Chromatographical analysis of Phenolic acids in different preparations of pea (Pisum sativum) and chickpea (Cicer arietinum)

P Tiwari, A Singh, U Singh, S Maurya, M Singh

Citation

P Tiwari, A Singh, U Singh, S Maurya, M Singh. *Chromatographical analysis of Phenolic acids in different preparations of pea (Pisum sativum) and chickpea (Cicer arietinum)*. The Internet Journal of Alternative Medicine. 2008 Volume 8 Number 1.

Abstract

Legumes represent one of the most important food components to cover the basic proteins and energy requirements for the health of human beings. The secondary metabolites of legumes reveal valuable medicinal properties. Among the secondary metabolites, phenolic acids have greater importance. Analysis of phenolic acids in pea (Pisum sativum) and three varieties of chickpea (Cicer arietinum) seeds and their different preparations consumed commonly in India was done through High Performances Liquid Chromatography (HPLC). In total nine phenolic acids, viz., ferulic, gallic, chlorogenic, O-coumaric, cinnamic, vanillic, caffeic, tannic acids and salicylic acid could be identified on the basis of their retention time with standard compounds and co-chromatography. Several preparations of seeds of both the legumes were made for the analysis of phenolic acids. Maximum amount of gallic and tannic acids was observed in dried mature pea seeds. Maximum caffeic acid was found in boiled mature seeds of pea while ferulic acid in immature pea seed extract in distilled water for 24 h while vanillic acid was found maximum in similar extract. Three varieties of chickpea were taken, viz., Kabuli, Radhey and Avarodhi. In chickpea maximum gallic acid was observed in seeds boiled in distilled water while maximum tannic acid was observed in seeds of Kabuli variety fried with salt. Maximum caffeic acid was observed in Radhey (chickpea) seeds soaked in distilled water for 24 h while ferulic and salicylic acids in dried Radhey seeds (crushed in 80% ethanol) and vanillic acid was found maximum in fried Kabuli variety of chickpea without salt. O-coumaric acid was observed maximum in Avarodhi (chickpea) seeds soaked in distilled water.

INTRODUCTION

Pea (Pism sativum) and chickpea (Cicer arietinum) occupy an important position in the orchestra of legumes [1] . Nearly 25 species of cultivated legumes provide food for human beings and domestic animals to a certain extent [2] . Protein is an important ingredient of human diet. Animal protein is costly for people in India, and also in other developing countries. Hence, legumes are the only alternative for supplementing the diet with protein. Chickpea is the third ranking [3] among the world's legumes and is grown in more than 80% semiarid zones of India [4] . Sweet pea (Pisum sativum) is second only to chickpea in acreage as well as production [5] .

With the development of various efficient, purification and identification techniques, many bioactive products have been isolated from a number of legume. Phenolics are an important group of natural compounds contributing significantly to the marked pharmacological properties of a number of plants including fruits and legumes [6] . They are important component, either in free or conjugated forms, of almost all the plants in which they are biosynthesized via Shikimic acid or phenylpropanoid pathway [7] . Legumes have been a rich source of various groups of natural products including phenolics. The enzyme especially phenylalanine ammonia lyase (PAL) is not found in animals and humans. This is why animals solely depend on plants for the requirement of phenols in their body. Phenolics have wide therapeutic and pharmacological properties against human and animal diseases and are, therefore considered to be safer in traditional and alternative medical systems over synthetic medicines (Table 1).

