

# HPLC Analysis of the Phenolic Profiles in Different Parts of Chilli (*Capsicum annum*) and Okra (*Abelmoschus esculentus* L.) Moench

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## Abstract

The nature of phenolic acids in the two important vegetable crops chilli (*Capsicum annum*, Family Solanaceae) and okra (*Abelmoschus esculentus* L. Family Malvaceae) was investigated and their medicinal significance was discussed. Presence of tannic, gallic, caffeic, vanillic, ferulic, chlorogenic and cinnamic acids was recorded in different parts of the plant and seeds of various varieties. Gallic acid was found to be the major constituent in almost all the plant parts of chilli and okra. Concentration of tannic, ferulic, chlorogenic and cinnamic acids varied significantly in seeds of okra varieties, namely, BO2, VRO5, IIVRO10 and Prabhani Kranti. Presence of caffeic acid could only be traced in different varieties of chilly while vanillic acid was found only in the fruits of sweet pepper.

## INTRODUCTION

Vegetables provide substantial amount of nutrients of important for human health because they are particularly important sources of micronutrients and vitamins, e.g., provitamin A, B6, C and E. as well as folic acid. Chilli (*Capsicum annum*, Solanaceae) and okra (*Abelmoschus esculentus* L. Malvaceae) are important vegetable crops in India and several other countries. Many varieties of both the crops varying in size, shape, flavour, colour and degree of pungency have been cultivated. While fruits of chilli are comparatively smaller in size and highly pungent (hence called hot or chilli pepper). Shimla mirch, one of the varieties of chilli, has nonpungent taste. Dehydrated okra is a processed product for preservation and export. Okra seeds are used as a nutritious ingredient of cattle feed and as source of vegetable oil. Roasted and grinded seeds are used as a substitute for coffee. Bland mucilage of plants is used as a clarifier in jaggery preparation. There exists certain important medicinal attribute for okra, which is given to people suffering from renal colic, leucorrhoea, spermatorrhoea, chronic dysentery and general weakness. Due to high iodine content, the fruits are useful for controlling goitre. Leaves of okra are used in Turkey for preparation of medicines to reduce inflammation <sup>1</sup>.

Phytochemical investigations showed that the chief constituents of chilli pericarp are crystalline colorless pungent principle known as capsaicin or capscutin, a condensation product of 3-Hydroxy-4-methoxybenzylamine and decylenic acid which produces highly irritating vapour on heating <sup>2</sup>. Green chillies are rich in vit. A and C and the seed contains traces of starch. The fruits also contain a fixed oil and nonpungent red coloring matter. Besides these, chilli fruits are reported to contain proteins, fat, minerals, fibres, carbohydrates, calcium, magnesium, riboflavin, oxalic acid and nicotinic acid <sup>2</sup>.

Except for a few report on the presence of the total or individual phenolics, there is a lack of information on the nature of phenolic acids in commercial varieties and plant parts of chilli and okra. Phenolic acids not only provide major quality attributes to the finished products <sup>3</sup>, they also contribute to the astringent taste and thus to overall flavor of the food. Besides, there exist reports showing pronounced physiological and biological properties of a number of phenolic acids, namely, tannic, gallic, caffeic and cinnamic acids <sup>4,5,6</sup>. It is reported that vegetables constitute an important source of dietary intake of polyphenols and phenolic acids account for almost one third of the total intake of phenolics <sup>7</sup>. This prompted us to study the phenolic

acid profile of the two important vegetable crops and results are presented here.

## **MATERIALS AND METHODS**

### **PHENOLIC STANDARDS**

Phenolic standards (tannic, gallic, caffeic, vanillic, ferulic, chlorogenic and cinnamic acids) were obtained from Sigma Chemical Co. (St. Louis, MO). Salicylic acid was obtained by Hi Media (Hi Media Laboratories Ltd., Mumbai, India). All the solvents used in this investigation were of HPLC grade.

