CHAPTER 3

TO DETERMINE THE PHYTOCHEMICAL CONTENT AND ANTIOXIDANT CAPACITIES IN SELECTED FRESH FRUITS AND COOKED VEGETABLES OF ASSAM.

3.1. Introduction

Fruits are natural source of micronutrients like minerals, vitamins as well as secondary metabolites like polyphenols. [1] Epidemiological studies had established that the polyphenols mainly phenolic acids and flavonoid present in fruits have a positive effect on the human health. A positive correlation has been reported between the intake of fruits and reduced risk of chronic degenerative diseases [2-4]. The polyphenols present in fruits can scavenge the free radicals and hence act as antioxidants and destroy the free radical initiated oxidation pathways like lipid peroxidation and DNA damage in the human body and thus lower the risk of cancer, cardiovascular diseases and ageing. [5] However, the effectiveness and activity against the free radicals depend on the polyphenol composition and content in the fruit. Different fruits contain different polyphenol content and their radical scavenging property varies accordingly. [6] There are number of factors which determine the polyphenol content in different fruits. Earlier studies had suggested that depending on the cultivars, environmental conditions, locations and agronomic factors, the composition and content of polyphenols in fruits vary. [7]

Varieties of fruits are available in Assam but their phytochemical content and antioxidant properties in the raw state have not been systematically studied. In addition to the common fruits that are available easily in the market of Assam, *poniol* (*Flacourtia jangomas* (Lour.) Raeusch), hogplum (*Spondias pinnata* L. Kurz), carambola (*Averrhoa* carambola *L.*), *leteku* (*Baccurea sapida Muell. Arg),* and different jamun (*Syzygium* sp.) varieties are often believed to have some therapeutic properties and are used in many traditional medicines. Study on their phytochemical properties is required to harness their goodness into the diet of the people.

Like in fruits, phenolic compounds constitute the major portion of the phytochemicals apart from carotenoids and vitamins in vegetables. The phenolic compounds

help in the destruction of free radicals and other toxic compounds in the human body. Although fruits are mostly consumed in raw form, vegetables need to be cooked to enhance their palatability and taste. However, cooking brings about a number of physical and chemical changes in the vegetables. [8] These changes could be both beneficial and detrimental depending on the extent and type of treatment conditions. Variety of effects like destruction, release and structural transformation of the phytochemicals take place during the cooking process. Cooking treatments like boiling, microwaving,[9] baking, frying and griddling lead to changes in texture and nutritional properties of the vegetables. Studies have reported that cooking softens the cell walls which lead to increase in the extraction of carotenoids. [10]. However, other studies have reported that cooking can also lead to loss in essential vitamins and antioxidants, mostly water soluble and heat labile compounds. The extent of loss is dependent on the type of cooking treatment [11] and the phytochemical composition of the cooked vegetable.

Therefore, based on the above aspects, a study was carried out to estimate the total phenolic content, flavonoid content, and antioxidant activities as well as to determine ascorbic acid and major phenolic acids present in the thirteen fresh fruit samples from Tezpur, Assam. The fruit samples were *bogi jamun (Syzygium jambos* L.), amla *(Emblica officinalis* Gaertn), Indian olive *(Elaeocarpus serratus* L.), *leteku* (*Baccurea sapida* Muell. Arg), carambola (*Averrhoa carambola L.)*, black jamun *(Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var *lanatus*), pineapple *(Ananas comosus L. Merr),* hog plum *(Spondias pinnata L. Kurz), pani jamun* or water apple (*Syzygium samarangense* (Blume) Merr. & Perry), Khasi mandarin orange (*Citrus reticulate* Blanco), *Poniol* or Coffee plum (*Flacourtia jangomas* (Lour.) Raeusch*) and* litchi (*Litchi* chinensis Sonn.).

Further, the effect of boiling, steaming and microwave cooking on the antioxidant activity of the phytochemicals of cauliflower (*Brassica oleracea* L. *var botrytis*), cabbage *(Brassica oleracea* L. *var captita)*, green pea (*Pisum sativa* L.), banana blossom (*Musa balbisiana* Colla, *ABB*), beetroot (*Beta vulgaris* L), teasel gourd *(Momordica dioica* Roxb.), black eyed pea (*Vigna unguiculata* subsp. *Unguiculata*), bottle gourd (*Lagenaria siceraria* (Molina) Standl.), tomato (*Solanum lycopersicum* L.), carrot *(Daucus carota subsp. Sativus), kharua* brinjal (*Solanum melongena* L.), radish (*Raphanus sativus* L.), knol-khol (*Brassica caulorapa L.)* and roselle leaves (*Hibiscus acetosella* Welw.) that are widely available and

consumed in North eastern India and Assam in particular were determined. Vegetables like banana blossom, roselle leaves, black eyed pea and teasel gourd are traditionally known to have health benefitting properties.

3.2. Materials and Methods

All the chemicals used were of analytical grade and supplied by Merck, India and Himedia Laboratories and Sigma chemicals, India.

3.2.1. Materials

3.2.2. Fruit samples

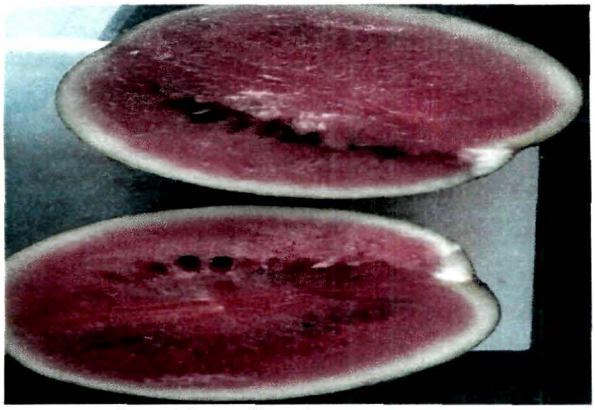
The fruit samples are *bogi jamun (Syzygium jambos* L.*),* amla *(Emblica officinalis* Gaertn), Indian olive *(Elaeocarpus serratus* L.*), leteku* (*Baccurea sapida* Muell. Arg), carambola (*Averrhoa carambola L.)*, black jamun *(Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var *lanatus*), pineapple *(Ananas comosus L. Merr),* hog plum *(Spondias pinnata L. Kurz), pani jamun* or water apple (*Syzygium samarangense* (Blume) Merr. & Perry), Khasi mandarin orange (*Citrus reticulate* Blanco), *poniol* or Coffee plum (*Flacourtia jangomas* (Lour.) Raeusch*)* and litchi (*Litchi* chinensis Sonn.) were procured from the local fruit market, Tezpur, Assam during the season (Fig. 3.1).

3.2.3. Vegetable samples

Freshly harvested cauliflower (*Brassica oleracea* Botrytis), cabbage *(Brassica oleracea captita)*, green pea(*Pisum sativa*), banana blossom (*Musa balbisiana ABB*), beetroot (*Beta vulgaris*) Teaselgourd *(Momordica dioica*), black eyed pea (*Vigna unguiculata* subsp. *Unguiculata*), bottlegourd (*Lagenaria siceraria*), tomato (*Solanum lycopersicum)*, carrot *(Daucus carota subsp. Sativus), kharua* brinjal (*Solanum sp.)*, radish (*Raphanus sativus*), knol-khol (*Brassica caulorapa L.)* and roselle leaves (*Hibiscus acetosella*) were purchased from the local market of Tezpur, Assam (Fig.3.2). All the vegetables were sorted, washed properly before use and cut into uniform pieces. Each vegetable batch was divided into four equal portions. One portion was retained as raw, and the remaining three were subjected to cooking treatments of boiling, steaming and microwave cooking, respectively.

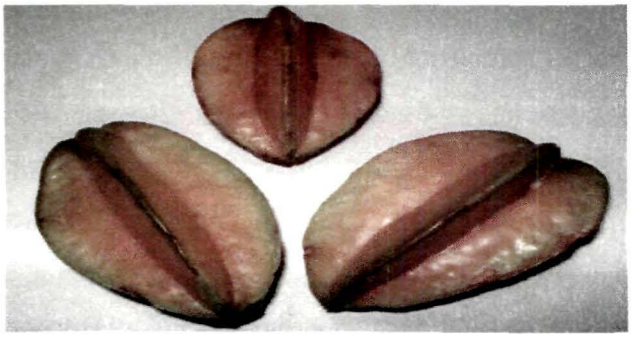
Bogi jamun (Syzygium jambos)

Pani jamun (*Syzygium samarangense*)



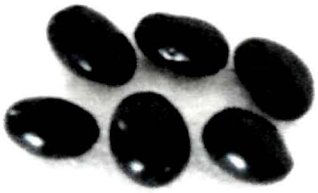
Watermelon (*Citrullus lanatus*)

Amla *(Emblica officinalis* Gaertn)

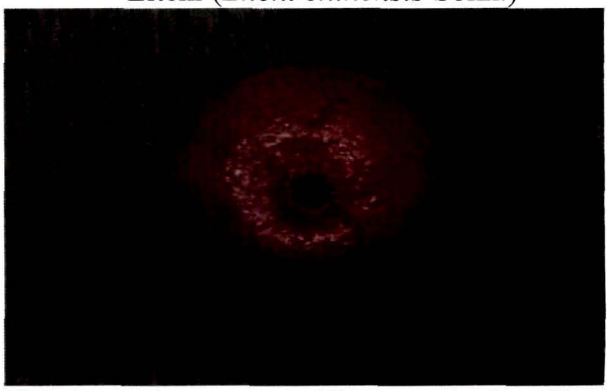


Carambola (*Averrhoa carambola)*

Litchi (*Litchi chinensis* Sonn.)



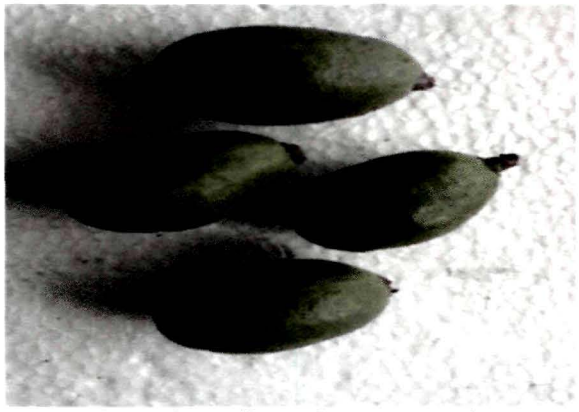
Black jamun *(Syzygium cumuni*)



Khasi mandarin orange (*Citrus reticulate* Blanco)

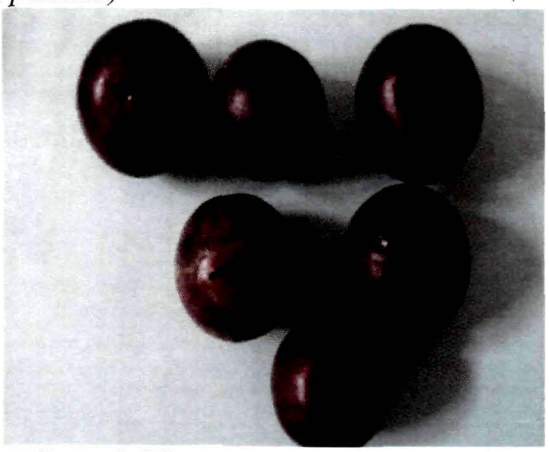
Pineapple *(Ananas comosus)*

Leteku (*Baccurea sapida*)



Hogplum *(Spondias pinnata)*

Indian olive *(Elaeocarpus serratus* L.*)*



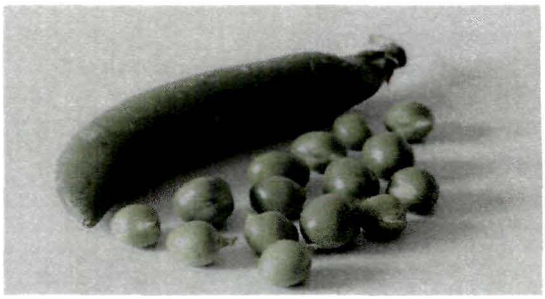
Poniol (Flacourtia catafracta Roxb)

Fig. 3.1. Photograph of the studied fruit samples

Beetroot (*Beta vulgaris* L)

Cabbage *(Brassica oleracea* L. *var captita)*

Knol-khol (*Brassica caulorapa L.)*



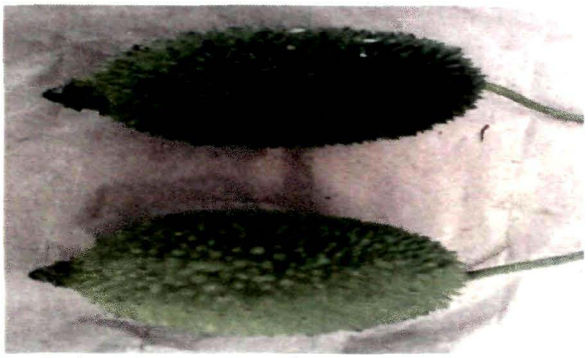
Green pea (*Pisum sativa* L.)

