Phytoingredients as a natural source of antioxidant in masala paneer

RR Saikia, T Borpuzari, M Hazarika, R Nath and AK Sharma

Abstract

The study was conducted to determine the antioxidant activity of phytoingredients in masala paneer. Fresh plant extracts of Paederia foetida (T1) and Houttuynia cordata (T1) were incorporated to the treatment groups @ 4% along with crushed cumin while the control (T0) was without any extract. The phytoingredients identified in Liquid Chromatography- Mass Spectrometry spectra of P. foetida extract were gallic acid, scandoside, asperuloside, paederoside, quercetin and kaempferol and those of H. cordata were vanillic acid, caffeic acid, houttuynamide A, quercetin hexoxide and chlorogenic acid. Total Phenolic Content and 2,2-diphenyl-1-picrylhydrazyl assay of the fresh samples showed highest value in H. cordata added group followed by that of P. foetida one. Thiobarbituric acid number of the product during refrigeration storage of 10d showed significant and highly significant differences between the treatment groups and days of storage, respectively. Observed that H. cordata was better than P. foetida in production of phyto-preserved masala paneer.

Keywords: Masala paneer, Paederia foetida, Houttuynia cordata, antioxidants, phytoingredients, TBA (Thiobarbituric acid), total phenolic content, DPPH (2,2-diphenyl-1-picrylhydrazyl)

Introduction

Phytochemicals are plant’s secondary metabolites, and phenolic compounds which are a potent source of antioxidant comprised a major class of these phytochemicals (Gioscarie et al., 2016) [1]. The phenolic compounds by chelating the transition metal ions inhibits free radical formation and propagation of free radical reactions and thereby it inhibits lipid oxidation (Brown et al., 1998) [3]. Moreover, plant ingredients (herbs and spices) are found to be more effective with lesser side effects. Paederia foetida and Houttuynia cordata are two indigenous medicinal plants of Assam since long. Paederia foetida (Bhedailata) is used in traditional medicine since long for its antibacterial effect (Uddin et al., 2009) [20], therapeutic value (Chanda et al., 2013) [5], antioxidant property (Chanda et al., 2013; Kumar et al., 2014) [5, 14] and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity (Soni et al., 2013) [19]. H. cordata (Musundori) extract also possesses antimicrobial (Isogai, 1952; Chikane et al., 2003; Kim et al., 2008; Sekita et al., 2016; Li et al., 2017) [12, 7, 13, 18, 15] and antioxidant properties (Choi et al., 2002; Chen et al., 2003; Nuengchamnong et al., 2009) [8, 6, 16]. Their proven antimicrobial and antioxidant properties offer a scope to conduct a study to incorporate them in masala paneer and to determine their antioxidant effect on milk lipid.

Materials and Methods

The study was undertaken in the laboratory of the Department of Livestock Products Technology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati. Fresh raw cow’s milk for the study was procured from the Instructional Livestock Farm (Cattle Unit), College of Veterinary Science, AAU, Khanapara-22. Cumin and plants (Paederia foetida and Houttuynia cordata) were procured from local market. Antioxidant compounds of plant extract prepared as per method of Demiray et al. (2009) [9] was analysed by using Liquid Chromatography-Mass Spectrometer by using UltiMate3000 (Thermo Scientific, United States) as per the method of Nuengchamnong et al. (2009) [16]. Paneer was prepared in the laboratory using standard method (Aneja et al., 2002) [1] with slight modification. The milk was first heated to 86°C (for 10min) and subsequently cooled to 76°C (within 10min) and allotted to the following groups viz., Control (T0) (0.25% crushed cumin), Treatment T2 (0.25% crushed cumin with 4% P. foetida fresh plant extract) and T3 (0.25% crushed cumin with 4% H. cordata fresh plant extract). Masala paneer samples were vacuum
packed in high density polyethylene (HDPE) films of 200gauge. Physicochemical analysis of masala paneer for TBA number was determined as per Witte et al. (1970) [23], the antioxidant activity of the plant extract in masala paneer was analyzed using DPPH (1,1-diphenyl-2-picyrylhydrazyl) radical inhibition assay and total phenolic content as per the method of Apostolidis et al. (2007) [22]. Experimental data obtained from the experiment were analyzed by using standard method of Repeated Measures Design, Analysis of Variance (ANOVA) technique, Honest Significant Difference (HSD) test in Jmp of SAS 9.3 and SPSS IBM Statistics.