### **PLANT MATERIALS**

Freshly harvested plant parts of okra and chilli were collected from the Vegetable Farm, BHU, India and after air drying at room temperature, stored at 4° C. Seeds of okra cultivars namely, BO2, VRO5, IIVRO10 and Prabhani kranti and chilli (green, red, sweet pepper) were obtained from Indian Institute of Vegetable Research, Varanasi (India). All solvents and reagents were of highest analytical grade and were redistilled before use.

### **EXTRACTION OF PHENOLIC ACIDS**

One g of freshly harvested plant parts were macerated in paste-mortar followed by suspending fine-crushed sample into 5 ml of ethanol-water (80:20, v/v) in glass tubes. Dry seeds of okra and chilli varieties (1 g) were crushed and suspended in the extraction solvent in a similar way. The suspension was subjected to ultra-sonication (Branson sonifier, USA) at 60 % duty cycles for 25 min at 4°C followed by centrifugation at 7,500 rpm for 15 min. The clear-greenish supernatant of each sample was subjected to charcoal treatment to remove pigments prior to evaporation under vacuo (Buchi Rotavapor Re Type). Dried samples were resuspended in 1.0 ml HPLC grade methanol by vortexing and stored at 4°C for further analysis.

### **HPLC ANALYSIS**

High performance liquid chromatography (HPLC) of the samples was performed with the HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable UV-VIS detector (Shimadzu SPD-10 AVP) and a Winchrom integrator (Winchrom). Reverse phase chromatographic analysis was carried out in isocratic conditions using RP C-18 HPLC column (250 x 4.6 mm id, particle size 5 µm, Luna 5µ C-18 (2), Phenomenex, USA) at 25°C. Running conditions included injection volume: 5 µl, mobile phase:

methanol-0.4 % acetic acid (80:20, v/v), flow rate: 1 ml/min, detection at 290 nm and attenuation response 0.03 AUFS. Samples were filtered through organic solvent compatible membrane filters (Pore size 0.20 µm, Millipore) prior to injection in sample loop. Tannic, gallic, caffeic, vanillic, ferulic, chlorogenic and cinnamic acids were used as internal and external standards. Phenolic compounds present in the samples were identified by comparing retention time (Rt) of the standards and by the co-injection. Contents of phenolic acids were calculated by comparing peak areas of reference compound with those in the samples run under similar elution conditions. Mean relative concentrations of phenolic compounds were statically analyzed by ANOVA and the normality of the results and homogeneity of the variances were tested.

## **RESULTS AND DISCUSSION**

HPLC analysis of different parts of okra (*A. esculentus*) and chilli (*C. annum*) revealed the presence of a number of phenolic acids, namely, tannic (Rt 2.76), gallic (Rt 2.94), caffeic (Rt 3.15), vanillic (Rt 3.26), ferulic (Rt 3.40), chlorogenic (Rt 4.15) and cinnamic (Rt 4.51 min) acids. Quantitative as well as qualitative variations have been observed in the content of these phenolics in different plant parts. Both the content and per cent composition varied significantly in all the the varieties of both the vegetables studied.

Gallic acid was found to be the major phenolic acid in all parts of green chilli. It is evident from Fig. 1 A and B that the highest concentration of the compound was detected in chilli collar (38.46 µg/g fresh wt) that was almost 19.42 per cent of the total phenolic composition as observed by the HPLC analysis. Significant amount of this phenolic acid also detected in other parts such as pulp, leaves, roots and seed but in stem, it was minimum (3.67 µg/g fresh wt).

Agronomic and genetic varietal differences in chilli (*C. annum*) also led to variations in their phenolic content. Highest amount of gallic acid was recorded in red chilli pulp (55.61 µg/g fresh wt) (Fig 1 B) followed by black pepper seed (54.84 µg/g), sweet pepper seed (43.93 µg/g) and fruit (17.79 µg/g), green chilli seed (8.5 µg/g) and chilli seed (2.54 µg/g). Per cent amount of gallic acid in total phenolics also varied significantly in plants and varieties (Fig. 1 C & D). In sweet pepper fruit, gallic acid constituted 41.66 per cent of the total phenolics followed by green chilli seed, red chilli pulp, black pepper seed, sweet pepper seed and red chilli seed.