Roselle leaves (*Hibiscus acetosella* Welw.)

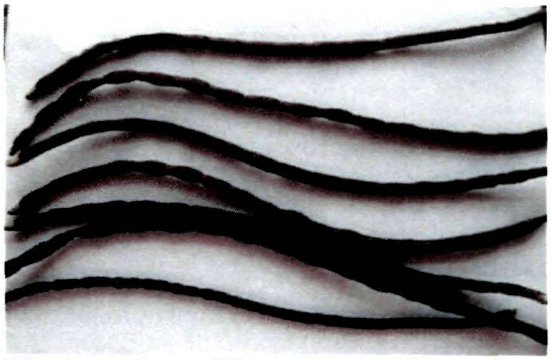
Radish (*Raphanus sativus* L.)

Kharua brinjal (*Solanum melongena* L.)

Carrot *(Daucus carota subsp. Sativus)*



Teasel gourd *(Momordica dioica* Roxb.)



Black eyed pea (*Vigna unguiculata* subsp. *Unguiculata*)

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.)

Banana blossom (*Musa balbisiana* Colla, *ABB*)

Cauliflower (*Brassica oleracea* L. *var botrytis*)

Tomato (*Solanum lycopersicum* L.*)*

Fig. 3.2. Photoghraph of the studied vegetable samples

3.2.4. Cooking treatments

The vegetables were subjected to three cooking treatments- conventional boiling, steaming and microwave cooking. Prior to choosing the best cooking time for the vegetables, the individual vegetables were cooked for different times and the best cooking time was determined by taking into consideration the surface appearance and tender texture felt both by fingers and teeth. The cooking conditions for each treatment are given in Table 3.1. Immediately after cooking, the vegetables were cooled in an ice bath to stop the process of cooking and then stored at -20°C until analysis for phytochemicals and antioxidant activities.

Boiling treatment: Vegetables were added to boiling water in a covered stainless steel container (1:2 sample/water) and cooked. Excess water was drained.

Steaming treatment: Vegetables were cooked in steam using an autoclave (Equitron Model 7407ST, India) under atmospheric pressure (760 mmHg).

Microwave treatment: The vegetables were cooked in a microwave oven (Samsung model) at 600W power level with water (1:1 sample/water).

3.2.5. Sample extraction

The different variants of fresh fruits and vegetables that were obtained were homogenized and extracted in 80% acetone for 90 min at 20°C in a ratio of 1:10 (sample:solvent) in a shaking incubator (Labtech) at 200 rpm and then centrifuged (Hettich centrifuge, Germany) at 970 xg. The supernatant was collected and stored at -20°C until further analysis of their total phenolics content, total flavonoid content, ferric reducing antioxidant potential, DPPH radical scavenging activity and metal chelation activity.

3.2.6. Phytochemical content and antioxidant activities

3.2.6.1. *Determination of total phenolic content*

Total phenolic content in the sample extracts was assessed using the Folin–Ciocalteau assay [12] with slight modification. For the analysis, 20 µL each of sample extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteau reagent, mixed well and within 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40°C. The absorbance

was then measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). The results were expressed in mg GAE/ 100g.

3.2.6.2. ***Determination of total flavonoid content***

The flavonoid content was determined by aluminium trichloride method. [13] Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium trichloride, 0.1 mL of 1M potassium acetate, and 2.8 ml of deionised water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against deionised water blank in a UV-Vis spectrophotometer (Cecil, Aquarius 7400). Results were expressed as quercetin equivalent (mgQE/100g) of sample.

3.2.6.3. ***Determination of ferric reducing antioxidant property (FRAP)***

FRAP activity of the samples was measured by the method of Benzie and Strain. [14] Briefly, a 40 µL aliquot of properly diluted sample extract was mixed with 3 mL of FRAP solution. The reaction mixture was incubated at 37°C for 4 min and the absorbance was determined at 593 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) against a blank that was prepared using distilled water. FRAP solution was pre warmed at 37°C and prepared freshly by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5 mL of 20 mM ferric chloride and 25 mL of 0.3 M acetate buffer (pH 3.6). A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1-10 mM). FRAP values were expressed as µM of ferrous equivalent Fe (II) per 100 g of sample.

3.2.6.4. ***Determination of DPPH radical scavenging activity***

Radical scavenging activity of the sample extracts was measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. [15] Precisely, 100 µL of extracts were added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). The absorbance at 517 nm was measured at 30 min against blank (100 µL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis Spectrophotometer (Cecil Aquarius 7400). The results were expressed in terms of radical scavenging activity.

Radical scavenging acitivity (%) = [(Ao-As)/ Ao] × 100 **Eq.3.1**

Where, Ao is absorbance of control blank, and As is absorbance of sample extract.

Table 3.1. Cooking treatments and cooking time for vegetables

Sl.no	Sample name	Treatment	Time (min)
1	Cauliflower	Steaming	8
		Microwave (600W)	8
		Boiling	9
2	Cabbage	Steaming	7
		Microwave (600W)	7
		Boiling	5
3	Green pea	Steaming	5
		Microwave (600W)	5
		Boiling	6
4	Banana blossom	Steaming	5
		Microwave (600W)	7
		Boiling	8
5	Beetroot	Steaming	7
		Microwave (600W)	9
		Boiling	8
6	Teasel gourd	Steaming	8
		Microwave (600W)	4
		Boiling	10
7	Black eyed pea	Steaming	6
		Microwave (600W)	5
		Boiling	7
8	Bottle gourd	Steaming	5
		Microwave (600W)	5
		Boiling	6
9	Tomato	Steaming	3
		Microwave (600W)	2
		Boiling	3
10	Carrot	Steaming	3
		Microwave (600W)	3
		Boiling	5
11	*Kharua* brinjal	Steaming	4
		Microwave (600W)	3
		Boiling	4
12	Radish	Steaming	5
		Microwave (600W)	4
		Boiling	5
13	Knol-khol	Steaming	5
		Microwave (600W)	4
		Boiling	5
14	Roselle leaves	Steaming	3
		Microwave (600W)	3
		Boiling	4

3.2.6.5. *Determination of metal chelating capacity*

Metal chelating capacity was determined based on the method of Dinis et al., [16] an aliquot of 100 µL sample extract was added to 100 µL of 1mM ferrous chloride and 3.7 mL of distilled water. The reaction was initiated by adding 200 µL of 5mM ferrozine. After 20 min incubation at room temperature, the absorbance at 562 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400) was recorded. The control contained all the reaction reagents except the extract. Decreased absorbance of the reaction mixture indicated increased activity.

Chelation activity [%] = [(Ao-As)/ Ao] ×100 **Eq.3.2**

Where, Ao is absorbance of control blank, and As is absorbance of sample extract

3.2.7. RP-HPLC study of the polyphenols

Sample extraction: The sample extract was prepared by extracting the respective fruits and four vegetable samples in 80% acetone and evaporated under vacuum, then redissolved in 1mL of HPLC grade methanol and filtered through a 0.22 µm nylon filter (Himedia, India).

RP-HPLC (Waters system) gradient elution method was used to identify the major phenolic acid composition of the studied samples. Symmetry 300^{TM} C_{18} (5 µm, 4.6 X 250 mm) column with a binary pump (Waters, 1525) and a UV-VIS detector (Waters, 2489) was used. Mobile phases used were acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method: 80% A (0-8 min), 65% A (9-12 min), 45% A (13-16 min), 30% A (17-20 min), 20% A (21-30 min), 10% of A (31-34 min) and then washing of the column with 65% A (35-39 min) and lastly, 80% A (40-45 min) was followed. Sample volume of 20 µL was used. The flow rate was maintained at 0.8 mL/min and wavelengths used for UV-Vis detector were 254 nm and 325 nm. The standards used for comparison and identification were gallic acid, ascorbic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, ferulic acid, coumaric acid, rutin hydrate, kaempferol , quercetin. The gallic acid and syringic acid belong to the hydroxybenzoic acid group of phenolic acids whereas ferulic acid, chlorogenic acid, coumaric acid and caffeic acid were the hydoxycinnamic acid derivatives. Catechin, rutin, kaempferol and quercetin were the members of the flavonoid group.

3.2.8. Statistical analysis

All experiments were carried out at least in triplicates and reported as mean ± standard deviation of mean (S.E.M) using SPSS version 11.5. The data were statistically analyzed by Duncan's multiple range tests at $p \leq 0.05$ significant levels.

3.3. Results and discussion

3.3.1. Phytochemical and antioxidant properties of thirteen fruit samples

The TPC and TFC results for the studied fresh fruits are given in Table 3.2. The highest TPC was observed in black jamun followed by litchi, *bogi jamun*, amla, hogplum, *pani jamun*, carambola, *poniol* and *leteku*. Lowest TPC value was observed in watermelon. The highest flavonoid content was observed in amla followed by hogplum, black jamun, *leteku*, olive and carambola.

