging and H₂O₂ scavenging of different extracts of Minor millets

<table>
<thead>
<tr>
<th>Species</th>
<th>Solvent</th>
<th>DPPH scavenging activity (IC₅₀ in µg/ml)</th>
<th>β-carotene bleaching effect (IC₅₀ in µg/ml)</th>
<th>H₂O₂ (IC₅₀ in µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinochloa crus-galli</td>
<td>686.0497±8.0304</td>
<td>140.165±2.498</td>
<td>26.947±0.55</td>
<td></td>
</tr>
<tr>
<td>Panicum miliaceum</td>
<td>1% Methanol</td>
<td>165.867±12.59</td>
<td>237.718±6.838</td>
<td>24.987±0.55</td>
</tr>
<tr>
<td>Panicum sumatrense</td>
<td>339.0027±12.51</td>
<td>163.65±21.80</td>
<td>22.130±0.24</td>
<td></td>
</tr>
<tr>
<td>Echinochloa crus-galli</td>
<td>226.467±13.068</td>
<td>143.65±1.960</td>
<td>21.587±0.52</td>
<td></td>
</tr>
<tr>
<td>Panicum sumatrense</td>
<td>Water</td>
<td>206.876±9.04656</td>
<td>227.735±3.013</td>
<td>19.934±1.54</td>
</tr>
<tr>
<td>Echinochloa crus-galli</td>
<td>199.5967±8.2838</td>
<td>130.67±4.8107</td>
<td>39.62±1.542</td>
<td></td>
</tr>
<tr>
<td>Panicum miliaceum</td>
<td>Water</td>
<td>300.8487±8.5480</td>
<td>254.625±4.056</td>
<td>21.46±0.98</td>
</tr>
<tr>
<td>Panicum sumatrense</td>
<td>Water</td>
<td>237.7147±9.0132</td>
<td>228.822±5.421</td>
<td>16.79±6.18</td>
</tr>
</tbody>
</table>
Acetone extract of Panicum sumatrense showed good activity against S. aureus with 17.7 mm zone of inhibition (Mahesh et al., 2008). Moreover, it was observed that acetone extract of Panicum sumatrense showed good activity against both gram-positive and gram-negative bacterial strains. The results of antifungal activity for both fungal pathogens were evaluated and as shown in the table 2, zone of inhibition for F. oxysporum species found predominant with maximum zone of inhibition was achieved for acetone extract of Panicum sumatrense. If we compare the results, the extracts of Panicum sumatrense were showed the highest activities for bacterial strains and fungal pathogens.

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The results obtained in this study indicate differences in antimicrobial assay between extracts depending on the species of minor millets as a function of the type of extracting solvents. Other authors (Cho et al., 1999) have observed similar differences. These results were in agreement with the suggestion of (Oloke et al., 2008) those phytochemicals of any medical plant have different solubility in different extracting solvents. The reason for weak activity of aqueous extracts was that phytochemicals present in the species of minor millets were insoluble or poorly soluble in water. The results also indicate that the efficacious interaction effect may be dependent on the precise concentrations of certain phytochemicals in an extract. In spite of the fact that most independent plant-derived extracts have shown weak potency against pathogenic bacteria compared to antibiotics, plants usually, fight infections successfully in their natural environment.

3.2 Total phenolic content

Phenolics are aromatic secondary plant metabolites and called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet, superoxide free radicals and hydroxyl radicals. Natural polyphenols have chain-breaking antioxidant activities and are believed to present many degenerative diseases, including cancer and scene diseases.

The crude extracts obtained from aerial parts of Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum were characterized by their contents of total phenolics expressed as gallic acid equivalent (GAE) for Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum seeds were use in three different solvents viz. 95% ethanol, 1% acidified methanol and water. Lowest total phenolics were obtained from the water extract of Panicum sumatrense 45.49 GAE/g and highest total phenolics were obtained from the 95% ethanolic extract of Panicum miliaceum 845.

33 GAE/g. The calculations of total phenolics were carried out by using standard GA curve with least square regression. Similarly, total flavonoid content equivalent to quercetin (QU. E) for crude of Echinochloa crus-galli, Panicum sumatrense, and Panicum miliaceum was carried out. The obtained yield was calculated from standard curve of QU with least square regression. The phenolic contents in different extracts varied significantly in both plants and as well as solvents used for extraction. The order of phenolic content in three solvents was 1% methanol > 95% ethanol > water for aerial parts of three gender of millets. Variations in phenolic contents of various extracts were attributed to polarities of different compounds present in the various parts of millet used for present study and such differences have been reported elsewhere. Similarly, the order of flavonoid content in three solvents was 95% ethanol> 1% methanol > water for this three spices of millet. The results of total phenolics and total flavonoids are summarized in Table 3.

3.3 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH is a stable free radical in aqueous or methanol and ethanol solution and accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is usually used as a substrate to evaluate the antioxidative activity of antioxidants. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm. The scavenging effect of extracts in the range of 50–500 µg/ml on the DPPH radical increased with an increasing concentration of each extract is depicted in figure 1.

The scavenging of by hydrogen donation caused the decrease in absorbance of DPPH radical caused by antioxidant. DPPH assay shows that extracts of in water had highest impact with 39.47% scavenging at low concentration of seed extract. IC₅₀ of extracts of Echinochloa crus-galli, Panicum miliaceum and Panicum sumatrense are summarized in table 3. The degree of discoloration indicates the scavenging capacity of the extract. The effect of antioxidants on the DPPH
Figure 1: Percentage scavenging of DPPH in 1% methanol, 95% ethanol and water extracts of Minor millets.

radical scavenging was thought to result from their hydrogen donating ability. IC_{50} value for DPPH scavenging by water extract of was lowest, which was found to be much higher than the standard butylated hydroxy toluene (BHT). Similarly, many authors reported that some plant extracts showed lower activity against the DPPH radical compared with butylated hydroxy toluene (BHT). In addition, there was a statistically significant correlation between the amount of phenolic compounds and DPPH scavenging activity in all the extract.