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A number of phenolic acids, namely, tannic, caffeic, ferulic, chlorogenic and cinnamic acids were detected in different parts of green chilli (Table 1). Except in fruits and seeds, which contained 80.89 and 50.97 µg/g of tannic acid, respectively, this compound could not be traced in other parts. Caffeic acid was highest in collar region (83.64 µg/g) followed by root (64.82 µg/g), pulp (15.97 µg/g) and stem (0.46 µg/g). Ferulic acid was recorded in almost all parts of green chilli except in pulp, but the concentration did not exceed above 1.00 µg/g fresh wt in any case. Chlorogenic acid was found only in pulp (0.83 µg/g) while cinnamic acid in pulp (0.54 µg/g) as well as seed (0.12 µg/g). Per cent amount of these phenolics varied significantly in the plant parts (Table 1). In chilli seeds, tannic acid constituted almost 53.76 per cent of the total phenolics while as high as 75.45 and 67.03 per cent of caffeic acid was present in root and collar region of chilli, respectively.

### Figure 1

Table 1: Some phenolic acids in different parts of chilli

Plant parts	Phenolic acids (µg/g fresh wt)				
	Tannic (2.76)*	Caffeic (3.15)*	Ferulic (3.40)*	Chlorogenic (4.15)*	Cinnamic (4.51)*
Leaf	ND	ND	0.49±0.06 (1.94±0.41)	ND	ND
Stem	ND	0.46±0.1 (1.94±0.41)	0.15±0.03 (13.99±0.8)	ND	ND
Collar	ND	83.64±2.8 (67.03±1.7)	0.68±0.10 (0.80±0.05)	ND	ND
Root	ND	64.82±3.2 (75.45±3.8)	0.39±0.08 (0.67±0.12)	ND	ND
Fruit	80.89±4.2 (34.17±3.6)	ND	0.96±0.14 (1.29±0.44)	ND	ND
Pulp	ND	15.97±1.3 (34.02±2.4)	ND	0.83±0.21 (0.64±0.15)	0.54±0.07 (1.46±0.2)
Seed	50.97±4.1 (53.76±1.5)	ND	0.27±0.07 (0.97±0.13)	ND	0.12±0.02 (0.79±0.0)

\* Retention time in min.

ND = Not Detected

Data in parenthesis represent per cent composition of total phenolics.

Table 2 shows the amount of various phenolic acids in different varieties of chilli. Seeds of red chilli contained as high as 105.79 µg/g tannic acid followed by 50.97 µg/g in seed of green chilli which constituted almost 81.11 and 53.76 per cent of the total phenolics, respectively. Caffeic acid was highest in sweet pepper seed (39.49 µg/g) followed by black pepper seed (30.39 µg/g) and red chilli pulp (25.18 µg/g). Interestingly, vanillic acid in trace (0.064 µg/g) was detected only in the fruit of sweet pepper (0.094 µg/g). Amount of ferulic acid ranged from as high as 2.39 µg/g in sweet pepper fruit to as low as 0.27 µg/g in green chilli seed. Chlorogenic acid was traced only in red chilli pulp (0.84 µg/g) and sweet pepper seed (0.46 µg/g) while in all other

varieties and plant parts, it was absent. On the other hand, cinnamic acid was constantly present in all varieties with varying amount and its amount in total phenolic also varied significantly (Table 2).