Table 3.2. Total phenolic and flavonoid content of the selected fresh untreated fruits (fresh weight)

Name	Total phenolic content (mgGAE/100g)	Total flavonoid content (mgQE/100g)
Black jamun	7185.15 ± 0.15^{m}	44.13 ± 0.21^{g}
Litchi	2525.00 ± 0.12^{l}	13.13 ± 0.13^{c}
Bogi jamun	2255.00 ± 0.45^{k}	18.85 ± 0.12^{d}
Amla	1923.00 ± 0.26^{j}	152.25 ± 0.21^{i}
Hogplum	1658.50 ± 0.13^{i}	65.63 ± 0.11^{h}
Pani jamun	1220.00 ± 0.41^{h}	10.94 ± 0.19^{b}
Carambola	652.50 ± 0.11^{g}	29.75 ± 0.17^{e}
Poniol	377.00 ± 0.45^{f}	6.66 ± 0.38^{f}
Leteku	305.50 ± 0.28^{e}	43.00 ± 0.11^{g}
Pineapple	92.00 ± 0.09^{d}	4.30 ± 0.07^{a}
Olive	68.00 ± 0.19^{c}	30.50 ± 0.23^{e}
Khasi mandarin	48.50 ± 0.32^{b}	5.13 ± 0.11^{a}
Watermelon	28.40 ± 0.12^{a}	11.25 ± 0.11^{b}

*results are mean±S.D of triplicates data with the same letter between the rows are not significantly different at $p\leq0.05$ by DMRT

The lowest TFC was observed in pineapple (4.30±0.07 mgQE/100g), Khasi mandarin (5.13±0.11 mgQE/100g) and *poniol* (6.66±0.38 mgQE/100g). The rest of the samples showed flavonoid content between 10.94 and 18.85 mgQE/100g. Highest FRAP

value was observed in amla followed by black jamun, hogplum, carambola, *poniol, bogi jamun* and *leteku* (Table 3.3.). Similarly, black jamun, litchi, amla, hogplum, *poniol* showed DPPH activity above 90% while the lowest activity of 22.30% was observed in pineapple. The MCC value was highest in *poniol* (18.55%), carambola (15.95%) and l*eteku* (11.54%). The rest of the samples showed metal chelation capacity in the range of 1.97-10.26%.

The studied fruit samples can be divided according to their phenolic content. This was suggested by Vasco et al. [1] and Rufino et al. [17] based on the total phenolic content of fruit samples from Equador and Brazil, respectively. They classified the fruits into three categories: low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g) and high (>500 mg GAE/100 g) for samples based on fresh matter. Therefore, based on the above classification pineapple, olive, Khasi mandarin and watermelon can be considered to have low total phenolic content; *poniol* and *leteku* contained medium total phenolic content and rest of the samples viz. carambola, *pani jamun,* hogplum, amla, *bogi jamun*, litchi and black jamun can be considered to have high total phenolic content.

Table 3.3. Ferric reducing antioxidant property (FRAP), DPPH radical scavenging activity and metal chelating capacity (MCC) of the selected fresh untreated fruits (fresh weight)

Name	FRAP (μM/100g)	DPPH (%)	MCC (%)
Black jamun	5149.31 ± 0.19^{l}	96.92 ± 0.21^{l}	1.97 ± 0.12^{a}
Litchi	1581.60 ± 0.13^{f}	94.12 ± 0.19^{h}	8.06 ± 0.09^{f}
Bogi jamun	2180.55 ± 0.19^{h}	58.31 ± 0.27^{e}	6.16 ± 0.13^{c}
Amla	6897.57 ± 0.09^{m}	97.17 ± 0.15^{l}	10.26 ± 0.09^{g}
Hogplum	4836.81 ± 0.17^{k}	92.19 ± 0.23^{g}	4.01 ± 0.11^{b}
Pani jamun	947.92 ± 0.32^{e}	51.31 ± 0.10^{d}	4.16 ± 0.14^{b}
Carambola	4468.75 ± 0.23^{j}	62.33 ± 0.19^{f}	15.95 ± 0.29^{i}
Poniol	3288.28 ± 0.46^{i}	91.97 ± 0.39^{g}	18.65 ± 0.27^{j}
Leteku	2128.47 ± 0.42^{g}	49.12 ± 0.22^{d}	11.54 ± 0.17^{h}
Pineapple	446.53 ± 0.37^{a}	22.30 ± 0.13^{a}	6.85 ± 0.21^{d}
Olive	654.51 ± 0.38^{b}	43.97 ± 0.19^{c}	9.93 ± 0.11^{g}
Khasi mandarin	743.06 ± 0.17^{c}	63.01 ± 0.12^{f}	7.55 ± 0.18^{e}
Watermelon	864.58 ± 0.27^{d}	25.93 ± 0.19^{b}	7.55 ± 0.17^{e}

*results are mean±S.D of triplicates data with the same letter between the rows are not significantly different at $p\leq0.05$ by DMRT.

Table 3.4. Pearson's correlation coefficient values for relation between TPC, TFC and antioxidant activity

	TFC	FRAP	DPPH	MCC
TPC	0.217	0.509	0.587*	-0.510
TFC		0.830**	0.553*	0.116
FRAP			0.741**	0.110
DPPH				0.048

*correlation is significant at 0.05 levels
**correlation is significant at 0.01 levels
#TPC-Total phenolic content; TFC-Total flavonoid content; FRAP- Ferric reducing antioxidant property; DPPH- DPPH radical scavenging activity; MCC-Metal chelating capacity

The Pearson correlation showed positive significant correlation between TPC and DPPH activity values (Table 3.4). Similarly, TFC was found to be positively correlated to FRAP and DPPH activity values. The FRAP values are in turn correlated to the radical scavenging activity. However, no significant correlation was observed between the phytochemical content and MCC values. Usually high antioxidant activity could be correlated to high phenolic content but in some cases this doesn't follow the same rule, [18-20] this is because certain phenolics have a higher redox potential than that of other phenolics and therefore can exhibit independent results irrespective of their total phenolic content. Apart from that, the variation in results could be due to presence of other reducing agents such as ascorbic acid, minerals and carotenoids in the fruits, [21, 22] genetic, agronomic and environmental factors. [23]

3.3.2. RP-HPLC analysis of the phenolic acids in the thirteen fruit samples

Nine phenolic acids along with ascorbic acid were identified the given samples by comparing with their standards (Sigma chemicals, India). Peak of the chromatographs obtained at 254 nm were only considered and reported (Table 3.5 and Fig 3.3). All the fruit samples showed presence of varied phenolic acids depending on the sample type. Ascorbic acid was present in all the samples except in *poniol* and carambola. It was found to be highest in amla (24.93±0.12 mg/100g) and hogplum (26.22±0.08 mg/100g) while, *bogi*

jamun, *leteku,* olive showed very low of ascorbic acid. The gallic acid was not present in *bogi jamun* and *poniol.* The rest of the samples showed gallic acid content that ranged between 0.94±0.02 and 43.77±0.15 mg/100g. Amla, hogplum, carambola and black jamun showed relatively good content. The catechin was present only in amla, hogplum, *poniol* and *leteku* and among them highest was present in amla and hogplum. Similarly, cholorgenic acid and caffeic acid was highest in carambola. The syringic acid was obtained only in cararmbola and *bogi jamun.* Likewise, litchi and carambola showed good ferulic acid content. The coumaric acid content ranged between 1.06±0.03 mg/100g and 11.51±0.07 mg/100g. Rutin was detected in six fruit samples viz. litchi, *bogi jamun, pani jamun, poniol,* olive and Khasi mandarin. Only olive and *bogi jamun* showed presence of kaempferol. While, Khasi mandarin, *leteku, poniol,* hogplum, amla, *bogi jamun* and black jamun did not show any quercetin peak, the rest of fruit samples showed low quercetin content that ranged between 0.40±0.02 mg/100g and 3.50±0.07 mg/100g.

The presence of gallic acid in fruits like carambola, black jamun and amla had been reported by previous researchers, [24-27] similarly the presence of quercetin in pani jamun was reported earlier by Reynertson et al.[28] While, Sivaprasad et al.[29] found the presence of ascorbic acid in hogplum.

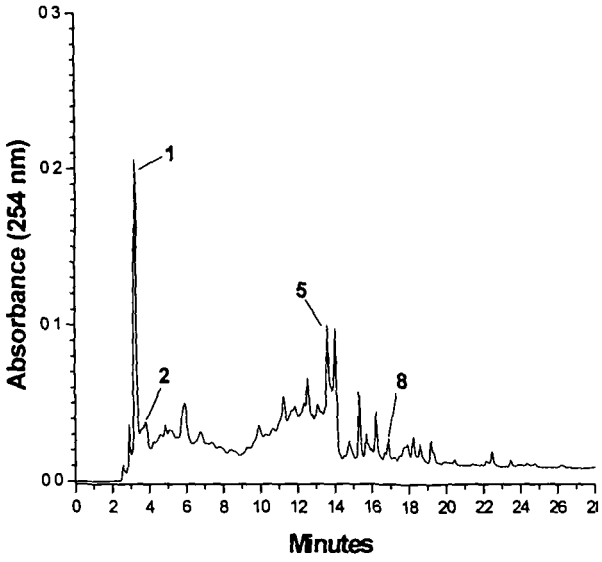
From the above results in can be inferred that, depending on the sample type, variety, agronomic and environmental factors (climate, soils and light exposure) the individual phenolic composition varies and hence some samples show absence while others show presence of a particular phenolic acids. Ikram et al. [30] Therefore, with exceptions in some cases, the rest of the fruit samples could be a naturally good source of phytochemicals and should be included in the diet more often for their health promoting properties.

Table 3.5. Ascorbic acid and phenolic acids in the selected fruit samples determined by RP-HPLC expressed in mg/100g (fresh weight)

Fruits	GA*	AA*	CTH*	CGA*	CFA*	SA*	FA*	CMA*	RTH*	KMF*	QTH*
Black jamun	20.34±0.11	5.55 ±0.07	ND	9.92±0.03	ND	ND	ND	2.74±0.03	ND	ND	ND
Litchi	2.05±0.03	6.08±0.09	ND	ND	ND	ND	33.00±0.07	ND	7.5±0.03	ND	1.01±0.03
Bogi jamun	ND	0.15±0.02	ND	ND	ND	0.28±0.02	1.27±0.06	1.14±0.02	0.49±0.04	0.76±0.02	ND
Amla	43.77±0.15	24.93±0.12	23.93±0.17	ND	ND	ND	7.97±0.10	ND	ND	ND	ND
Hogplum	42.02±0.03	26.22±0.08	26.95±0.11	ND	ND	ND	9.59±0.06	ND	ND	ND	ND
Pani jamun	4.04±0.06	2.41±0.03	ND	ND	ND	ND	ND	ND	1.15±0.03	ND	1.05±0.01
Carambola	38.12±0.18	ND	ND	33.27±0.14	30.03±0.07	12.13±0.09	20.45±0.14	11.51±0.07	ND	ND	3.50±0.07
Poniol	ND	ND	4.02±0.03	ND	ND	ND	ND	4.39±0.05	1.89±0.11	ND	ND
Leteku	3.62±0.10	0.28±0.02	1.24±0.07	ND	0.45±0.01	ND	ND	1.06±0.03	ND	ND	ND
Pineapple	4.12±0.03	1.04±0.01	ND	1.82±0.06	0.92±0.07	ND	ND	ND	ND	ND	1.06±0.03
Olive	2.46±0.05	0.51±0.05	ND	ND	ND	ND	ND	5.51±0.07	0.51±0.08	0.41±0.05	0.40±0.02
Khasi mandarin	1.83±0.07	3.56±0.04	ND	ND	ND	ND	ND	3.39±0.12	6.90±0.09	ND	ND
Watermelon	0.94±0.02	3.08±0.05	ND	1.28±0.03	ND	ND	ND	ND	ND	ND	0.49±0.03

* Results are mean ±S.D of duplicate values;

