Chapter 4
Results
Chapter 4

The study was undertaken in accordance with the objective, and experimental design and results of the study are described as follows.

### 4.1 Proximate composition

The results of the proximate analysis of selected gourd vegetables are presented in table 4.1. The moisture content of all the selected gourd vegetables ranged from 8.9 to 14.7 g/100 g (dwb) and being maximum in *C. pepo* and minimum in *T. dioica*. The results showed that total ash and acid insoluble ash content varied from 5.5 to 8.9 g /100 g (dwb) and 1.4 to 2.6 g /100 g (dwb) respectively with less amount estimated in the *L. cylindrica*. The crude protein content varied from 9.1 to 22.8 g /100 g (dwb) in the selected vegetables and a significantly (p<0.05) higher concentration was estimated in *C. pepo* in comparison to other vegetables. The crude fat analysis showed that vegetables were deficient in fat content and the fat content in *L. acutangula* and *C. maxima* (2.9 g /100 g dwb) was higher than the rest of the studied vegetables. The crude fiber content in *L. siceraria* was reported as 24.0 g /100 g (dwb) and its concentration was significantly higher (p<0.05) than the rest of the selected vegetables. The carbohydrate content as measured by difference method ranged from 35.9 to 63.6 g /100g (dwb) and it was found maximum in *T. dioica*, whereas its minimum concentration was reported in *L. siceraria*.

### 4.2 Yield of extracts

The yield of different extracts prepared from raw as well as thermally processed gourd vegetables is depicted in Fig. 4.1. Three extracting solvents namely methanol, ethanol and butanol were evaluated for their effectiveness to extract phytochemicals from raw and cooked gourd vegetables. The inconsistent effect on extraction concentration was observed after the various cooking treatments. The extraction yield from raw and cooked vegetables significantly (p<0.05) varied from 2.62 to 6.00 g /100g and 1.06 to 8.66 g/100g (fwb) respectively. The extraction yield was also considerably affected by the solvent. However, this effect of extraction solvent on the extraction yield varied among different crops. Regardless of cooking treatments, methanol was most effective in recovering the phytochemicals in case of *T. dioica, C. pepo* and *C. pepo*; ethanol was most effective solvent in case of *L. siceraria, L. acutangula* and *L. cylindrica*, whereas butanol was effective in case of *M. charantia* and *L. cylindrica* in extracting out the phytochemicals.
Table 4.1 Proximate composition of gourd vegetables (g/100g of dry weight of vegetables)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Moisture ±SD</th>
<th>Ash ±SD</th>
<th>AIA ±SD</th>
<th>Protein ±SD</th>
<th>Fat ±SD</th>
<th>Crude Fiber ±SD</th>
<th>Carbohydrate ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. charantia</em></td>
<td>12.8±0.07c</td>
<td>5.7±0.10bc</td>
<td>1.8±0.20ab</td>
<td>16.3±0.58c</td>
<td>2.6±0.06b</td>
<td>11.0±1.00c</td>
<td>49.7±0.56c</td>
</tr>
<tr>
<td><em>L. siceraria</em></td>
<td>9.6±0.52ab</td>
<td>8.7±0.22cd</td>
<td>2.6±0.13c</td>
<td>17.4±1.04cd</td>
<td>1.7±0.12a</td>
<td>24.0±1.00e</td>
<td>35.9±2.50a</td>
</tr>
<tr>
<td><em>L. cylindrica</em></td>
<td>14.2±0.91c</td>
<td>5.5±0.09a</td>
<td>1.4±0.19a</td>
<td>18.2±0.91d</td>
<td>2.7±0.07bc</td>
<td>8.9±0.12b</td>
<td>49.1±1.15c</td>
</tr>
<tr>
<td><em>L. acutangula</em></td>
<td>10.3±1.02ab</td>
<td>7.9±0.06c</td>
<td>2.2±0.25bc</td>
<td>14.4±0.56b</td>
<td>2.9±0.10c</td>
<td>13.8±0.28d</td>
<td>48.3±0.86c</td>
</tr>
<tr>
<td><em>T. dioica</em></td>
<td>8.9±0.71a</td>
<td>5.7±0.17bc</td>
<td>2.2±0.12bc</td>
<td>9.1±0.03a</td>
<td>2.6±0.03b</td>
<td>7.9±0.06c</td>
<td>63.6±0.87d</td>
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<tr>
<td><em>C. maxima</em></td>
<td>12.9±0.84c</td>
<td>8.9±0.52d</td>
<td>1.5±0.10a</td>
<td>20.1±0.04e</td>
<td>2.9±0.10c</td>
<td>5.2±0.25d</td>
<td>48.4±0.76c</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>14.7±0.54c</td>
<td>6.2±0.25b</td>
<td>2.2±0.25b</td>
<td>22.8±0.63f</td>
<td>1.7±0.10a</td>
<td>9.20±0.70b</td>
<td>43.0±0.79b</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD (n=3) and referred to the dry weight.

Means in the same column with different letters are significantly different (p < 0.05)

*A1A- Acid insoluble ash

Irrespective of extraction solvent, *L. siceraria* and *L. cylindrica* showed increased yield after different cooking treatments. Pressure cooking exerted an increasing effect on the extraction yield from all the vegetables whereas, microwave cooking was effective in increasing the extraction yield from *L. siceraria, L. cylindrica, T. dioica, C. pepo* and *C. maxima*.

4.3 Phytochemicals screening

The phytochemicals screening of the selected gourd vegetables as shown in table 4.2, showed the presence of considerable amounts of flavonoids, tannins, saponins, terpenoids and alkaloids. The results of phytochemical screening revealed flavonoids, tannins and terpenoids as the prominent phytochemicals in most of the extracts in raw as well as cooked vegetables. Among all the selected vegetables *L. siceraria, L. cylindrica, T. dioica, C. pepo* and *C. maxima* were the richest in flavonoid compounds whereas, moderate amount of flavanoids were screened out in remaining vegetables. The Tannin contents were also reported in most of the raw and cooked vegetables of gourd family. However, the higher results were revealed in *M. charantia, L. siceraria* and *C. pepo* as compared to the other selected vegetables. Interestingly, as indicated in case of flavanoids the tannin contents were also absent in raw state of all the extracts of *C. maxima* but after the cooking treatments the presence was observed. The saponins were also tested positive in most of
g. 4.1 Yield of vegetable extracts (A) *M. charantia* (B) *L. siceraria* (C) *L. cylindrica*

1) *L. acutangula* (E) *T. dioica* (F) *C. maxima* (G) *C. pepo*
Table 4.2 Phytochemicals screening of gourd vegetables

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Saponin</th>
<th>Terpenoids</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td><em>M. charantia</em></td>
<td>Raw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
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<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Microwave Cooked</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fried</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>L. siceraria</em></td>
<td>Raw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Microwave Cooked</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fried</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. cylindrica</em></td>
<td>Raw</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td></td>
<td>Microwave Cooked</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fried</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>L. acutangula</em></td>
<td>Raw</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
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<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fried</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>T. dioica</em></td>
<td>Raw</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>Microwave Cooked</td>
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<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>Fried</td>
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<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. maxima</em></td>
<td>Raw</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Microwave Cooked</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fried</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>Raw</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Pressure Cooked</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Microwave Cooked</td>
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<td>+</td>
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</tr>
<tr>
<td></td>
<td>Fried</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Present moderately; ++ present strongly; - Absent; ME-methanolic extract, EE-ethanolic extract, BE-butanolic extract
the ME and EE of all the samples except the fried samples of all selected gourd vegetables except the *C. pepo*. The terpenoids were also present in most of the extracts of selected vegetable samples especially *L. siceraria*, *T. dioica* and *C. pepo*. The alkaloids were tested positive in some of the extracts and these were almost absent in ME of all the vegetables except the *M. charantia*.

### 4.4 Phytochemicals and antioxidant potential of raw vegetables

#### 4.4.1 Antioxidant compounds

The phytochemicals screening of the selected raw gourd vegetables showed the presence of considerable amounts of flavonoids, tannins, saponins, terpenoids and alkaloids as shown in table 4.2. The study revealed the presence of flavonoids and tannins in most of the extracts of the selected vegetables except *C. maxima*. Flavonoids recorded their strong presence in ME and EE of *T. dioica* whereas, the same extracts of *M. charantia* and *L. siceraria* exhibited strong presence of tannins. The saponins were tested positive only in the ME and EE of selected vegetables with *M. charantia* extracts revealing the strong presence of saponins. Terpenoids were present in considerable amounts in *L. acutangula* and *M. charantia* whereas in other vegetables terpenoids were present in moderate concentrations. The results of the study indicated that among all the selected vegetables, *M. charantia* was the richest in phytochemicals including polyphenolic compounds whereas, *C. maxima* revealed absence of these phytochemicals except the presence of alkaloids and saponins to some extent. The results of the present study on phytochemicals screening are also indicative of presence of flavonoids as the most prominent phytochemicals in almost all the selected gourd vegetables.

#### 4.4.2 Quantitative testing of phytochemicals

The total phenols, flavonoids, tannins and carotenoids content measured for the selected gourd vegetables are illustrated in table 4.3 and 4.4. A wide variation in relation to the mentioned phytochemicals was observed among the selected raw vegetables. The total phenol content was significantly different among almost all the vegetables in their respective extracts (p<0.05) and regardless of extraction solvent it was observed in the order of *M. charantia* > *L. siceraria* > *C. pepo* > *L. cylindrica* > *L. acutangula* > *T. dioica* > *C. maxima*. The effect of different solvents on content of polyphenols is also evident from the results as given in table 4.3. In most of the crops methanol was most effective in extracting the total phenols. However, the average recovery of total phenolics content was
### Table 4.3 Total phenols, flavonoids and tannins content of different extracts of raw gourd vegetables

<table>
<thead>
<tr>
<th>Vegetables crops</th>
<th>Total phenols (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannin content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td><em>M. charantia</em></td>
<td>355.1±0.9</td>
<td>463.6±1.37</td>
<td>658.9±2.41</td>
</tr>
<tr>
<td><em>L. siceraria</em></td>
<td>501.7±2.52</td>
<td>440.7±2.12</td>
<td>451.8±3.49</td>
</tr>
<tr>
<td><em>L. cylindrica</em></td>
<td>216.3±2.09</td>
<td>300.0±2.51</td>
<td>275.1±2.62</td>
</tr>
<tr>
<td><em>L. acutangula</em></td>
<td>228.6±1.77</td>
<td>156.2±2.02</td>
<td>251.1±1.19</td>
</tr>
<tr>
<td><em>T. dioica</em></td>
<td>163.2±1.53</td>
<td>118.2±1.11</td>
<td>100.5±1.00</td>
</tr>
<tr>
<td><em>C. maxima</em></td>
<td>62.5±0.92</td>
<td>56.1±0.69</td>
<td>53.1±0.80</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>519.8±2.35</td>
<td>336.2±1.52</td>
<td>272.1±1.57</td>
</tr>
</tbody>
</table>

Mean 292.4 267.3 294.7 36.8 41.5 97.8 7.3 9.7 10.4

F-Statistics at df=6.14 (p=0.000)

Values are presented as mean±SD (n=3) and referred to the dry weight. Means in columns followed by different letters differed significantly at 5% level of significance (p<0.05)

GE= gallic acid equivalent, QE= quercetin equivalent, CE= catechin equivalent

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
Table 4.4 Carotenoids content of different extracts of raw gourd vegetables

<table>
<thead>
<tr>
<th>Vegetables crops</th>
<th>Carotenoids content (mg β-carotene/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
</tr>
<tr>
<td>M. charantia</td>
<td>7.3±0.03 e</td>
</tr>
<tr>
<td>L. siceraria</td>
<td>15.7±0.11 f</td>
</tr>
<tr>
<td>L. cylindrica</td>
<td>4.6±0.10 d</td>
</tr>
<tr>
<td>L. acutangula</td>
<td>2.4±0.05 b</td>
</tr>
<tr>
<td>T. dioica</td>
<td>3.8±0.06 e</td>
</tr>
<tr>
<td>C. maxima</td>
<td>0.5±0.03 a</td>
</tr>
<tr>
<td>C. pepo</td>
<td>3.8±0.08 e</td>
</tr>
<tr>
<td>Mean</td>
<td>5.4</td>
</tr>
</tbody>
</table>

F-Statistics at F=14.37.4, df=6,14 (p=0.000)

Values are presented as mean ±SD (n=3) and refer to the dry weight. Means in columns followed by different letters differed significantly at 5% level of significance (p<0.05).

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

the highest in BE it varied from 53.1 to 658.9 mg GE/100g (dwb) whereas; the average yield of total phenolics was lowest in EE varying from 56.1 to 463.6 GE/100g (dwb).

The flavonoid content in ME, EE and BE varied from 3.7 to 64.3, 3.7 to 84.8 and 10.4 to 278.9 mg QE/100g (dwb) respectively and it differed significantly (p<0.05) among all the vegetables in their respective extracts. C. pepo, M. charantia and again M. charantia exhibited the highest concentration of flavonoids in ME, EE and BE respectively. Regardless of extraction solvents the maximum concentration of flavonoids was observed in M. charantia and the minimum was in C. maxima.

The tannin content varied from 1.8 to 24.4 mg CE/100g (dwb) in all the extracts of selected gourd vegetables. Regardless of extraction solvent the highest tannin content was measured in L. siceraria, whereas its lowest concentration was in C. maxima. The average tannin content of the selected vegetables in different extracts was in order of ME (7.3 mg CE/100g) < EE (9.7 mg CE/100g) < BE (10.4 mg CE/100g).

The total carotenoids content of the selected raw gourd vegetables showed wide variation from 0.2 to 22.0 mg β-carotene/100 g (dwb) in various extracts (Table 4.4). The carotenoids content of L. siceraria, M. charantia and L. cylindrica measured as β-carotene was much significantly higher when compared to remaining gourd vegetables.
Regardless of the vegetable the carotenoids content was in the order of BE > EE > ME.

4.4.3 Antioxidant activity of raw vegetables extracts

The antioxidant capacity might be influenced by several factors and could not be fully described by a single assay. In addition, most natural antioxidants are multifunctional and therefore, a reliable antioxidant evaluation protocol requires different antioxidant activity assessments to take into account various mechanisms of antioxidant action. The results of total antioxidant activity of different raw vegetable extracts as determined using different assays are described in table 4.5 and 4.6.

4.4.3.1 Percent inhibition as measured by FTC and TBA Methods

The FTC method was used to measure the peroxide level during the initial stage of lipid (linoleic acid) oxidation. Low absorbance values indicated high level of antioxidant activity (Appendix-II). From the results of percent inhibition of ME, EE and BE as determined by FTC assay it was evident that the magnitude of antioxidative potency varied with the type of extract as ME of the selected vegetables showed maximum percent inhibition (41.2%) followed by BE (28.8%) and EE (24.2%). Regardless of extraction solvents the percent inhibition as determined by FTC method was found in the order of M. charantia > L. siceraria > T. dioica > C. pepo > L. cylindrica > C. maxima and this average percent inhibition was lower when compared to that of vitamin E (74.6%) and BHT (88.8%) used as standard. The results of correlation studies as shown in table 4.7 revealed a highly significant and positive correlation of the FTC assay results with phenols content (r= 0.577, p<0.01) as well as carotenoids content (r= 0.584, p<0.01) suggesting that phenols and carotenoids were the main compounds responsible for the antioxidative activity of the gourd vegetables as described by FTC method.

During the oxidation process, peroxide is gradually decomposed to malonaldehyde, which was measured by TBA method on the final day of the incubation period. Percent inhibition as measured by TBA method in different extracts ranged from 17.7 to 83.8% and irrespective of vegetable it was found maximum in ME followed by BE and EE. Regardless of extraction solvents the percent inhibition was maximum in M. charantia followed by L. siceraria and T. dioica. As determined by TBA method all the vegetables revealed lower antioxidant activity than the standard BHT (90.9%) and vitamin E (78.3%). As also observed in case of FTC method, a highly significant and positive
The correlation of the TBA results with phenols content \((r= 0.574, p<0.01)\) as well as carotenoids content \((r= 0.571, p<0.01)\) was observed indicating that phenols and carotenoids were the main phytochemicals responsible for the antioxidant activity of the gourd vegetables as described by TBA method. A very high correlation \((r= 0.999, p<0.01)\) between FTC and TBA assay showed that the increase in peroxide level caused formation of malonaldehyde compounds.

### 4.4.3.2 Ferric reducing antioxidant power (FRAP)

FRAP assay is based on the ability of antioxidant to reduce ferric (III) ions to ferrous (II) ion. As indicated by the results of FRAP assay given in table 4.5, significant differences were observed in the FRAP values among most of the vegetables in their respective extracts \((p<0.05)\). The average FRAP values of the all the selected raw vegetables, varied from 816.6 to 1015.4 \(\mu\)M FeSO₄/100g being highest in BE followed by EE and ME. The antioxidative activity of all the three types of extracts of *L. siceraria, M. charantia* and *L. cylindrica* as determined with FRAP method was significantly higher \((p<0.05)\) when compared to remaining gourd vegetables in their respective extracts. In contrast all the extracts of *C. maxima* showing the lowest antioxidative activity. The results of correlation studies also indicated highly significant positive correlations of FRAP values with total phenols \((r= 0.854, p<0.01)\), flavonoids \((r= 0.692, p<0.01)\) and carotenoids \((r= 0.915, p<0.01)\) suggesting that FRAP assay best described the antioxidative activity of raw gourd vegetables and the antioxidative activity was mainly ascribed to the phenols and carotenoids.

### 4.4.3.3 DPPH free radical scavenging activity

The DPPH method is used to estimate the radical scavenging activity of antioxidant compounds. Free radical scavenging activity for DPPH radical was expressed as IC\(_{50}\) value (the concentration required to scavenge 50% of DPPH). The percent scavenging at different concentration of extracts are presented in Appendix III. Regardless of extraction solvents the antioxidant activity as adjudged by IC\(_{50}\) values of different vegetables was in order of *L. acutangula > C. maxima > C. pepo > T. dioica > L. cylindrica > M. charantia > L. siceraria*. The data regarding DPPH radical scavenging activity as given in table 4.6 showed that the IC\(_{50}\) values of all the raw vegetables in different extracts were higher than the ascorbic acid (5.05 \(\mu\)g/ml) indicating lower antioxidant activity in comparison to ascorbic acid.
### Table 4.5 Antioxidant activity of different extracts of raw gourd vegetables as determined by FTC, TBA and FRAP assays

<table>
<thead>
<tr>
<th>Vegetable crops</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP (µM FeSO4/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td><em>L. cylindrica</em></td>
<td>14.7±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.9±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.6±0.90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>L. acutangula</em></td>
<td>35.4±0.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.9±0.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.9±0.78&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. dioica</em></td>
<td>37.5±0.57&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.6±0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.3±0.62&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. maxima</em></td>
<td>19.3±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.9±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td>30.4±0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.7±1.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.0±0.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>41.2</td>
<td>24.2</td>
<td>28.8</td>
</tr>
</tbody>
</table>

F-Statistics at df=6,14, p=0.00

Values are presented as mean ±SD (n=3) and referred to the dry weight. Means in columns followed by different letters differed significantly at 5% level of significance (p<0.05)

FTC % Inhibition of BHT = 88.8±6.28, Vit. E=74.6±16.16

TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
Table 4.6 Free radical scavenging activity of different extracts of raw gourd vegetables as measured by DPPH assay

<table>
<thead>
<tr>
<th>Vegetables crops</th>
<th>DPPH (% Scavenging at 30 µg/ml)</th>
<th>DPPH (IC_{so}) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td>M.charantia</td>
<td>89.8±0.11</td>
<td>27.3±0.29</td>
</tr>
<tr>
<td>L.siceraria</td>
<td>79.3±0.06</td>
<td>36.0±0.13</td>
</tr>
<tr>
<td>L.cylindrica</td>
<td>20.5±0.23</td>
<td>9.6±0.12</td>
</tr>
<tr>
<td>L.acutangula</td>
<td>14.7±0.06</td>
<td>9.9±0.07</td>
</tr>
<tr>
<td>T.dioica</td>
<td>17.9±0.13</td>
<td>6.4±0.07</td>
</tr>
<tr>
<td>C.maxima</td>
<td>9.5±0.13</td>
<td>7.7±0.07</td>
</tr>
<tr>
<td>C.pepo</td>
<td>13.6±0.11</td>
<td>9.2±0.12</td>
</tr>
</tbody>
</table>

Mean 35.0 15.1 12.6 258.9 175.7 294

Values are presented as mean ±SD (n=3) and referred to the dry weight.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

IC value of ascorbic acid= 5.05µg/ml

% Scavenging of ascorbic acid at 30 µg/ml = 92.0 ± 0.06

The average free radical scavenging activity of the EE was the highest whereas reverse was true for BE. Statistical correlations as given in table 4.7 revealed significant positive correlations of DPPH results with total phenols (r= 0.480, p<0.05) and carotenoids (r= 0.639, p<0.01) indicating that scavenging activity of the gourd vegetables extracts was ascribed mainly to their phenols and carotenoids.