### Figure 2

Table 2: Some phenolic acids in different varieties and plant parts of chilli

Varieties/ Parts	Phenolic acids (µg/g fresh wt)					
	Tannic (2.76)*	Caffeic (3.15)*	Vanillic (3.26)*	Ferulic (3.40)*	Chlorogenic (4.15)*	Cinnamic (4.51)*
Green chilli seed	50.97±2.1 (53.76±3.2)	ND	ND	0.27±0.06 (0.97±0.05)	ND	0.12±0.02 (0.79±0.07)
Red chilli seed	105.79±6.2 (81.11±3.9)	ND	ND	0.29±0.02 (0.78±0.07)	ND	0.55±0.04 (1.37±0.65)
Red chilli pulp	ND	25.18±2.9 (24.03±1.6)	ND	ND	0.84±0.07 (0.33±0.04)	2.37±0.9 (6.09±1.07)
Sweet pepper seed	ND	39.49±2.3 (41.49±3.8)	ND	ND	0.46±0.5 (0.17±0.3)	0.025±0.1 (0.34±0.01)
Sweet pepper fruit	ND	ND	0.064±0.01 (0.094±0.02)	2.39±0.45 (11.14±1.1)	ND	1.05±0.1 (0.91±0.07)
Black pepper seed	ND	30.39±4.3 (27.39±1.7)	ND	2.13 (0.28)	ND	0.043 (0.049)

\* Retention time in min.

ND = Not Detected

Data in parenthesis represent per cent composition of total phenolics.

There exist certain reports on the phytochemical investigation of green chilli. Presence of caffeic acid in the fruits of selected pepper cultivars was reported by Haword et al., along with other compounds such as capxanthin and zeaxanthin. Bandyopadhyay et al., reported total phenolics and phenolic acids in five varieties of green pepper and found variation on the level of total phenolics and also in different phenolic acids in different varieties. Capsaicinoids and their phenolic intermediates in various lines of *C. annuum* have been reported by Isuzugawa et al.,. Sukrasano and Yeoman<sup>10</sup> studied phenylpropanoid metabolism during growth and development of *Capsicum* fruits where they reported presence of a few phenolic compounds. On the contrary, this investigation deals with a profile of certain important phenolic acids in various parts as well as some varieties of chilli.

Concentration of tannic, gallic, ferulic and chlorogenic acids also varied significantly in various parts of okra (Fig. 2). The amount of gallic acid was significantly lower in all plant parts as compared to ferulic, chlorogenic and tannic acids. Maximum content of gallic acid was recorded in okra seed (2.13 µg/g fresh wt) followed by root (0.55 µg/g) while in other parts it was below 0.15 µg/g. Okra leaf and stem were rich in tannic acid (12.19 and 104.42 µg/g, respectively) while ferulic acid was maximum (10.96 µg/g) in stem tissues followed by leaf, root, pulp and seed. Chlorogenic acid was maximum in okra pulp (9.62 µg/g) followed by the leaf (9.15

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µg/g). In stem and seed only minor amount of chlorogenic acid was observed, whereas it could not be traced in root (Fig. 2).

**Figure 3**

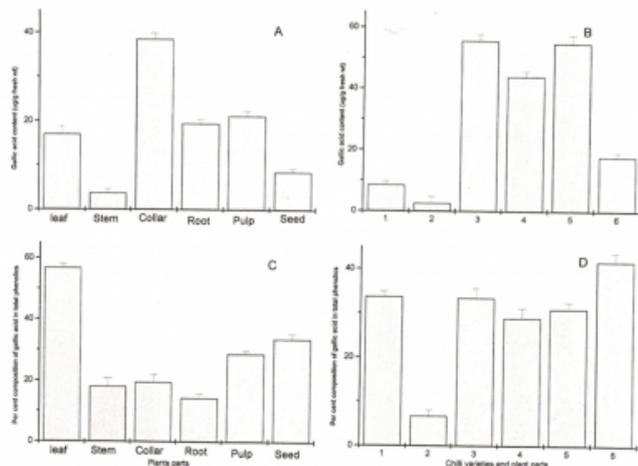


Fig. 1 Gallic acid content and per cent composition in total phenolics of various plant parts (A and C), different varieties and parts (B and D) of chilli; 1. green chilli seed, 2. red chilli seed, 3. red chilli pulp, 4. sweet pepper seed, 5. sweet pepper fruit, 6. black pepper seed