Footnote: GA- gallic acid; AA- ascorbic acid; CTH- catechin; CGA- chlorogenic acid; CFA- caffeic acid; SA- syringic acid; CMA- coumaric acid; FA- ferulic acid; RTH- rutin hydrate; KF- kaempferol ; QTH- quercetin.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Minutes
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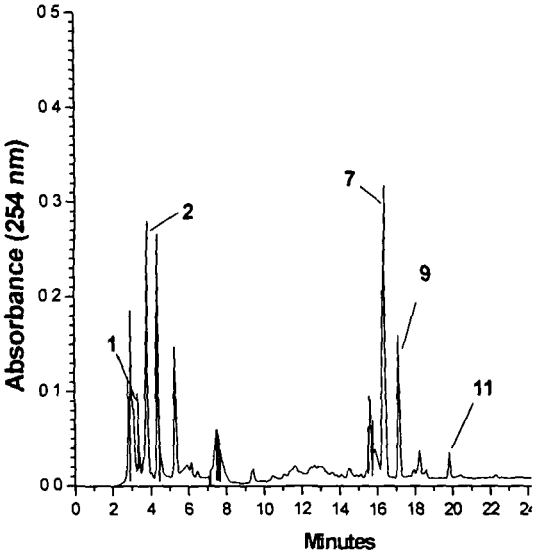
(a)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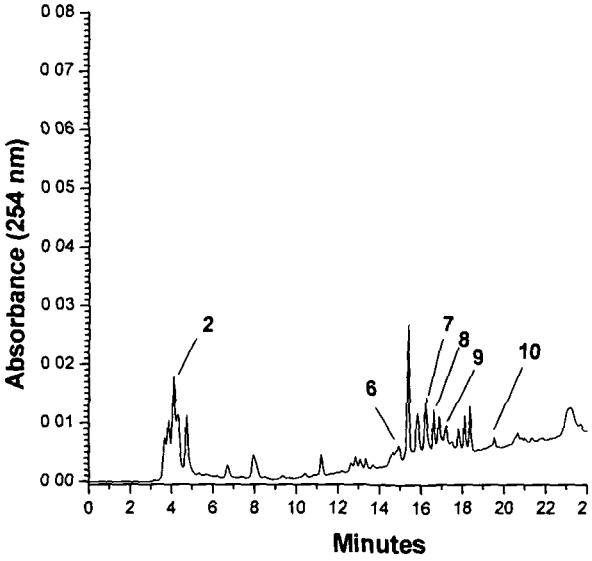
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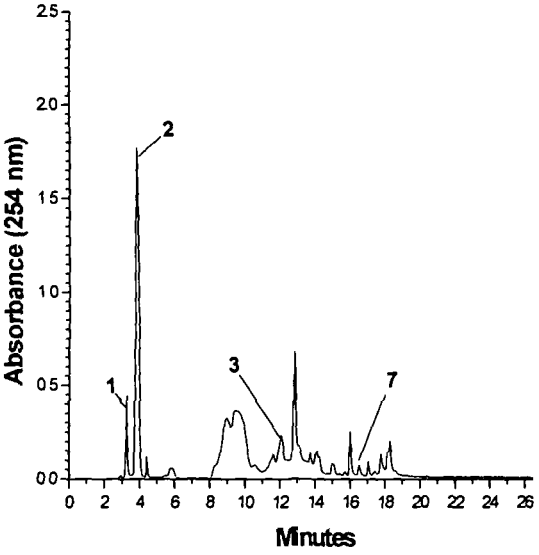
(c)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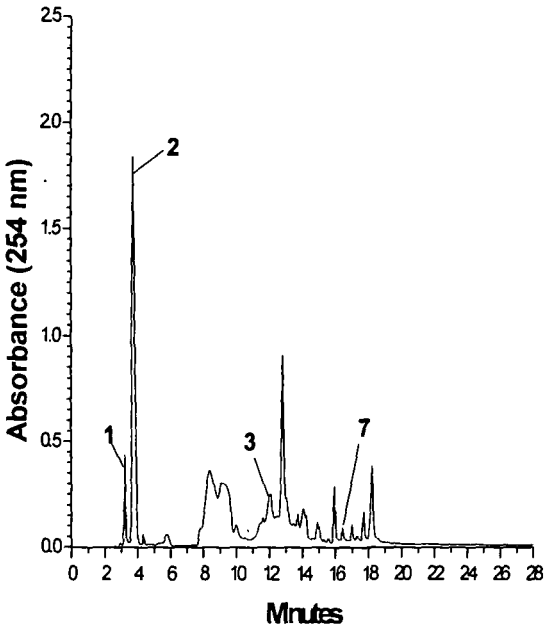
(d)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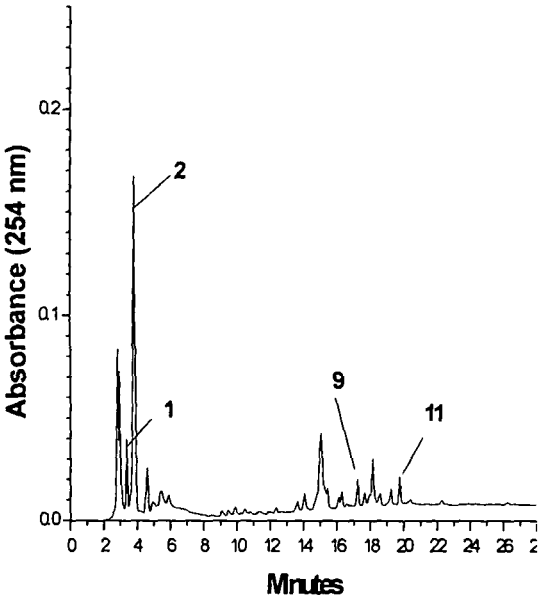
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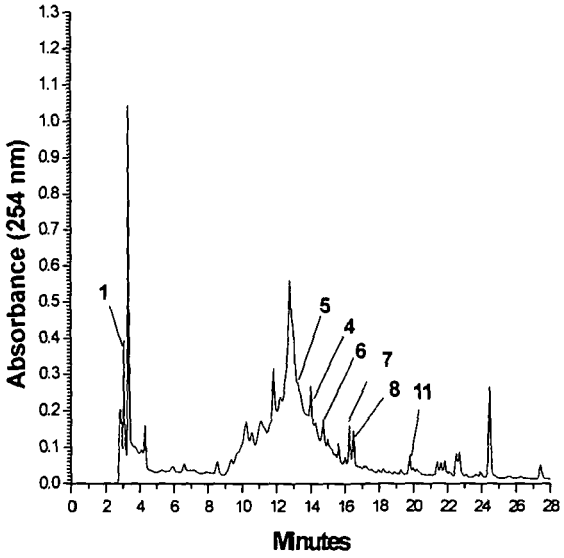
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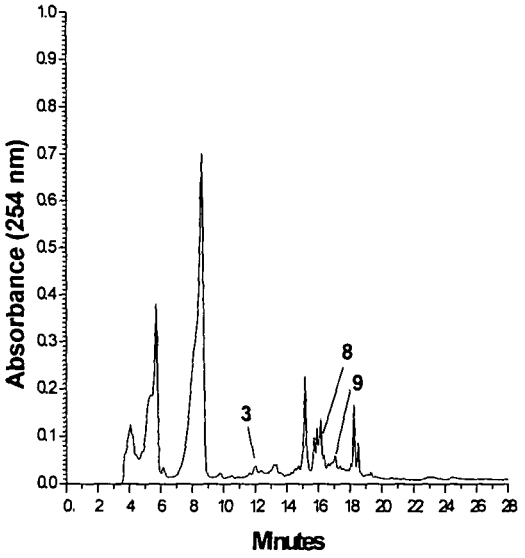
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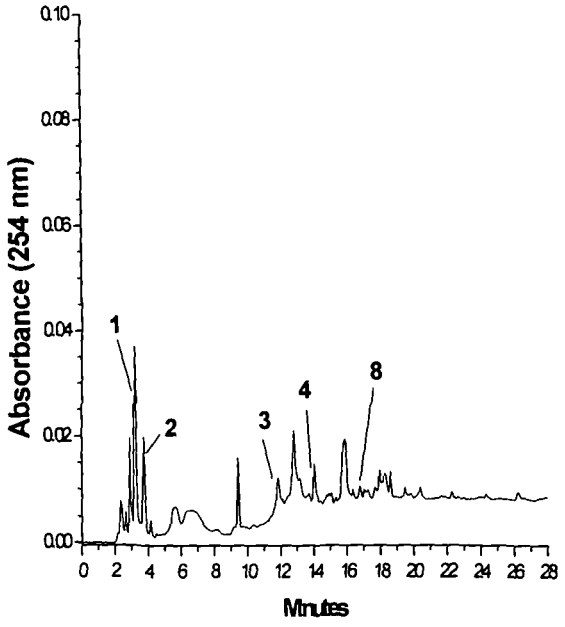
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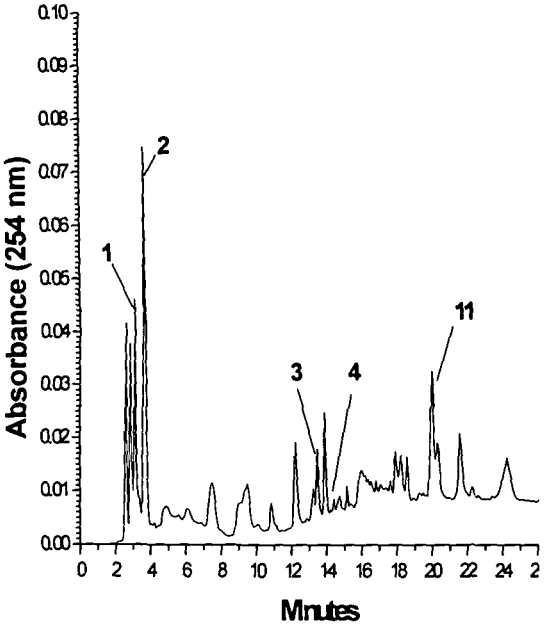
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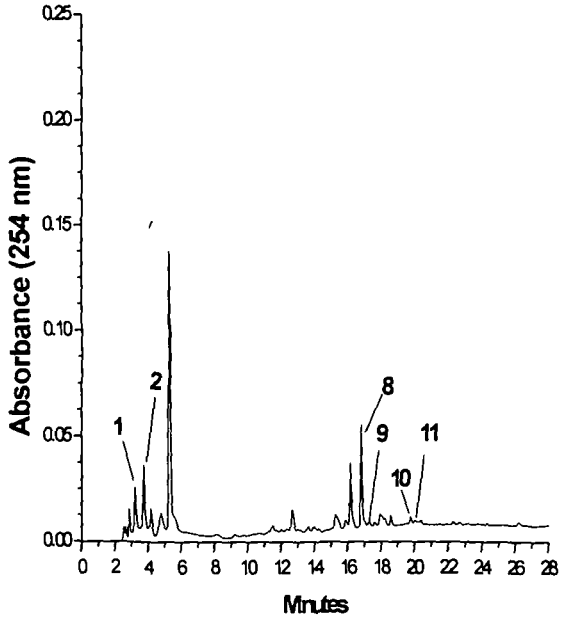
Absorbance (254 nm)
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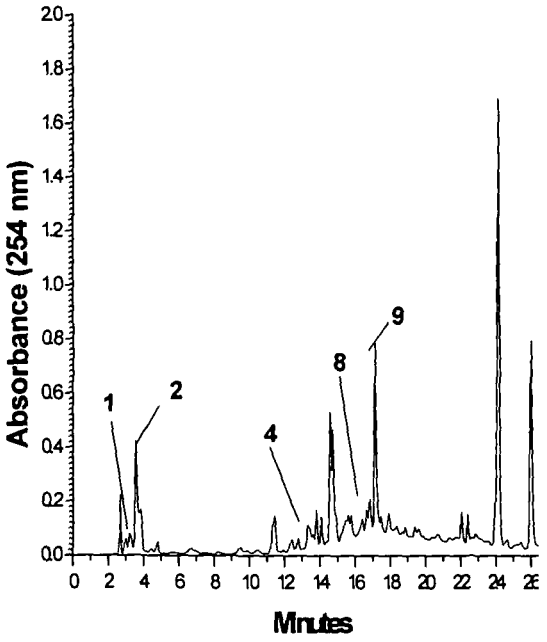
Absorbance (254 nm)
Mnutes
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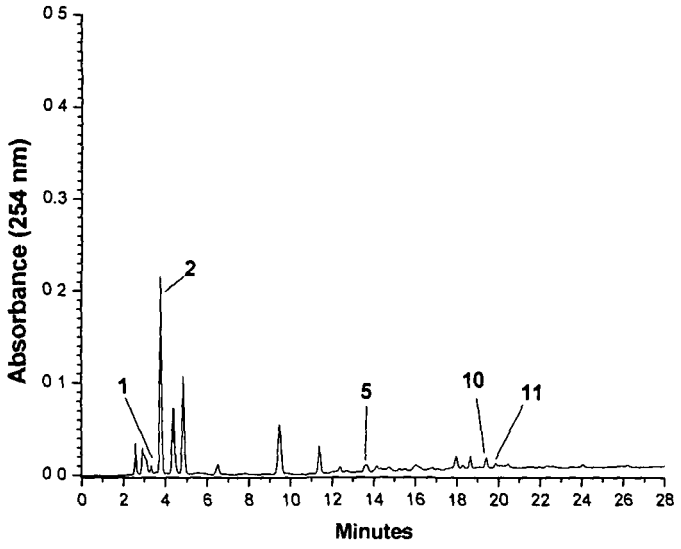
(j)