Figure 1

Table 1. Therapeutic uses of phenolic acids

Phenolic acid	Biological activity	References
Monomeric and polymeric polyphenols	Growth inhibition of *Erwinia caratovora in vitro*	Lyon *et al.* (1988)[23]
Gallic acid and its ester derivatives	Anti-inflammatory and selective induction of cell death in cancer cells	Inoue *et al.* (1995)[19]
	Scavengers of superoxide radicals	Fernandes *et al.* (1999)[21]
	Low density lipoprotein (LDI)	Laranjinha *et al.* (1994)[10]
	Antibacterial against gram-negative and gram-positive bacteria	Binutu *et al.* (2001)[20]
Ferulic acid	Antioxidant	Graf (1992)[18]
	Antifungal	Mehrotra (1997)[24]
		Sarma and Singh [26]
Cinnamic acids and its derivatives	Natural protection against infections by pathogenic micro-organisms, growth and on plasma membrane H^+-ATPase activity of *Saccharomyces cerevisae*	Champbel *et al.* (1999)[15]
	Superoxide scavenging	Fernandes *et al.* (1999) [21]
4-propoxycinnamic acid	Antimalarial activity	Weisner *et al.* (2001)[17]
Chlorogenic acid and iso-chlorogenic acid	Develop resistance in plants, inhibitor of IAA oxidase, inhibits HSV-1 replication without any cytotoxicity	Johnson and Schaal (1952)[12]
	Antifungal activity	Mehrotra *et al.* (1997) [23]
Caffeic acid	Antifungal and antifungal activity	Ravn *et al.* (1989)[12]

Being natural hydrogen-donors, they are strong antioxidants, and natural free-radical scavengers because of their structural characteristics and hydrogen donating properties of the phenol moiety [8]. Their role in the protection of both humans and plants against oxidative stress, which has adverse effects on the tissues, has been reported widely. They also contribute to wound healing and their glycosylated analogs provide a vehicle for the carbohydrate transport and storage, which is relatively osmotically inactive. Individually, phenolics may possess pharmacological uses and find wide application and description in the traditional medical systems.

The present study was undertaken to estimate phenolic acids in two major legumes (chickpea and pea) used regularly as food supplements in India by high performance liquid chromatography (HPLC). The results are presented here.

MATERIALS AND METHODS

SAMPLE PREPARATION

Two legumes (three varieties of chickpea and sweet pea) were purchased from authentic legume shops. One gram (dry weight) of each legume was weighed and powdered in a pestle-mortar followed by suspending fine crushed sample in 5 ml of ethanol: water (80:20 v/v). These samples were collected in screw-capped specimen tubes (10 ml) and the suspension was subjected to ultrasonication by Branson Sonifiers 450 (Branson Ultrasonic Corps, Danury CT, USA) for 15 min at 4 ° C followed by centrifugation at 7,500 g for 15 min. The clear greenish supernatant was subjected to charcoal treatment to remove pigments from each sample and was then transferred to glass tubes. The residue was re-extracted twice and the supernatant was pooled prior to evaporation under vacuum (Buchi Type Rotavapor). Dried samples were re-suspended in 1.0 ml HPLC grade methanol by vortexing and filtered through membrane filter (pore size 0.45µm, Millipore) and stored at 4 ° C for further analysis by HPLC.

THE REAGENTS

Standard phenolic acids, viz., tannic acid, gallic (3, 4, 5-trihydroxybenzoic), vanillic (4-hydroxy-3 methoxybenzoic) cinnamic, caffeic (3,4-dihydroxycinnamic), O-coumaric (4-hydroxycinnamc), ferulic (4-hydroxy-3 methoxycinnamic) and salicylic acids were obtained from Merck, Himedia and Sigma companies. Solvents used during HPLC analysis (e.g. methanol, distilled water etc.) were of the HPLC grade (Merck, Germany).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSIS

High performance liquid chromatography (HPLC) of the samples was performed on HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable UV-VIS detector (Shimadzu SPD-10 AVP UV-VIS) and Rheodyne model 7725 in factor with a loop size of 20 µl, and integrator and CLASS-VP software for data recording and processing (Shimadzu). Reverse phase chromatographic analysis was carried out in isocratic conditions using C-18 reverse phase HPLC column {(250 X 4.6 mm i.d., particle size 5 µm) Luna 5 µ C-18 (2), Phenomenex, Torrance USA at 25 ° C. Running conditions included mobile phase methanol-0.4% acetic acid (80:20, v/v), flow rate 1.0 ml / min, injection volume 5 µl and detection at 290 nm [9]. Sample was injected thrice in the sample loop and the means of the peak areas of individual compounds were taken for quantification. Tannic, caffeic, vanillic, ferulic, cinnamic and salicylic acids were used as internal and external standards. Phenolic compounds present in the sample were identified by comparing retention time (Rt.) of the standards tannic (TA, Rt.2.94 min), gallic (GA, Rt. 3.10 min), caffeic (Caf-A, Rt. 3.48 min), vanillic (VA, Rt. 3.76 min), ferulic (FA, Rt. 4.02 min), O-coumaric (O-Cou-A Rt. 4.64 min) cinnamic (CA, Rt. 6.67 min) and salicylic acids (SA, Rt. 7.86 min). These phenolic acids were identified by co-injection of internal and external standards

for their confirmation. Amounts of individual compounds were calculated by comparing peak areas of reference compounds with those in the samples run under similar elution conditions.

QUANTITATIVE ESTIMATION OF PHENOLIC ACIDS AND PREPARATION OF STANDARD CURVE

Phenolic acid standards were accurately weighted (1 mg/ml) and dissolved in HPLC grade methanol. Concentrations ranging from 1 µg/ml to 10 µg/ml were prepared by serial dilution. Quantitative estimation of phenolic acids was conducted by reverse phase C-18 column under similar running conditions as has been described for the analysis of the samples. The analysis was carried out in triplicate and the detection was monitored at 290 nm. Calibration curves were plotted showing a linear correlation and peak areas for phenolic acids separately.

RESULTS

Several phenolic acids in both the legumes (pea and chickpea) were detected (Tables 2a, b and 3).

Figure 2

Table 2a. Chickpea () varieties and their preparations

Chickpea (*Cicer arietinum*) varieties	Material used		Sample No.
Radhey	Dried seed (crushed in 80% ethanol)		1
	Soaked in distilled water for 24 hrs	Seed	2
		Water (extract)	
	Boiled (seed) in distilled water	Seed	4
		Water (extract)	5
	Fried (seed)	With salt	6
		Without salt	7
Avarodhi	Dried seed (crushed in 80% ethanol)		8
	Soaked in distilled water for 24 hrs	Seed	9
		Water (extract)	10
	Boiled (seed) in distilled water	Seed	11
		Water (extract)	12
	Fried (seed)	With salt	13
		Without salt	14
Kabuli	Dried seed (crushed in 80% ethanol)		15
	Soaked in distilled water for 24 hrs	Seed	16
		Water (extract)	17
	Boiled (seed) in distilled water	Seed	18
		Water (extract)	19
	Fried (seed)	With salt	20
		Without salt	21
	Boiled seed (Rahdey)	Seed peel	22
		Seed pulp	23
	Boiled seed (Avarodhi)	Seed peel	24
		Seed pulp	25
	Boiled seed (Kabuli)	Seed peel	26
		Seed pulp	27

Figure 3

Table 2b. Phenolic acid content in different preparations of chickpea ()