Results and Discussion
Phytoingredients of plant extract

The phytoingredients of P. foetida extract identified in LC-MS spectra in the peak Nos. 1, 2, 3, 4, 5 and 6 were gallic acid, scandoside, asperuloside, paederoside, quercetin and kaempferol given in Table 1 (Fig no. 1a,1b,1c and 1d) and those of H. cordata extract identified in peak Nos. 1, 2, 3, 4 and 5 were vanillic acid, caffeic acid, houttuynamide A, quercetin hexoside and chlorogenic acid (Fig no. 2a and 2b).

Table 1: Major phytoingredients identified by lc-ms in methanol: aqueous extract of P. Foetida and h. Cordata

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant</th>
<th>Compound</th>
<th>Molecular Weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P. foetida</td>
<td>Gallic acid</td>
<td>171.15</td>
</tr>
<tr>
<td>2</td>
<td>Scandoside</td>
<td>391.28</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Asperuloside</td>
<td>413.27</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Paederoside</td>
<td>445.19</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Quercetin</td>
<td>302.14</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Kaempferol</td>
<td>285.04</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Vanillic acid</td>
<td>167.03</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Caffeic acid</td>
<td>181.05</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Houttuynamide A</td>
<td>273.19</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Quercetinhexoside</td>
<td>463.13</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Chlorogenic acid</td>
<td>354.09</td>
<td></td>
</tr>
</tbody>
</table>

Fig 1a: lc-ms spectra of the methanol: aqueous extract of p. Foetida*

* Peak No.

Fig 1b: lc-ms spectra of the methanol: aqueous extract of p. Foetida*

* Peak No.
Fig 1c: lc-ms spectra of the methanol: aqueous extract of *P. Foetida*

Fig 1d: lc-ms spectra of the methanol: aqueous extract of *P. Foetida*

Fig 2a: lc-ms spectra of the methanol: aqueous extract of *H. Cordata*
Thiobarbituric acid number

The effect of P. foetida and H. cordata on TBA number during storage period showed significant differences ($P<0.05$) between the treatment groups and highly significant variations ($P<0.01$) between days of storage. A gradual increase in TBA number were noticed in all the treatment groups from 0 to 10d of storage (Table 2 and Fig. 3). Minimum TBA value of 0.230±0.009 mg malonaldehyde/kg was recorded for T$_3$ sample on 10d of storage. The values ranged between 0.136, 0.120 and 0.120 on 0d to 0.237, 0.211 and 0.191 on 5d and 0.285, 0.243 and 0.230 mg malonaldehyde/kg on 10d of refrigeration storage for T$_1$, T$_2$ and T$_3$ samples, respectively. Maximum TBA number was observed in control group during entire storage period. However, interaction effect between treatments and day of storage did not show any significant variation ($P>0.05$).

This might be due to the potential antioxidant activity of the phenolic substances present in the plant extracts of P. foetida (Osman et al., 2009; Chanda et al., 2013) and H. cordata (Nuengchamnong et al., 2009; Cai et al., 2012; Fu et al., 2013) added to masala paneer which in turn prevented lipid oxidation. Results of the present study are supported by the findings of Wanjari (2016).