(m)

Fig. 3.3. RP-HPLC chromatogram of the fruit samples at 254 nm. (a) black jamun (b) litchi, (c) *bogi jamun* (d) amla, (e) hogplum, (f) *pani jamun*, (g) carambola, (h) *poniol*, (i) *leteku*, (j) pineapple, (k) olive, (l) Khasi mandarin and (m) watermelon.

**1=gallic acid; 2= ascorbic acid; 3= catechin; 4= caffeic acid; 5= chlorogenic acid; 6=syringic acid; 7= ferulic acid;8= coumaric acid; 9= rutin hydrate; 10= kaempferol and 11= quercetin

3.3.3. Changes in the phytochemical content after processing in the vegetable samples

3.3.3.1. Changes in total phenolic content (TPC) and total flavonoid content (TFC)

The cooking treatments caused significant changes in the total phenolic content in the selected vegetables (Table 3.6). But cooking processes were not always detrimental to the phytochemical properties. It depended in some cases on the method used and species considered for cooking. [31] Steaming of banana blossom and cauliflower floret caused an increase in TPC value but had a negative effect on microwave and boiling treatments. Similarly, in beetroot and teasel gourd, steaming and boiling had positive effects on the phenolics content compared to raw samples. In black eyed pea, while steaming drastically reduced TPC, boiling was found to increase it. Likewise, cabbage showed an increased TPC on steaming and microwave cooking. Moreover, the remaining vegetables viz. tomato, *kharua* brinjal, knol-khol and carrot exhibited increased TPC during all the three methods of cooking. However, not all the samples followed an increasing effect on phenolics upon cooking. Green pea, bottle gourd, radish and roselle leaves showed negative effect of

thermal treatments on their TPC values. Most importantly, among the studied vegetables, banana blossom (5481.48 mg GAE/100g), beetroot (1063.89 mg GAE/100g), teasel gourd (1166.67 mg GAE/100g), black eyed pea (2059.52 mg GAE/100g), *kharua* brinjal (1516.13 mg GAE/100g) and roselle leaves (3118.11 mg GAE/100g) were found to be rich in TPC and could be exploited for their phenolic content in the food industries and should be included in the diet as a good source of phenolic compounds.

Cooking had both positive and negative effects on TFC depending on the type of vegetables (Table 3.7). Banana blossom, cauliflower, green pea, black eyed pea, bottlegourd and roselle leaves exhibited a lowering trend while, knol-khol, cabbage, tomato and carrot showed increased TFC values upon cooking. Apart from that, beetroot showed an increased TFC in steamed and boiled samples. Radish also showed maximum increase in steamed sample although microwaved and boiling treatment led to destruction of flavonoids. Lastly, in *kharua* brinjal, boiling caused lowering of TFC but steaming and microwave cooking had a positive effect.

Application of heat during cooking involves changes in the structural integrity and cellular matrix of the vegetables and this causes both positive and negative effects on the phytochemical properties. It was observed that cooking caused a significant change in the phenolic and flavonoid content in the selected vegetables. Usually, thermal treatments have destructive effect on the flavonoid and phenolic compounds as they are highly unstable compounds. [32] The black eyed pea and roselle leaves which has anthocyanin, a class of flavonoid as its major pigment showed decreased TPC and TFC upon cooking as these are heat labile. But again, the pattern of change in phenolics depends on the severity of the heat treatments, exposure to air and light, leaching of soluble phenolics [33], the bioactive structures of the studied vegetables, the cutting, chopping and cooking method, and bioavailability and heat stability of the present phenolics. [34] Moreover, heat treatment usually leads to inactivation of the polyphenol oxidase and other oxidising enzymes which in turn slows down the phenolic destruction by oxidation on exposure to the surrounding environment. [35] In some cases, an increasing trend in phenolic and flavanoid content was observed upon thermal treatment. These could be due to breakdown of the cellular matrix which helped in the binding of the total phenolics with pectin or cellulose networks and making them more extractable into the solvents. Moreover, in some instances, application of

heat could cleave the phenolic-sugar glycosidic bonds resulting in the formation of phenolic aglycones, which have high reactivity with Folin- Ciocalteau reagent and thus lead to an increased value of total phenolic content. [36] Also cooking could lead to decomposition of some polyphenols bound to dietary fibre of vegetables releasing free phenolic compounds that increase their detection. [37]

Table 3.6. Total phenolics content (mg GAE/100g DW) in acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	5481.48 ± 0.29^{c}	6070.00 ±0.21^{d}	5100.00 ± 0.28^{b}	2320.51 ± 0.21^{a}
Cauliflower floret	583.33 ± 0.12 ^{c}	684.68 ± 0.29 ^{d}	209.09 ± 0.17 ^{a}	446.33 ± 0.29^{b}
Beetroot	1063.89 ± 0.19^{b}	2003.03 ± 0.11 ^{d}	866.67 ± 0.15^{a}	1434.27 ± 0.22^{c}
Green pea	184.06 ± 0.11^{d}	110.10 ± 0.07^{b}	144.7 ± 0.15^{c}	105.21 ± 0.16^{a}
Teasel gourd	1166.67 ± 0.18^{b}	1230.77 ± 0.15^{c}	1146.67 ± 0.17^{a}	1912.12 ± 0.19^{d}
Black eyed pea	2059.52 ± 0.22^{c}	1420.37 ± 0.27^{a}	1878.79 ± 0.18^{b}	2381.82 ± 0.15^{d}
Cabbage	266.64 ± 0.23^{b}	567.14 ± 0.29^{d}	272.07 ± 0.11^{c}	250.00 ± 0.09^{a}
Bottle gourd	406.25±0.29^{d}	319.15±0.27^{a}	393.94±0.19^{c}	386.36±0.29^{b}
Radish	837.50±0.16^{d}	647.73±0.23^{c}	493.51±0.22^{b}	337.35±0.17^{a}
Tomato	443.66±0.11^{a}	633.33±0.19^{d}	577.59±0.07^{c}	485.50±0.17^{b}
Kharua brinjal	1516.13±0.12^{a}	2449.44±0.11^{c}	2617.65±0.34^{d}	1623.29±0.11^{b}
Knol-khol	199.47±0.22^{a}	386.36±0.23^{c}	564.36±0.13^{d}	340.52±0.23^{b}
Carrot	206.52±0.31^{a}	326.61±0.23^{c}	508.47±0.11^{d}	253.33±0.27^{b}
Roselle leaves	3118.11±0.17^{d}	2178.57±0.25^{c}	1723.14±0.33^{b}	1487.80±0.29^{a}

* * Means with the same letter within row are not significantly different at p≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

Apart from that, the phenolics can be hydrophilic or lipophilic depending on their solubility pattern. The overall difference in the results of the total phenolics and flavonoids of the selected vegetables could be due to the presence of different phenolic groups in the vegetables and their susceptibility to change or destruction during the three cooking treatments. [38] Cooking treatments altered the TPC and TFC of the vegetables although the

direction of change and extent of change was not uniform across all vegetables and across all treatments.

Table 3.7. Total flavonoid content (mgQE/100g DW) of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	359.26± 0.10^{d}	180.83± 0.13^{b}	273.96± 0.13^{c}	67.31± 0.15^{a}
Cauliflower floret	482.46 ± 0.11^{d}	102.48 ± 0.09^{b}	87.12 ± 0.13^{a}	142.66 ± 0.18^{c}
Beetroot	200.69±0.19^{b}	358.33± 0.17^{d}	137.91± 0.10^{a}	303.99± 0.16^{c}
Green pea	32.97 ± 0.11^{d}	26.77± 0.11^{c}	19.05± 0.11^{a}	23.18± 0.11^{b}
Teasel gourd	87.03 ± 0.06^{c}	74.36 ± 0.11^{b}	41.67 ± 0.03^{a}	87.88 ± 0.15^{c}
Black eyed pea	495.83± 0.20^{d}	169.44± 0.19^{a}	293.18± 0.19^{c}	246.59± 0.10^{b}
Cabbage	35.14±0.19^{a}	85.45±0.25^{d}	69.88±0.19^{c}	46.41±0.17^{b}
Bottle gourd	125.00±0.12^{d}	58.51±0.22^{b}	45.45±0.32^{a}	73.86±0.13^{c}
Radish	45.31±0.17^{b}	60.61±0.11^{c}	19.48±0.14^{a}	22.59±0.05^{a}
Tomato	112.68±0.13^{a}	216.67±0.24^{c}	213.36±0.19^{c}	182.97±0.21^{b}
Kharua brinjal	446.24±0.08^{b}	529.49±0.34^{c}	527.57±0.11^{c}	375.00±0.09^{a}
Knol-khol	15.29±0.25^{a}	40.72±0.11^{c}	45.79±0.22^{d}	35.56±0.21^{b}
Carrot	40.76±0.23^{a}	81.65±0.22^{c}	133.47±0.13^{d}	59.17±0.29^{b}
Roselle leaves	269.75±0.20^{c}	190.63±0.34^{b}	116.75±0.12^{a}	109.50±0.23^{a}

* * Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

3.3.4. Changes in the antioxidant activities after processing in the vegetable samples

3.3.4.1. *Changes in ferric reducing antioxidant potential (FRAP)*

The vegetables showed varied results for ferric reducing antioxidant potential upon cooking compared to the raw uncooked vegetables (Table 3.8). The FRAP values upon cooking showed an increased and positive effect on beetroot, green pea, black eyed pea, radish, tomato, *kharua* brinjal and knol-khol for all the three cooking treatments. Banana blossom showed high FRAP value in microwaved and boiled samples but low value in steamed blossom. Cabbage showed increased value during steam cooking.