**Figure 4**

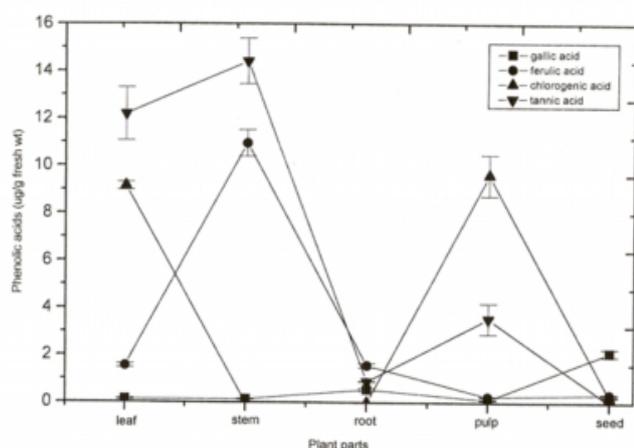


Fig. 2 Concentration of gallic, ferulic, chlorogenic and tannic acids in various parts of okra

**Figure 5**

Figure 3: reveals the presence of gallic, ferulic and chlorogenic acids in the seeds of various okra varieties namely, BO2, VRO6, VRO5, IIVRO10 and Prabhani kranti.

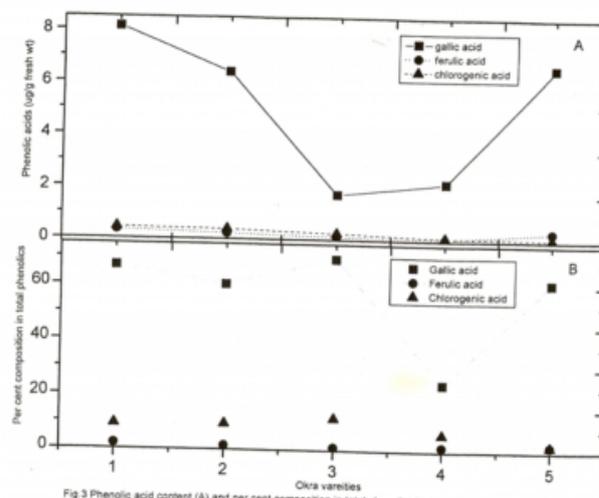


Fig. 3 Phenolic acid content (A) and per cent composition in total phenolics in the seeds of various okra varieties. 1. BO2, 2. VRO6, 3. VRO5, 4. IIVRO10, 5. Prabhani kranti

Among the three phenolics, gallic acid was the major constituent in the seeds of all the varieties. Maximum content of the compound (8.12 µg/g) was recorded in BO2 variety with 66.9 per cent followed by Prabhani kranti, VRO6, IIVRO10 and VRO5. Ferulic acid content was quite low as it reached only 0.43 µg/g in variety BO2 while in all other varieties, the amount was quite less. The per cent composition of ferulic acid varied from as high as 11.68 per cent of the total phenolics in variety VRO5 to as less as 2.22 per cent in Prabhani kranti. Chlorogenic acid content was found to be even less than ferulic and gallic acids. Maximum content of chlorogenic acid was recorded in the VRO6 variety (0.32 µg/g). The compound, however, could not be traced in varieties VRO5 and IIVRO10. Estimation of total phenolics in okra seeds has been reported by Supotra and Mukharji<sup>11</sup> but phenolic compounds have not been estimated from various parts of okra. Sharma and Rai<sup>12</sup> studied the antagonism between abscisic and phenolic compound in rooting of *A. esculentus* using cinnamic and gallic acids. Kulshrestha and Chauhan<sup>13</sup> reported inhibitory effect of distilled water kept on okra leaves for 72 h to demonstrate fungal suppression and estimated phenolics as the probable cause of spore germination inhibition.

Vegetables are one of the important sources of dietary intake of polyphenols<sup>7</sup>. Phenolic acids play a vital role not only in maintaining taste, flavor and quality of the vegetables, but are also able to add important medicinal

values to the foods is well documented<sup>3</sup>. Reports on the selective cytotoxicity of gallic acid<sup>9</sup> and antimicrobial activities of gallic and tannic acids<sup>4</sup> indicate medicinal significance of these phenolic acids. Interestingly, these compounds were significantly reported in this study. In view of the above facts, these results themselves point out vital significance of these phenolic acids in the two important vegetable crops, chilli and okra.

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