Sample No.	Phenolic Acid (µg/ml dry wt.)							
	Tannic acid	Gallic acid	Caffeic acid	Vanillic acid	O-Coumaric acid	Ferulic acid	Cinnamic acid	Salicylic acid
1	UDL*	4.686	0.5923	0.687	0.017	10268	UDL*	4.1024
2	1.2188	1.674	2.087	0.3018	UDL*	0.650	0.0828	1330
3	UDL	UDL*	UDL*	1.145	UDL	0.6239	0.0276	0.0205
4	1.2055	5.519	UDL	UDL	UDL	0.54	0.0057	1.8063
5	UDL	1.6605	0721	1.027	0.460	1.375	0.0191	0.0201
6	0.9664	5.1919	UDL	UDL	0.978	UDL*	0.0245	1.123
7	5.170	UDL	UDL	0.792	UDL	0.5869	UDL	0.736
8	UDL	11.6266	UDL	0.309	UDL	0.691	0.0166	0.3051
9	UDL	2.3994	UDL	UDL	2.0858	UDL	UDL	1.4563
10	UDL	0.5219	UDL	0.984	0.4509	1.430	0.0276	0.6103
11	UDL	1.5470	UDL	0.3307	0.0589	UDL	UDL	0.8356
12	UDL	UDL	1.6524	1.288	0.5296	UDL	0.0852	1.2143
13	5.053	UDL	UDL	0.790	0.0320	0.261	0.00027	0.9937
14	UDL	7.740	UDL	1.181	.7122	UDL	0.0448	0.615
15	1.2967	UDL	UDL	0.398	1.3928	UDL	0.0172	1.6268
16	UDL	11.326	UDL	1.642	0.676	UDL	UDL	0.7477
17	UDL	0.524	0.5776	1.364	0.448	0.351	.1076	0.489
18	1.678	14.548	0.858	1.370	UDL	UDL	UDL	0.3468
19	UDL	0.3212	UDL	0.725	0.326	UDL	0.0384	0.2951
20	0.3597	2.586	UDL	0.220	0.162	UDL	0.0122	2.4776
21	UDL	UDL	UDL	1.664	0.474	UDL	0.0117	2.4167
22	0.9360	5.165	UDL	0.676	0.1387	UDL	0.0022	0.0011
23	UDL	0.538	0.0202	0.0411	UDL	0.3042	0.0071	0.6086
24	1.1073	UDL	0.3162	UDL	UDL	0.1944	UDL	20.2286
25	UDL	4.688	0.8085	UDL	0.302	0.561	.0100	0.7808
26	UDL	13981	UDL	0.080	0.0055	0.0258	0.0059	0.5312
27	UDL	4.860	UDL	UDL	0.1369	0.278	UDL	0.8627

*** UDL = under detectable level**

Figure 4

Table 3. Phenolic acid content in different preparations of pea ()

Different Preparations	Phenolic Acid (µg/ml dry wt.)							
Sample No.*	Ferulic acid	Gallic acid	Chlorogenic acid	Cinnamic acid	Vanillic acid	Caffeic acid	Tannic acid	Salicylic acid
1	0.878	16.2429	UDL**	UDL**	UDL**	UDL**	UDL**	UDL**
2	8.966	20459	UDL	UDL	UDL	UDL	43.146	0.259
3	1842	21.9095	UDL	UDL	UDL	UDL	UDL	UDL
4	UDL**	2.6902	UDL	UDL	UDL	0.735	0.0614	UDL
4a	30.4059	UDL	0.0398	0.0643	UDL	2.328	UDL	UDL
4b	0.2399	11.3295	UDL	UDL	UDL	UDL	UDL	UDL
5a	3.7173	20.949	UDL	UDL	10.1113	UDL	UDL	UDL
5b	UDL	10.339	UDL	UDL	UDL	UDL	1.808	UDL
6	3.6076	2.909	UDL	UDL	UDL	0.144	UDL	UDL
7	UDL	5.026	UDL	UDL	UDL	53.836	0.6765	UDL
8	UDL	9.213	UDL	UDL	UDL	UDL	UDL	UDL
9	UDL	8.408	UDL	UDL	UDL	UDL	3.0044	UDL
10	2.9027	19.949	UDL	UDL	UDL	UDL	4.842	UDL
11	0.028	44.94	0.0082	UDL	UDL	UDL	16.20	UDL