Carrot retained the FRAP value found in raw but exhibited an increase in microwaved and boiled samples. Likewise, cauliflower floret showed no significant change in FRAP on steaming. However, in the remaining vegetables viz. teasel gourd, bottlegourd and roselle leaves a decrease in FRAP value was observed.

Table 3.8. Ferric reducing antioxidant potential (μM Fe (II)/100-1g) of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	16319.44± 0.33^{b}	14956.27± 0.13^{a}	39570.47± 0.19^{c}	55825.62± 0.29^{d}
Cauliflower floret	3944.32± 0.13^{c}	3941.32± 0.23^{c}	2725.99± 0.19^{a}	2756.46± 0.11^{b}
Beetroot	4480.72± 0.13^{a}	7215.18± 0.15^{c}	7892.03± 0.17^{d}	7154.27± 0.27^{b}
Green pea	417.67± 0.04^{a}	603.86± 0.13^{b}	649.15± 0.11^{c}	1036.63± 0.10^{d}
Teasel gourd	3665.12± 0.27^{d}	2864.58± 0.15^{b}	3067.13± 0.19^{c}	2372.69± 0.25^{a}
Black eyed pea	7155.26± 0.31^{a}	21192.96 ± 0.12^{c}	16319.44± 0.29^{b}	22656.25± 0.33^{d}
Cabbage	1512.93± 0.28^{c}	2235.45±0.12^{d}	425.75±0.14^{a}	1103.12±0.28^{b}
Bottle gourd	3356.72±0.17^{d}	1548.19±0.19^{a}	3018.75±0.29^{c}	2913.75±0.19^{b}
Radish	1862.44±0.11^{a}	3176.25±0.39^{d}	2182.50±0.29^{c}	2066.48±0.11^{b}
Tomato	2151.80±0.17^{a}	4652.78±0.23^{c}	3621.89±0.11^{b}	5308.98±0.27^{d}
Kharua brinjal	8923.32±0.26^{a}	19505.24±0.37^{c}	22165.83±0.32^{d}	14049.88±0.41^{b}
Knol-khol	331.76±0.11^{a}	1561.88±0.33^{c}	2984.71±0.42^{d}	1209.76±0.26^{b}
Carrot	1148.72±0.21^{a}	1131.71±0.35^{a}	2995.17±0.17^{c}	1755.60±0.37^{b}
Roselle leaves	4482.64±0.27^{d}	3281.25±0.23^{c}	2434.03±0.39^{b}	1906.25±0.21^{a}

* * Means with the same letter within row are not significantly different at $p \leq 0.05$ by DMRT. Superscript of DMRT describes significant difference between the treatments

3.3.4.2. *Changes in DPPH radical scavenging activity*

The DPPH radical scavenging effect of the selected vegetables was affected significantly during the cooking treatments (Table 3.9). Banana blossom retained the DPPH activity in boiled sample but the activity increased on steaming (91.19%). Similarly, black

eyed pea and knol khol showed increased activities during microwave cooking but steaming had a destructive effect.

Table 3.9. DPPH activity (%) of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	91.19± 0.31^{b}	92.89± 0.21^{c}	90.27± 0.18^{a}	91.45± 0.26^{b}
Cauliflower floret	7.30± 0.17^{a}	19.53± 0.18^{c}	11.77± 0.19^{b}	11.00± 0.12^{b}
Beetroot	24.96± 0.23^{a}	51.38± 0.30^{d}	35.21± 0.26^{b}	41.48± 0.27^{c}
Green pea	7.35± 0.07^{a}	10.30± 0.16^{c}	10.23± 0.11^{c}	8.51± 0.17^{b}
Teasel gourd	0.33± 0.03^{a}	19.10± 0.28^{b}	35.52± 0.37^{c}	48.65± 0.30^{d}
Black eyed pea	91.06± 0.11^{c}	75.82± 0.23^{a}	80.43± 0.27^{b}	91.34± 0.25^{c}
Cabbage	30.62± 0.18^{d}	6.16± 0.14^{c}	3.59± 0.12^{b}	2.37± 0.13^{a}
Bottle gourd	3.77±0.09^{a}	25.58±0.15^{d}	18.17±0.13^{b}	22.03±0.16^{c}
Radish	21.72±0.07^{d}	2.86±0.05^{b}	0.52±0.03^{a}	3.96±0.06^{c}
Tomato	16.18±0.10^{a}	44.31±0.15^{b}	44.78±0.13^{b}	43.49±0.19^{b}
Kharua brinjal	47.79±0.18^{a}	85.50±0.21^{c}	61.22±0.21^{b}	44.58±0.39^{a}
Knol-khol	4.79±0.10^{c}	1.72±0.03^{b}	6.93±0.20^{d}	0.86±0.05^{a}
Carrot	3.87±0.15^{a}	11.06±0.13^{d}	7.30±0.09^{c}	6.23±0.22^{b}
Roselle leaves	64.15±0.21^{d}	48.79±0.22^{c}	34.26±0.09^{a}	41.31±0.09^{b}

* * Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments

In *kharua* brinjal, maximum increase in DPPH activity was observed in steamed samples (85.50%) compared to raw (47.79%). The remaining vegetables viz. cauliflower florets, beetroot, teasel gourd, green pea, bottle gourd and carrot showed increase in radical scavenging activity on cooking while a decrease in activity was observed in cabbage, radish, roselle leaves and tomato. There was decrease in activity in tomato on cooking compared to raw; however, there was no significant difference between the treatments. The DPPH activity was highest in banana blossom (above 90% activity) and black eyed pea (above 75% activity) followed by *kharua* brinjal (above 45% activity) on cooking by the three treatments.

3.3.4.3. *Changes in metal chelation capacity (MCC)*

The MCC values of the raw and cooked vegetable samples are given in Table 3.10. Cooking caused an increase in MCC in banana blossom in all the three cooked forms. Green pea and radish retained MCC in boiled samples, while an increase in steamed and microwaved samples was observed. Likewise, black eyed pea and carrot showed an increased activity in microwaved samples but showed no change in steamed and boiled vegetables except in carrot where a decrease was observed on boiling. Similarly, steaming had a positive effect on bottle gourd and knol khol. However, a decrease in activity was observed in tomato, *kharua* brinjal and roselle during steaming and boiling. Teasel gourd showed reduction in MCC on cooking.

Table 3.10. MCC (%) values of acetone extracts of raw and cooked vegetables

Samples	Raw	Steamed	Microwaved	Boiled
Banana blossom	6.08± 0.12^{a}	7.35± 0.13^{b}	8.20± 0.08^{c}	9.23± 0.10^{d}
Cauliflower floret	5.79± 0.15^{b}	5.89± 0.16^{b}	5.17± 0.12^{a}	4.97± 0.14^{a}
Beetroot	ND	ND	ND	ND
Green pea	6.26± 0.12^{a}	8.36± 0.15^{c}	7.28± 0.13^{b}	6.60± 0.17^{a}
Teasel gourd	14.42± 0.07^{c}	7.34± 0.13^{b}	7.43± 0.15^{b}	3.51± 0.10^{a}
Black eyed pea	2.72±0.10^{a}	2.11± 0.09^{a}	4.61± 0.10^{b}	3.18± 0.14^{a}
Cabbage	7.39± 0.19^{b}	6.28± 0.10^{a}	7.55± 0.17^{b}	5.98± 0.15^{a}
Bottle gourd	3.89±0.07^{b}	5.73±0.11^{c}	2.28±0.10^{a}	7.06±0.10^{d}
Radish	3.18±0.05^{a}	8.28±0.10^{c}	6.55±0.09^{b}	3.69±0.10^{a}
Tomato	4.66±0.08^{c}	2.63±0.04^{a}	4.86±0.10^{c}	3.26±0.16^{b}
Kharua brinjal	4.46±0.07^{b}	3.14±0.04^{a}	5.14±0.10^{c}	3.37±0.09^{a}
Knol-khol	4.08±0.09^{c}	5.75±0.04^{d}	1.92±0.07^{a}	2.76±0.11^{b}
Carrot	4.29±0.09^{b}	4.60±0.10^{b}	5.37±0.06^{c}	2.22±0.10^{a}
Roselle leaves	6.11±0.20^{b}	4.88±.06^{a}	7.43±0.02^{c}	5.03±0.10^{a}

* * Means with the same letter within row are not significantly different at P≤0.05 by DMRT. Superscript of DMRT describes significant difference between the treatments. ND- not detected

Therefore, depending on the type of vegetables and cooking method, the MCC values varied. In majority of the vegetables, MCC activity was retained or was enhanced. The possible reason for the above results could be that there are many hundreds of different phytochemicals present in food, and each has different characteristics of reacting to the changes in their cellular matrix caused by heat treatments or cooking. This could lead to an increase or decrease in the antioxidant activities of the vegetables. The phenolic content and antioxidant activity have a strong relationship between them [39] and thus phenolic content is a significant factor in most of the cases for increase or decrease in antioxidant activity.

In beetroot, tomato, *kharua* brinjal, knol khol, and carrot an increase in both TPC and antioxidant activity was observed in most of the cases with some exceptions. The increase in antioxidant activity in tomato could be due to the increased bioavailability and accessibility of its lycopene content. In some cases, increase in antioxidant activity was observed due to transformation of phytochemicals into more active compounds like deglycosylation of some flavonoids.

Other factors like polymerization of polyphenols during cooking may result in higher antioxidant activities. [40] During steaming increase in antioxidants activity for all the selected vegetables with the exception of one or two vegetables was observed. This effect was perhaps due to production of redox-active secondary plant metabolites or breakdown products, but is highly likely to be related to release of antioxidants from intercellular proteins, changes in plant cell wall structure and matrix modification. [41] Apart from these, inactivation of oxidative enzymes which are responsible for increase in oxidation of phenolic compounds could lead to an increased activity. Moreover, enhanced antioxidant activity could also be witnessed due to the production of novel compounds due to Maillard reaction. [42]

3.3.5. Changes in the phenolic acids composition in the processed four selected vegetables

The changes in phenolic acid content due to processing effects were studied only for four vegetables viz. banana blossom, roselle leaves, black eyed pea and *kharua* brinjal due to their good total phenolic content compared to the rest of the samples. Here also the chromatogram obtained at 254 nm was only considered as the peak intensities obtained at

325 nm were not significant. The four vegetables showed changes in their composition after various processing treatments (Table 3.11). In some cases, disappearance of a particular phenolic acid was observed while in other, newer phenolic acid peaks were identified in the processed samples (Fig 3.4, 3.5, 3.6 & 3.7).

In raw *kharua* brinjal, only caffeic acid peak was identified. In the microwaved and steamed brinjal, small quantity of gallic acid was obtained which was originally absent in the raw sample. The caffeic acid content showed an increasing trend. Compared to steam and boiling treatments, increase was more in microwaved brinjal.

Similarly, in banana blossom gallic acid was the predominant phenolic acid. The other phenolic acids present were syringic acid, rutin and quercetin. However, after processing treatments the quercetin got destroyed in all the samples while steaming destroyed rutin. But steaming and boiling might have caused release of catechin from its bound form into the extracting medium. The phenolic acid content in black eyed pea like in *kharua* brinjal, only caffeic acid peak was identified and it was observed that heat had detrimental effect. Highest degradation was observed in microwaved black eyed pea.

Catechin, ferulic acid and rutin were predominantly present in the roselle leaves. Processing had detrimental effect on these three phenolics. While, kaempferol was present in the raw leaves, processing caused its destruction. However, small amount of syringic acid was observed in boiled roselle sample.