Table 4.7 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity of raw vegetables

<table>
<thead>
<tr>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.708**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>0.342</td>
<td>0.145</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.762**</td>
<td>0.640**</td>
<td>0.337</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>0.577**</td>
<td>0.236</td>
<td>0.034</td>
<td>0.584**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>0.574**</td>
<td>0.222</td>
<td>0.040</td>
<td>0.571**</td>
<td>0.999**</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>0.854**</td>
<td>0.692**</td>
<td>0.383</td>
<td>0.915**</td>
<td>0.561**</td>
<td>0.559**</td>
<td>1.000</td>
</tr>
<tr>
<td>DPPH</td>
<td>0.480*</td>
<td>0.237</td>
<td>-0.51</td>
<td>0.639**</td>
<td>0.598**</td>
<td>0.590**</td>
<td>0.593**</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=21
** Significantly correlated at p<0.01, n=21
4.4.4 Identification and quantification of phenolic acids and flavonoids by HPTLC in raw samples

Considering the fact that the average antioxidant activity of ME of the gourd vegetables was reasonably higher, the ME of gourd vegetables were identified and quantified for their phenolic acids and flavonoids by comparing their Rf values. The Rf value and standard curve of different phenolic acids and flavonoids standard compounds as presented in Appendix IV were: 0.08 (chlorogenic acid); 0.29 (gallic acid); 0.63 (quercetin); 0.72 (caffeic acid); 0.87 (kaempferol); 0.90 (apigenin) in solvent system I while 0.08 (chlorogenic acid); 0.13 (rutein); 0.22 (ellagic acid); 0.37 (catechin); 0.72 (caffeic acid); 0.79 (leutolin); 0.91 (p-coumaric acid) in solvent system II and 0.73 (vanillic acid); 0.74 (ferulic acid); 0.75 (benzoic acid); 0.94 (cinnamic acid) in solvent system III. The HPTLC profiles of ME of all the raw vegetables in solvent system I, II and III are as shown in Fig. 4.2(A-C) to 4.8(A-C). The distribution of phenolic acids and flavonoids as detected from HPTLC profiles is presented in table 4.8. The HPTLC profile of L. siceraria and M. charantia revealed the presence of many of the phenolic acids and flavonoids in comparison to all other gourd vegetables. Caffeic acid, p-coumaric acid, ferulic acid, ellagic acid, quercetin, apigenin, kaempferol and leutolin were found in M. charantia with ellagic acid and p-coumaric acid being present in maximum (291.2 µg/ml) and minimum (19.1 µg/ml) concentrations respectively. Caffeic acid, p-coumaric acid, vanillic acid, quercetin and myrecetin in concentrations of 31.1, 19.1, 172.6, 35.5 and 43.1 µg/ml respectively were detected in L. siceraria. In L. cylindrica, a concentration of 26.8, 18.4, 49.6, 8.6 and 35.7 µg/ml was reported for gallic acid, caffeic acid, ferulic acid, cinnamic acid and myrecetin respectively. Interestingly, L. acutangula showed only benzoic acid as phenolic acid present in significantly high concentration (1817.5 µg/ml). Myrecetin and rutein in concentrations of 28.7 and 0.8 µg/ml respectively were present as flavonoid compounds in T. dioica. Chlorogenic acid (66.5 µg/ml) and ferulic acid (37.3 µg/ml) were detected as phenolic acids in ME of C. maxima whereas; no flavonoid amongst the studied flavonoid compounds was detected in C. maxima extract. C. pepo revealed the presence of chlorogenic acid, cinnamic acid, myrecetin, apigenin, rutein and catechin in concentrations of 303.3, 8.5, 53.1, 3.7, 727.8 and 263.8 µg/ml respectively. Rutein, one of the primary flavonoids present in C. pepo.
Table 4.8 Identification and quantification of phenolic acids and flavonoids (µg/ml of extract) in ME of raw gourd vegetables as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M. charantia</th>
<th>L. siceraria</th>
<th>L. cylindrica</th>
<th>L. acutangula</th>
<th>T. dioica</th>
<th>C. maxima</th>
<th>C. pepo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>nd</td>
<td>31.1</td>
<td>26.8</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>55.0</td>
<td>nd</td>
<td>18.4</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>66.5</td>
<td>303.3</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>21.1</td>
<td>19.1</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>198.8</td>
<td>172.6</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>nd</td>
<td>nd</td>
<td>49.6</td>
<td>nd</td>
<td>nd</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>nd</td>
<td>nd</td>
<td>8.6</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>8.5</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>1817.5</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>291.2</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>76.2</td>
<td>35.5</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>nd</td>
<td>43.2</td>
<td>35.7</td>
<td>nd</td>
<td>28.7</td>
<td>nd</td>
<td>53.1</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>3.8</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>12.3</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Leutolin</td>
<td>39.7</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutein</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.8</td>
<td>nd</td>
<td>727.8</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>263.8</td>
</tr>
</tbody>
</table>

nd = not detected

4.5 Antioxidant potential of bitter gourd (*Momordica charantia*)

4.5.1 Quantitative estimation of phytochemicals

The total phenol content (TPC) of raw and cooked vegetable of *M. charantia* was determined in relation with standard gallic acid and the results were expressed in terms of mg GE/100g dwb (Table 4.9). The average TPC of various extracts regardless of cooking method was in order of ME > EE > BE. F- value statistics (7425.1 at df= 2, p= 0.000) also indicated highly significant impact of extraction solvents on the extraction yield of total phenols. The value of TPC irrespective of extracting solvents showed a significant reduction (p<0.05) in their TPC after cooking and this reduction was observed to be maximum (45.2%) in microwaved sample. However, in terms of interactive effects of cooking treatments and extracting solvents the significant interaction effect (F= 21316.9 at df= 6, p= 0.000) was observed and various cooking treatments exerted inconsistent effect on the recovery of TPC. The BE of the raw vegetable sample showed maximum amount of total phenols, whereas in case of cooked samples the maximum recovery of total phenols was found in ME of pressure cooked sample followed by ME of fried and EE of pressure cooked samples.
The values for total flavonoids content of raw and cooked vegetable samples are presented in table 4.9. The average flavonoids content of the raw sample irrespective of extracting solvents was 139.4 mg QE/100g (dwb), whereas in cooked samples this value varied from 71.4 to 88.8 mg QE/100g (dwb) indicating decreased flavonoids content after different cooking treatments. Regardless of cooking treatments the effect of extracting solvents was also significant (F= 27181.6 at df= 2, p= 0.000) and the flavonoids content of various extracts was in order of BE > ME > EE showed that the significant interaction effect (F= 43028 at df= 6, p= 0.000) of heat processing treatments and the extraction solvents showed that methanol was the most effective solvent for extracting out maximum yield of flavonoids from pressure cooked and fried samples in comparison to their respective raw sample. However, the total flavonoids were found to have declined markedly (p<0.05) after microwave cooking in all extracts as compared to their raw counterpart and the maximum drop was observed in EE (58.5%) followed by BE (48.6%) and ME (34.4%) respectively.

The average tannin content in the cooked samples irrespective of the extraction solvent varied from 5.5 to 8.8 mg CE/100g when compared to the tannin content of raw sample (8.5 mg CE/100g) again suggesting the inconsistent effect of heat processing treatments on tannin content (Table 4.9). Regardless of cooking treatments the average value of tannin content ranged from 7.2–7.8 mg CE/100g. The significant interaction effect (F= 16.6 at df= 6, p= 0.000) of the heat processing treatments and the extraction solvents showed that butanol, methanol, butanol and ethanol were the most effective solvents in extracting out the tannins from raw, pressure cooked, microwave cooked and fried vegetable samples respectively.

Table 4.10 summarises the total carotenoids content of raw and cooked vegetable of M. charantia measured as β carotene. The average value of carotenoids content of cooked vegetable samples regardless of the extraction solvent ranged from 9.7 to 11.7 mg β-carotene/100g (dwb) in comparison to 13.9 mg β-carotene /100g (dwb) of raw vegetable sample. The results showed significant (p<0.05) reduction in carotenoids recovery from cooked samples and the order of decrease in carotenoids content was pressure cooking (30.2%) > frying (20.9%) > microwave cooking (15.8%). Butanol was found to be most efficient solvent for extracting the carotenoids (18.5 mg β-carotene /100g, dwb) as
### Table 4.9 Total phenols, flavonoids, and tannins content of various extracts of cooked vegetable of *M. charantia*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenols (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannins content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>355.1±0.90&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>463.7±1.37&lt;sup&gt;cB&lt;/sup&gt;</td>
<td>659.0±2.41&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>433.9±2.71&lt;sup&gt;cB&lt;/sup&gt;</td>
<td>349.6±2.42&lt;sup&gt;cB&lt;/sup&gt;</td>
<td>255.7±1.32&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>333.3±1.00&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>337.6±0.58&lt;sup&gt;cB&lt;/sup&gt;</td>
<td>138.8±1.01&lt;sup&gt;cB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>423.7±1.43&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>293.0±0.93&lt;sup&gt;cB&lt;/sup&gt;</td>
<td>215.8±0.92&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>386.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>361.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>309.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 7425.19 at df = 2, p=0.000
F-Statistics for cooking treatments = 34628.34 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 21316.96 at df = 6, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

GE = gallic acid equivalent, QE = quercetin equivalent, CE = catechin equivalent.

ME = methanolic extract, EE = ethanolic extract, BE = butanolic extract.
compared to methanol and ethanol, regardless of cooking methods. The significant interaction effect ($F= 180.2$ at $df= 6$, $p= 0.000$) indicated the inconsistent effect of cooking treatments in regards to the recovery of carotenoids in different solvents. The ME of the pressure cooked sample revealed an increased recovery of carotenoids in comparison to their raw counterparts while, the EE and BE of pressure cooked sample showed decrease concentration. However, in microwaved and fried sample most abrupt increase in the carotenoids content in respect to their raw vegetable sample was measured in BE, which showed an increased level of 34.5% and 4.6% correspondingly.

4.5.2 The antioxidant activity as measured by using FTC, TBA, FRAP and DPPH radical scavenging methods

4.5.2.1 Percent inhibition as measured by FTC method

Antioxidant activity of ME, EE and BE of raw and cooked vegetable samples of *M. charantia* in terms of measurement of inhibition of peroxidation is as shown in table 4.11. Different extracts of various samples of *M. charantia* inhibited 15.3-82.1% peroxidation of linoleic acid after an incubation period of 96h. This wide variation in the inhibition level could be because of differences in the type and concentration of antioxidative compounds in these extracts. Nevertheless, the antioxidant activity of all extracts, in terms of measurement of percent inhibition was significantly ($p<0.05$) lower than BHT (88.8%). Despite of cooking treatments the significant difference ($F= 13737.9$ at $df = 2$, $p=0.000$) was observed in percent inhibition and it was found to be maximum in ME (70.7%) followed by EE (57.1%) and BE (22.9%). Regardless of extraction solvent, the percent inhibition was observed to decrease significantly ($p<0.05$) after various cooking treatments and the major loss was found in microwave cooking (37.9%) followed by pressure cooking (29.7%) and frying (13.3%). The interaction effect of cooking treatments and the extraction solvents was also significant ($F= 1211.8$ at $df= 6$, $p= 0.000$). The results showed that percent inhibition in pressure cooked and fried samples were significantly higher ($p<0.05$) in ME, while it was found to be lower in EE and BE respectively as compared to their respective raw vegetable sample. The microwave cooked samples exhibited decreased value of peroxide inhibition in all types of extracts as compared to their raw counterparts.

4.5.2.2 Percent inhibition as measured by TBA method

Table 4.11 shows the inhibition values of various extracts of raw and cooked vegetable samples of *M. charantia*. Regardless of cooking treatments, ME revealed the
Table 4.10 Carotenoids content of various extracts of raw and cooked vegetable of *M. charantia*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoids content (mg β- Carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>7.3 ± 0.03bA</td>
<td>14.9 ± 0.10bB</td>
<td>19.4 ±0.18bc</td>
<td>13.9c</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>13.9 ± 0.35cB</td>
<td>7.1 ± 0.12bA</td>
<td>8.2 ± 0.05aA</td>
<td>9.7a</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>4.3 ± 0.07aA</td>
<td>4.7 ± 0.10aA</td>
<td>26.1 ± 2.87cB</td>
<td>11.7b</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>6.8 ± 0.04bA</td>
<td>5.8 ± 0.07bA</td>
<td>20.3 ±0.04bB</td>
<td>11.0b</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>8.1A</td>
<td>8.1A</td>
<td>18.5B</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD (n=3) and referred to the dry weight.
F-Statistics for extraction solvents = 615.84 at df = 2, p=0.000
F-Statistics for cooking treatments = 38.76 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 180.21 at df = 6, p=0.000

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

highest percent inhibition (74.8%) followed by BE (56.7%) and EE (32.5%). F- value statistics (29125.7 at df = 2, p=0.000) also indicated highly significant impact of extraction solvents on the percent inhibition. The main effect of the cooking treatments was also significant (F= 8816 at df= 3, p= 0.000). The average value of percent inhibition irrespective of extracting solvents was observed to be decreased after various cooking treatments and this was in the order of microwave cooking > pressure cooking > frying. There was significant interaction effect between the extracting solvents and the heat treatments on the percent inhibition (F= 3350 at df= 6, p= 0.000). The results showed that ME of raw sample was most effective in inhibiting the oxidation. However, in cooked samples the maximum increased level of inhibition was revealed by ME of fried sample followed by ME of pressure cooked and EE of fried sample. Overall, frying was most effective to retain the percent inhibition by various extracts of *M. charantia*.

4.5.2.3 Ferric reducing antioxidant power (FRAP)

The results of the FRAP assay are reported in Table 4.11. The antioxidant activities were expressed as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1μM of FeSO₄. Significant differences were observed in the FRAP values among all the cooking treatments in their respective extracts (p<0.05). The average FRAP values as shown by various extracts regardless of cooking treatments varied from

90
994.3 to 1258.6 μM FeSO₄/100g being the highest in ME followed by EE and BE (F= 847.2 at df= 2, p= 0.000). Regardless of extraction solvents, the significant effect (F= 7750.6 at df= 3, p= 0.000) of cooking treatments on ferric reducing power was observed when compared to raw vegetable sample and it was found to be in the order of pressure cooking > frying > microwave cooking. The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 4437.3 at df= 6, p= 0.000).

As measured by FRAP assay, the BE of raw sample was most effective in reducing the Fe³⁺ ion. In case of cooked sample maximum increase in the reducing power over the raw sample was shown by ME of pressure cooked sample (104.9%) followed by ME of fried (43.8%) and EE of pressure cooked sample (27.9%).

4.5.2.4 DPPH free radical scavenging activity

The scavenging effect of DPPH free radical is shown as relative activity against ascorbic acid (Table 4.12). The percent scavenging activity of 30 μg/ml ascorbic acid (92.0%) was observed to be highest with respect to various extracts of raw and cooked vegetable samples of M. charantia. The average percent scavenging of the raw samples irrespective of extracting solvents was 43.5%, whereas in cooked samples this value varied from 44.9 to 52.3% and increase in the scavenging activity was found after various cooking methods. Regardless of cooking treatments the effect of extracting solvents was significant (F= 106716 at df= 2, p= 0.000) and the percent scavenging at 30 μg/ml of various extracts was in order of EE > ME > BE. The interactive effect of heat processing treatments and the extraction solvents was also significant (F= 27311 at df= 6, p= 0.000). The results showed that average percent scavenging of pressure cooked, microwaved and fried samples were significantly higher (p<0.05) in EE and BE, while it was observed to be lower in ME of all cooked samples as compared to raw vegetable sample.

Free radical scavenging activity for DPPH radical was also expressed in terms of IC₅₀ value. The IC₅₀ value was estimated by using the percent scavenging at different concentration (Appendix-III). The average free radical scavenging activity as assessed by IC₅₀ values regardless of the extraction solvent increased by 1.9% after pressure cooking, whereas it decreased by 35.4% and 15.2% after microwave cooking and frying respectively (Table 4.12). Regardless of cooking treatments the average IC₅₀ value was observed to be highest in BE (125.4 μg/ml) followed by ME (29.4 μg/ml) and EE (23.7 μg/ml). Various extracts of raw vegetable sample showed IC₅₀ value ranging from 6.0 to 157.3 μg/ml. The ME of all the cooked samples showed an increase IC₅₀ value in
Table 4.11 Antioxidant activity of various extracts of cooked vegetable of *M. charantia* as determined by FTC, TBA, FRAP assays

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP μM FeSO₄/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>79.7±0.58&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>77.8±0.70&lt;sup&gt;db&lt;/sup&gt;</td>
<td>31.7±0.63&lt;sup&gt;da&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>81.3±0.84&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>36.3±1.00&lt;sup&gt;db&lt;/sup&gt;</td>
<td>15.3±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>39.7±0.70&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>53.6±0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>23.9±0.70&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>82.1±0.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.7±0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.9±0.61&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>70.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 13737.9 at df = 2, p=0.000
F-Statistics for cooking treatments = 1946.5 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 1211.79 at df = 6, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively
FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16
TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

F-Statistics for extraction solvents = 29125.7 at df = 2, p=0.000
F-Statistics for cooking treatments = 8816.2 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 1211.79 at df = 6, p=0.000
F-Statistics for cooking treatments & extraction solvents = 4437.3 at df = 6, p=0.000
Table 4.12 Free radical scavenging activity of various extracts of cooked vegetable of *M. charantia* as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH (Mean ± SE)</th>
<th>IC-50 (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME (mg/ml)</td>
<td>EE (mg/ml)</td>
</tr>
<tr>
<td>Raw</td>
<td>89.8±0.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>27.3±0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>45.3±0.23&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>82.1±0.13&lt;sup&gt;cC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>71.8±0.23&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>68.3±0.13&lt;sup&gt;bcB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>27.5±0.28&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>86.6±0.19&lt;sup&gt;bcC&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>58.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>66.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD (n=3)

F-Statistics for extraction solvents = 106716 at df = 2, p=0.000

F-Statistics for cooking treatments = 1695.4 at df = 3, p=0.000

F-Statistics for cooking treatments & extraction solvents = 27311 at df = 6, p=0.000

% Scavenging of ascorbic acid at 30 μg/ml = 92.0 ± 0.06

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

IC<sub>50</sub> value of ascorbic acid=5.05 μg/ml

Comparison to their respective extracts of raw sample and the highest increased IC<sub>50</sub> value was observed in fried sample (875%) followed by pressure cooked (471%) and microwave cooked (213%) sample.

4.5.3 Correlation studies

Statistical correlations have been studied between phytochemicals content and antioxidant activity determined by different assays, as shown in table 4.13. The results of correlation studies have indicated highly significant positive correlations of FRAP values with total phenols (r=0.872, p<0.01) suggesting that FRAP assay best described the antioxidative activity of *M. charantia* and the antioxidative activity was mainly ascribed to the phenols content. The FRAP value showed the positive correlation (r= 0.429) with total flavonoids, concluding that phenols and flavonoids may be the main compounds responsible for reduction of the ferrous ions into ferric ions in their respective extracts of *M. charantia*. The percent inhibition as measured by TBA assay showed positive correlation (r= 0.432) with the total phenols. This again indicated that the phenols may be the phytochemicals which inhibit malonaldehyde formation and responsible for the antioxidant activity of *M. charantia*. Interestingly, a strong correlation (r =0.997, p<0.01) between FTC and TBA assay showed that the increase in peroxide level caused formation.
Table 4.13 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity

<table>
<thead>
<tr>
<th></th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.416</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>0.230</td>
<td>0.165</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>-0.069</td>
<td>0.669*</td>
<td>0.223</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>0.417</td>
<td>-0.405</td>
<td>0.254</td>
<td>-0.362</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>0.432</td>
<td>-0.263</td>
<td>0.331</td>
<td>-0.203</td>
<td>0.957**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>0.872**</td>
<td>0.429</td>
<td>0.246</td>
<td>0.034</td>
<td>0.310</td>
<td>0.402</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.070</td>
<td>0.857**</td>
<td>0.112</td>
<td>0.549</td>
<td>-0.640</td>
<td>-0.534</td>
<td>0.096</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=12

** Significantly correlated at p<0.01, n=12

of malonaldehyde compounds. Similarly, a highly significant and positive correlation of the DPPH value with flavonoids content (r= 0.857, p<0.01) indicated that the change in free radical scavenging activity of processed sample may be due to the change in flavonoids content after the cooking process.