UDL** = under detectable level

* Sample No. 1 Immature seed (green pea) crushed in 80% ethanol water

2 Oven dried (60 ° C/24h)

3 Dried mature seed crushed in 80% ethanol

4 15 minutes boiled mature seed

4 (a, b) Mature seed soaked in distilled water for 24 hours

(a) – Extract (b) – Seed

5 Immature seed soaked in distilled water for 24 hours

6 Extract of immature seed (15 minutes boiled)

7 Extract of mature seed (15 minutes boiled)

8 Seed of boiled immature seed

9 Fried immature seed with salt

10 Fried immature seed without salt

11 Flour of pea

Analysis revealed that maximum amount of tannic acid (43.416 µg/g) was detected in dried mature sweet pea seeds crushed in 80% ethanol followed by flour of sweet pea (16.20 µg/g) in dried mature sweet pea seeds crushed in 80% ethanol followed by fried Radhey variety of chickpea without salt (5.170 µg/g), avarodhi variety of chickpea with salt (5.053 µg/g), fried immature sweet pea seed without salt (4.842 µg/g) and fried immature sweet pea seeds with salt (3.004 µg/g). In remaining sweet pea preparations the amount was less than 3.0 µg/g. Gallic acid was maximum in dried mature sweet pea seeds (204.59 µg/g), followed by flour of sweet pea (44.94 µg/g), 15 min boiled mature sweet pea seeds (21.99 µg/g), extract of immature sweet pea seeds soaked in distilled water for 24 h (20.948 µg/g), fried immature sweet pea seeds without salt (19.949 µg/g), avarodhi variety of chickpea dried seeds crushed in 80% ethanol (11.626 µg/g), kabuli variety of chickpea seeds boiled in distilled water (14.54 µg/g), avarodhi variety of chickpea dried seeds crushed in 80% ethanol (11.626 µg/g). In remaining legume preparations the amount was less than 11.50 µg/g. Ferulic acid was maximum in extract of mature sweet pea soaked in distilled water for 24 h (30.405 µg/g), followed by radhey variety of dried chickpea seeds crushed in 80% ethanol (10.268 µg/g) followed by 15 min boiled mature sweet pea seeds (8.966 µg/g). In remaining legume preparations, the amount was less than 5.0 µg/g. Similarly, cinnamic acid was seen only in extract of avarodhi variety of boiled chickpea seeds in distilled water (0.085 µg/g).

Similarly cinnamic acid was detected only in extract of avarodhi variety of boiled chickpea seeds soaked in distilled water (0.085 µg/g), followed by radhey variety of chickpea seeds soaked in distilled water for 24 h (0.082 µg/g), extract of mature sweet pea seeds soaked in distilled water for 24 h (0.0643 µg/g), while other preparations of legume showed very little amount of cinnamc acid. The caffeic acid was maximum in extract of 15 min boiled mature sweet pea seeds (5.836 µg/g) followed by extract of mature sweet pea soaked in distilled water for 24 h (2.328 µg/g), radhey variety of chickpea seeds soaked in distilled water for 24 h (2.087 µg/g), while in other preparations caffeic acid was found in traces. Vanillic acid was maximum in extract of immature sweet pea seeds soaked in distilled water for 24 h (10.113 µg/g) followed by kabuli variety of fried chickpea seeds without salt (1.664 µg/g), kabuli variety of chickpea seeds soaked in distilled water for 24 h (1.642 µg/g), while in other preparations vanillic acid was found in traces. The salicylic acid was maximum in radhey variety of chickpea dried seeds crushed in 80% ethanol (4.102 µg/g) followed by kabuli seeds fried with salt (2.146 µg/g) while in other preparations it was found in traces. O-coumaric acid was found only in chickpea but not in sweet pea. It was maximum in avarodhi chickpea seeds soaked in distilled water for 24 h (2.085 µg/g) while in other preparations it was found in traces (Tables 2b, c).

