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RR Saikia
M.V.Sc, Department of Livestock Products Technology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

T Borpuzari
Professor, Department of Livestock Products Technology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

M Hazarika
Professor and Head, Department of Livestock Products Technology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

R Nath
Professor, Department of Veterinary Biochemistry, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

AK Sharma
Professor, Department of Livestock Production and Management, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

Corresponding Author:
RR Saikia
M.V.Sc, Department of Livestock Products Technology, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

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* (T_2) and *Houttuynia cordata* (T_3) were incorporated to the treatment groups @ 4% along with crushed cumin while the control (T_1) 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 hexoside 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 (Gioxarie *et al.*, 2016) [11]. 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*., 2007) [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, Ghy-22. 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 preparaed 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 86^0C (for 10min) and subsequently cooled to 76^0C (within 10min) and allotted to the following groups *viz.,* Control (T_1) (0.25% crushed cumin), Treatment T_2 (0.25% crushed cumin with 4% *P. foetida* fresh plant extract) and T_3 (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) [22], the antioxidant activity of the plant extract in masala paneer was analyzed using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical inhibition assay and total phenolic content as per the method of Apostolidis *et al*. (2007) [2]. Experimental data obtained from the experiment were analysed 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.

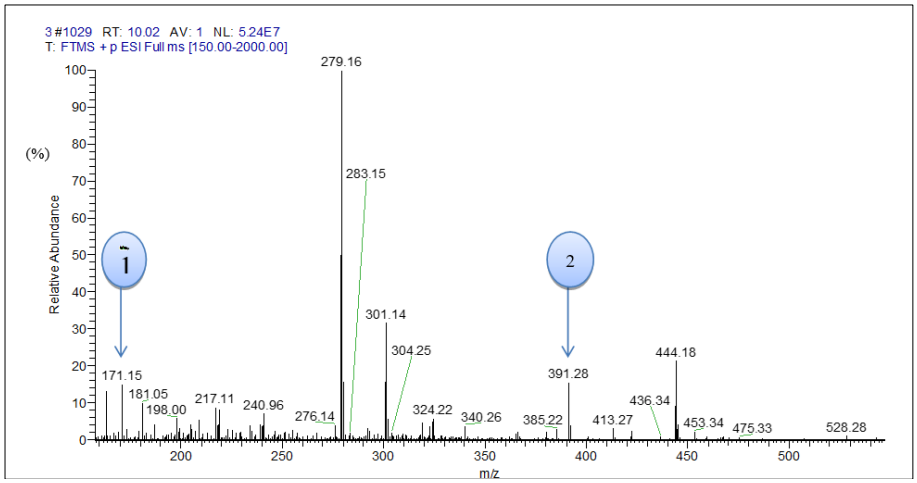
Results and Discussion

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).

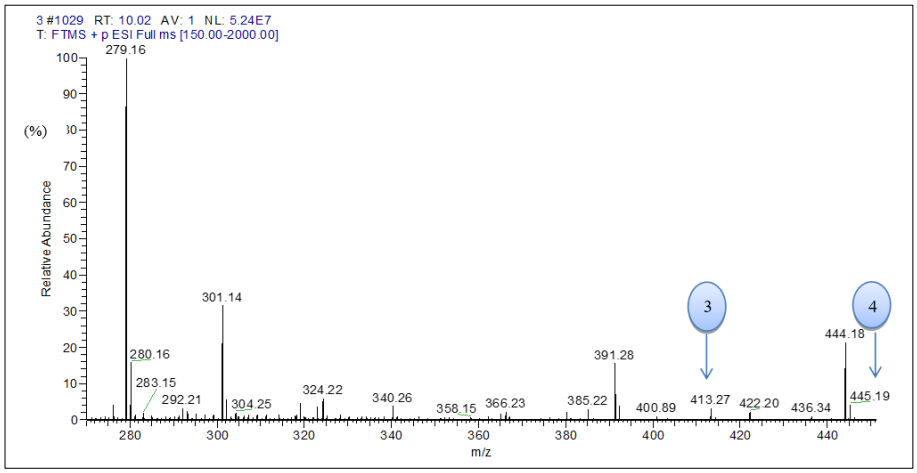
Table 1: Major phytoingredints identified by lc-ms in methanol: aqueous extract of *p. Foetida* and *h. Cordata*