Loss in phenolic could be due to their breakdown during cooking. [43] Another reason for loss or decrease may be due to the covalent binding between oxidized phenols and proteins or amino acids as well as the polymerization of oxidized phenols. [44]

Table 3.11. Phenolic acids in raw and processed vegetables identified by RP-HPLC expressed as mg/100g

Sample	GA*	CTH*	CFA*	SA*	FA*	RTH*	KF*	QTH
Kharua brinjal								
Raw	ND	ND	1.07±0.03	ND	ND	ND	ND	ND
Microwaved	0.53±0.05	ND	7.00±0.21	ND	ND	ND	ND	ND
Steamed	0.63±0.01	ND	4.47±0.19	ND	ND	ND	ND	ND
Boiled	ND	ND	4.12±0.08	ND	ND	ND	ND	ND
Banana blossom								
Raw	58.70±0.11	ND	ND	3.96±0.11	ND	5.14±0.09	ND	4.77±0.06
Microwaved	4.44±0.3	ND	ND	3.33±0.18	ND	4.44±0.01	ND	ND
Steamed	117.93±0.09	3.21±0.06	ND	4.76±0.09	ND	ND	ND	ND
Boiled	58.38±0.11	5.54±0.11	ND	2.46±0.06	ND	4.31±0.07	ND	ND
Black eyed pea								
Raw	ND	ND	3.56±0.07	ND	ND	ND	ND	ND
Microwaved	ND	ND	0.75±0.02	ND	ND	ND	ND	ND
Steamed	0.52±0.09	ND	2.67±0.07	ND	ND	ND	ND	ND
Boiled	ND	ND	3.51±0.08	ND	ND	ND	ND	ND
Roselle leaves								
Raw	ND	85.71±0.09	ND	ND	77.68±0.8	30.96±0.09	2.75±0.09	ND
Microwaved	ND	35±0.04	ND	ND	46.95±0.05	16.35±0.07	ND	ND
Steamed	ND	27.78±0.01	ND	ND	42.02±0.07	25.11±0.13	ND	ND
Boiled	ND	54.55±0.01	ND	4.55±0.07	47.73±0.05	24.14±0.01	ND	ND

*Results are mean±S.D of duplicate values; GA- gallic acid; CTH- catechin; CFA- caffeic acid; SA- syringic acid; FA- ferulic acid; RTH- rutin hydrate; KF- kaempferol; QTH- quercetin.

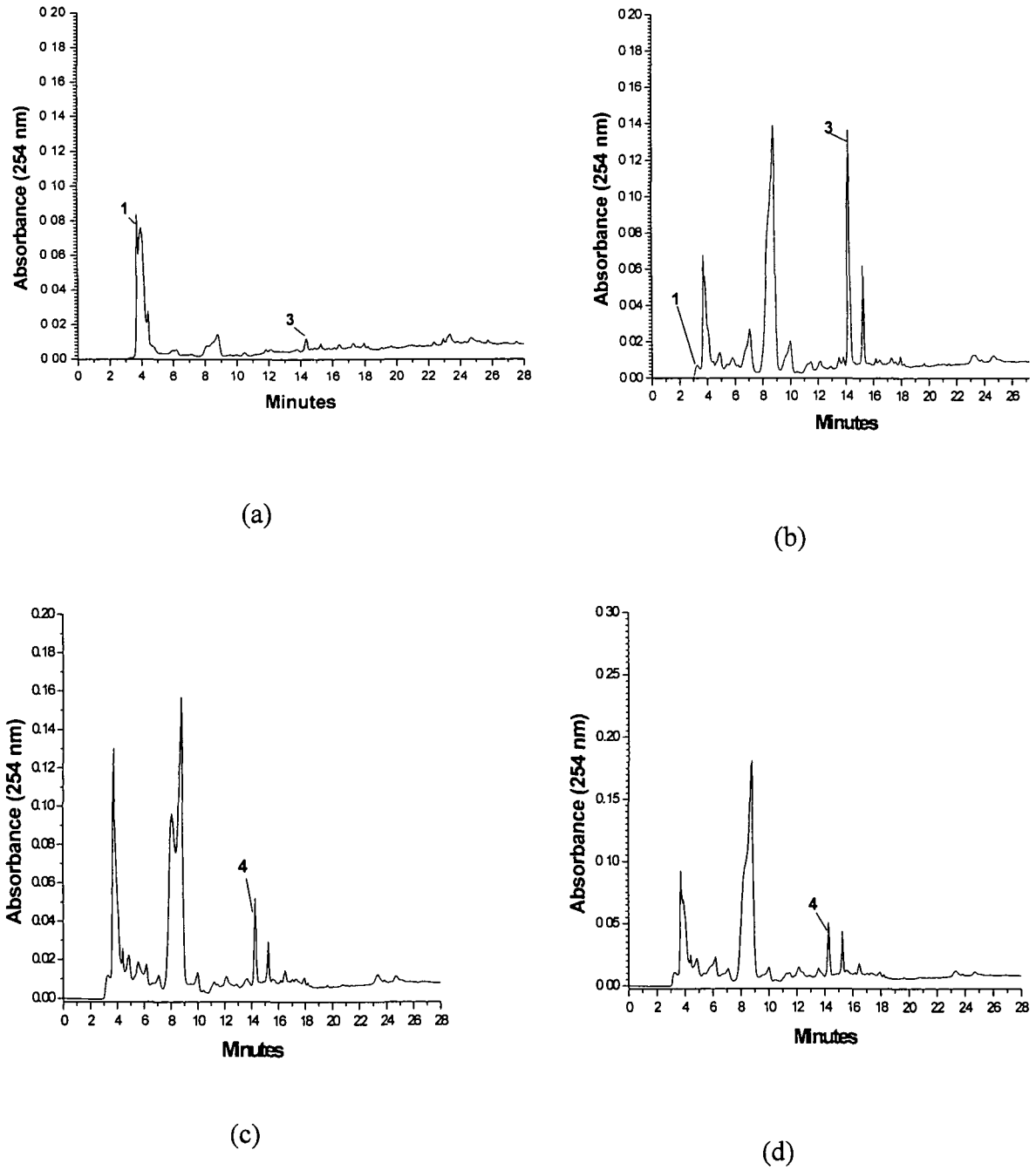


Fig. 3.4. RP-HPLC chromatograms of the raw and processed *kharua* brinjal samples at 254 nm. (a) raw, (b) microwave treated, (c) steam treated and (d) boiled; 1=gallic acid; 2= catechin; 3= caffeic acid; 4= syringic acid; 5= ferulic acid;6= rutin hydrate; 7= kaempferol and 8= quercetin.

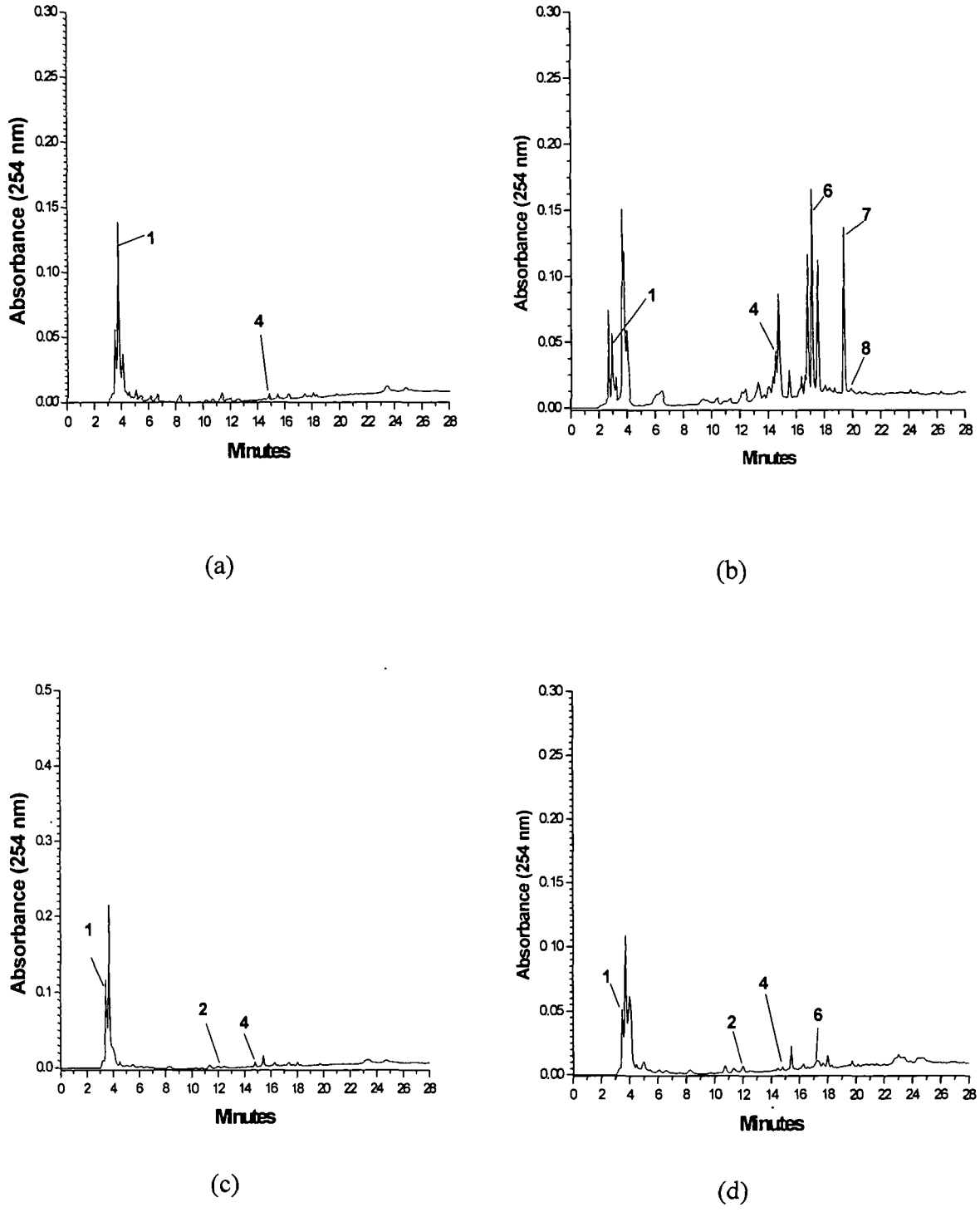


Fig. 3.5. RP-HPLC chromatograms of the raw and processed banana blossom samples at 254 nm. (a) raw, (b) microwave treated, (c) steam treated and (d) boiled; 1=gallic acid; 2= catechin; 3= caffeic acid; 4= syringic acid; 5= ferulic acid;6= rutin hydrate; 7= kaempferol and 8= quercetin.