4.5.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

Considering the fact that the average value of total phenols and antioxidant activity (as measured by FTC, TBA and FRAP assay) of ME of *M. charantia* was reasonably higher than the EE and BE and since the methanol is considered as universal extraction solvent, the ME were selected for identification and quantification of phenolic acids and flavonoids. The Rf value and standard curve of different phenolic acids and flavonoid standard compounds with three different solvent systems are presented in Appendix-IV. The polyphenol distribution and chromatogram of ME of *M. charantia* is presented in table 4.14 and Fig. 4.2 (A-C) respectively. The gallic acid as a phenolic acid was not identified in the raw sample extract, but it was detected in pressure cooked and microwave cooked sample. Caffeic acid (55.01 µg/ml) was reported in raw vegetable of *M. charantia* and its concentration was found to decrease after pressure cooking and microwave cooking whereas, in fried sample caffeic acid was not detected. The p-coumaric acid (21.13 µg/ml) was detected in raw sample and its retention was observed only in microwaved sample (21.31 µg/ml). Moreover, vanillic acid was found only in pressure cooked sample and its concentration was although lower in comparison to raw
Table 4.14 Identification and quantification of phenolic acids and flavonoids (µg/ml of extract) in ME of *M. charantia* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>nd</td>
<td>23.17</td>
<td>73.35</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>55.01</td>
<td>20.83</td>
<td>11.93</td>
<td>nd</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>21.13</td>
<td>nd</td>
<td>21.31</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>198.88</td>
<td>46.19</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>nd</td>
<td>559.41</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>291.21</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>76.28</td>
<td>54.59</td>
<td>86.58</td>
<td>41.89</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>nd</td>
<td>nd</td>
<td>65.70</td>
<td>nd</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>6.55</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>12.33</td>
<td>5.19</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Leutolin</td>
<td>39.71</td>
<td>16.41</td>
<td>nd</td>
<td>26.44</td>
</tr>
<tr>
<td>Rutin</td>
<td>nd</td>
<td>73.42</td>
<td>nd</td>
<td>163.51</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>89.46</td>
<td>201.32</td>
<td>97.16</td>
</tr>
</tbody>
</table>

nd = not detected

sample (198.88 µg/ml). Benzoic acid (559.41 µg/ml) was identified as most prominent phenolic acid and found after the microwave cooking, however it was not detected in rest of the extracts including the raw vegetable. Ellagic acid was identified only in raw sample of *M. charantia* and there was no retention of ellagic acids after the various cooking treatments. Quercetin as a flavonoid was revealed in all the samples and its concentration was observed to decrease after the cooking treatments except the microwave cooking. This suggested that the microwave cooking was the most efficient cooking treatment to retain the quercetin. Myricetin (65.70 µg/ml) was observed only after the microwave cooking and apigenin was observed after frying, however these were not detected in rest of the extracts including the raw vegetable. The retention of kaempferol as flavonoid was observed in pressure cooked sample. Leutolin was observed to be present in *M. charantia*. However, its concentration decreased drastically in the heat processed samples and in microwave cooked sample leutolin was not identified. Rutin was although not identified in the raw sample extract, but it was detected in pressure cooked (73.42 µg/ml) and fried (163.51 µg/ml) samples. Interestingly, the catechin was newly formed compound
Fig. 4.2A HPTLC profile of ME of *M. charantia* as developed in chloroform: hexane: methanol: formic acid (6.4: 3.9: 2.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.2B HPTLC profile of ME of *M. charantia* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.2C HPTLC profile of ME of *M. charantia* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried.
observed only after the cooking treatments. The results presented here showed that heat processing treatments could affect the makeup of phenolic acids and flavonoids in cooked vegetable samples. Surprisingly, no phenolic acid was observed after the frying whereas a variety of phenolic acids were reported in different cooking treatments. The most destructive effect of heat processing was on ellagic acid, which was prominently detected in raw sample, but after various cooking treatments its presence was not identified.

4.6 Antioxidant potential of bottle gourd (*Lagenaria siceraria*)

4.6.1 Quantitative estimation of phytochemicals

The total phenol content (TPC) of raw and cooked vegetable of *L. siceraria* as determined by Folin-Ciocalteu Reagent (FCR) is reported in table 4.15. The average TPC of various extracts regardless of cooking method was in order of ME > EE > BE. The main effect of the cooking treatments was highly significant ($F= 7300.6$ at df= 3, $p= 0.000$). The average value of TPC irrespective of extracting solvents increased after pressure cooking and frying, whereas it decreased after microwave cooking. However, in terms of interactive effects of cooking treatments and extracting solvents, although there was significant interaction effect ($F= 7634.3$ at df= 6, $p= 0.000$), but various cooking treatments exerted inconsistent effect on the TPC of various extracts. Different extracts of the pressure cooked samples revealed an increased recovery of TPC in comparison to their raw counterparts. However, the effect of microwave cooking and frying was observed to be inconsistent in regard to the recovery of total phenols in various extracts as EE and BE of microwaved samples showed increased concentrations of TPC, contrary to decreased concentration in ME, when compared to their raw counterparts. Whereas, in case of frying ME and EE revealed an increased concentration in contrast to BE where a decrease of 79% in concentration of TPC was observed.

Table 4.15 shows the total flavonoids content of raw and cooked vegetable samples. The average flavonoids content of the raw samples irrespective of extracting solvents was 66.3 mg QE/100g (dwb), whereas in cooked samples this value varied from 63.2 to 158.1 mg QE/100g (dwb) indicating that the effect of different heat processing treatments on the flavonoids content was although significant ($F= 22229$ at df= 3, $p= 0.000$) but inconsistent as microwave cooking decreased the flavonoids content whereas pressure cooking and frying resulted in an increase in the flavonoids content. The flavonoids content of various extracts irrespective of the heat processing treatments was in order of
Table 4.15 Total phenols, flavonoids and tannins content of various extracts of cooked vegetable of *L. siceraria*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenol (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannin content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>501.8±2.52bC</td>
<td>440.8±2.12aa</td>
<td>451.9±3.49bB</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>660.4±5.57dC</td>
<td>507.8±3.82aA</td>
<td>558.8±3.79bB</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>394.5±2.81ab</td>
<td>473.0±4.02bC</td>
<td>160.5±2.65aA</td>
</tr>
<tr>
<td>Fried</td>
<td>726.7±5.0aB</td>
<td>758.8±3.67bC</td>
<td>94.6±0.89aa</td>
</tr>
<tr>
<td>Mean</td>
<td>570.8c</td>
<td>545.1b</td>
<td>316.4a</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 18836 at df = 2, p=0.000
F-Statistics for cooking treatments = 7300.6 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 7634.3 at df = 6, p=0.000
F-Statistics for extraction solvents = 8535.9 at df = 2, p=0.000
F-Statistics for cooking treatments = 2229.7 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 11151.5 at df = 6, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

GE= gallic acid equivalent, QE= quercetin equivalent, CE= catechin equivalent
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
EE > ME > BE. The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 11151 at df= 6, p= 0.000). The BE of raw sample revealed maximum recovery of flavonoids, whereas in case of cooked vegetable samples EE of fried sample followed by ME of fried and EE of pressure cooked sample showed the maximum recovery of total flavonoids, which showed an increase upto 444% recovery when compared to raw counterparts.

The average tannin content in the cooked samples irrespective of the extraction solvent varied from 11.0 to 19.5 mg CE/100g when compared to the tannin content of raw sample (13.5 mg CE/100g) (Table 4.15) suggesting again the inconsistent effect of heat processing treatments on tannin content. The microwave cooking exerted pronounced decreasing effect on the tannin content. Among all the extracts, ethanol was found to be the most effective solvent for extracting tannins. The significant interaction effect (F= 9.7 at df= 6, p= 0.000) of the heat processing treatments and the extraction solvents showed the increased tannin content after all the cooking treatments in ME and BE when compared to their raw counterparts. However, the EE revealed an inconsistent effect of heat treatment on the recovery of tannin content and the maximum recovery (24.5% increase) was found in fried sample.

The carotenoids content of raw and cooked samples of L. seceraria measured as β-carotene is presented in Table 4.16. The carotenoids content of cooked vegetable samples regardless of the extraction solvent ranged from 9.1 to 27.6 mg β-carotene /100g (dwb) in comparison to carotenoid content of 19.4 mg β-carotene /100g (dwb) in the raw vegetable sample. The results showed significant (p<0.05) change in carotenoids recovery from cooked samples. Recovery of carotenoids from microwaved sample increased significantly (42.2% increase), whereas a marginal decrease of 1.5% and a significant decrease of 53.1% was observed in pressure cooked and microwaved samples. Butanol was found to be most efficient for extracting the carotenoids (25.7 mg β-carotene /100g, dwb) followed by ethanol and methanol. The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 11988.3 at df= 6, p= 0.000). The maximum carotenoids contents in raw sample was found in its BE, whereas in case of cooked samples, BE of microwaved followed by BE of pressure cooked and EE of fried samples showed the highest content of carotenoids with increases ranging from 3.8 to 127.7% over their respective native counterparts.
Table 4.16 Carotenoids content of various extracts of cooked vegetable oil of L. siceraria

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoid content (mg β-Carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>15.7 ± 0.11&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>20.4 ± 0.13&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>22.0 ± 0.08&lt;sup&gt;bcC&lt;/sup&gt;</td>
<td>19.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>16.3 ± 0.43&lt;sup&gt;dB&lt;/sup&gt;</td>
<td>10.8 ± 0.14&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>30.3 ± 0.13&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>19.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>13.6 ± 0.22&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>19.1 ± 0.29&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>50.1 ± 0.30&lt;sup&gt;dC&lt;/sup&gt;</td>
<td>27.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>0.5 ± 0.10&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>26.5 ± 0.19&lt;sup&gt;dB&lt;/sup&gt;</td>
<td>0.5 ± 0.03&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>11.5&lt;sup&gt;A&lt;/sup&gt;</td>
<td>19.2&lt;sup&gt;B&lt;/sup&gt;</td>
<td>25.7&lt;sup&gt;C&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD (n=3) and referred to the dry weight.

F-Statistics for extraction solvents = 13867.4 at df = 2, p=0.000
F-Statistics for cooking treatments = 11787.3 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 11988.3 at df = 6, p=0.000

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

4.6.2 The antioxidant activity as measured by using FTC, TBA, FRAP and DPPH radical scavenging methods

4.6.2.1 Percent inhibition as measured by FTC method

Antioxidant activity of ME, EE and BE of raw and cooked vegetable samples of L. siceraria in terms of measurement of inhibition of peroxidation is as shown in table 4.17. For all the cooked samples, significant differences (p<0.05) in percent inhibition were observed among different extraction solvents. These results indicated the possible influence of extracting solvent on percent inhibition but not all in the same way. The various extracts of different samples of L. siceraria inhibited 26-87.7% peroxidation of linoleic acid after incubation for 96h. Nevertheless, the antioxidant activity of all extracts, in terms of measurement of percentage inhibition was significantly (p<0.05) lower than BHT (88.8%). In general, ME revealed significantly higher antioxidant activity followed by BE and EE (F= 3756.3 at df= 2, p= 0.000). Regardless of extraction solvent, the percent inhibition was observed to be inconsistent after various cooking treatments and it was found to be inferior in pressure cooked and microwave cooked samples in comparison to raw sample. The interaction effect of cooking treatments and the extraction solvents was also significant (F= 1625.9 at df= 6, p= 0.000). The results showed that percent inhibition by the extracts of pressure cooked samples was significantly higher.
(p<0.05) in ME and EE, while it was lower by 19.1% in BE when compared to respective raw vegetable extracts. The microwave cooking exerted a decreasing effect on peroxide inhibition in all types of extracts except the EE. However, all the three types of extracts of fried samples revealed an increased percent inhibition in comparison to their raw counterparts.

4.6.2.2 Percent inhibition as measured by TBA method

Percent inhibition values of various extracts of raw and cooked vegetable samples of *L. siceraria* are presented in table 4.17. Regardless of cooking treatments, ME revealed highest percent inhibition (76.4 %) followed by BE (70.7%) and EE (55.1%). The main effect of the cooking method was also highly significant (F= 6628.5 at df= 3, p= 0.000). The average value of percent inhibition irrespective of extracting solvent decreased only after microwave cooking while after pressure cooking and frying the percent inhibition was observed to increase. The significant interaction effect of cooking treatments and extraction solvents suggested that there was an inconsistent effect of heat treatments on inhibition power as measured by TBA assay. The BE of raw vegetable sample showed maximum percent inhibition, whereas the maximum percent inhibition in case of cooked sample was found in fried samples of EE followed by BE and ME. As determined by TBA method all the extracts revealed lower antioxidant activity than the standard BHT (90.9%). Similarly, all the extracts revealed lower antioxidant activity than the standard vitamin E (78.3%) except the raw sample of BE and fried sample of all the extracts.

4.6.2.3 Ferric reducing antioxidant power (FRAP)

As indicated by the results of FRAP assay given in table 4.17, significant differences were observed in the FRAP values among all the cooking treatments in their respective extracts (p<0.05). The average FRAP values as shown by various extracts regardless of cooking methods varied from 1645.0 to 2620.9 μM FeSO₄/100g being highest in EE followed by ME and BE. Irrespective of extraction solvent ferric reducing power was observed to increase after all the cooking treatments when compared to raw vegetable sample and it was found in the order of microwave cooked < fried < pressure cooked. The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 4425.9 at df= 6, p= 0.000). All the extracts of pressure cooked samples were observed to be more effective in reducing the Fe³⁺ when compared to their raw counterpart and this increased reducing power was maximum in ME (85.1%)
Table 4.17 Antioxidant activity of various extracts of cooked vegetable of *L. siceraria* as determined by FTC, TBA and FRAP assays

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP µM FeSO₄/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>71.7±0.70b</td>
<td>26.03±0.79a</td>
<td>76.68±0.62c</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>74.9±0.87c</td>
<td>35.7±0.66a</td>
<td>62.01±0.57b</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>65.1±0.87c</td>
<td>39.6±0.54a</td>
<td>40.9±0.70ab</td>
</tr>
<tr>
<td>Fried</td>
<td>74.6±0.73c</td>
<td>87.75±0.64bc</td>
<td>77.6±0.68c</td>
</tr>
<tr>
<td>Mean</td>
<td>71.6c</td>
<td>47.3a</td>
<td>64.3b</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 3756.3 at df = 2, p=0.000
F-Statistics for cooking treatments = 3236.4 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 1625.9 at df = 6, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.
FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16
TBA % Inhibition of BHT= 90.4±0.47, Vit. E=78.3±0.19
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
followed by EE (35.2%) and BE (31.3%). The effect of microwave cooking and frying was observed to be inconsistent in regard to their reducing power as their ME and EE showed increased FRAP value in contrast to decreased FRAP value shown by BE, when compared to their respective raw counterparts.

4.6.2.4 DPPH free radical scavenging activity

The percent scavenging at a concentration of 30 µg/ml of various extracts are reported in table 4.18. Regardless of cooking treatments the effect of extracting solvents was significant (F= 56278 at df= 2, p= 0.000) and the percent scavenging of various extracts was in order of ME > BE > EE. The inconsistent effect of heat treatments was observed on radical scavenging activity of various extracts. The percent scavenging of raw samples irrespective of extracting solvents was 55.1%, whereas in cooked samples this value varied from 36.3 to 66.6%. The results showed that percent scavenging activity was significantly higher (p<0.05) in EE and BE of microwaved and BE of fried samples, whereas rest of the samples showed decreased scavenging activity when compared to their raw counterparts. Moreover, the percent scavenging activity of ascorbic acid (92.0%) was observed to be highest in comparison to the various extracts of *L. siceraria*.

Free radical scavenging activity for DPPH radical was also expressed as IC₅₀ value. The IC₅₀ values and percent scavenging with respect to various concentrations of different extracts of *L. siceraria* in raw as well as heat processed forms are presented in table 4.18 and Appendix-III respectively. The average free radical scavenging activity as assessed by IC₅₀ values regardless of the extraction solvent increased by 27.4% after pressure cooking and 29.3% after frying, whereas it decreased by 34% after microwave cooking. Regardless of cooking treatments the average IC₅₀ value was observed to be highest in EE (39.4 µg/ml) followed by BE (32.5 µg/ml) and ME (28.7 µg/ml). The present results suggested that there was significant interaction effect between the cooking treatments and the extraction solvents. Various extracts of raw vegetable sample showed IC₅₀ value ranging from 22.5 to 39.0 µg/ml, whereas all the extracts of pressure cooked sample showed an increase IC₅₀ value in comparison to their respective extracts of raw sample. Conversely, the effect of microwave cooking and frying was inconsistent in respect to their IC₅₀ value of various extracts as EE and BE of microwaved sample showed decreased IC₅₀ value in contrast to increased IC₅₀ in ME when compared to their raw counterparts.
Table 4.18 Free radical scavenging activity of various extracts of cooked vegetable of *L. siceraria* as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH (% Scavenging at 30 μg/ml)</th>
<th>IC-50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td>Raw</td>
<td>79.3±0.06&lt;sup&gt;dc&lt;/sup&gt;</td>
<td>36.0±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>44.4±0.06&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>26.2±0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>61.4±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>80.1±0.29&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>47.0±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.6±0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>58.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SE (n=3).

F-Statistics for extraction solvents = 56278 at df = 2, p<0.000
F-Statistics for cooking treatments = 93871 at df = 3, p<0.000
F-Statistics for cooking treatments & extraction solvents = 47365 at df = 6,

% Scavenging of ascorbic acid at 30 μg/ml = 92.0 ± 0.06

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME = methanolic extract, EE = ethanolic extract, BE = butanolic extract.