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



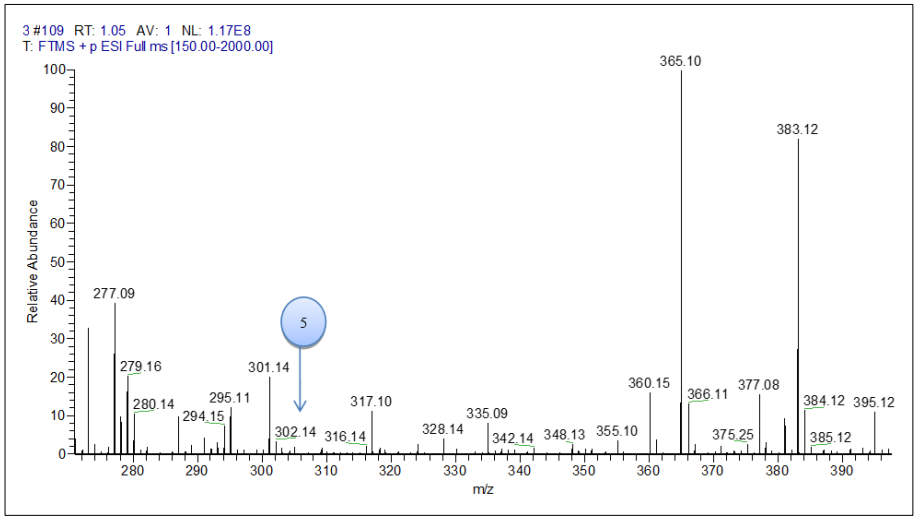
* Peak No.

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



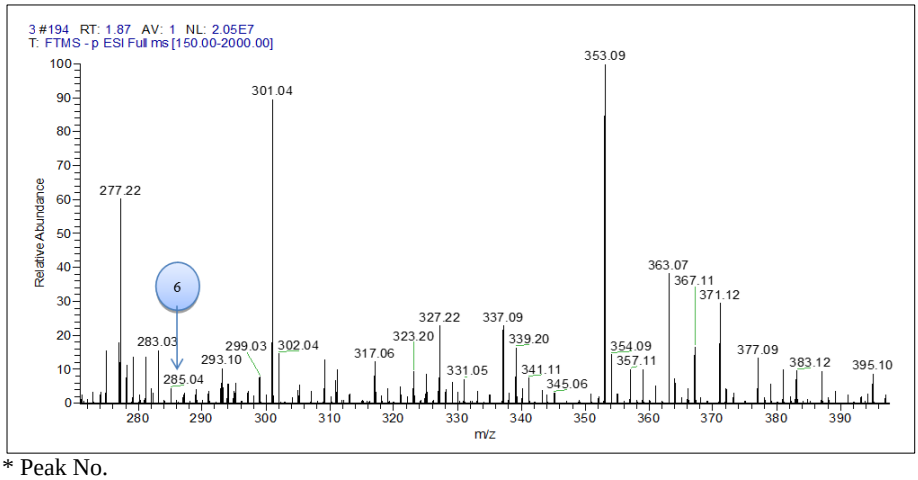
* Peak No.

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



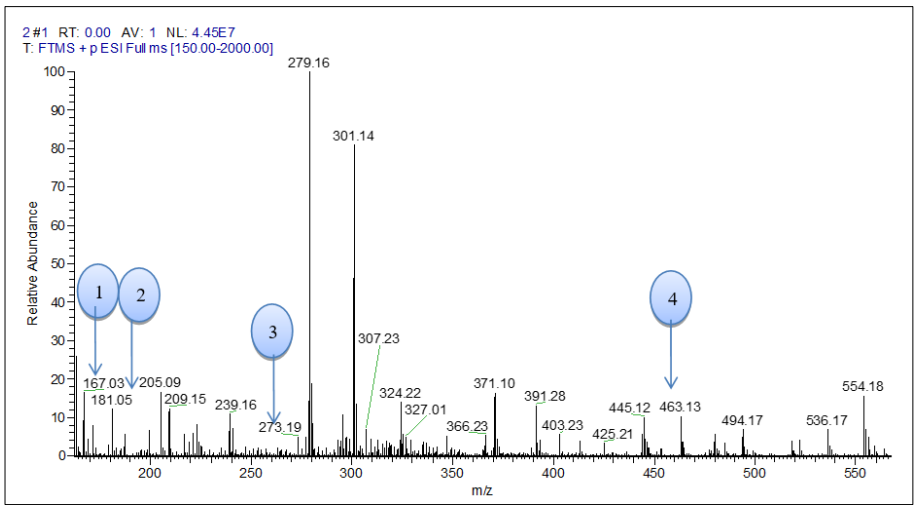
* Peak No.

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



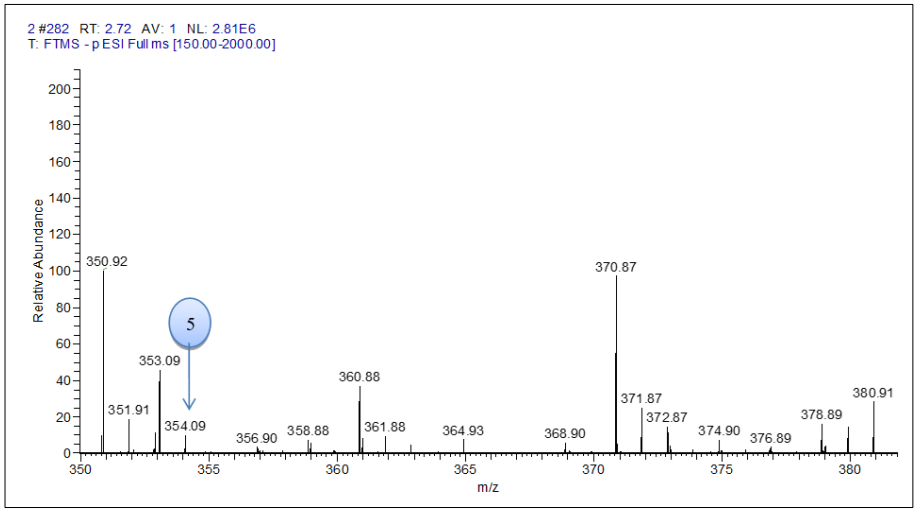
* Peak No.