### Table 2: Effect of plant extracts on TBA number (mg malonaldehyde/kg) of masala paneer during refrigerated storage*

<table>
<thead>
<tr>
<th>Day(s)</th>
<th>Treatment(s)</th>
<th>0</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T$_1$</td>
<td>0.136±0.004$^a$</td>
<td>0.237±0.013$^a$</td>
<td>0.285±0.022$^a$</td>
</tr>
<tr>
<td></td>
<td>T$_2$</td>
<td>0.120±0.005$^b$</td>
<td>0.211±0.013$^b$</td>
<td>0.243±0.010$^b$</td>
</tr>
<tr>
<td></td>
<td>T$_3$</td>
<td>0.120±0.003$^b$</td>
<td>0.191±0.007$^{ab}$</td>
<td>0.230±0.009$^b$</td>
</tr>
</tbody>
</table>

*Significant at $P<0.05$; **Significant at $P<0.01$

NS- Non Significant

Effect of plant extracts on antioxidant activity of masala paneer

**Total Phenolic Content**

Results of total phenolic content (TPC) of masala paneer incorporated with P. foetida and H. cordata extracts showed highly significant differences ($P<0.01$) between the treatments and are presented in Table 3 (Fig. 4). From the table, it could be seen that the amount of TPC were significantly higher in T$_2$ and T$_3$ treatment groups of masala paneer with P. foetida and H. cordata extracts, respectively. Maximum TPC was exhibited by the T$_3$ group (0.8960±0.002 mgGAE/g) followed by T$_2$ (0.7890±0.003 mgGAE/g) and T$_1$ samples (0.2068±0.001 mgGAE/g).

**DPPH Radical Scavenging Activity**

The effect of P. foetida and H. cordata extract on DPPH radical scavenging activity of masala paneer also showed highly significant differences ($P<0.01$) between the treatments Table 3 (Fig. 5). Results in the table indicate that masala paneer with H. cordata extract exhibited maximum DPPH radical scavenging activity of 29.63±0.39% followed by T$_2$ group (24.35±0.59%) with P. foetida extract and the
least antioxidant activity was noticed in control group T1 (5.10±0.39%).

Table 3: Effect of Plant Extracts on Antioxidant Activity of Masala Paneer*

<table>
<thead>
<tr>
<th>Treatment (s)</th>
<th>TPC (mg GAE/g)</th>
<th>DPPH Activity (Inhibition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.2068±0.00139*</td>
<td>5.10±0.39*</td>
</tr>
<tr>
<td>T2</td>
<td>0.7890±0.00310*</td>
<td>24.35±0.50*</td>
</tr>
<tr>
<td>T3</td>
<td>0.8960±0.00253*</td>
<td>29.63±0.39*</td>
</tr>
</tbody>
</table>

n=5
*Mean ± SE
Means with common superscripts column wise does not differ significantly.

Fig 4: effect of p. foetida and h. cordata on total phenolic content (mg gae/g) of masala paneer

Fig 5: effect of p. Foetida and h. Cordata on antioxidant activity (dpph % inhibition) of masala paneer

Conclusion
From the present study it was found that incorporation of fresh plant extracts in masala paneer preparation have significant effect on the total phenolic content (TPC) and DPPH activity of the masala paneer. Plant extract also have a significant effect on TBA which gives the indication of lower lipid oxidation in plant incorporated groups of masala paneer. However, extract of H. cordata was found to be better than P. foetida in production of phyto-preserved masala paneer from cow’s milk. Thus, masala paneer (T2 and T3) with plant extracts may be recommended for its health beneficial effects like antimicrobial, anti-inflammatory, antioxidant etc.

Acknowledgement
The authors express sincere gratitude and thankfulness to the Dean, Faculty of Veterinary Science, AAU, Khanapara, Guwahati – 22, Dr B.N. Saikia for providing the necessary facilities and financial aid to carry out the research programme successfully, Dr R.N. Borpuzari, professor and Dr. M. Raquib, Asst. Professor, Deppt. of Livestock Technology and Dr. J. Hussain, Professor, Deptt. of Livestock Production and Management, College of Veterinary science, AAU, Khanapara, Ghy-22 for extending help in writing of this manuscript. I am also very much grateful to Mrs. Juri Pathak, Asst. Technician, IASST, Boragaon, Assam for extending help in pursuing my research study at IASST.

References
12. Isogai Y. An antimicrobial substance isolated from the rhizome of Houttuynia cordata. Scientific papers of the College of General Education, University of Tokyo, Tokyo, 1952.