IC<sub>50</sub> value of ascorbic acid = 5.05μg/ml

4.6.3 Correlation studies

Statistical correlations have been studied between phytochemicals content and antioxidant activity determined by different assays, as shown in table 4.19. The percent inhibition as measured by TBA assay was shown to provide the positive association (r=0.402) with the total flavonoids. This indicates that the flavonoids may be the phytochemicals which inhibit malonaldehyde formation and responsible for the antioxidant activity of *L. siceraria*. Interestingly, a strong correlation (r=0.999, p<0.01) between FTC and TBA assay showed that the increase in peroxide level caused formation of malonaldehyde compounds. A highly significant and positive correlation of the FRAP value with phenols content (r=0.892, p<0.01) and flavonoids content (r=0.809, p<0.01) concluded that phenols and flavonoids may be the main compounds responsible for reduction the ferrous ions into ferric ions in their respective extracts. Similarly, a highly significant and positive correlation of the DPPH value with flavonoids content (r=0.743, p<0.01) and tannin content (r=0.852, p<0.01) concluded that the change in free radical scavenging activity of processed sample may be due to the change in flavonoids and tannins content after the cooking process.
### Table 4.19 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity

<table>
<thead>
<tr>
<th></th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.737**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>0.363</td>
<td>0.621*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>-0.164</td>
<td>-0.006</td>
<td>0.087</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>0.309</td>
<td>0.392</td>
<td>-0.111</td>
<td>-0.292</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>0.319</td>
<td>0.402</td>
<td>-0.108</td>
<td>-0.283</td>
<td>0.999**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>0.892**</td>
<td>0.809**</td>
<td>0.419</td>
<td>0.081</td>
<td>0.242</td>
<td>0.254</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.435</td>
<td>0.743**</td>
<td>0.852**</td>
<td>0.085</td>
<td>0.185</td>
<td>0.190</td>
<td>0.468</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=12
** Significantly correlated at p<0.01, n=12

#### 4.6.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

The Rf value of different phenolic acids and flavonoid standard compounds with three different solvent systems are presented in Appendix-IV. The distribution of phenolic acids and flavonoids in ME of *L. siceraria* is presented in table 4.20 and Fig. 4.3 (A-C). The different phenolic acids identified and quantified were gallic acid (31.11 µg/ml), p-coumaric acid (19.08 µg/ml), vanillic acid (172.7 µg/ml), quercetin (35.52 µg/ml) and myricetin (43.1 µg/ml) in raw sample extract; gallic acid (24.8 µg/ml), ellagic acid (34 µg/ml), quercetin (34 µg/ml), myricetin (28.8 µg/ml) and rutin (199.5 µg/ml) in pressure cooked sample extract; only ellagic acid (67.3 µg/ml) and rutin (264.9 µg/ml) in microwaved sample extract and gallic acid (25.8 µg/ml), ellagic acid (101.3 µg/ml), rutin (247.7 µg/ml) and catechin (63.2 µg/ml) in the fried sample extract. Gallic acid was identified as most prominently present phenolic acid in *L. siceraria*. However, its concentration decreased drastically in the heat processed samples and in microwave cooked sample gallic acid was not identified. The p-coumaric acid and vanillic acid were only identified in the raw sample of *L. siceraria* and there was no retention of these phenolic acids after the various cooking treatments. Interestingly, the ellagic acid and rutin were newly formed compounds observed after all the cooking treatments although; these were not identified in raw *L. siceraria*. The flavonoid such as quercetin and myricetin were only found in pressure cooked sample and their concentration was lower in comparison to raw sample. Rutin was although not identified in the raw sample extract, but it was detected in all the cooked samples. The catechin (63.18 µg/ml) was observed only in the fried sample, however, it was not detected in rest of the extracts including the...
Table 4.20 Identification and quantification of phenolic acids and flavonoids (µg/ml of extract) in ME of *L. siceraria* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>31.11</td>
<td>24.83</td>
<td>nd</td>
<td>25.83</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>19.08</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>172.69</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>nd</td>
<td>34.04</td>
<td>67.36</td>
<td>101.34</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>35.52</td>
<td>34.04</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>43.17</td>
<td>28.85</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Leutolin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>nd</td>
<td>199.51</td>
<td>264.91</td>
<td>247.76</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>63.18</td>
</tr>
</tbody>
</table>

nd = not detected

raw vegetable sample. The results presented here showed that heat processing treatments could make the phenolic acids and flavonoids different from that of uncooked form. The most destructive effect of heat processing was on p-coumaric acid and vanillic acid, which was not identified at all in the cooked samples.

4.7 Antioxidant potential of sponge gourd (*Luffa cylindrica*)

4.7.1 Quantitative estimation of phytochemicals

The significant impact of solvents on the extraction of total phenols was observed (F = 216.0 at df = 2, p = 0.000) (Table 4.21). The average TPC of various extracts of *L. cylindrica* regardless of cooking method was in order of EE > BE > ME. The TPC of cooked samples regardless of the extracting solvents was in order of frying > pressure cooking > microwave cooking and the TPC of pressure cooked sample and fried sample was higher when compared to raw sample. Nonetheless, there was highly significant interaction effect (F = 585.1 at df = 6, p = 0.000), suggesting that various cooking treatments exerted inconsistent effect on the TPC of different extracts. The EE of the raw vegetable sample revealed maximum recovery of phenols, whereas in case of cooked
Fig. 4.3A HPTLC profile of ME of *L. siceraria* as developed in chloroform: hexane: methanol: formic acid (6.4: 3.9: 2.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.3B HPTLC profile of ME of *L. siceraria* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried.
Fig. 4.3C HPTLC profile of ME of *L. siceraria* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
vegetable samples the TPC was observed to be maximum in case of fried sample following the order of BE > EE > ME.

The flavonoids content of the raw sample irrespective of extracting solvents was 72.1 mg QE/100g (dwb), whereas in cooked samples this value varied from 43.4 to 262.7 mg QE/100g (dwb) suggesting the inconsistent but significant (F= 93918.3 at df= 3, p= 0.000) effect of different cooking treatments on the flavonoids content (Table 4.21). The effect of solvents in extracting out the flavonoids was also significant (F= 20822.3 at df= 2, p= 0.000) and the flavonoids content of various extracts was in the order of BE > EE > ME. The significant interaction effect of cooking treatments and extraction solvents showed that the total flavonoids content have declined markedly (p<0.05) after microwave cooking in all the extracts as compared to their raw counterparts and the maximum drop was observed in EE (46.4%) followed by BE (41.0%) and ME (21.5%). However, in case of fried sample, all the extracts were observed to be proficient in extracting the flavonoids as compared to their respective raw counterparts.

The average tannin content of the cooked samples irrespective of the extraction solvents varied from 12.1 to 14.8 mg CE/100g when compared to the tannin content of raw sample (24.1 mg CE/100g) suggesting the declining effect of heat processing treatments on tannin content (Table 4.21). Regardless of cooking treatments the average value of tannin content of different extracts ranged from 15.2–17.4 mg CE/100g. The significant interaction effect (F= 10.2 at df= 6, p= 0.000) of the cooking treatments and the extraction solvents showed that the tannin content varied among different extracts in relation with the cooking treatments. All types of extracts of differently cooked vegetable samples exhibited decreased tannin content over their respective raw counterparts. The extent of the decrease was maximum in ME of microwaved sample (79.6%) followed by ME of fried (67.3%) and EE of pressure cooked samples (61.2%).

Table 4.22 summarises the total carotenoids content of raw and cooked vegetable of L. cylindrica measured as β carotene. The average value of carotenoids content of cooked vegetable samples regardless of the extraction solvent ranged from 15.0 to 18.2 mg β-carotene/100g (dwb) when compared to carotenoids content of 9.2 mg β-carotene/100g (dwb) in the raw vegetable sample. Pressure cooking was the most effective cooking treatment in retaining the carotenoids (97.8% increase) followed by frying (85.9% increase) and microwave cooking (63.0% increase). Butanol was found to be most...
Table 4.21 Total phenols, flavonoids, and tannins content of various extracts of cooked vegetable of *Z. cylindrica*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenols (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannin content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>216.4±2.09&lt;sup&gt;A&lt;/sup&gt;</td>
<td>300.1±2.51&lt;sup&gt;B,C&lt;/sup&gt;</td>
<td>275.1±2.62&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>339.4±4.17&lt;sup&gt;B&lt;/sup&gt;</td>
<td>311.0±5.60&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>258.7±3.89&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>219.6±2.35&lt;sup&gt;B&lt;/sup&gt;</td>
<td>207.2±3.01&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>178.7±2.58&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>368.6±4.79&lt;sup&gt;A&lt;/sup&gt;</td>
<td>441.9±2.66&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>490.3±2.89&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>286.0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>315.1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>300.7&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 216 at df = 2, p<0.000
F-Statistics for extraction solvents = 20822.3 at df = 2, p<0.000
F-Statistics for cooking treatments = 7753.4 at df = 3, p<0.000
F-Statistics for cooking treatments = 93918.3 at df = 3, p<0.000
F-Statistics for cooking treatments & extraction solvents = 585.1 at df = 6, p<0.000
F-Statistics for cooking treatments & extraction solvents = 1360.1 at df = 6, p<0.000
F-Statistics for cooking treatments & extraction solvents = 20822.3 at df = 2, p<0.000
F-Statistics for cooking treatments & extraction solvents = 93918.3 at df = 3, p<0.000
F-Statistics for cooking treatments & extraction solvents = 585.1 at df = 6, p<0.000
F-Statistics for cooking treatments & extraction solvents = 1360.1 at df = 6, p<0.000
F-Statistics for cooking treatments & extraction solvents = 20822.3 at df = 2, p<0.000
F-Statistics for cooking treatments & extraction solvents = 93918.3 at df = 3, p<0.000
F-Statistics for cooking treatments & extraction solvents = 585.1 at df = 6, p<0.000
F-Statistics for cooking treatments & extraction solvents = 1360.1 at df = 6, p<0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.
GE= gallic acid equivalent, QE= quercetin equivalent, CE= catechin equivalent
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
### Table 4.22 Carotenoids content of various extracts of cooked vegetable of *L. cylindrica*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoid content (mg β-carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>4.6 ± 0.10^A^</td>
<td>10.0 ± 0.16^B^</td>
<td>12.9 ± 0.21^AC^</td>
<td>9.2^a^</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>11.0 ± 0.21^C^</td>
<td>12.4 ± 0.22^BB^</td>
<td>31.3 ± 0.16^AC^</td>
<td>18.2^d^</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>6.0 ± 0.13^BA^</td>
<td>16.9 ± 0.19^BB^</td>
<td>22.2 ± 0.13^C^</td>
<td>15.0^b^</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>14.7 ± 0.17^dA^</td>
<td>17.0 ± 0.21^dC^</td>
<td>19.7 ± 0.07^dC^</td>
<td>17.1°</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>9.1^A^</td>
<td>14.1^B^</td>
<td>21.5^C^</td>
<td></td>
</tr>
</tbody>
</table>

*Values are presented as mean ±SD (n=3) and referred to the dry weight.*

F-Statistics for extraction solvents = 16274.4 at df = 2, p=0.000

F-Statistics for cooking treatments = 5039.1 at df = 3, p=0.000

F-Statistics for cooking treatments & extraction solvents = 2206.3 at df = 6, p=0.000

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract.

Efficient solvent for extracting the carotenoids (21.5 mg β-carotene/100g, dwb) from cooked vegetable samples. Nonetheless, in terms of interactive effects of cooking treatments and the extraction solvents there was a significant interaction effect (F= 2206.3 at df= 6, p= 0.000). Different extracts of the pressure cooked sample revealed an increased recovery of carotenoids in comparison to their raw counterparts and this recovery was maximum in BE (142.6 % increased) follow by ME (139.1% increased) and EE (24% increased). However, in microwave cooking most abrupt retention in the carotenoid content was reported in BE followed by EE and ME as compared to their raw vegetable sample.

### 4.7.2 Total antioxidant activity as measured by using different assays is as given in table 4.23

#### 4.7.2.1 Percent inhibition as measured by FTC method

The extracts of different samples of *L. cylindrica* inhibited 6.1-35.4% peroxidation of linoleic acid after an incubation period of 96h as measured by FTC assay. However, the antioxidant activity of all the extracts in terms of measurement of percent inhibition
was significantly (p<0.05) lower than synthetic antioxidant BHT (88.8%) and vitamin E (74.6%). Irrespective of cooking treatments, significant differences (F= 777.1 at df= 2, p=0.000) were observed in percent inhibition and it was found maximum in ME (25.6%) followed by EE (20.2%) and BE (14.3%). Regardless of extraction solvents, the percent inhibition was observed to be inconsistent after various cooking treatments and it was found to be superior in pressure cooked and fried samples in comparison to raw sample. The interactive effect showed that percent inhibition in pressure cooked sample was significantly higher (p<0.05) in ME and EE by 169.4% and 3.9% respectively, while it was lower by 66.1% in BE as compared to raw vegetable extracts. The extracts of microwaved samples showed decreased peroxidation inhibition as compared to their raw counterparts. However, in case of fried sample, all the extracts revealed an increased percent inhibition in comparison to their raw counterparts.

4.7.2.2 Percent inhibition as measured by TBA method

Regardless of cooking treatments, the percent inhibition by various extracts followed the same order as measured by TBA assay. The main effect of the cooking methods was although significant (F= 2844.5 at df= 3, p= 0.000) but the contradictory results were observed after different cooking treatments. The interactive effects of cooking treatments and extracting solvents showed that the EE of raw sample was most effective in inhibiting the oxidation. However, in cooked samples, the maximum increased level of inhibition was revealed by ME of pressure cooked sample followed by ME of fried and EE of fried sample. In general, frying was most effective in increasing the percent inhibition by various extracts of L. cylindrica. After frying the percent inhibition of various extracts varied from 38.6 to 44.6% in comparison to 25.8 to 29.0% of various extracts of raw sample.

4.7.2.3 Ferric reducing antioxidant power (FRAP)

Significant differences were observed in the FRAP values among all the cooking treatments in their respective extracts (p<0.05). The average FRAP values as shown by various extracts regardless of cooking treatments varied from 905.5 to 1313.0 μM FeSO₄/100g being highest in EE followed by BE and ME (F= 626.1 at df = 2, p=0.000). Regardless of extraction solvents, the significant effect of cooking on ferric reducing power was observed when compared to raw vegetable sample and it was found in the order of fried > pressure cooked > microwave cooked (F= 2950 at df= 3, p= 0.000
Table 4.23 Antioxidant activity of various extracts of cooked vegetable of *L. Cylindrica* as determined by FTC, TBA and FRAP assays

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP µM FeSO₄/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.7±0.58&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>17.9±0.53&lt;sup&gt;B&lt;/sup&gt;</td>
<td>18.6±0.90&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>39.6±0.73&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>18.6±0.55&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.3±0.54&lt;sup&gt;AC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.8±0.81&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>15.6±0.54&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>6.1±0.87&lt;sup&gt;AC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.4±0.87&lt;sup&gt;C&lt;/sup&gt;</td>
<td>28.6±0.54&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.3±0.76&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>25.6&lt;sup&gt;C&lt;/sup&gt;</td>
<td>20.2&lt;sup&gt;B&lt;/sup&gt;</td>
<td>14.3&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 777.1 at df = 2, p=0.000  
F-Statistics for cooking treatments = 1121.5 at df = 3, p=0.000  
F-Statistics for cooking treatments & extraction solvents = 417.6 at df = 6, p=0.000  

Values are presented as mean ±SD (n=3) and referred to the dry weight.  
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.  
FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16  
TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19  
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
The interaction effect of cooking treatments and the extraction solvents was also significant (F = 346.5 at df = 6, p = 0.000). The effect of pressure cooking was observed to be inconsistent in regard to their reducing power as ME was more effective to retain the antioxidant activity than the EE and BE as compared to their respective raw vegetable. As compared to the raw samples, all the extracts of fried samples were more effective with respect to their reducing power and the increased FRAP value was in order of BE > ME > EE.

4.7.2.4 DPPH free radical scavenging activity

The percent scavenging activity of 30 μg/ml ascorbic acid (92.0%) was observed to be highest in respect to various raw and cooked samples of L. cylindrica (Table 4.24). The percent scavenging activity of the raw samples irrespective of extracting solvents was 12.1%, which varied from 5.7 to 20.5% in cooked samples, suggesting that the effect of different heat processing treatments on the percent scavenging was highly significant (F = 1693.3 at df = 3, p = 0.000) but inconsistent. Regardless of cooking treatments, the percent scavenging at 30 μg/ml of various extracts was in order of BE > EE > ME. The interaction effect of heat processing treatments and the extraction solvents was also significant (F = 751.8 at df = 6, p = 0.000) and the results showed that percent scavenging activity of pressure cooked and microwave cooked samples was significantly higher (p<0.05) in their EE and BE in comparison to the respective extracts of raw vegetable. However, the fried sample exhibited decreased scavenging activity in all types of extracts over their raw counterparts.

The percent scavenging at various concentrations are presented in Appendix-III. Table 4.24 summarises the results of IC50 values of raw and cooked vegetable samples of L. cylindrica. Regardless of the extraction solvent, free radical scavenging activity as assessed by IC50 values decreased by 14.3% and 52.9% after pressure cooking and microwave cooking respectively, whereas it increased by 111.9% after frying. Regardless of cooking treatments the average IC50 value was observed to be highest in ME (212.6 μg/ml) followed by BE (200.5 μg/ml) and EE (164.4 μg/ml). Nevertheless, the IC50 values of all extracts were significantly (p<0.05) higher than ascorbic acid (5.05 μg/ml).

4.7.3 Correlation studies

The results of correlation studies showed highly significant positive correlations of FTC assay with total phenols (r=0.711, p<0.01) (Table 4.25) suggesting that phenol
Table 4.24 Free radical scavenging activity of various extracts of cooked vegetable of *L. Cylindrica* as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH ( % Scavenging at 30 µg/ml )</th>
<th>IC-50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>20.5±0.23&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.6±0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>8.1±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.9±0.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>12.4±0.26&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>19.1±0.07&lt;sup&gt;bd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>5.4±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4±0.19&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD (n=3)

F-Statistics for extraction solvents = 133.4 at df = 2, p=0.000  
F-Statistics for cooking treatments = 1693.3 at df = 3, p=0.000  
F-Statistics for cooking treatments & extraction solvents = 751.8 at df = 6, p=0.000  
% Scavenging of ascorbic acid at 30 µg/ml=92.0 ± 0.06

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract  
IC<sub>50</sub> value of ascorbic acid=5.05µg/ml

may be the main phytochemical responsible for the oxidation of linolic acid. Similarly, the positive correlation was observed between percent inhibition (as measured by TBA assay) and phenol content (r= 0.737, p<0.01) indicating that the high concentration of phenol inhibits malonaldehyde formation and was responsible for the antioxidant activity of *L. cylindrica*. Interestingly, a strong correlation (r= 0.989, p<0.01) between FTC and TBA assay showed that the increase in peroxide level caused the formation of malonaldehyde compounds. A highly significant and positive correlation of the FRAP value with phenols content (r= 0.909, p<0.01) and flavonoids content (r = 0.702, p<0.01) concluded that phenols and flavonoids may be the main compounds responsible for the reduction the ferrous ions into ferric ions in their respective extracts. Moreover, a highly significant and positive correlation of the DPPH value with phenol content (r= 0.880, p<0.01) and flavonoids content (r= 0.756, p<0.01) indicated that the change in free radical scavenging activity of heat processed sample may be due to the change in phenol and flavonoids content after the cooking treatments.
Table 4.25 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity

<table>
<thead>
<tr>
<th></th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>.811**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>.365</td>
<td>.296</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>.117</td>
<td>.437</td>
<td>.443</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>.711**</td>
<td>.471</td>
<td>.317</td>
<td>-.271</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>.737**</td>
<td>.478</td>
<td>.378</td>
<td>-.241</td>
<td>.989*</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>.909**</td>
<td>.702*</td>
<td>.378</td>
<td>.119</td>
<td>.562</td>
<td>.600*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>.880**</td>
<td>.756**</td>
<td>.224</td>
<td>-.039</td>
<td>.712**</td>
<td>.740**</td>
<td>.783**</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Significantly correlated at $p<0.05$, n=12
** Significantly correlated at $p<0.01$, n=12

4.7.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

The Rf value of different phenolic acids and flavonoid standard compounds in three different solvent systems are presented in Appendix-IV. The distribution of phenolic acids and flavonoids in ME of L. cylindrica is presented in table 4.26 and Fig. 4.4 (A-C). Gallic acid (26.8 µg/ml) as a phenolic acid was identified only in the raw sample extract. Caffeic acid (18.4 µg/ml) and cinnamic acid (8.6 µg/ml) were reported in raw vegetable sample of L. cylindrica and its concentration decreased in all the cooked sample extracts. The ferrulic acid (49.6 µg/ml) was detected in raw sample extract and its retention was observed after pressure cooking and microwave cooking. Ellagic acid (78.8 µg/ml) and rutin (79.3 µg/ml) were observed as most prominently present phenolic acid in pressure cooked samples. Myricetin (35.8 µg/ml) was detected in raw sample extracts and its retention was observed after microwave cooking (20.9 µg/ml) and frying (30.9 µg/ml). Quercetin was although not identified in the raw sample extract, but it was detected in microwave cooked (55.4 µg/ml) and fried (45.2 µg/ml) samples. Interestingly, catechin was newly formed compounds in the cooked samples as it was not identified in raw sample of L. cylindrica.
Table 4.26 Identification and quantification of phenolic acids and flavonoids (µg/ml of extract) in ME of *L. cylindrica* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>26.85</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>18.43</td>
<td>6.87</td>
<td>1.04</td>
<td>0.23</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>49.62</td>
<td>9.31</td>
<td>27.22</td>
<td>nd</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>8.63</td>
<td>7.74</td>
<td>6.50</td>
<td>1.52</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>nd</td>
<td>78.84</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>nd</td>
<td>nd</td>
<td>55.42</td>
<td>45.18</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>35.79</td>
<td>nd</td>
<td>20.95</td>
<td>30.89</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Leutolin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>nd</td>
<td>79.32</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>62.24</td>
<td>77.87</td>
<td>57.94</td>
</tr>
</tbody>
</table>

nd= not detected

4.8 Antioxidant potential of ridge gourd (*Luffa acutangula*)

4.8.1 Quantitative estimation of phytochemicals

The results of quantitative testing of phytochemicals including TPC, flavonoids and tannin contents are shown in table 4.27. The TPC of various extracts regardless of the cooking treatments was in the order of BE > ME > EE. F- value statistics (F= 431 at df= 2, p= 0.000) also indicated highly significant impact of extraction solvents on the yield of total phenols. The average value of TPC irrespective of extracting solvents increased in pressure cooked sample, whereas it decreased after microwave cooking and frying. Significant interaction effect (F= 2267 at df= 6, p= 0.000) of cooking treatments and extraction solvent showed that the TPC of a particular sample depend on nature of solvent. The effect of pressure cooking was observed to be inconsistent in regard to the recovery of total phenols in various extracts as EE showed increased concentration of TPC, contrary to decreased concentration in ME and BE, when compared to their raw counterparts. Different extracts of microwave cooked samples revealed reduction in recovery of TPC as compared to their raw counterparts. However, the effect of frying on the recovery of TPC in various solvents was also inconsistent.
Fig. 4.4A HPTLC profile of ME of *L. cylindrica* as developed in chloroform: hexane: methanol: formic acid (6:4:3:9; 2:0:5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.4B HPTLC profile of ME of *L. cylindrica* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.4C HPTLC profile of ME of *L. cylindrica* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
The average flavonoids content of the raw samples irrespective of extracting solvents was 42.0 mg QE/100g (dw basis), whereas in cooked samples this value varied from 33.2 to 60.9 mg QE/100g (dwb) (Table 4.27) suggesting that the effect of different heat processing treatments on the flavonoids content was although significant (p<0.05) but inconsistent as microwave cooking decreased the flavonoids content whereas pressure cooking and frying resulted in an increase in the flavonoids content. The main effect of extracting solvents was also significant (F= 779.4 at df= 2, p= 0.000) and the flavonoids content of various extracts irrespective of the heat processing treatments was in order of BE > ME > EE. The interactive effect of heat processing treatments and the extraction solvents was also significant (F= 5852.7 at df= 6, p= 0.000). Ethanol was observed to be most effective solvent for extracting out maximum yield of flavonoids from pressure cooked sample. All the solvents were observed to be inferior in extracting the flavonoids from microwave cooked sample. The recovery of flavonoids was observed to increase in ME and EE of fried samples in contrast to decreased recovery of flavonoids in BE when compared to respective extract of the raw vegetable sample. In case of fried sample, methanol was found to be most effective solvent for extracting flavonoids.