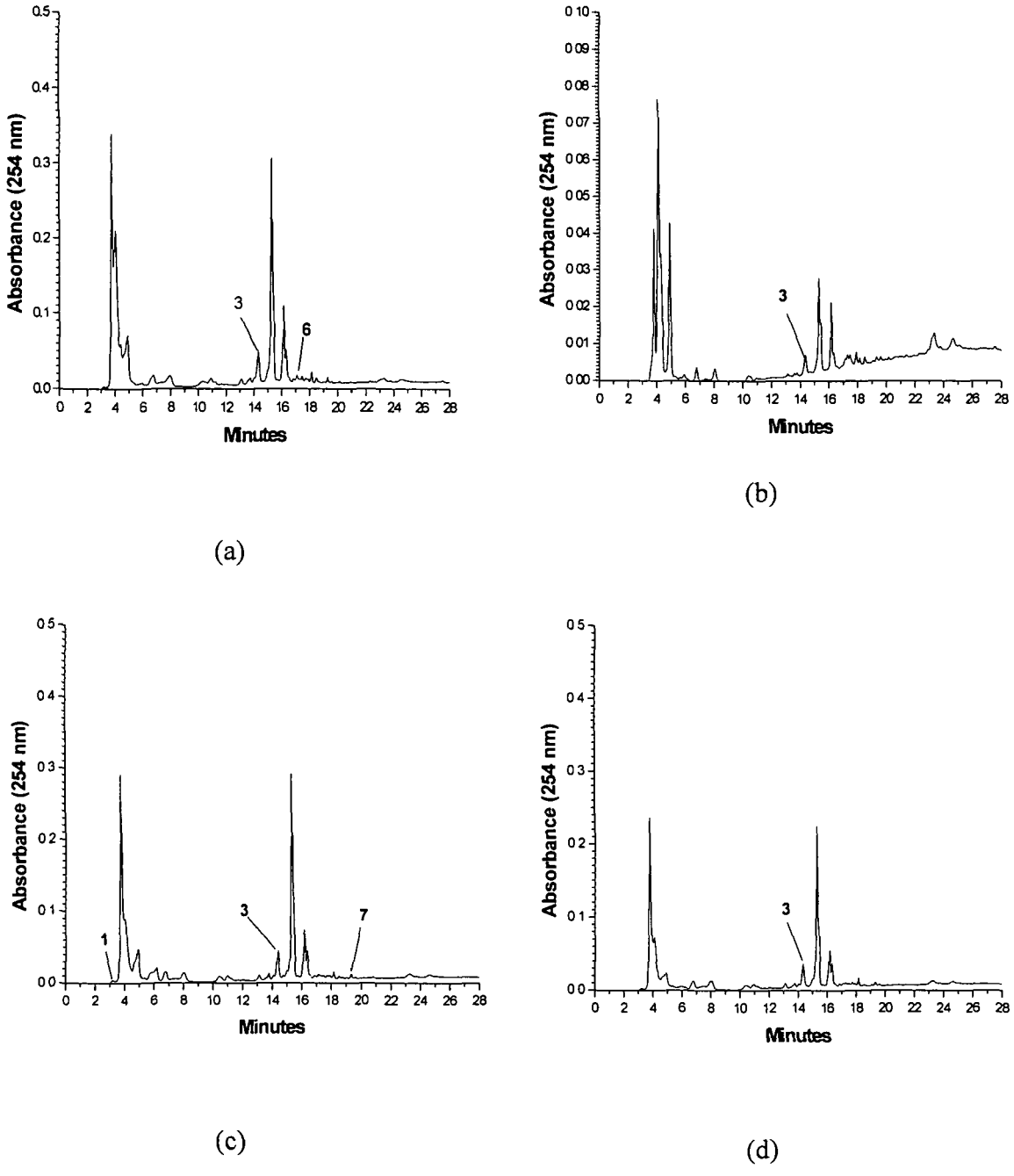


Fig. 3.6. RP-HPLC chromatogram of the raw and processed black eyed pea samples at 254 nm. (a) raw, (b) microwave treated, (c) steam treated and (d) boiled; 1=gallic acid; 2= catechin; 3= caffeic acid; 4= syringic acid; 5= ferulic acid;6= rutin hydrate; 7= kaempferol and 8= quercetin

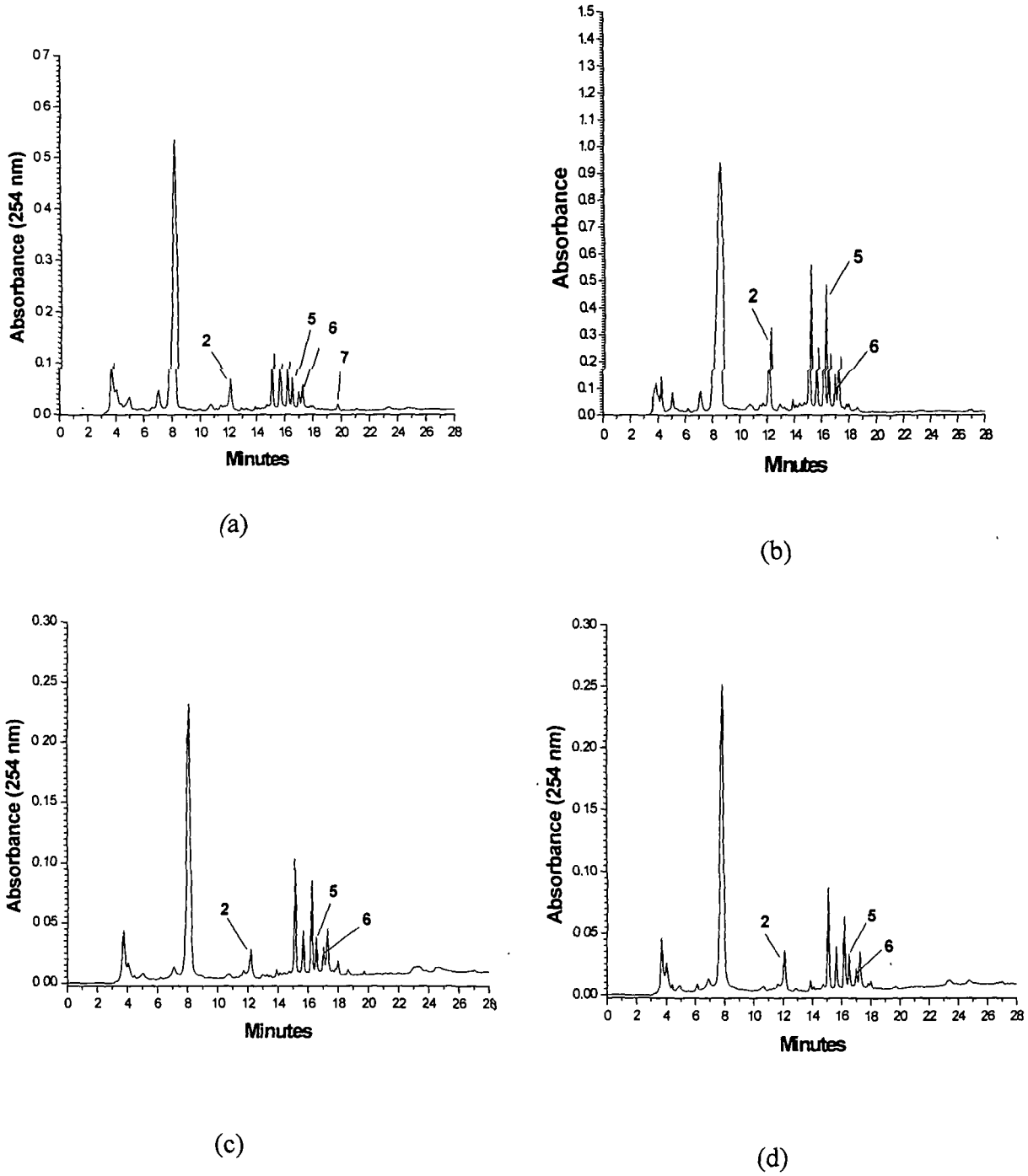


Fig. 3.7. RP-HPLC chromatogram of the raw and processed roselle leaves samples at 254 nm. (a) raw, (b) microwave treated, (c) steam treated and (d) boiled; 1=gallic acid; 2= catechin; 3= caffeic acid; 4= syringic acid; 5= ferulic acid;6= rutin hydrate; 7= kaempferol and 8= quercetin

Processing treatments like, application of heat might cause disruption of cell wall matrix which in turn facilitates the release of the bound phenolic from the cellular structures like lignin and other polysaccharides [45] and thus, increases the solubilization as free or esterified or glycosylated forms in suitable solvents. [46] Granado and Olmedikkla [46] also suggested that processing treatments and time may cause chemical changes through polymerization and degradation, for example, degradation of complex phenolics like tannins and flavonoids into simple forms. Ewald et al. [47] reported decrease in flavonoids like quercetin and kaempferol during blanching, boiling or microwave cooking. Other authors studied and reported flavonoid decrease during boiling of onion. [48]

In case of flavonoids, processing in general has detrimental effect. [49] Depending on the type of processing and nature of heat applied to the vegetables, higher or lower cleavage of phenolic bonds might occur resulting in the different results observed with variation in sample type and processing methods. [50] In case of flavanols, baking and sautéing lead to a 7–25% gain in quercetin concentration, whereas boiling leads to a decrease. Makris and Rossiter [51] reported in onion that, the type of flavonols present differed with processing methods and appearance of novel flavonol substance was observed.

3.4. Conclusion

The highest TPC was observed in black jamun followed by litchi, *Bogi jamun,* amla, hog plum, *pani jamun* and carambola. Similarly, the above fruits showed good ferric reducing antioxidant potential and radical scavenging activities compared to the rest of the studied fruits. Usually high antioxidant activity could be due to high phenolic content but in some cases this may vary. On comparison across the thirteen fruit samples pineapple, olive, Khasi mandarin and watermelon were low in total phenolic content; *poniol* and *leteku* had medium total phenolic content and rest of the samples viz. carambola, *pani jamun,* hogplum, amla, *bogi jamun*, litchi and black jamun were high in total phenolic content. Certain phenolics have a higher redox potential than other phenolics and therefore can exhibit independent results irrespective of their TPC. Khasi mandarin, litchi, *pani jamun*, amla, hog plum, watermelon showed good ascorbic acid content. Carambola showed good amounts of gallic acid, chlorogenic acid, caffeic acid, syringic acid, ferulic acid and quercetin. Amla and hog plum showed high content of gallic acid, catechin and ferulic acid.

In case of vegetable samples, cooking can make the phenolics and antioxidants of cooked vegetables quite different from that of uncooked form. This is probably due to variety of effects like destruction, release and transformation of the phytochemicals. Cooking enhanced the antioxidant activity of the selected vegetables in most of the cases. Overall, steaming was the most preferred method for cooking. But, in case of cooked banana blossom and black eyed pea, a decrease in flavonoid content was observed. Among the vegetables, banana blossom, beetroot, teasel gourd, black eyed pea, *kharua* brinjal and roselle leaves were found to be rich in TPC and antioxidant properties. In banana blossom, gallic acid was the predominant phenolic acid. The other phenolic acids present are syringic acid, rutin and quercetin. Steaming and boiling treatments to blossom released catechin from its bound form into the extracting medium. Catechin, ferulic acid and rutin were predominantly present in the roselle leaves. Processing had detrimental effect on these three phenolics. In some cases, disappearance of a particular phenolic acid was observed while in other, newer phenolic acid peaks were identified in the processed samples.

Therefore, from the above discussed results, it could be inferred that depending on the sample type, variety, agronomic and environmental factors (climate, soils and light exposure) the individual phenolic composition varies and hence some samples show absence while others show presence of a particular phenolic acid. In vegetables processing treatments had both positive and negative impact on the phytochemicals and antioxidant activities on the vegetables. In most cases, cooking increased the release of phenolics into the extraction medium and among the three cooking methods employed steaming emerged as the most suitable method followed by microwave cooking in most of the cases.

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