DISCUSSION

Legumes are the most important food category that have been extensively used as stable food to cover basic proteins and energy requirements throughout the history of human civilization. Besides adding nutrient, legumes also possess valuable medicinal properties because of the presence of several potential bioactive secondary metabolites, viz., enzyme inhibitors, lectins, phylates, oxalates, polyphenols, phytosterols and saponins that prevent humans from various diseases, viz., mainly coronary heart disease and diabetes [10]. The legumes under investigation are traditionally known to have pronounced effect on human health. Phenolics constitute and important group of natural products contributing significantly to the medicinal value of a number of plants including legumes.

Chlorogenic and isochlorogenic acids produce anti-lipoxygenase and anti-cycloxygenase activity and have been suggested to possess anti-inflammatory property [11]. Chlorogenic acid has also been found to significantly inhibit HVS-1 replication without any cytotoxicity [12]. Caffeic acid

is the most prominent cinnamate that provides protection against genotoxic agents. It has been demonstrated to possess anti-carcinogenic properties in experimental animals [13] . It has also been reported that caffeic acid in the form of an extract of the artichoke (rich in chlorogenic and caffeic acids) can be used to lower serum cholesterol level in human beings [[[14.15]]] . Several reports indicate that the presence of pre-existing and inducible phenolics acids in chickpea (C.arietinum) prevent the infection of collar rot caused by Scletorium rolfsii [1617] .

HPLC analysis of Lathyrus martimus showed the presence of (+) catechin and (-) epicatechin as the main low-molecular-weight phenolic compounds [18] . Singh et al. [9] reported that the induced phenolic acids in pea by plant growth promoting rhizobacteria (PGPR) prevent powdery mildew (Erysiphe pisi) infection and disease development in pea (P. sativum).

Cinnamic and hydroxycinnamic acids are rather more abundant and diverse groups of phenolics with higher dietary intake [10] . In terms of dietary load, total cinnamic acid intake in different populations ranges up to 1000 mg/day. Cinnamic acid and analogs provide natural protection against infections caused by pathogenic microorganisms [19] . 4-propoxycinnamic acid residue shows antimalarial acctivity [20] . Ferulic acid is present as natural dietary supplements with pronounced anti-inflammatory and antioxidant activity and is a pharmacological agent used as photoprotectants in skin lotions [21] . Gallic acid has anti-inflammatory and cytoxic property against all cancer cell lines studied in vitro [22] . It also possesses hepatoprotective effects at fairly high concentrations corresponding to its level in plasmas that might only be achieved by dietary means [23] . Ellagic acid, a dimmer of gallic acid, is a potent antioxidant (molar antioxidant activity in terms of Trolox Equivalent Antioxidant Capacity: TEAR = 3.0), the antioxidant activity of which is three times that of vitamim C or vitamin E [24] . Derivatives of gallic acid with a number of free hydroxyl groups having free radical scavenging property are also powerful antioxidants and possess antibacterial activity against gram-negative and gram-positive bacteria [2526] . Similarly, ferulic acid is known to possess antifungal and antimicrobial properties [27] .

Review of literature reveals that very little work has been done on the estimation of individual phenolic acids in pea and chickpea. Phenolic compounds of seed coats of white and coloured variety of pea and their antioxidant activity have been studied by Transisca and Ciska [28] . They found protocatechuic, genticic and vanillic acids in coloured seed coat while ferulic and coumaric acids in the white seed coat. Wang et al., [29] estimated total phenolics in field pea. Cherit et al., [30] found that gallic, cinnamic, ferulic and chlorogenic acids were associated with the protection of chickpea from fungal attributes through induced resistance. Recently, Dilis and Trichopoulon [31] , estimated total phenolics in pulses. However, the detailed phenolic acid analysis in different preparation of three varieties of chickpea and one of pea are being reported for the first time.