Table 4.27 summarizes the tannin contents of cooked vegetables of L. acutangula. The average tannin content in the cooked samples irrespective of the extraction solvent varied from 10.7 to 24 mg CE/100g incompared to the tannin content of 9.0 mg CE/100g (dwb) in raw sample suggesting the increasing effect of heat processing treatments on tannin content. The microwave cooking exerted most pronounced increasing effect on the tannin content followed by frying and pressure cooking. Among all the extracts, butanol was found to be the most effective solvent for extracting tannins. The significant interaction effect (F= 41.9 at df= 6, p= 0.000) of the heat processing treatments and the extraction solvents showed about 13 fold and 6 fold increase in the tannin content of ME of microwaved and fried vegetable samples respectively over their respective native counterparts. However, this increasing effect was not consistent in other cooked samples and BE of pressure cooked and EE of microwaved vegetable samples revealed a decreased recovery of tannins in comparison to their respective raw counterparts.

The carotenoids content of raw and cooked samples of L. acutangula measured as B carotene are presented in table 4.28. The average value of carotenoids content of cooked vegetable samples regardless of the extraction solvent ranged from 12.6 to 16.8 mg B carotene /100g (dwb) in comparison to an amount of 2.8 mg B carotene /100g (dwb) in
Table 4.27 Total phenols, flavonoids, and tannins content of various extracts of cooked vegetable of *L. acutangula*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenols (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannin content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>228.7±1.77&lt;sup&gt;B&lt;/sup&gt;</td>
<td>156.3±2.02&lt;sup&gt;A&lt;/sup&gt;</td>
<td>251.1±1.19&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>174.8±3.35&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>273.7±3.75&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>221.1±2.50&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>114.1±1.35&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>121.8±1.89&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>203.4±1.06&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>262.8±2.11&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>167.4±1.84&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>146.2±1.15&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean 195.1<sup>B</sup> 179.8<sup>A</sup> 205.5<sup>C</sup> 44.8<sup>B</sup> 41.3<sup>A</sup> 50.7<sup>C</sup> 18.0<sup>B</sup> 10.5<sup>A</sup> 21.1<sup>C</sup>

F-Statistics for extraction solvents = 431 at df = 2, p = 0.000
F-Statistics for cooking treatments = 2223.4 at df = 3, p = 0.000
F-Statistics for cooking treatments & extraction solvents = 2267 at df = 6, p = 0.000

Values are presented as mean ± SD (n = 3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p < 0.05) among cooking methods and extracts respectively.

GE = gallic acid equivalent, QE = quercetin equivalent, CE = catechin equivalent
ME = methanolic extract, EE = ethanolic extract, BE = butanolic extract
the raw vegetable sample. The results showed significant (p<0.05) increase in carotenoids recovery from cooked samples and this increased recovery was in order of microwave cooked > pressure cooked > fried sample. Among all the solvents, butanol was found to be most efficient for extracting the carotenoids (18.2 mg β carotene /100g, dwb) followed by ethanol and methanol. The interaction effect of heat processing treatments and the extraction solvents was highly significant (F= 19282.5 at df= 6, p= 0.000). The maximum carotenoids content in raw sample was found in its BE whereas, in case of cooked samples, BE of microwaved followed by BE of pressure cooked and ME of fried samples respectively showed the highest content of carotenoids with quantum of increase ranging from 556 to 874% over their respective native counterparts.

4.8.2 The antioxidant activity as measured by using FTC, TBA, FRAP and DPPH radical scavenging methods

4.8.2.1 Percent inhibition as measured by FTC method

Antioxidant activity of ME, EE and BE of raw and cooked samples of *L. acutangula* in terms of measurement of inhibition of peroxidation is as shown in table 4.29. The results indicated the possible influence of extracting solvent on percent inhibition but not all in the same way. The various extracts of different samples of *L. acutangula* inhibited 7.3-36.4% peroxidation of linoleic acid after incubation for 96h. Nevertheless, the antioxidant activity of all extracts, in terms of measurement of percentage inhibition was significantly (p<0.05) lower than BHT (88.8%) and vitamin E (74.6%). In general, ME revealed significantly higher antioxidant activity followed by BE and EE (F= 2294.9 at df= 2, p= 0.000). Regardless of extraction solvent, the percent inhibition was observed to decrease after various cooking treatments in comparison to raw sample and maximum reduction was measured in microwave cooked sample followed by pressure cooked and fried sample. The interaction effect of cooking treatments and the extraction solvents was also significant (F= 107.7 at df= 6, p= 0.000). The results showed that percent inhibition by the extracts of sample, pressure cooking was significantly higher (p<0.05) in EE, while it was observed to be lower in ME and BE when compared to raw *L. acutangula*. All the three types of extracts of microwave cooked samples revealed decreased percent inhibition in comparison to their respective raw vegetable samples. However, the frying exhibited significantly (p<0.05) increased value of peroxide inhibition in all types of extracts except the BE where a decrease of 56.5% was observed when compared to raw counterparts.
Table 4.28 Carotenoids content of various extracts of cooked vegetable of *L. acutangula*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoid content (mg β-Carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>2.5 ± 0.05ᵃᴬ</td>
<td>2.3 ± 0.10ᵃᴬ</td>
<td>3.5 ± 0.05ᵇᴮ</td>
<td>2.8ᵃ</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>5.2 ± 0.12ᵇᴬ</td>
<td>15.1 ± 0.14ᵈᴮ</td>
<td>24.6 ± 0.07ᶜᶜ</td>
<td>15.0ᶜ</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>5.3 ± 0.07ᵇᴬ</td>
<td>10.9 ± 0.09ᵇᴮ</td>
<td>34.1 ± 0.18ᵈᶜ</td>
<td>16.8ᵈ</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>17.2 ± 0.15ᶜᶜ</td>
<td>10.0 ± 0.07ᶜᴬ</td>
<td>10.5 ± 0.09ᵇᴮ</td>
<td>12.6ᵇ</td>
</tr>
</tbody>
</table>

Mean: 7.5ᵃ 9.6ᵇ 18.2ᶜ

Values are presented as mean ±SD (n=3) and referred to the dry weight.
F-Statistics for extraction solvents = 33993.6 at df = 2, p=0.000
F-Statistics for cooking treatments = 31415.4 at df= 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 19282.5 at df= 6, p=0.000

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

4.8.2.2 Percent inhibition as measured by TBA method

Regardless of cooking treatments, ME revealed highest percent inhibition (42.2%) followed by BE (26.4%) and EE (24.3%). F- value statistics (F= 6312.2 at df= 2, p= 0.000) as given in table 4.29 also indicated highly significant impact of extraction solvent on the percent inhibition suggesting marked influence of solvent on the extraction of phytochemicals. The value of percent inhibition irrespective of extracting solvents decreased after all the cooking treatments. However, in terms of interactive effect of cooking treatments and extracting solvents, various cooking treatments exerted contradictory effect on the percent inhibition. Among three extracts ME of raw vegetable showed maximum percent inhibition level. In case of cooked samples maximum decrease in percent inhibition over the respective extracts of raw counterparts was observed in BE of fried, microwaved and pressure cooked sample respectively.

4.8.2.3 Ferric reducing antioxidant power (FRAP)

As indicated by the results of FRAP assay given in table 4.29, significant differences were observed in the FRAP values among all the cooking treatments in their respective extracts (p<0.05). The average FRAP values as shown by various extracts regardless of cooking methods varied from 430.7 to 506.7 μM FeSO₄/100g being highest.
Table 4.29 Antioxidant activity of various extracts of cooked vegetable of *L. acutangula* as determined by FTC, TBA and FRAP assays

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP μM FeSO₄/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>35.4±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.9±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.9±0.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>29.3±0.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>13.8±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.1±0.84&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>29.9±0.70&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.3±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.4±1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>36.4±1.00&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.2±0.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>10.4±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>32.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 2994.9 at df = 2, p<0.000
F-Statistics for cooking treatments = 122.2 at df = 3, p<0.000
F-Statistics for cooking treatments & extraction solvents = 107.7 at df = 6, p<0.000

Mean ± SD (n=3) are reported. Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16
TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19
ME= methanolic extract, EE= ethanolic extract, BE= buatanolic extract.
in BE followed by EE and ME (F= 66.7 at df= 2, p= 0.000). Irrespective of extraction solvent, the ferric reducing power was observed to increase after pressure cooking in contrast to decreased reducing power of fried and microwaved samples in comparison to raw sample extracts. The significant interaction effect of heat processing treatments and the extraction solvents (F= 454.6 at df= 6, p= 0.000) indicated inconsistent effect of various cooking treatments on the FRAP value of different extracts.

4.8.2.4 DPPH free radical scavenging activity

The percent scavenging activity irrespective of extracting solvents was found to increase after various cooking treatments (Table 4.30). Regardless of cooking treatments the significant (p<0.05) effect of extracting solvents was observed and the maximum value was observed in ME followed by EE and BE. However, the scavenging activity of all the extracts was observed to be lower in comparison to ascorbic acid (92.0%) with same concentration (30 µg/ml).

The IC50 value was calculated from percent scavenging at different concentration as presented in Appendix-III. The IC50 values of various extracts of L. acutangula in raw as well as heat processed forms are presented table 4.30. The average free radical scavenging activity as assessed by IC50 values regardless of the extraction solvent decreased by 7.4% after pressure cooking, 46.3% after microwave cooking and 17.5% after frying. Regardless of cooking treatments the average IC50 value was observed to be highest in EE (375.7 µg/ml) followed by BE (338.3 µg/ml) and ME (308.8 µg/ml). Various extracts of raw vegetable sample showed IC50 value ranging from 275.6 to 658.4 µg/ml. The effect of pressure cooking was inconsistent in respect to their IC50 value of various extracts as EE of pressure cooked sample showed increased IC50 values in contrast to decreased IC50 in ME and BE, when compared to their raw counterparts. Nonetheless, all the extracts of microwave cooked sample showed decreased IC50 value in comparison to their respective extracts of raw sample. The IC50 value was observed to increase in ME (50.2%) and EE (45.1%) of fried sample in contrast to decreased IC50 value in BE (75.6%), when compared to the respective extracts of the raw vegetable sample.

4.8.3 Correlation studies

Statistical correlations have been studied between phytochemicals content and antioxidant activity determined by different assays as shown in table 4.31.

129
Table 4.30 Free radical scavenging activity of various extracts of cooked vegetable of *L. acutangula* as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH (% Scavenging at 30 µg/ml)</th>
<th>IC-50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>14.7±0.06&lt;sup&gt;C&lt;/sup&gt;</td>
<td>9.9±0.07&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>18.4±0.11&lt;sup&gt;B&lt;/sup&gt;C</td>
<td>5.3±0.13&lt;sup&gt;B&lt;/sup&gt;A</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>19.7±0.13&lt;sup&gt;C&lt;/sup&gt;C</td>
<td>7.8±0.26&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>14.6±0.13&lt;sup&gt;C&lt;/sup&gt;C</td>
<td>4.7±0.07&lt;sup&gt;BA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>9.3&lt;sup&gt;C&lt;/sup&gt;C</td>
<td>5.2&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD (n=6).
F-Statistics for extraction solvents = 13507.1 at df = 2, p=0.000
F-Statistics for cooking treatments = 2059.4 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 1627.1 at df = 6, p=0.000
% Scavenging of ascorbic acid at 30 µg/ml= 92.0 ± 0.06

Table 4.31 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity

<table>
<thead>
<tr>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td>0.801**</td>
<td>-0.354</td>
<td>0.269</td>
<td>0.337</td>
<td>0.359</td>
<td>0.916**</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td>1.000</td>
<td>-0.354</td>
<td>0.406</td>
<td>0.252</td>
<td>0.254</td>
<td>0.813**</td>
</tr>
<tr>
<td>Tannin</td>
<td></td>
<td></td>
<td></td>
<td>0.172</td>
<td>0.019</td>
<td>-0.043</td>
<td>0.995**</td>
</tr>
<tr>
<td>Carotenoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.306</td>
<td>-0.304</td>
<td>1.000</td>
</tr>
<tr>
<td>FTC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.250</td>
<td>0.163</td>
</tr>
<tr>
<td>TBA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.195</td>
</tr>
<tr>
<td>FRAP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>DPPH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.792**</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=12
**Significantly correlated at p<0.01, n=12

A highly significant and positive correlation of the FRAP value with phenols content (r= 0.916, p<0.01) and flavonoid content (r= 0.813, p<0.01) indicated that phenols and flavonoids might be the main compounds responsible for reduction the ferrous ions into ferric ions in their respective extracts. Similarly, a highly significant and positive correlation of the DPPH value with phenol (r= 0.735, p<0.01) and flavonoids content (r=
concluded that the change free radical scavenging activity of cooked sample may be due to the change in phenol and flavonoids content after the cooking process. Interestingly, a strong correlation \( r = 0.995, p < 0.01 \) between FTC and TBA assay showed that the increase in peroxide level caused the formation of malonaldehyde compounds.

4.8.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

The Rf value of different phenolic acids and flavonoids standard compounds with three different solvent systems are presented in Appendix-IV. The distribution of phenolic acids and flavonoids in ME of *L. acutangula* is presented in table 4.32 and Fig. 4.5(A-C). Different phenolic acids identified and quantified were benzoic acid (1817.6 \( \mu \)g/ml) in raw sample extract; vanillic acid (60.3 \( \mu \)g/ml), ellagic acid (17.3 \( \mu \)g/ml), rutin (383.3 \( \mu \)g/ml) and catechin (101.2 \( \mu \)g/ml) in pressure cooked sample extract; vanillic acid (43.9 \( \mu \)g/ml), kaempferol (1.4 \( \mu \)g/ml), rutin (352.3 \( \mu \)g/ml) and catechin (273.8 \( \mu \)g/ml) in microwaved sample extract and ferulic acid (1.5 \( \mu \)g/ml), ellagic acid (19.4 \( \mu \)g/ml), kaempferol (1.3 \( \mu \)g/ml) and rutin (363.3 \( \mu \)g/ml) in the fried sample extract. The vegetable had most destructive effect on benzoic acid as it was only identified in raw sample of *L. acutangula* and there was no retention of this phenolic acid in the cooked samples. However, new phenolics like vanillic acid and catechin were observed after different cooking treatments. The vanillic acid and catechin were in pressure cooked and microwave cooked samples. Ferulic acid (1.5 \( \mu \)g/ml) was observed only in fried sample extract. Ellagic acid was although not identified in the raw sample extract, but it was identified in pressure cooked and fried samples. Nevertheless, kaempferol was observed after microwave cooking and frying in weak concentration as compared to other phenolics present in various extracts of *L. acutangula*. Interestingly, the rutin was newly formed compounds observed after all the cooking treatments.
Table 4.32 Identification and quantification of phenolic acids and flavonoids (µg/ml of extract) in ME of *L. acutangula* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>nd</td>
<td>16.30</td>
<td>43.99</td>
<td>nd</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>1.54</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>1817.56</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>nd</td>
<td>17.29</td>
<td>nd</td>
<td>19.40</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>nd</td>
<td>nd</td>
<td>1.37</td>
<td>1.33</td>
</tr>
<tr>
<td>Leutolin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>nd</td>
<td>383.33</td>
<td>352.34</td>
<td>363.26</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>101.16</td>
<td>273.78</td>
<td>nd</td>
</tr>
</tbody>
</table>

*nd* = not detected
Fig. 4.5A HPTLC profile of ME of *L. acutangula* as developed in chloroform: hexane: methanol: formic acid (6.4: 3.9: 2.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.5B HPTLC profile of ME of *L. acutangula* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.5C HPTLC profile of ME of *L. acutangula* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried.
4.9 Antioxidant potential of pointed gourd (*Tricosanthes dioica*)

4.9.1 Quantitative estimation of phytochemicals

The TPC of raw and cooked vegetable of *T. dioica* was expressed as milligram of gallic acid equivalents per 100 gram of dry *T. dioica* (mg GE/100g dwb) and the values are presented in table 4.33. Regardless of cooking treatments, different solvent extracts showed significant (F= 84.1 at df= 2, p= 0.000) difference in their TPC, which was in the order of ME > EE > BE. Irrespective of extraction solvents, the effect of various cooking methods was inconsistent as pressure cooking decreased the TPC, whereas a marginal decrease in TPC was observed in the fried sample. In contrary, the increased in TPC was measured in fried sample. The interactive effect of cooking treatments and extracting solvents was also significant (F= 745.2 at df= 6, p= 0.000). ME of the raw vegetable sample showed the maximum amount of total phenols, whereas the maximum recovery of total phenols in case of cooked sample was found in ME of microwaved sample followed by EE of fried and BE of fried sample respectively. However, the most pronounced effect of heating on TPC was observed in case of frying as observed in BE of fried sample (44.2% increase).

The flavonoids content was expressed in terms of quercetin equivalent (QE). As indicated in table 4.33, the average flavonoids content of the raw sample irrespective of extracting solvents was 30.2 mg QE/100g (dwb), whereas in cooked samples this value varied from 22.2 to 46.6 mg QE/100g (dw basis) suggesting that the effect of different heat processing treatments on the flavonoids content was significant (F= 4511.1 at df= 3, p= 0.000) and the inconsistent effect on the recovery of flavonoids was observed after various cooking treatments. Regardless of the cooking treatments, different extracting solvents showed significant (F= 414.2 at df= 2, p= 0.000) differences in their flavonoids content being highest in the BE and lowest in ME. The interaction effect of cooking treatments and the extraction solvents was also significant (F= 1899.8 at df= 6, p= 0.000). The BE of the raw vegetable sample revealed maximum recovery of flavonoids, whereas in case of cooked vegetable sample BE of fried sample followed by BE of microwaved and EE of fried sample showed the maximum recovery of total flavonoids, which showed an increase up to 99.2% in recovery when compared to raw counterparts.