3.4 Reducing power
The results showed that the reducing power of all the three extracts was concentration dependent. Five different concentrations ranging from 20 µg/ml to 100 µg/ml were used to see the effectiveness of concentrations of all three extracts in three solvents as shown in figure 2. The results were compared against the standard BHT and BHA solutions. Water extract of millet showed significantly higher reducing power than other extracts in three different solvents. In addition, it was reported that the antioxidant activity of reductions is believed to break radical chains by donation of a hydrogen atom, indicating that the antioxidative properties are concomitant with the development of the reducing power. Various authors have reported that the reducing power of bioactive compounds (mainly low and high molecular phenolics), extracted from peanut hulls and stem bark of Indian laburnum, was associated with antioxidant activity, specifically scavenging of free radicals. Reductions are also reported to react with certain precursors of peroxide thus, preventing peroxide formation. The results obtained by us do support these claims and there were positive correlation between total antioxidant activity and reducing power of all five extracts in three solvents. Different studies have indicated that the reducing power of bioactive compounds is associated with antioxidant activity. Therefore, the antioxidant activity of the tested samples might partially be a result of their reducing power.

The reducing capacity of tannins prevented liver injury by inhibiting the formation of lipid peroxides. Furthermore, the reducing compounds can react directly with peroxides and with certain precursors and thereby prevent peroxide formation.
The reducing capacity of various examined extracts might be due to their hydrogen donating ability. Therefore, the examined samples might contain reducing compounds, which can react with free radicals to stabilize and terminate radical chain reactions.

3.5 H₂O₂ scavenging activity
The results of H₂O₂ scavenging activity indicated that all the extracts showed excellent H₂O₂ scavenging activities at a concentration range from 10µg /ml to 50µg /ml in the reaction mixture and increased steadily with the increased concentration. The percentage inhibition of standard ascorbic acid was varied between 30% to 50% at five different doses used for present study, while all five extracts in three different solvents had similar effect with percentage scavenging ranging from 5% to 49% at five different doses used for present study. IC₅₀ for standard ascorbic acid was 30.9573 ± 2.306 µg /ml. IC₅₀ for 95% ethanolic extract of Panicum miliaceum was very close to standard, which indicates that water extract of Echinochloa crus-galli had very high impact H₂O₂ scavenging. Similarly, water extract of little has IC₅₀ value 52.07 ± 0.41 µg /ml with linear regression; while 95% ethanolic extract of proso has similar kind of effect on H₂O₂ scavenging effect as IC₅₀ was found 53.75 ± 0.84 µg /ml with linear regression.

3.6 β-carotene bleaching
The bleaching effect was measured by the peroxidation of β-Carotene and the effect of peroxidation of β-Carotene is shown in figure. Antioxidants can reduce the extent of β-Carotene destruction by neutralizing the linoleate-free radical and other free radicals formed in the system. Accordingly, the absorbance deceased rapidly in reaction mixtures without extracts, whereas in the presence of extracts the reaction mixtures retained their colour and thus absorbance for a longer time. The IC₅₀ of different extracts in inhibiting the bleaching of β-Carotene suggest that bleaching can be moderately inhibited by all three extracts in three different solvents. This can be attributed to the fact that the presence of different antioxidant molecules in extracts might be responsible for inhibition of β-Carotene destruction by neutralizing the effect of linoleate-free radical and other free radicals formed in the system. The representative data are summarized in Table 3.

Conclusion
Our results are encouraging as the sources for NSAID drug are natural and hence, it may not have side effects like synthetic molecules. The traditional methods of extraction are promising since the yield extracts are devoid of acute toxicity on rats. In order to go toward a valuation of these traditional extracts in the form of phyto-drugs, follow-up studies must be carried out in particular on their stability and the evolution of the hematological and biochemical parameters.

Research Highlights
1. Plants are natural source for phytochemicals, which can be useful as therapeutic agents.
2. Extracts of minor millets are known for their antioxidant activities but are useful as safe antimicrobial agents.
3. Phytochemicals from Panicum sumatrense were showed good antimicrobial activities as compared to two other genders of minor millets

Limitations
Due to shortage of some standard phenolics and flavonoids, we are unable to identify all extracted phytochemicals from three genders of minor millets. This lack of standard chemicals is due to lack of funding at or place and hence it was not possible to procure all necessary chemicals for qualitative and quantitative analysis of each active ingredient.

Isolation of single component from crude extract is also challenging task and this part is partially achieved with the help of conventional column chromatographic technique but complete isolation was not possible, as we do not have flash chromatographic technique in our department.

Recommendations
I personally recommend that one should have collaborative research with well-established institute to carry out in-depth study of natural products. This will enable teachers like us those have little facilities or lack of funding to work for complete isolation of single component from crude extract of plants.

Funding and Policy Aspects
We have various funding agencies, which are generous for providing funding for such work. DST, New Delhi, UGC, New Delhi, DBT, New Delhi are few Govt. of India’s leading funding agencies in our country.

Unfortunately, for this particular work I was unable to receive fund from any of the agency.
Author’s Contribution and Competing Interests

We are working in this field since last five years and published seven papers in various national and international journals, which shows that our work is widely accepted in the scientific community worked in the similar field. Moreover, as a reviewer, I have reviewed many papers of same kind in well-reputed journals. This is again showed the contribution of our team in the field of natural product and medicinal plant chemistry.

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