

Full Length Research Paper

Phenolic acids in some Indian cultivars of *Momordica charantia* and their therapeutic properties

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Bitter gourd (*Momordica charantia*) is an important medicinal plant consumed mostly as vegetable. Often the whole plant is used in different forms for improving human health. Phenolic acid analysis by high performance liquid chromatograph (HPLC) of three cultivars of *M. charantia* (viz., Pusa Vishesh, Kalyanpur Barasati and Priya) has been done. Kalyanpur Barasati was rich in some phenolic acids (six phenolic acids) followed by Pusa Vishesh and Priya (five phenolic acids). Gallic, caffeic, chlorogenic and ferulic acids were detected in fruit parts of all the three varieties where gallic acid was in maximum amount. Caffeic acid was maximum in Pusa Vishesh and Kalyanpur Barasati. In roots and leaves, phenolic acids were detected in traces. The importance of fruits which are consumed as vegetable in human diet has been discussed in the light of phenolic acid content.

Key words: Indian cultivars, *Momordica charantia*, phenolic acids, high performance liquid chromatograph.

INTRODUCTION

Momordica charantia (Bitter gourd; family Cucurbitaceae) is an important medicinal vegetable crop. In practice the whole plant, including fruits and seeds are consumed by human beings. Its fruits contain riboflavin, thiamin, ascorbic acid, corbegenin, luteolin whereas bitter glycosides, cucurbitins are mostly found in seeds (Wealth of India, 1987). In ancient Indian medicine (Ayurveda), the plant is reported to be hypoglycemic with antidiabetic properties (Chunekar, 1999; Patel and Srinivasan, 1995, 1997). The fruits are antistomachache, carminative, purgative, emetic and the roots have abortifacient activity (Chunekar, 1999). Fruits and seeds are used for the treatment of rheumatism, gout, diseases of liver and spleen. Dry fruit powder is effective in healing wounds, leprosy and malignant ulcers and raw fruit juice has hypoglycemic activity (Chunekar, 1999; Wealth of India, 1987). It is also reported to have antihelmintic, antibacterial, antibiotic, antitumor, antiviral, antileukemic, antimicrobial, antimutagenic, aphrodisiac, astringent, cavivative, cytostatic, cytotoxic, depuratives, immunostimulant, insecticidal, lactagogue, laxative,

pergative, refrigerant, stomatic, styptic, tonic and vermifuge properties (alpha and beta momorcharin). Recently, two proteins have been isolated from the seeds of bitter gourd which have shown to act as immunosuppressive without having any cytotoxic effect. They also modulate the activity of both α and β lymphocytes and significantly suppress the macrophage activity (Demida, 2003).

The phenolic acids are abundantly present in plants and have wide range of pharmacological properties. They may contribute in immunostimulating activity in human beings due to anti-oxidant property (Bors et al., 2001). Plants monomeric and polymeric phenols can strengthen the gastric mucosal barrier and 4-propoxycinnamic acid residue shows antimalarial activity (Weisner et al., 2001). Chlorogenic acid has been found inhibitory against HSV-1 replication without any cytotoxicity. Isochlorogenic acid has antilipoxygenase and anticyclogenase enzymes that have been suggested to have anti-inflammatory property (Duarete et al., 2000). Gallic acid and its derivative are active against Gram-negative and Gram-positive bacteria (Binutu and Cardel, 2000). Seeing such properties of phenolic acids, different parts of three cultivars of *M. charantia* were analyzed with the help of high performance liquid chromatograph (HPLC). The results

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Table 1. Phenolic acid content ($\mu\text{g/g}$ fresh wt) in different parts of some Indian cultivars of *Momordica charantia*