The tannin content of raw and cooked vegetable of *T. dioica* expressed in terms of catechin equivalent (CE) is shown in table 4.33. Average tannin content in the cooked
Table 4.33 Total phenols, flavonoids, and tannins content of various extracts of cooked vegetable of *T. dioica*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenols (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannin content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>163.2±1.53&lt;sup&gt;dc&lt;/sup&gt;</td>
<td>118.2±1.11&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>100.6±1.00&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>97.7±1.85&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>125.3±1.65&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>110.4±3.21&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>156.0±2.25&lt;sup&gt;bC&lt;/sup&gt;</td>
<td>98.7±1.89&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>125.6±3.06&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>104.4±1.21&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>154.8±0.60&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>145.1±1.46&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>130.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>124.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>120.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 84.1 at df = 2, p<0.000  
F-Statistics for cooking treatments = 248.7 at df = 3, p<0.000  
F-Statistics for cooking treatments & extraction solvents = 745.2 at df = 6, p<0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.  
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.  
GE = gallic acid equivalent, QE = quercetin equivalent, CE = catechin equivalent.  
ME = methanolic extract, EE = ethanolic extract, BE = butanolic extract.
samples irrespective of the extraction solvents varied from 10.0 to 12.5 mg CE/100g when compared to the tannin content of raw sample (12.3 mg CE/100g) suggesting the inconsistent effect of cooking treatments on tannin content. The main effect of extraction solvent was significant (F= 18.8 at df= 2, p= 0.01). Among all the extracts, butanol was found to be most effective solvent for extracting tannins followed by ethanol and methanol. The significant interaction effect (F= 39.1 at df= 6, p= 0.000) of the heat processing treatments and the extraction solvents showed that butanol, ethanol, methanol and again butanol were the most effective solvents in extracting out the tannin from the raw, pressure cooked, microwave cooked and fried vegetable samples respectively.

The carotenoids content of raw and cooked samples of T. dioica measured as β-carotene is presented in table 4.34. The average value of carotenoid content of the cooked vegetable sample regardless of the extraction solvents ranged from 3.3 to 8.3 mg β-carotene/100g (dw) in comparison to the carotenoid content of 3.4 mg β-carotene/100g (dw) in the raw vegetable sample. Irrespective of extraction solvents, microwave cooking was the most effective treatment in retaining the carotenoids (144.1% increase) followed by frying (8.8% increase) and pressure cooking (2.9% decrease). The significant effect of extraction solvents was observed on the carotenoids concentration of extracts (F= 3256.7 at df= 2, p= 0.000). Butanol was found to be most efficient for extracting the carotenoids (6.7 mg β-carotene/100g) followed by ethanol and methanol. The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 3959 at df = 6, p=0.000). The recovery of carotenoids in different extracting solvents was found to be inconsistent after various cooking treatments. The EE of fried sample followed by BE and EE of the microwaved samples showed the maximum enhanced recovery of carotenoids when compared to their respective raw counterparts.

4.9.2 The antioxidant activity as measured by using FTC, TBA, FRAP and DPPH radical scavenging methods

4.9.2.1 Percent inhibition as measured by FTC method

The FTC method was used to measure the inhibition of linoleic acid oxidation by the ME, EE and BE of T. dioica as affected by different cooking treatments (Table 4.35). The results pointed out the possible influence of extracting solvent on percent inhibition but not all in the same manner. The various extracts of different samples of T. dioica inhibited 9.9-38.7% peroxidation of linoleic acid after incubation for 96h.
### Table 4.34 Carotenoids content of various extracts of cooked vegetable of *T. dioica*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoid content (mg β-Carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>3.8 ± 0.06&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>1.4 ± 0.04&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>5.0 ± 0.09&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>1.7 ± 0.08&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>3.9 ± 0.14&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>4.3 ± 0.09&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>4.8 ± 0.07&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>4.1 ± 0.15&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>16.2 ± 0.26&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>8.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>2.2 ± 0.06&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>7.5 ± 0.07&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>1.3 ± 0.02&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;B&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD (n=3) and referred to the dry weight.

F-Statistics for extraction solvents = 3256.7 at df = 2, p=0.000

F-Statistics for cooking treatments = 4279.5 at df = 3, p=0.000

F-Statistics for cooking treatments & extraction solvents = 3959 at df = 6, p=0.000

Small and capital capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract.

This wide variation in the inhibition level could be because of differences in the type and concentration of antioxidative compounds in these extracts. The present data were also compared with that of the synthetic antioxidant BHT and vitamin E (reference compounds), which exhibited higher inhibition of linoleic acid oxidation i.e. 88.8% and 74.6% respectively. In general, ME revealed significantly higher antioxidant activity followed by BE and EE (F= 1565.5 at df = 2, p=0.000). Regardless of extraction solvent, the inconsistent effect of various cooking treatments on percent inhibition was observed. The percent inhibition was observed to be increased in fried sample by 29.3%, whereas reductions in percent inhibition were measured in pressure cooked and microwave cooked samples by 29.3% and 27.8% respectively, when compared to the raw counterparts. The interaction effect of cooking treatments and the extraction solvents was also significant (F= 227.2 at df= 6, p= 0.000). After pressure cooking and microwave cooking, a decrease in percent inhibition was observed in all the extracts when compared to their respective raw vegetable sample. However, the effect of frying was observed to be inconsistent in regard to their percent inhibition as EE and BE showed increased inhibition in contrast to decreased inhibition shown by ME when compared to their raw counterparts.
Table 4.35 Antioxidant activity of various extracts of cooked vegetable of *T. dioica* as determined by FTC, TBA and FRAP assays

| Treatments      | FTC (% Inhibition) | TBA (% Inhibition) | FRAP μM FeSO₄/100g | ME | BE | EE | BE | EE | ME | BE | EE | BE | EE | ME | BE | EE | ME | BE | EE | ME | BE | EE | ME | BE | EE | ME | BE | EE | ME | BE | EE | ME | BE | EE | ME | BE |
|-----------------|--------------------|--------------------|---------------------|-------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Raw (Control)   | 37.5±4.087EC       | 16.7±4.056BC       | 140.8±3.042BC      | 28.5±0.395BC      | 46.8±0.515EC       | 10.2±0.692BC       | 12.3±0.107BC       | 40.1±0.425BC       |
| Pressure        | 30.5±4.07643E      | 10.2±0.69604E      | 16.3±0.70012E      | 22.8±0.39904E      | 41.0±0.47021E       | 19.0±0.70904E      | 22.2±0.42504E      | 22.2±0.44404E      |
| Microwave cooked| 32.8±4.05845E      | 9.9±4.01765E       | 16.4±0.60245E      | 22.0±0.59545E      | 41.7±0.35904E       | 19.0±0.70904E      | 22.2±0.44404E      | 22.2±0.44404E      |
| Fried           | 32.8±4.05845E      | 33.0±4.05845E      | 16.4±0.60245E      | 22.0±0.59545E      | 41.7±0.35904E       | 19.0±0.70904E      | 22.2±0.44404E      | 22.2±0.44404E      |
| Mean            | 33.5±4.03554E      | 17.4±4.02545E      | 24.4±0.54354E      | 24.4±0.54354E      | 43.4±0.35904E       | 29.0±0.54354E      | 29.0±0.54354E      | 29.0±0.54354E      |

Values are presented as mean ± SE (n=1) and referred to the dry weight.

FTC % Inhibition of BHT = 88.8±15.3, V. E = 70.6±15.7
TBA % Inhibition of BHT = 90.6±14

ME = methanolic extract, EE = ethanolic extract, BE = butanolic extract

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.
4.9.2.2 Percent inhibition as measured by TBA method

Percent inhibition levels, as measured by TBA assay, are as presented in table 4.35. Regardless of cooking treatments, ME revealed highest percent inhibition (43.4%) followed by BE (35.2%) and ME (29.0%). F- value statistics (F= 3416.8 at df= 2, p= 0.000) also indicated a highly significant impact of extraction solvent on the percent inhibition. The main effect of the cooking methods was also highly significant (F= 2363.6 at df= 3, p= 0.000). The average value of percent inhibition irrespective of extracting solvent decreased after the pressure cooking and microwave cooking, in contrast the increased inhibition after frying. Nevertheless, the antioxidant activity of all extracts, in terms of the capacity of malonaldehyde formation was significantly (p<0.05) lower than BHT (90.9%) and vitamin E (78.3%). The significant interaction effect of cooking methods and extraction solvent suggested that there was a decrease in percent inhibition level of all the extracts of pressure cooked and microwave cooked vegetable sample and the extent of decrease varied from 10.8 to 23.4% over their respective extracts from raw counterparts. However, the EE and BE of fried samples were observed to be efficient in retaining the percent inhibition while ME was ineffective in retaining the percent inhibition as compared to the raw vegetable sample.

4.9.2.3 Ferric reducing antioxidant power (FRAP)

As indicated by the results of the FRAP assay given in table 4.35, the average FRAP value of the raw samples irrespective of extracting solvents was 675.8 μM FeSO₄/100g (dw) whereas in cooked samples this value varied from 418.0 to 555.3 μM FeSO₄/100g (dw) suggesting that the effect of different heat processing treatments on the FRAP value was significant (F= 414.3 at df= 3, p= 0.000) and decreased value of FRAP were observed after various cooking treatments. Regardless of cooking methods various extracts showed significant (p<0.05) difference in their reducing power, the maximum reduction power was measured in ME (712.3 μM FeSO₄/100g) followed by EE (471.2 μM FeSO₄/100g) and BE (429.8 μM FeSO₄/100g). The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 303.7 at df= 6, p= 0.000). All types of extracts of differently cooked vegetable sample except EE of fried sample exhibited decreased FRAP value over the respective raw counterparts. The extent of the decrease was maximum in BE of fried sample (86%) followed by ME of pressure cooked and fried sample (35%).
4.9.2.4 DPPH free radical scavenging activity

The DPPH radical scavenging activities of different extracts from raw and cooked vegetable samples of T. dioica are shown in table 4.36. The percent scavenging activity of 30 µg/ml ascorbic acid (92.0%) was observed to be highest with respect to various raw and cooked extracts of T. dioica. The percent scavenging of the raw samples irrespective of extracting solvents was 10.3%, whereas in cooked samples this value varied from 11.7 to 19.8% indicating the increased scavenging activity after various cooking treatments. Regardless of cooking treatments the effect of extracting solvents was also significant (F= 35493 at df= 2, p= 0.000) and the percent scavenging at 30 µg/ml of various extracts was in order of ME > BE > EE. The pressure cooking exhibited significantly (p<0.05) increased scavenging activity in all the extracts, as compared to their raw counterparts and this retention was maximum in BE (287.9%) followed by ME (49.4%) and EE (12.5%). The increased in percent radical scavenging activity was also reported in all extracts of microwave cooked samples when compared to their respective raw T. dioica. However, in case of frying ME and BE revealed an increasing percent scavenging activity by 22.9% and 15% respectively as compared to their respective extracts from the raw sample of T. dioica.

The percent scavenging with respect to various concentrations are presented in Appendix-III. The average free radical scavenging activity as assessed by IC50 values regardless of the extraction solvent decreased by 37.5, 30.8 and 40.9% after pressure cooking, microwave cooking and frying respectively (Table 4.36). Regardless of cooking treatments, the average IC50 value was observed to be highest in ME (429.8 µg/ml) followed by BE (222.7 µg/ml) and EE (163.4 µg/ml). Nevertheless, the IC50 values of all extracts were significantly (p<0.05) higher than ascorbic acid (5.05 µg/ml). The various extracts of the raw vegetable sample showed IC50 value ranging from 202.3 to 624.7 µg/ml. All the extracts of pressure cooked and microwaved samples showed decreased IC50 value in comparison to their respective extracts of the raw sample. However, the effect of frying was inconsistent with respect to their IC50 value of various extracts as EE and BE of fried sample showed increased IC50 value, in contrast to the decreased IC50 value in BE, when compared to their raw counterparts.
Table 4.36  Free radical scavenging activity of various extracts of cooked vegetable of *T. dioica* as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH (% Scavenging at 30 µg/ml)</th>
<th>IC-50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>17.9±0.13&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>6.4±0.07&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>26.6±0.13&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>7.2±0.07&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>18.0±0.06&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>23.7±0.07&lt;sup&gt;bC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>22.0±0.07&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>5.4±0.07&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>21.1&lt;sup&gt;C&lt;/sup&gt;</td>
<td>10.6&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD (*n*=3)

F-Statistics for extraction solvents = 35493 at df = 2, *p*=0.000
F-Statistics for cooking treatments = 19591 at df = 3, *p*=0.000
F-Statistics for cooking treatments & extraction solvents = 15281 at df = 6, *p*=0.000

% Scavenging of ascorbic acid at 30 µg/ml = 92.0 ± 0.06

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
IC50 value of ascorbic acid = 5.05 µg/ml

4.9.3 Correlation study

The results of correlation studies showed positive correlations of FTC assay with total phenols (r= 0.516) (Table 4.37) suggesting that phenol may be the main phytochemical responsible for the oxidation of linolic acid. Similarly, the positive correlation was observed between percent inhibition (as measured by TBA assay) and phenol content (r= 0.489) indicating that the high concentration of phenol inhibits malonaldehyde formation and was responsible for the antioxidant activity of *T. dioica*. Interestingly, a strong correlation (r= 0.997, p<0.01) between FTC and TBA assay showed that the increase in peroxide level caused the formation of malonaldehyde compounds. A positive correlation of the DPPH value with phenols content (r= 0.554) concluded that the change in free radical scavenging activity of heat processed sample may be due to the change in phenols content after the cooking treatments.
### Table 4.37 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity

<table>
<thead>
<tr>
<th></th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.458</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>0.524</td>
<td>0.375</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.148</td>
<td>0.447</td>
<td>0.066</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>0.516</td>
<td>0.325</td>
<td>0.157</td>
<td>-0.248</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>0.489</td>
<td>0.305</td>
<td>0.129</td>
<td>-0.257</td>
<td>0.997**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>0.215</td>
<td>-0.458</td>
<td>-0.083</td>
<td>0.129</td>
<td>0.242</td>
<td>0.244</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.554</td>
<td>0.030</td>
<td>0.174</td>
<td>-0.116</td>
<td>0.680*</td>
<td>0.639*</td>
<td>0.529</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=12
** Significantly correlated at p<0.01, n=12

### 4.9.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

The Rf value of different phenolic acids and flavonoid standard compounds with three different solvent systems are presented in Appendix-IV. A typical HPTLC chromatogram of ME of *T. dioica* is shown in Fig. 4.6(A-C), and the content of individual phenolic compounds is summarised in table 4.38. The flavonoids namely myricetin (28.7 μg/ml) and rutin (0.79 μg/ml) were identified and quantified in the raw sample extract, whereas no phenolic acids were identified in the raw sample of *T. dioica*. Whereas, caffeic acid (1.73 μg/ml), benzoic acid (135.62 μg/ml), quercetin (39.64 μg/ml) and rutin (50.1 μg/ml) were identified in pressure cooked sample; ellagic acid (1.07 μg/ml), quercetin (38.7 μg/ml) and catechin (35.5 μg/ml) were identified in microwaved sample and caffeic acid (7.01 μg/ml) and ellagic acid (4.27 μg/ml) were identified in the fried sample. No phenolic acid was reported in raw *T. dioica* but some new phenolic compounds such as caffeic acid, benzoic acid, and ellagic acid were observed after various cooking treatments. The prominent amount of benzoic acid was reported after pressure cooking of *T. dioica*. The ellagic acid was the only phenolic acid observed in the microwaved sample and its concentration was found to be increased after frying. Interestingly, quercetin was the newly formed compound observed after pressure cooking and microwave cooking although; this was not identified in raw *T. dioica*. There was prominent destructive effect of heat processing on myricetin, which was not identified in the cooked sample extracts. The rutin was only found in the pressure cooked sample and its concentration was very much elevated (50.08 μg/ml) in comparison to raw sample.
(0.79 μg/ml). Catechin (35.5 μg/ml) was observed only after the microwave cooking, however; it was not detected in the rest of the extracts including the raw sample.

Table 4.38 Identification and quantification of phenolic acids and flavonoids (μg/ml of extract) in ME of *T. dioica* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>nd</td>
<td>1.73</td>
<td>nd</td>
<td>7.01</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>nd</td>
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<td>nd</td>
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<tr>
<td>Ferulic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>135.62</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>nd</td>
<td>nd</td>
<td>1.07</td>
<td>4.27</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>nd</td>
<td>39.64</td>
<td>36.31</td>
<td>nd</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>28.73</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Leutolin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.79</td>
<td>50.08</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>nd</td>
<td>35.50</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd= not detecte
Fig. 4.6A HPTLC profile of ME of *T. dioica* as developed in chloroform: hexane: methanol: formic acid (6.4: 3.9: 2.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.6B HPTLC profile of ME of *T. dioica* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.6C HPTLC profile of ME of *T. dioica* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried.
4.10 Antioxidant potential of pumpkin (*C. maxima*)

4.10.1 Quantitative estimation of phytochemicals

Table 4.39 indicates the TPC of the raw and cooked vegetable of *C. maxima*. The average TPC of various extracts irrespective of cooking methods ranged from 66.0 to 101.5 mg GE/100g (dwb) and was in the order of EE > ME > BE. Regardless of extraction solvents all cooking methods exerted a significant (p<0.05) increasing effect in the amount of TPC over the raw sample. The average value of TPC increased maximum in pressure cooked sample (109.1%) followed by microwaved (66.3%) and fried sample (44.4%). The interactive effect of cooking treatments and extracting solvents was also significant (F= 784.6 at df= 6, p= 0.000). Different extracts of the pressure cooked and microwaved samples revealed an increased recovery of TPC in comparison to their respective raw counterparts with maximum increase in recovery in EE (145.8%) followed by BE (94.3%) and ME (88.2%) in case of pressure cooked samples. However, in case of microwaved samples the order of increase in concentration of total phenol was EE > ME > BE. However, the effect of frying was observed to be inconsistent in regard to the TPC of various extracts. Butanol was found to be an inferior solvent for the extraction of total phenol from fried sample of *C. maxima* as compared to methanol and ethanol.

