CHAPTER 5

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF FINGER MILLET POLYPHENOLS

5.1 MATERIALS AND METHODS

5.1.1 Antimicrobial Activity

The pathogenic/spoilage strains used as indicators for the inhibition study were *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*, *Aspergillus niger* and *Aspergillus fumigatus*. These strains, which were laboratory isolates obtained from Sri Ramachandra Medical Centre and Hospital, Chennai are food-borne pathogens that may cause gastrointestinal disease.

The indicator bacteria and yeast strains were routinely propagated in nutrient broth (NB) (30°C for 24 h) and potato dextrose broth (PDB) (30°C for 36 h) respectively. The LAB strains were grown in de Man Rogosa Sharpe (MRS) broth and incubated at 30°C for 24 h. The strains were maintained in a mixture of respective media (800 µL) and glycerol (200 µL) at -80°C until further use.

5.1.2 Antioxidant Activity

The methanolic extract of samples obtained for measuring total polyphenols were taken for the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) antioxidant assay which measures their ability to reduce the DPPH free radical and subsequent decrease in the absorbance is expressed as
% inhibition using trolox as reference standard (Blois 1958). This method is rapid, simple, inexpensive and widely used in assay of polyhydroxy aromatic compounds (Nishizawa et al. 2005).

5.2 RESULTS AND DISCUSSION

5.2.1 Antimicrobial Activity of Fermented Millet

The polyphenol extract of finger millet at 18 h of fermentation was taken for antimicrobial activity due its higher content of polyphenols. The antibacterial activity was highest against *Micrococcus luteus* (25 mm) as evident from the zone of inhibition (Table 5.1 and Figure 5.1). Further, the extract showed high antifungal activity against *Aspergillus niger* (14 mm).

**Table 5.1 Antimicrobial activity of 18 h natural fermented CO13 extract**

<table>
<thead>
<tr>
<th>Clinical pathogens</th>
<th>Phenolic extract (µg g⁻¹ GAE)</th>
<th>Norfloxacin (10 µg)</th>
<th>Tetracycline (30 µg)</th>
<th>Vancomycin (30 µg)</th>
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<tbody>
<tr>
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<tr>
<td>Bacteria</td>
<td></td>
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<tr>
<td><em>Bacillus subtilis</em></td>
<td>18±0.03</td>
<td>21±0.11</td>
<td>17±0.16</td>
<td>9±0.02</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>15±0.22</td>
<td>20±0.05</td>
<td>19±0.14</td>
<td>7±0.01</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>25±0.05</td>
<td>19±0.18</td>
<td>18±0.06</td>
<td>11±0.07</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12±0.08</td>
<td>23±0.22</td>
<td>15±0.03</td>
<td>-</td>
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<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Aspergillus niger</em></td>
<td>14±0.13</td>
<td>7±0.05</td>
<td>15±0.16</td>
<td>-</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>11±0.03</td>
<td>11±0.01</td>
<td>13±0.08</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 5.1  Zone of inhibition of 18 h natural fermented CO13 extract against Micrococcus luteus

The polyphenol extract of GPU-28 showed no activity against the tested fungal strain *Aspergillus flavus*, while a zone of inhibition (13 mm) was observed against *Bacillus cereus* (Viswanath et al. 2009).

Simango & Rukure (1992) showed bactericidal and/or bacteriostatic properties of *mahewu* against *Aeromonas*, *Salmonella*, *Shigella*, *Campylobacter jejuni* and *Escherichia coli*. This suggests that traditional fermented foods are an unlikely vehicle for the transmission of enteric pathogens.
CO9 variety had low levels of polyphenols which was reduced further during fermentation and therefore the antimicrobial assay was not carried out in this case.

5.2.2 Antioxidant Activity of Fermented Millet

The antioxidant activity showed similar trend as the total phenolics content. It decreased with increase in fermentation time. At 18 h of natural fermentation, the polyphenols inhibited 72.42% of free radicals in the brown variety. The white variety showed less antioxidant activity (53.42%) which may be due to the low content of polyphenols. Germination decreased the antioxidant activity (38.93%) and starter-culture fermentation lowered the activity further (24.16%) (Figures 5.2 and 5.3).

Although several researchers have studied the antioxidant properties of polyphenols, information on the antioxidant activity of finger millet polyphenols during fermentation have not been studied extensively. The antioxidant activity of native and malted *ragi* seed coat polyphenols (Subba Rao & Muralikrishna 2002) and that of germinated and fermented finger millet polyphenols by ESR method (Sripriya et al. 1996) have been reported so far.

The results of these studies on the antioxidant properties of finger millet polyphenols in the whole and processed flour were varied based on the assay method and variety. Sripriya et al. (1996) reported antioxidant activity of finger millet (brown variety) to be 94% by DDPH radical quenching assay and 77% by hydroxyl quenching action. Antioxidant activity of about 27% was reported by Viswanath et al. (2009) in the polyphenol extracts of GPU-28 using β-carotene – linoleic acid assay. The higher DDPH scavenging property observed for the polyphenols extract may contribute to the nutraceutical properties of the millet polyphenols. The antioxidant activity of any
compound is normally dose-dependent. Since, appreciable amount of polyphenols are found in finger millet, especially in the brown variety, they can be considered as a rich source of antioxidants among cereals. Miyake & Shibamoto (1997) suggested that the level of antioxidant activity of phenolics depends on the position and extent of hydroxylation of the phenolic rings.

Figure 5.2 Antioxidant activities of fermented CO13 and CO9
Figure 5.3  Antioxidant activities of germinated and fermented CO13 and CO9
Health benefits are mainly attributed to the content of antioxidant compounds such as polyphenols (Crozier et al. 2009). Although ellagic acid is believed to function as both \textit{in vitro} and \textit{in vivo} antioxidant, its efficiency depends on chemical structure, mostly the number of hydroxyl groups (Pfundstein et al. 2010). Also ellagic acid has the highest antioxidant activity as measured by its ability to neutralize the free radical DPPH and it is attributed to the fact that ellagic acid has two dihydroxyl groups (Zafrilla et al. 2001).

Blood glucose level of alloxan-induced diabetic rats was controlled by whole grain flour diet rich in phenolic antioxidants, suggesting that millets can provide valuable health protective properties against diet-related chronic disease (Hegde et al. 2005). The levels of enzymatic (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and non-enzymatic antioxidants (glutathione, vitamins E and C) and lipid peroxides were significantly reduced in diabetic rats and restored to normal levels in the millet-fed groups. Antioxidants inhibit glycation by scavenging reactive oxygen species and superoxide dismutase (SOD) and metal chelators protect against alloxan-induced diabetes in animals (Chattopadhyay et al. 1997).

Although, the antioxidant activity decreased with increase in fermentation time, 72% inhibition of free radicals was still observed at 18 h of NF. Thus, natural antioxidants equal to or greater than those of existing synthetic antioxidants can be developed from the finger millet extracts. Further, polyphenols with antimicrobial and antioxidant potential can be utilized as food preservatives.