Plant part	Phenolic acid ($\mu\text{g/g}$ fresh wt)					
	Pusa Vishesh C					
	TA (Rt-2.76 Min)	GA (Rt-2.88 Min)	Caff-A (Rt-3.14 Min)	FA (Rt-3.42 Min)	Chl-A (Rt-4.16 Min)	CA (Rt-4.45 Min)
Root	ND	2.56 \pm 0.14	0.554 \pm 0.45	0.438 \pm 0.56	ND	ND
Stem	ND	2.67 \pm 0.56	ND		ND	ND
Leaf	3.48 \pm 0.24	7.036 \pm 0.32	ND	0.797 \pm 0.18	ND	ND
Fruit peel	ND	6.325 \pm 0.53	0.238 \pm 0.53	5.425 \pm 0.85	ND	ND
Fruit pulp	ND	14.94 \pm 0.18	0.103 \pm 0.58	2.85 \pm 0.62	ND	ND
Fruit seed	ND	27.16 \pm 0.18	0.192 \pm 0.14	2.058 \pm 0.53	ND	ND
Kalyanpur Barasati C						
Root	0.015 \pm 0.44	1.118	0.559 \pm 0.18	ND	ND	ND
Stem	2.029 \pm 0.92	0.725	1.412 \pm 0.14	ND	0.073 \pm 0.2	0.012 \pm 0.14
Leaf	ND		0.719 \pm 0.56	ND		
Fruit peel	ND	2.143 \pm 0.32	ND	ND	ND	ND
Fruit pulp	ND	ND	ND	ND	ND	ND
Fruit seed	43.08 \pm 1.53	ND	0.282 \pm 0.12	ND	ND	ND
Priya C						
Root	ND	1.142 \pm 0.36	ND	ND	ND	ND
Stem	ND	6.669 \pm 0.51	ND	ND	ND	0.102 \pm 0.40
Leaf	ND	6.235 \pm 0.21	ND	ND	ND	0.028 \pm 0.16
Fruit peel	0.985 \pm 0.19	1.008 \pm 0.12	ND	1.113 \pm 0.14	ND	0.153 \pm 0.11
Fruit pulp	ND	11.67 \pm 0.4	ND	0.6438 \pm 0.14	ND	ND
Fruit seed	ND	113.05 \pm 1.56	ND	6.366 \pm 0.14	ND	ND

ND = Not detectable, \pm = Standard error, TA = Tannic, GA = Gallic, Caff-A = Caffeic, FA = Ferulic, Chl-A = Chlorogenic and CA = Cinnamic acid, C = Cultivar.

are presented here.

MATERIALS AND METHODS

Randomly selected three plants of the same age were harvested and pooled together to make one sample each of roots, stems, leaves and fruits. One gram of freshly harvested leaves, stems, roots and fruit parts (peel, pulp and seeds) were finely crushed in 5 to 10 ml of ethanol water (80 to 20; v/v) followed by ultrasonication (Branson Sonifier, USA) for 15 min at 4°C. The samples were centrifuged at 7500 rpm for 15 min. The clear greenish supernatant was treated with charcoal to remove pigments. The residues were re-extracted twice and supernatant was pooled and evaporated under vacuum. Dried samples were re-suspended in 1.0 ml HPLC grade methanol, filtered through membrane filter (pore size 0.45 μm , Millipore) and analyzed by HPLC (Singh et al., 2002). The HPLC (Shimadzu Corporation, Kyoto, Japan) was equipped with UV-VIS detector (Shimadzu SPD-10 AVP), C-18 reverse phase column [(250 X 4.6 mm id, particle size 5 μm) Luna 5 μm C-18 (2), Phenomenex, USA] at 25°C, mobile phase, methanol; 0.4% aqueous acetic acid (80:20, v/v), flow rate 1 ml/min, injection volume 5 μl and detection at 290 nm. Samples were injected thrice in the sample loop and mean of the peak area of the individual compounds was taken by quantification. Tannic (TA), gallic (GA), chlorogenic (Chl.A), caffeic (Caff.A), vanillic (VA), ferulic (FA) and cinnamic (CA) acids were used as internal and external standards.