The concentration of total flavonoids of raw and cooked vegetable of *C. maxima* is shown in table 4.39. The average flavonoids content of the raw samples irrespective of extracting solvents was 6.0 mg QE/100g (dwb), whereas in cooked samples this value varied from 9.8 to 24.2 mg QE/100g (dwb) suggesting that there was a significant effect of cooking treatments on the flavonoids content (F= 4515.9 at df= 3, p= 0.000). The main effect of extracting solvents was also significant (F= 414.2 at df= 2, p= 0.000) and the flavonoids content of various extracts regardless of the cooking treatments varied from 11.8 to 15.6 mg QE/100g (dwb) being highest in ME and the lowest in BE. The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 2684.7 at df= 6, p= 0.000). All the solvents were observed to be more effective in extracting the flavonoids from pressure cooked and microwaved samples. However, the effect of frying was inconsistent and methanol and ethanol were observed to be more effective in their ability to extract out flavonoids from fried sample in comparison to raw sample.
Table 4.39 Total phenols, flavonoids, and tannins content of various extracts of cooked vegetable of *C. maxima*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total phenols (mg GE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>Tannin content (mg CE/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>62.5±0.92&lt;sup&gt;ab&lt;/sup&gt; 56.1±0.69&lt;sup&gt;ab&lt;/sup&gt; 53.1±0.80&lt;sup&gt;ab&lt;/sup&gt; 57.2&lt;sup&gt;a&lt;/sup&gt; 3.8±0.20&lt;sup&gt;a&lt;/sup&gt; 3.7±0.22&lt;sup&gt;a&lt;/sup&gt; 10.5±0.39&lt;sup&gt;b&lt;/sup&gt; 6.0&lt;sup&gt;a&lt;/sup&gt; 2.9±0.20&lt;sup&gt;a&lt;/sup&gt; 1.8±0.76&lt;sup&gt;a&lt;/sup&gt; 7.6±1.34&lt;sup&gt;ab&lt;/sup&gt; 4.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>117.6±1.26&lt;sup&gt;b&lt;/sup&gt; 137.9±1.27&lt;sup&gt;b&lt;/sup&gt; 103.2±1.62&lt;sup&gt;b&lt;/sup&gt; 119.6&lt;sup&gt;d&lt;/sup&gt; 19.4±0.40&lt;sup&gt;c&lt;/sup&gt; 17.8±0.32&lt;sup&gt;b&lt;/sup&gt; 14.1±0.68&lt;sup&gt;c&lt;/sup&gt; 17.1&lt;sup&gt;c&lt;/sup&gt; 4.1±0.40&lt;sup&gt;a&lt;/sup&gt; 6.4±2.09&lt;sup&gt;b&lt;/sup&gt; 9.1±1.57&lt;sup&gt;b&lt;/sup&gt; 6.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>97.9±1.08&lt;sup&gt;ab&lt;/sup&gt; 113.1±1.43&lt;sup&gt;c&lt;/sup&gt; 74.3±1.03&lt;sup&gt;c&lt;/sup&gt; 95.1&lt;sup&gt;b&lt;/sup&gt; 4.2±0.26&lt;sup&gt;a&lt;/sup&gt; 7.0±0.41&lt;sup&gt;b&lt;/sup&gt; 18.2±0.37&lt;sup&gt;c&lt;/sup&gt; 9.8&lt;sup&gt;b&lt;/sup&gt; 3.7±1.05&lt;sup&gt;a&lt;/sup&gt; 5.6±2.08&lt;sup&gt;b&lt;/sup&gt; 9.2±0.99&lt;sup&gt;b&lt;/sup&gt; 6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fried</td>
<td>115.4±1.25&lt;sup&gt;c&lt;/sup&gt; 98.9±1.30&lt;sup&gt;b&lt;/sup&gt; 33.4±0.83&lt;sup&gt;a&lt;/sup&gt; 82.6&lt;sup&gt;c&lt;/sup&gt; 35.1±0.31&lt;sup&gt;c&lt;/sup&gt; 33.0±0.28&lt;sup&gt;ab&lt;/sup&gt; 4.6±0.22&lt;sup&gt;a&lt;/sup&gt; 24.2&lt;sup&gt;d&lt;/sup&gt; 1.9±0.06&lt;sup&gt;a&lt;/sup&gt; 1.3±0.00&lt;sup&gt;a&lt;/sup&gt; 15.2±1.30&lt;sup&gt;b&lt;/sup&gt; 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>98.3&lt;sup&gt;b&lt;/sup&gt; 101.5&lt;sup&gt;c&lt;/sup&gt; 66.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.6&lt;sup&gt;b&lt;/sup&gt; 15.4&lt;sup&gt;b&lt;/sup&gt; 11.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt; 3.8&lt;sup&gt;a&lt;/sup&gt; 10.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 3465.9 at df = 2, p=0.000
F-Statistics for cooking treatments = 4541.6 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 784.6 at df = 6, p=0.000
F-Statistics for cooking treatments & extraction solvents = 3465.9 at df = 2, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.
GE= gallic acid equivalent, QE= quercetin equivalent, CE= catechin equivalent
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
The average tannin content in the cooked samples irrespective of the extraction solvent varied from 6.1 to 6.5 mg CE/100g (dwb) when compared to the tannin content of raw sample (4.1 mg CE/100g, dwb) suggesting the increasing effect of heat processing treatments on tannin content. Among all the extracts, butanol was found to be most effective solvent for extracting tannins followed by ethanol and methanol. The significant interaction effect ($F=6.6$ at $df=6$, $p=0.000$) of the heat processing treatments and the extraction solvents showed that the tannin content varied greatly among different extracts, which indicated the possible influence of extracting solvent on tannin contents. All the three types of extracts of pressure cooked samples revealed an increased recovery of tannin contents in comparison to their raw counterpart and this increased recovery was maximum in EE (255.5%) followed by ME (41.4%) and BE (19.7%). Similarly, the increased recovery of tannin content was found in all the extracts after the microwave cooking with in order of EE > ME > BE. However, in case of frying the inconsistent effect on the recovery of tannin content was observed among various extracts.

The carotenoids content of raw and cooked vegetable samples of *C. maxima* measured as β-carotene is presented in table 4.40. The average value of carotenoids content of cooked vegetable samples regardless of the extraction solvent ranged from 1.1 to 5.2 mg β-carotene /100g (dwb) in comparison to carotenoid content of 0.3 mg β-carotene /100g (dwb) in the raw vegetable sample. The results showed significant ($p<0.05$) change in carotenoids recovery from cooked samples. Irrespective of extraction solvents, all the cooking treatments were observed to be effective in retaining the carotenoids content and the maximum retention was observed in fried sample followed by pressure cooked and microwave cooked sample. Among all the solvents, ethanol was found to be most efficient for extracting the carotenoids (3.0 mg β-carotene /100g, dwb) followed by methanol and butanol, regardless of cooking methods. The interaction effect of heat processing treatments and the extraction solvents was also significant ($F=4387.1$ at $df=6$, $p=0.000$). The recovery of carotenoid content was found to be increased after the cooking treatments. As in case of phenolics, all the extracts of pressure cooked sample were found to be effective to retain the carotenoid content and this retention was maximum in EE followed by BE and ME. However, in microwave cooking most abrupt increase in the carotenoids content was measured in BE (550%) followed by EE (200%) and ME (116.6%). The increased recovery of carotenoids was also observed in all the extracts of fried samples as compared to their raw counterparts.
Table 4.40 Carotenoids content of various extracts of raw and cooked vegetable of *C. maxima*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoid content (mg β-Carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>0.6 ± 0.03&lt;sup&gt;4h&lt;/sup&gt;</td>
<td>0.2 ± 0.05&lt;sup&gt;8A&lt;/sup&gt;</td>
<td>0.2 ± 0.03&lt;sup&gt;6A&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>1.9 ± 0.04&lt;sup&gt;6A&lt;/sup&gt;</td>
<td>3.4 ± 0.06&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>2.3 ± 0.05&lt;sup&gt;6B&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>1.3 ± 0.06&lt;sup&gt;bH&lt;/sup&gt;</td>
<td>0.6 ± 0.06&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>1.3 ± 0.03&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>6.3 ± 0.04&lt;sup&gt;4dB&lt;/sup&gt;</td>
<td>7.9 ± 0.06&lt;sup&gt;dC&lt;/sup&gt;</td>
<td>1.3 ± 0.02&lt;sup&gt;dA&lt;/sup&gt;</td>
<td>5.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD (n=3) and referred to the dry weight.

F-statistics for extraction solvents = 4451.3 at df = 2, p=0.00

F-statistics for cooking treatments = 19334.1 at df = 3, p=0.046

F-statistics for cooking treatments & extraction solvents = 4387.1 at df = 6, p=0.000

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

4.10.2 The antioxidant activity as measured by using FTC, TBA, FRAP and DPPH radical scavenging methods

4.10.2.1 Percent inhibition as measured by FTC method

The antioxidant activity of ME, EE and BE of raw and cooked vegetable sample of *C. maxima* in terms of measurement of inhibition of peroxidation is as shown in table 4.41. The results pointed out the possible influence of extracting solvent on percent inhibition. The various extracts of different samples of *C. maxima* inhibited 5.0 - 25.2% peroxidation of linoleic acid after incubation for 96 h as measured by FTC assay. This wide variation in the inhibition level could be because of differences in the type and concentration of antioxidative compounds in these extracts. Nevertheless, the antioxidant activity of all extracts, in terms of measurement of percentage inhibition was significantly (p<0.05) lower than BHT (88.8%) and vitamin E (74.6%). In general, ME revealed significantly higher antioxidant activity followed by EE and BE (F= 885.5 at df= 2, p= 0.000). Regardless of extraction solvent, the extracts of cooked samples revealed significantly increased (p<0.05) percent inhibition in order of pressure cooked > fried > microwave cooked samples. The interaction effect of cooking treatments and the
### Table 4.41 Antioxidant activity of various extracts of cooked vegetable of *C. maxima* as determined by FTC, TBA and FRAP assays

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP μM FeSO₄/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>19.3±0.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.0±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure Cooked</td>
<td>21.2±0.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.6±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.8±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave Cooked</td>
<td>19.8±0.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.5±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>25.2±0.97&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.0±0.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.3±0.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>21.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- F-Statistics for extraction solvents = 885.5 at df = 2, p=0.000
- F-Statistics for cooking treatments = 199.7 at df = 3, p=0.000
- F-Statistics for cooking treatments & extraction solvents = 75.6 at df = 6, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight. Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

- FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16
- TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19
- ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
extraction solvents was also significant ($F= 75.6$ at $df= 6$, $p= 0.000$). The results showed that percent inhibition by various extracts of pressure cooked and microwaved samples was significantly higher ($p<0.05$) and it increased by 9.8, 232 and 177.2% and 2.6, 30 and 36.8% respectively in pressure cooked and microwaved samples in their respective ME, EE and BE. However, the effect of frying was observed to be inconsistent in regard to their percent inhibition as ME and EE showed increased inhibition in contrast to decreased inhibition shown by BE, when compared to their respective raw counterparts.

### 4.10.2.2 Percent inhibition as measured by TBA method

Percent inhibition as measured by TBA assay of various extracts of raw and cooked *C. maxima* samples are presented in table 4.41. Regardless of cooking treatments, ME revealed highest percent inhibition (28.9%) followed by EE (23.4%) and BE (21.5%). F-value statistics ($942.2$ at $df = 2$, $p=0.000$) also indicated highly significant impact of extraction solvent on the percent inhibition of the extracts. Regardless of the extraction solvent, the various extracts of the cooked sample extracts showed increased percent inhibition. There was significant interaction effect between the extracting solvents and the heat treatments on the percent inhibition ($F= 376.5$ at $df= 6$, $p= 0.000$). The results showed that the ME of the raw sample was most effective in inhibiting the oxidation. However, in cooked samples, the maximum increased level of inhibition was revealed by EE of fried sample followed by EE and BE of pressure cooked sample respectively. Overall, pressure cooking was effective in increasing the percent inhibition by various extracts of *C. maxima*. After pressure cooking the percent inhibition of various extracts varied from 28 to 34.7% in comparison to 17.8 to 30.2% of various extracts of raw sample.

### 4.10.2.3 Ferric reducing antioxidant power (FRAP)

As indicated by the results of FRAP assay given in table 4.41, the average FRAP value of the raw samples irrespective of extracting solvents was 80 $\mu$M FeSO$_4$/100g (dwb) whereas in cooked samples this value varied from 116.3 to 232.0 $\mu$M FeSO$_4$/100g (dwb) suggesting that the effect of different heat processing treatments on the FRAP value of sample extracts was significant ($F= 3519.5$ at $df= 3$, $p= 0.000$) and the increased concentration of FRAP was observed after various cooking treatments. Regardless of cooking methods, the average FRAP values as shown by various extracts varied from 41.7 to 213.1 $\mu$M FeSO$_4$/100g being highest in ME followed by EE and BE ($F= 9553$ at
The interaction effect of heat processing treatments and the extraction solvents was also significant (F= 1304.5 at df= 6, p= 0.000). As measured by FRAP assay, like that of TBA assay, the methanolic extracts of raw sample was most effective in reducing the Fe$^{3+}$ ion. In case of cooked samples maximum increase in the reducing power was shown by EE of fried sample (375%) followed by EE of pressure cooked (195%) and ME of fried sample (142%).

**4.10.2.4 DPPH free radical scavenging activity**

The scavenging effects of DPPH free radical are shown as relative activities against ascorbic acid (Table 4.42). The percent scavenging activity of 30 µg/ml ascorbic acid (92.0%) was observed to be highest with respect to various raw and cooked sample extracts of *C. maxima*. The average percent scavenging activity of the raw samples irrespective of extracting solvents was 6.5%, whereas in cooked samples this value varied from 4.1 to 7.3%, suggesting that the effect of different heat processing treatments on the percent scavenging was highly significant (F= 751.2 at df= 3, p= 0.000) and inconsistent effect on scavenging activity was observed after various cooking treatments. Regardless of cooking treatments the effect of extracting solvents was also significant (F= 5189.8 at df= 2, p= 0.000) and the percent scavenging of 30 µg/ml of various extracts was in order of ME > EE > BE. Pressure cooking resulted in significantly (p<0.05) decreased scavenging activity in various extracts, as compared to their raw counterparts and this decreased activity was maximum in EE (51.9%) followed by BE (12.5%) and ME (3.2%). However, in case of microwave cooked sample ME and BE revealed an increased percent scavenging activity. The decrease in percent radical scavenging activity was also observed in all the extracts of fried samples, when compared to their respective extracts from raw sample of *C. maxima*.

The IC$_{50}$ values of various extracts raw as well as heat processed samples are presented in table 4.42. The average free radical scavenging activity as assessed by IC$_{50}$ values regardless of the extraction solvent increased by 68.3, 21.5 and 125.4% after pressure cooking, microwave cooking and frying respectively. Regardless of cooking treatments the average IC$_{50}$ value was observed to be highest in ME (723.1 µg/ml) followed by BE (476.2 µg/ml) and EE (378.7 µg/ml). Nevertheless, the IC$_{50}$ values of all extracts were significantly (p<0.05) higher than ascorbic acid (5.05 µg/ml).
Table 4.42 Free radical scavenging activity of various extracts of cooked vegetable of *C. maxima* as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH (% Scavenging at 30 μg/ml)</th>
<th>IC-50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
</tr>
<tr>
<td>Raw</td>
<td>9.5±0.13&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.7±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>9.2±0.30&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.7±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>10.6±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.2±0.26&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>7.7±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.3±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>9.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD (n=3)

- F-Statistics for extraction solvents = 5189.8 at df = 2, p=0.000
- F-Statistics for cooking treatments = 751.2 at df = 3, p=0.000
- F-Statistics for cooking treatments & extraction solvents = 169.5 at df=6, pO.000

% Scavenging of ascorbic acid at 30 μg/ml=92.0 ± 0.06

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract

IC<sub>50</sub> value of ascorbic acid=5.05μg/ml

### 5.10.3 Correlation studies

Statistical correlations have been studied between phytochemicals content and antioxidant activity determined by different assays, as shown in table 4.43. The percent inhibition as measured by FTC assay showed the significant (p<0.05) positive correlation (r= 0.619) with the total phenol, which indicated that the phenols might be the phytochemicals responsible for the antioxidant activity in *C. maxima*. The positive association between percent inhibition as measured by TBA assay and phenol content (r=0.510) also designated that the high concentration of phenol inhibits malonaldehyde formation and responsible for the antioxidant activity of *C. maxima*. Interestingly, a strong correlation (r= 0.719, p<0.01) between FTC and TBA assay showed that the increase in peroxide level caused the formation of malonaldehyde compounds. A highly significant and positive correlation of the FRAP value with phenol content (r= 0.681, p<0.05), flavonoid content (r = 0.768 p<0.01) and carotenoid content (r= 0.834, p<0.01) concluded that phenols, flavonoids and carotenoids may be the main compounds responsible for reduction the ferrous ions into ferric ions in their respective extracts. Moreover, a highly significant and positive correlation of the DPPH value with flavonoid...
Table 4.43 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity

<table>
<thead>
<tr>
<th></th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.526</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>-0.404</td>
<td>-0.331</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.478</td>
<td>0.897**</td>
<td>-0.364</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>0.619*</td>
<td>0.527</td>
<td>-0.527</td>
<td>0.563</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>0.510</td>
<td>0.148</td>
<td>-0.333</td>
<td>0.214</td>
<td>0.719**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>0.681*</td>
<td>0.768**</td>
<td>-0.682*</td>
<td>0.834**</td>
<td>0.729**</td>
<td>0.466</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.405</td>
<td>0.653*</td>
<td>-0.223</td>
<td>0.503</td>
<td>0.585</td>
<td>-0.054</td>
<td>0.421</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=12
** Significantly correlated at p<0.01, n=12

content (r= 0.653, p<0.05) concluded that the change in free radical scavenging activity of processed sample of *C. maxima* may be due to the change in flavonoids content after the cooking treatments.

4.10.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

The Rf value of different phenolic acids and flavonoid standard compounds with three different solvent systems are presented in Appendix-IV. The distribution of phenolic acids and flavonoids in ME of *C. maxima* is presented in table 4.44 and the typical chromatogram is shown in Fig. 4.7(A-C). The phenolic acids, namely chlorogenic acid (66.5 µg/ml) and ferulic acid (37.3 µg/ml) were identified and quantified in raw sample extract, whereas no flavonoids were identified in uncooked vegetable of *C. maxima*. Chlorogenic acid (39.9 µg/ml), vanillic acid (4.2 µg/ml), ellagic acid (40.7 µg/ml), quercetin (40.9 µg/ml) and myricetin (35.5 µg/ml) were identified in pressure cooked sample. Chlorogenic acid (34.8 µg/ml), ferulic acid (7.4 µg/ml), ellagic acid (37.5 µg/ml) and quercetin (38.7 µg/ml) were found to be present in microwaved sample extract. Chlorogenic acid (12.9 µg/ml), vanillic acid (0.4 µg/ml), ellagic acid (129.2 µg/ml) and quercetin (41.9 µg/ml) were observed in the fried sample extract. Chlorogenic acid was identified as most significantly present phenolic acid in *C. maxima*. However, its concentration decreased drastically in all the heat processed samples and the maximum destruction was found in fried sample. Interestingly, vanillic acid was newly formed compound observed after the pressure cooking and frying, although this was not
Table 4.44 Identification and quantification of phenolic acids and flavonoids (µg/ml of extract) in ME of *C. maxima* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>66.52</td>
<td>39.92</td>
<td>34.82</td>
<td>12.96</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>nd</td>
<td>4.23</td>
<td>nd</td>
<td>0.43</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>37.35</td>
<td>nd</td>
<td>7.43</td>
<td>nd</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>nd</td>
<td>40.75</td>
<td>37.48</td>
<td>129.24</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>nd</td>
<td>40.96</td>
<td>38.69</td>
<td>41.92</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>nd</td>
<td>35.55</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Apigenin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Leutolin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Catechin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd = not detected

identified in raw *C. maxima*. Ferulic acid was only found in microwave cooked sample and its concentration was lower in comparison to raw sample (37.3 µg/ml). Phenolics such as ellagic acid and quercetin were newly formed compounds in all the cooked sample extracts, although these were not identified in extracts of raw sample. The myricetin (35.5 µg/ml) was observed only after the pressure cooking, however it was not detected in rest of the extracts including the raw vegetable. The most retained phenolic acids were chlorogenic acid, which was present in all the cooked samples. However, the destructive effect of heat processing was most pronounced on ferulic acid, which was not identified in pressure cooked and fried samples.
Fig. 4.7A HPTLC profile of ME of *C. maxima* as developed in chloroform: hexane: methanol: formic acid (6.4: 3.9: 2.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.7B HPTLC profile of ME of *C. maxima* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried.
Fig. 4.7C HPTLC profile of ME of *C. maxima* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
4.11 Antioxidant potential of summer squash (*Cucurbita pepo*)

4.11.1 Quantitative estimation of phytochemicals

The TPC of raw and cooked *C. pepo* vegetable samples are reported in table 4.45. ME revealed the highest concentration of TPC (393.3 mgGE/100g dwb) followed by EE (390.7 mgGE/100g dwb) and BE (199.2 mgGE/100g dwb). F-value statistics (31315.9 at df= 2, p= 0.000) also indicated highly significant impact of extraction solvent on the extraction yield of total phenols. The effect of cooking treatments was also highly significant (F= 1981.6 at df= 3, p= 0.000) Regardless of extraction solvent the TPC was found to be decreased in all the cooked vegetable samples with frying showing the most pronounced effect. There was significant interaction effect between the extracting solvent and the heat treatment on the TPC (F= 7332.3 at df= 6, p= 0.000). However, this effect was inconsistent and the results showed a decreased concentration of TPC in the cooked samples except EE of microwaved and fried samples where an increase in the TPC was observed. The maximum reduction in TPC was obtained in case of BE of the microwaved sample.

The concentration of total flavonoids content of raw and cooked vegetable samples is as shown in table 4.45. The average flavonoids content of the raw samples was 55.4 mg QE/100g (dwb), whereas in cooked samples this value significantly varied from 40.4 to 57.4 (F= 1480.2 at df= 3, p= 0.000) suggesting the inconsistent effect of cooking treatments. Pressure cooking and microwave cooking decreased the flavonoid content, whereas frying resulted in an increase of the flavonoids content. The main effect of extracting solvents was also significant (F= 7871.9 at df= 2, p= 0.000) and the flavonoids content was highest in EE (68.6 mg QE/100g, dwb). The interaction effect of cooking treatments and the extraction solvent was also significant (F= 2002 at df= 6, p= 0.000). The results showed a maximum reduction of 65% in the flavonoids content of ME of microwaved sample followed by BE of pressure cooked (44.2%) and BE of microwave cooked sample (36.9%). As also observed in case of TPC, the EE of microwave cooked and fried samples showed significant increase in the flavonoids content.