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



* Peak No.

Fig 2a: lc-ms spectra of the methanol: aqueous extract of *h. Cordata*



* Peak No.

Fig 2b: 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) [17, 5] and *H. cordata* (Nuengchamnong *et al.*, 2009; Cai *et al.*, 2012; Fu *et al.*, 2013) [16, 4, 10] added to masala paneer which in turn prevented lipid oxidation. Results of the present study are supported by the findings of Wanjari (2016) [21].

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

Day(s) Treatment(s)	0	5	10
T_1	$_c$0.136± 0.004^a	$_b$0.237± 0.013^a	$_a$0.285± 0.022^a
T_2	$_c$0.120± 0.005^b	$_b$0.211± 0.013^{ab}	$_a$0.243± 0.010^{ab}
T_3	$_c$0.120± 0.003^b	$_b$0.191± 0.007^b	$_a$0.230± 0.009^b

*Significant at $P<0.05$; **Significant at $P<0.01$
NS- Non Significant

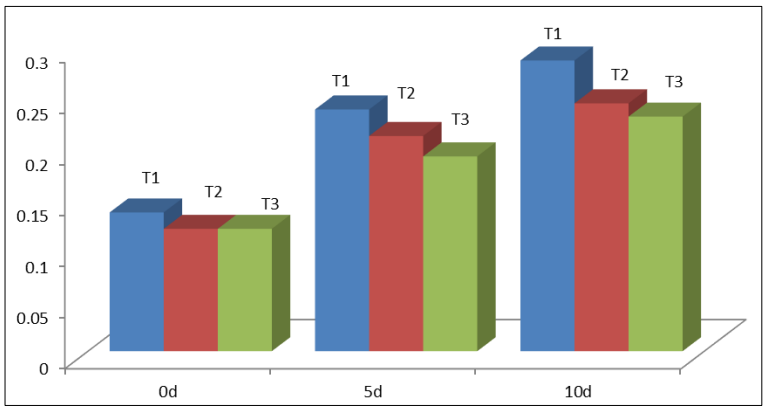


Fig 3: effect of *p. foetida* and *h. cordata* on tba number (mg malonaldehyde/kg) of masala paneer during refrigerated storage

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 T_1 (5.10±0.39%).

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

Treatment (s)	TPC (mg GAE/g)	DPPH Activity (Inhibition %)
T_1	0.2068±0.00139[a]	5.10±0.39[a]
T_2	0.7890±0.00316[b]	24.35±0.59[b]
T_3	0.8960±0.00253[c]	29.63±0.39[c]

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

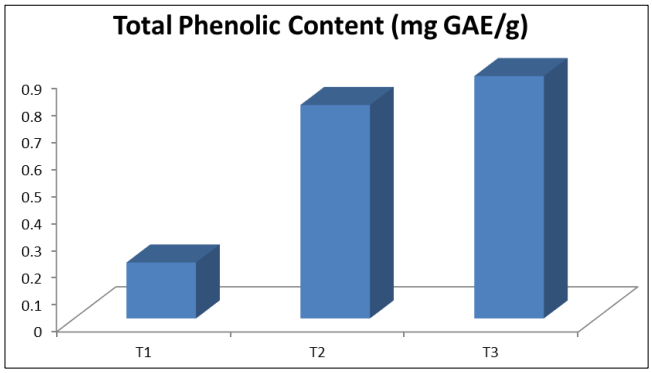


Fig 4: effect of *p. foetida* and *h. cordata* on total phenolic content (mg gae/g) of masala paneer

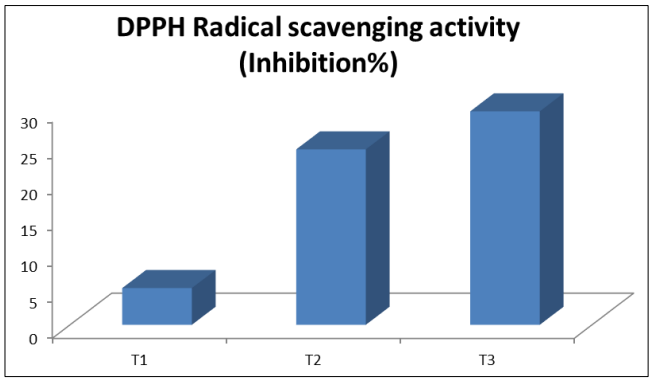


Fig 5: effect of *p. Foetida* and *h. Cordata* on antioxidant activity (dpph % inhibition) of masala paneer

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 (T_2 and T_3) with plant extracts may be recommended for its health beneficial effects like antimicrobial, anti-inflammatory, antioxidant etc.

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