Phenolic compounds present in the samples were identified by comparing retention time (Rt) of standards as well as co-injection. All the phenolic acids were shown as per gram fresh weight unless otherwise stated.

RESULTS AND DISCUSSION

The roots, stems, leaves and fruits of the three cultivars of *M. charantia*, that is Pusa Vishesh, Kalyanur Barasati and Priya, were analyzed for the presence of phenolic acids.

Pusa Vishesh (small fruits) had maximum amount of gallic acid (27.154 μg fresh wt) in the seeds followed by pulp (14.949 μg), leaf (7.036 μg), peel (6.325 μg) and in traces in stem and root. Ferulic acid was maximum in fruit peel (5.425 μg) followed by pulp (2.85 μg), seed (2.058 μg), leaf (0.797 μg) and root (0.438 μg). Caffeic acid was maximum in root (0.554 μg) followed by peel (0.238 μg), seed (0.192 μg) and pulp (0.103 μg). Tannic acid was detected only in leaves (3.48 μg) and stem (3.015 μg) (Table 1).

In Kalyanpur Barasati (long thin fruits) TA was maximum in seeds (43.08 μg), followed by stem

(2.029 µg) and root (0.015 µg). GA was maximum in peel (2.143 µg) and then in root (1.118 µg) and stem (0.725 µg). Caff.A was maximum in stem (1.412 µg) followed by leaves (0.719 µg), root (0.559 µg) and seed (0.282 µg). FA was detected only in fruit pulp (0.689 µg) and seed (0.486 µg) and CA only in stem (0.012 µg). Chl.A was detected only in seed (0.073 µg) (Table 1).

In Priya (bold fruits) GA was present in every part, maximum in seeds (113.053 µg) followed by pulp (11.666 µg), stem (6.669 µg), leaves (6.235 µg) and in traces in root (1.142 µg) and peel (1.008 µg). FA was maximum in seed (6.366 µg), followed by peel (1.113 µg) and pulp (0.644 µg). CA was seen only in stem (0.102 µg) and leaves (0.028 µg). TA and Chl.A were detected only in peel in traces (Table 1). The cultivar Priya is rich in GA and FA followed by Pusa Vishesh and Kalyanpur Barasati. It is interesting that Caff.A is present in fruits of Pusa Vishesh and Kalyanpur Barasati but other parts showed phenolic acids in traces.

Horax et al. (2006) studied the total phenolics, phenolic acids and their antioxidant properties in the extracts of four varieties of *M. charantia*. They also reported phenolic acid contents in seeds, inner tissue and flesh and found gallic acid as the main compound besides gallic, catechin, chlorogenic and epigallocatechin. Kubola and Siriamornpun (2008) investigated the antioxidant property and total phenolic acid content in water extract of leaf, stem and fruit fraction of *M. charantia*. The phenolic acid analysis revealed the presence of gallic acid followed by caffeic acid and catechin.

Gallic, ferulic, caffeic, cinnamic acids and their derivatives are known for various biological functions (Inoue, 1995; Fernandes et al., 1998; Graf, 1992; Ravn et al., 1989; Chambel et al., 1999). The presence of phenolic acids in the fruits of *M. charantia* indicates that the fruits are highly medicinal than other plant parts. However, taking the cumulative therapeutic properties in account, the entire plant has medicinal values which have already been described in ancient Indian medicine (Ayurveda) where fruits in particular have been recommended for diabetic patients (Chunekar, 1999). However, the mechanism of action in lowering down the blood sugar needs further study.

The presence of gallic acid as the main phenolic acid in all the three cultivars is in conformity with the result of earlier workers. Taking into account the number and amount of phenolic acids in regular edible part of *M. charantia* that is, fruits, it is suggested that Pusa Vishesh and Priya are better for human health. The detailed analysis of phenolic acids in different parts of *M. charantia* in three Indian cultivars is being reported for the first time.

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