The tannin content in cooked vegetable samples varied from 6.9 to 11.5 mg CE/100g (dwb) in comparison to 10.9 mg CE/100g of the raw vegetable sample (Table 4.45). Regardless of extraction solvent microwave cooking exerted most pronounced decreasing effect on the tannin content. Irrespective of cooking treatments methanol was
Table 4.45: Total phenols, flavonoids, and tannins content of various extracts of cooked vegetable of *C. pepo*

| Treatments         | Total phenols (mg GE/100g) | Mean ME | ME | EE | BE | Mean | Total flavonoids (mg QE/100g) | Mean ME | ME | EE | BE | Mean | Tannin content (mg CE/100g) | Mean ME | ME | EE | BE | Mean |
|--------------------|-----------------------------|---------|----|----|----|------|-------------------------------|---------|----|----|----|------|-------------------------------|---------|----|----|----|------|-------------------------------|---------|----|----|----|------|
| **Raw (Control)**  |                             |         |    |    |    |      |                               |         |    |    |    |      |                               |         |    |    |    |      |                               |         |    |    |    |      |
| Microwave cooked   |                             | 376.1<sup>a</sup> | 519.8±2.35<sup>bc</sup> | 336.2±1.52<sup>ab</sup> | 272.1±1.57<sup>da</sup> | 64.4±1.37<sup>bc</sup> | 56.8±0.59<sup>ab</sup> | 45.0±0.90<sup>ab</sup> | 55.4<sup>b</sup> | 18.1±4.80<sup>ab</sup> | 7.73±1.47<sup>aa</sup> | 6.88±3.36<sup>ab</sup> | 10.92<sup>b</sup> |
| Fried              |                             | 102.4±0.93<sup>aa</sup> | 453.8±2.98<sup>c</sup> | 310.9<sup>a</sup> | 22.5±0.75<sup>aa</sup> | 70.3±0.69<sup>cc</sup> | 28.4±0.34<sup>bb</sup> | 40.4<sup>c</sup> | 16.6±2.85<sup>bb</sup> | 8.17±2.32<sup>aa</sup> | 9.7±2.38<sup>bb</sup> | 11.51<sup>b</sup> |
| **Mean**           |                             | 199.2<sup>a</sup> | 390.7<sup>b</sup> | 44.5<sup>b</sup> | 68.6<sup>c</sup> | 32.1<sup>a</sup> | 11.77<sup>bb</sup> | 9.37<sup>ab</sup> | 7.90<sup>a</sup> |

F-Statistics for extraction solvents = 31315.9 at df = 2, p<0.000
F-Statistics for cooking treatments = 1981.6 at df = 3, p<0.000
F-Statistics for cooking treatments & extraction solvents = 7332.3 at df = 6, p<0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

GE = gallic acid equivalent, QE = quercetin equivalent, CE = catechin equivalent.

ME = methanolic extract, EE = ethanolic extract, BE = butanolic extract.
most effective solvent in recovering the tannins followed by ethanol and butanol. The significant interaction effect (F=8.84 at df= 6, p= 0.000) of the cooking method and the extraction solvent suggested that the tannin content of the ME of pressure cooked and fried sample was decreased only in ME. However, after microwave cooking the ME and BE was ineffective to retained the tannin contents.

The carotenoids content measured as β carotene of raw and cooked vegetable samples of *C. pepo* is presented in table 4.46. Regardless of extraction solvent the carotenoids content of cooked vegetable samples varied from 5.1 to 12.5 mg CE/100g (dwb) in comparison to 2.8 mg CE/100g (dwb) in the raw vegetable sample. Results showed that after cooking carotenoids content increased significantly (p<0.05) in order of fried > pressure cooked > microwave cooked. In general, ethanol was most effective solvent in extracting carotenoids (7.7 mg CE/100g, dwb) followed by butanol and methanol. The most abrupt increase in the carotenoid content was observed in fried samples, being maximum in EE (14.6 times) followed by BE and ME.

4.11.2 The antioxidant activity as measured by using FTC, TBA, FRAP and DPPH radical scavenging methods

4.11.2.1 Total antioxidant activity as measured by FTC assay

The antioxidant activity of different samples in terms of measurement of inhibition of peroxidation as measured by FTC assay is as shown in table 4.47. The extracts from different samples of vegetable inhibited 5.1–30.4% peroxidation of linoleic acid after incubation for 96h as measured by FTC assay. Nevertheless, the antioxidant activity of all extracts was significantly (p<0.05) lower than BHT (88.8%) and vitamin E (74.6%) used as standards. In general, ME revealed significantly higher antioxidant activity followed by EE and BE (F= 504.3 at df= 2, p= 0.000). Regardless of extraction solvents, the percent inhibition was found to be decreased after all the cooking treatments and the highest loss of antioxidant activity was observed in pressure cooked samples followed by microwaved and fried samples. The significant interaction effect of cooking treatments and extraction solvents suggested that there was a decreased percent inhibition level of all the extracts of pressure cooked sample and the extent of decrease varied from 15.4 to 69.4% over their respective raw samples. However, the EE of microwaved and fried samples showed increased percent inhibition while ME and BE resulted decreased value when compared to their raw counterparts.
Table 4.46 Carotenoids content of various extracts of cooked vegetable of C. pepo

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carotenoid content (mg β-Carotene/100g)</th>
<th>ME</th>
<th>EE</th>
<th>BE</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw (Control)</td>
<td></td>
<td>3.8 ± 0.08&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>1.2 ± 0.01&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>2.4 ±0.08&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td></td>
<td>8.9 ± 0.14&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>1.9 ± 0.29&lt;sup&gt;DB&lt;/sup&gt;</td>
<td>12.9±0.12&lt;sup&gt;CD&lt;/sup&gt;</td>
<td>7.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td></td>
<td>2.4 ± 0.11&lt;sup&gt;AA&lt;/sup&gt;</td>
<td>10.2 ± 0.17&lt;sup&gt;CC&lt;/sup&gt;</td>
<td>2.8 ± 0.04&lt;sup&gt;BB&lt;/sup&gt;</td>
<td>5.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td></td>
<td>9.9 ± 0.14&lt;sup&gt;AD&lt;/sup&gt;</td>
<td>17.5 ± 0.31&lt;sup&gt;DD&lt;/sup&gt;</td>
<td>9.9 ±0.09&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean: 6.3<sup>A</sup> 7.7<sup>c</sup> 7.0<sup>B</sup>

Values are presented as mean ±SD (n=3) and referred to the dry weight.

F-Statistics for extraction solvents = 250.4 at df = 2, p=0.00
F-Statistics for cooking treatments = 6476.6 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 2720.4 at df = 6, p=0.000

Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively.

ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract.

4.11.2.2 Total antioxidant activity as measured by TBA assays

As determined by TBA method, all the extracts revealed lower antioxidant activity than the standard BHT (90.9%) and vitamin E (78.3%) (Table 4.47). Regardless of cooking treatments, the percent inhibition of various extracts of raw and cooked C. pepo samples as measured by TBA method revealed highest percent inhibition in ME (36.5%) followed by EE (27.8%) and BE (25.8%). The average percent inhibition of the raw samples irrespective of extracting solvents was 32.9%, whereas in cooked samples this value varied from 27.3 to 31.4% indicating the decrease in percent inhibition after various cooking treatments as compared to their raw counterparts. There was a significant interaction effect between the extracting solvents and the cooking treatments on the percent inhibition of various extracts. After pressure cooking, the percent inhibition level of all the extracts decreased significantly (p<0.05). The EE of microwaved and fried samples showed increased percent inhibition, contrary to decreased percent inhibition in ME and BE when compared to their raw counterparts.

4.11.2.3 Ferric reducing antioxidant power (FRAP)

As indicated by the results of FRAP assay given in table 4.47, the average FRAP values as shown by various extracts regardless of cooking treatments varied from 360.6 to 959.4 µM FeSO₄/100g being highest in EE followed by ME and BE (F= 2571.1 at df = 2,
Table 4.47 Antioxidant activity of various extracts of cooked vegetable of *C. pepo* as determined by FTC, TBA and FRAP assays

<table>
<thead>
<tr>
<th>Treatments</th>
<th>FTC (% Inhibition)</th>
<th>TBA (% Inhibition)</th>
<th>FRAP (μM FeSO₄/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME</td>
<td>EE</td>
<td>BE</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>30.4±0.73&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>16.7±1.27&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>19.0±0.87&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>25.7±0.70&lt;sup&gt;BC&lt;/sup&gt;</td>
<td>5.1±1.17&lt;sup&gt;BA&lt;/sup&gt;</td>
<td>15.8±0.76&lt;sup&gt;BD&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>23.7±0.59&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>8.6±0.58&lt;sup&gt;BE&lt;/sup&gt;</td>
<td>9.3±1.17&lt;sup&gt;BA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fried</td>
<td>23.8±0.87&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>26.4±1.07&lt;sup&gt;AC&lt;/sup&gt;</td>
<td>11.5±1.41&lt;sup&gt;BA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>25.9&lt;sup&gt;B&lt;/sup&gt;</td>
<td>16.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.9&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-Statistics for extraction solvents = 504.3 at df = 2, p=0.000
F-Statistics for extraction solvents = 1748.3 at df = 2, p=0.000
F-Statistics for cooking treatments = 85.5 at df = 3, p=0.000
F-Statistics for cooking treatments = 270.3 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 127.6 at df = 6, p=0.000
F-Statistics for cooking treatments & extraction solvents = 499.6 at df = 6, p=0.000

Values are presented as mean ±SD (n=3) and referred to the dry weight.
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively
FTC % Inhibition of BHT = 88.8±0.28, Vit. E=74.6±0.16
TBA % Inhibition of BHT= 90.9±0.47, Vit. E=78.3±0.19
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
p=0.000). Irrespective of extraction solvents the ferric reducing power was observed to be increased only in fried sample, although a decrease in FRAP value was measured after pressure cooking and microwave cooking suggesting significant (F=470.7 at df= 3, p= 0.000) inconsistent effect of different cooking treatments on the FRAP value. The interaction effect of heat processing treatments and the extraction solvents showed significant differences in the FRAP values among all the cooking treatments in their respective extracts (p<0.05). Pressure cooking significantly (p<0.05) decreased the reducing power by 22% and 14.2% in ME and EE respectively while in case of BE it was found to increase by 3.7%. However, microwave cooking and frying exhibited a significant (p<0.05) decrease in reduction power of all the extract except EE.

4.11.2.4 DPPH free radical scavenging activity

The scavenging effects of DPPH free radical of raw and cooked vegetables are presented in table 4.48. The percent scavenging of the raw samples irrespective of extracting solvents was 9.3%, whereas in cooked sample extracts this value varied from 11.9 to 13.3%, indicating the increased scavenging activity after various cooking treatments. Regardless of cooking treatments, the percent scavenging of 30 µg/ml of various extracts was in order of ME > EE > BE. The interaction effect of heat processing treatments and the extraction solvents was significant (F= 41.9 at df= 6, p= 0.000). The results showed inconsistent effect of heat treatments on radical scavenging activity of various extracts. The EE of pressure cooked sample showed maximum radical scavenging activity followed by ME of microwaved and ME of fried sample.

Table 4.48 shows the IC$_{50}$ values of various extracts of C. pepo in raw as well as heat processed forms. The average free radical scavenging activity of extracts as assessed by IC$_{50}$ values regardless of the extraction solvent was found to be decreased in cooked samples and the maximum loss of 58.3% was found in microwave cooked sample followed by fried and pressure cooked sample extracts. Regardless of cooking treatments the average IC$_{50}$ value was observed to be highest in BE (259.7 µg/ml) followed by ME (208.8 µg/ml) and EE (159.1 µg/ml). The present results suggested that there was significant interaction effect between the cooking treatments and the extraction solvents. The IC$_{50}$ value was found to be decreased in all the extracts after pressure cooking and the maximum reduction were observed in EE (41.1%) followed by ME (28.3%) and BE (9.5%) as compared to their raw counterparts. Whereas, after microwave cooking and
Table 4.48 Free radical scavenging activity of various extracts of cooked vegetable of C. pepo as measured by DPPH assay

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DPPH Scavenging at 30 μg/ml</th>
<th>IC-50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ME Scavenging at 30 ng/ml</td>
<td>ME Scavenging at 30 ng/ml</td>
</tr>
<tr>
<td>Raw (Control)</td>
<td>13.6±0.11bC 9.2±0.12bB 4.9±0.14aA 9.3a 357.5 129.8 367.1 284.8</td>
<td></td>
</tr>
<tr>
<td>Pressure cooked</td>
<td>12.9±0.06aB 21.3±0.19cC 4.7±0.13aA 13.0b 256.4 76.4 332.2 221.7</td>
<td></td>
</tr>
<tr>
<td>Microwave cooked</td>
<td>20.6±0.17bC 6.1±0.19aA 13.3±0.18bB 13.3b 122.5 165.2 68.4 118.7</td>
<td></td>
</tr>
<tr>
<td>Fried</td>
<td>20.2±0.17bC 3.6±0.38aA 11.9±0.13bB 11.9b 98.9 264.7 271.2 211.6</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16.8b 10.1A 8.7A 208.8 159.1 259.7</td>
<td></td>
</tr>
</tbody>
</table>

All data are reported as mean ± SD (n=3)
F-Statistics for extraction solvents = 71.8 at df = 2, p=0.000
F-Statistics for cooking treatments = 9.5 at df = 3, p=0.000
F-Statistics for cooking treatments & extraction solvents = 41.9 at df = 6, p=0.000
% Scavenging of ascorbic acid at 30 μg/ml=92.0 ± 0.06
Small and capital letters in superscripts indicate significant differences (p<0.05) among cooking methods and extracts respectively
ME= methanolic extract, EE= ethanolic extract, BE= butanolic extract
IC<sub>50</sub> value of ascorbic acid=5.05μg/ml

frying the decreased IC<sub>50</sub> value was observed in ME and BE, while in EE it was observed to be increased.

4.11.3 Correlation studies

A high correlation between FTC and total phenols (0.759, p<0.01) as given in table 4.49, suggested that phenols were the main compounds responsible for the antioxidative activity, therefore the decrease in TPC in cooked sample extracts caused the decreased percent inhibition as measured by FTC methods. As also observed in case of FTC method, a highly significant and positive correlation of the TBA results with phenols content (r= 0.737, was observed, indicating that phenols were the main phytochemicals which inhibited malonaldehyde formation and responsible for the antioxidant activity of C. pepo as described by TBA method. A very high correlation (r= 0.997, p<0.01) between FTC and TBA assay showed that the increase in peroxide level caused formation of malonaldehyde compounds. A highly significant and positive correlation of the FRAP value with phenols content (r= 0.828, p<0.01) and flavonoids content (r= 0.893, p<0.01) concluded that lower activity of cooked sample extracts may be due to the loss of phenols and flavonoids after the cooking process. In contrast, the increase in FRAP value might
Table 4.49 Pearson correlation coefficients (r) between phytochemicals and antioxidant activity as determined by different assays

<table>
<thead>
<tr>
<th></th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Tannin</th>
<th>Carotenoids</th>
<th>FTC</th>
<th>TBA</th>
<th>FRAP</th>
<th>DPPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenols</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.733**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>0.616*</td>
<td>0.372</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carotenoids</td>
<td>0.236</td>
<td>0.415</td>
<td>0.265</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTC</td>
<td>0.759**</td>
<td>0.446</td>
<td>0.497</td>
<td>0.292</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA</td>
<td>0.737**</td>
<td>0.411</td>
<td>0.483</td>
<td>0.303</td>
<td>0.997**</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>0.828**</td>
<td>0.893**</td>
<td>0.385</td>
<td>0.511</td>
<td>0.617*</td>
<td>0.599*</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.303</td>
<td>0.195</td>
<td>0.597*</td>
<td>0.306</td>
<td>0.431</td>
<td>0.453</td>
<td>0.202</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Significantly correlated at p<0.05, n=12
** Significantly correlated at p<0.01, n=12

be attributed to the fact that the cooking treatments bruised the tissue and expose the antioxidant components.

4.11.4 Identification and quantification of phenolic acids and flavonoids by HPTLC

The Rf value of different phenolic acids and flavonoid standard compounds were: 0.08 (chlorogenic acid); 0.29 (gallic acid); 0.63 (quercetin); 0.72 (cafeic acid); 0.87 (kaempferol); 0.90 (apigenin) in solvent system I while 0.08 (chlorogenic acid); 0.13 (rutin); 0.22 (ellagic acid); 0.37 (catechin); 0.72 (cafeic acid); 0.79 (leutolin); 0.91 (p-coumaric acid) in solvent system II and 0.73 (vanillic acid); 0.74 (ferulic acid); 0.75 (benzoic acid); 0.94 (cinnamic acid) in solvent system III (Appendix-IV). The distribution of phenolic acids and flavonoids in ME of C. pepo is presented in table 4.50 and HPTLC chromatogram is shown in Fig. 4.8 (A-C). The different phenolic acids identified and quantified were chlorogenic acid (303.3 µg/ml) and cinnamic acid (8.5 µg/ml) in raw sample extract; cinnamic acid (10.7 µg/ml) and benzoic acid (192.25 µg/ml) in pressure cooked sample extract; again cinnamic acid (7.18 µg/ml) and benzoic acid (180.61 µg/ml) in microwaved sample extract and cinnamic acid (0.14 µg/ml) in the fried sample extract. Rutin (727.89 µg/ml) and catechin (263.85 µg/ml) were identified as two most prominently present flavonoids in C. pepo. However, their concentration decreased drastically in the heat processed samples with frying and pressure cooking influencing the maximum decrease in the concentration of rutin (470.98 µg/ml) and catechin (59.18 µg/ml) respectively. Quercetin was although not identified in the raw sample extract, but it was detected in the extracts of pressure cooked (31.87 µg/ml) and microwaved (45.95...
Table 4.50 Identification and quantification of phenolic acids and flavonoids (μg/ml of extract) in ME of *C. pepo* as determined by HPTLC

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw</th>
<th>Pressure Cooked</th>
<th>Microwave cooked</th>
<th>Fried</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phenolic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>nd</td>
<td>nd</td>
<td>19.32</td>
<td>nd</td>
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<tr>
<td>Chlorogenic acid</td>
<td>303.34</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>Vanillic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>8.58</td>
<td>10.70</td>
<td>7.18</td>
<td>0.14</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>nd</td>
<td>192.25</td>
<td>180.61</td>
<td>nd</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>nd</td>
<td>31.87</td>
<td>45.95</td>
<td>nd</td>
</tr>
<tr>
<td>Myrecetin</td>
<td>53.11</td>
<td>nd</td>
<td>nd</td>
<td>42.09</td>
</tr>
<tr>
<td>Apigenin</td>
<td>3.78</td>
<td>2.69</td>
<td>2.91</td>
<td>nd</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>4.96</td>
</tr>
<tr>
<td>Leutolin</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Rutin</td>
<td>727.89</td>
<td>500.75</td>
<td>552.76</td>
<td>470.98</td>
</tr>
<tr>
<td>Catechin</td>
<td>263.85</td>
<td>59.18</td>
<td>59.39</td>
<td>72.61</td>
</tr>
</tbody>
</table>

nd = not detected

μg/ml) samples. After different heat processing such as pressure cooking, microwave cooking and frying some new compounds were formed which were not identified in raw vegetable. In pressure cooked sample extract, benzoic acid (192.2 μg/ml) and quercetin (31.8 μg/ml) were detected. However, in microwave cooking three new phenolics, namely caffeic acid, benzoic acid and quercetin were found. While, after frying only kaempferol (4.96 μg/ml) was investigated.
Fig. 4.8A HPTLC profile of ME of C. pepo as developed in chloroform: hexane: methanol: formic acid (6.4: 3.9: 2.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.8B HPTLC profile of ME of *C. pepo* as developed in chloroform: hexane: methanol: formic acid (4.0: 1.0: 1.0: 1.0) (a) raw (b) pressure cooked (c) microwave cooked (d) fried
Fig. 4.8C HPTLC profile of ME of *C. pepo* as developed in acetonitrile: methanol: water (4.5: 1.0: 0.5) (a) raw (b) pressure cooked (c) microwave cooked (d) fried