References

1. Hernanez Bermejo J E, Leon J. Neglected crops: 1492 from a different prospective. 1992. Food and Agricultural Organization of the United Nations (FAO).
2. Kafka B. Vegetative love. New York: Artison, 2005, ISBN 978-1-57965-168-8.
3. Chadha K L. Handbook of Horticulture. Published by Directorate of Information and Publication of Agriculture. ICAR, Krishi Anusandhan Bhavan (PUSA), New Delhi, 2001, pp.688-726.
4. Dutta S K, Nima V K, Bhardwaj R J. Physical properties of gram. J. Agrl. Eng. Res. 1988, 39:259-268.
5. The wealth of India. Raw materials. Council of Scientific and Industrial Research, 1962, CSIR, India.
6. Chrungoo N, Koul K K, Farooq S. Phenolics compounds in corms of saffron (Crocus sativus L.) during bud development. Pl. Phys. Biochem. 1986, 3:79-81.
7. Harborne J B. Methods in Plant Biochemistry: Plant phenolics. Academic Press, 1989, London.
8. Miller N J, Ruiz B, Larea, M. Flavonoids and other plant phenols in the diet: Their significances as antioxidant. J. Nutri Envt. Med. 2002, 12:39-51.
9. Singh U P, Sarma B K, Singh D P, Bahadur A. Plant growth-promoting rhizobacteria-mediated induction of phenols in pea (Pisum sativum) following infection with Erysiphe pisi. Curr. Microbiol. 2002, 44:396-400.
10. Laraninha Kan, Almeida L M, Madeira V M C. Reactivity of dietary Phenolic acids with peroxyl radicals: antioxidant activity upon low density lipoprotein peroxidation. Biochem. Pharmaacol. 1994, 48:487-494.
11. Clifford M N. Clorogenic acid and other cinnamgates-nature, occurrence, dietary burden, absorption and metabolism. J. Sci. Food Agric. 2000, 80-1033-1043.
12. Johnson G, Schaal L A. Relation of chlorogenic acid to scab resistance. Pot. Sci. 1952, 115:627.
13. Ravn H, Andary C, Kovacs G, Molgaard P. Caffeic acid as in vitro inhibitors of plant pathogenic bacteria and fungi. Biochem System and Ecpl. 1989, 17:174-184.
14. Bors W, Miche C, Stettmaier, K. Structure- activity relationships governing antioxidant capacities of plant polyphenols. Methods Enzymology, 2001, 335:166-180.
15. Clifford M N, Scalabert A. Elligitanius- nature, occurrence and dietary burden. J. Sci. Food Agric. 2000, 80:1118-1125.
16. Maurya S, Singh U P, Singh D P, Singh K P, Srivastava J S. Secondary metabolites of chickpea (Cicer arietinum) and their role in pathogenesis after infection by Sclerotium rolfsii. J. Pl. Dis. Prot. 2005, 112:118-123.
17. Sarma B K, Singh U P. Ferulic acid may prevent infection by Sclerotium rolfsii in Cicer arietinum. W. J.

Micr. Bio. 2003, 19:123-127.
18. Shahidi F, Chavan U D, Naczk M, Amarowicz R. Nutrient distribution and Phenolic antioxidants in air-classified fractions of beach pea (Lathyrus maritimus L.). J. Agric. Food Chem. 2001, 49:926-933.
19. Chambel A, Viegas C A, Sa-correia I. Effect of cinnamic acid on the growth an on plasma membrane H+-ATPase activity of Saccharomyces cerevisiae. Inter. J. Food Microbiol. 1999, 50:173-179.
20. Wiesner J, MItsch A, Wissner P, Jomaa H, Schlitzer M. Structure-activity relationships o novel anti-malarial agents. Part 2: Cinnamic acid derivatives. Bioorg. Med. Chem. Lett. 2001, 11:423-424.
21. Graf E. Antioxidant potential of ferulic acid. Free Rad. Biol. Med. 1992, 13:435\448.
22. Inoue M. Gallic acid found in grapes seeds kills cancer cells. Biol. Pharma. Bull. 1995, 18:1526-1530.
23. Binutu O A, Cordet G A. Gallic acid derivatives from Mezoeuron benthamiamum leaves. Pharma. Bio. 2000, 38:286-294.
24. Donovan J L, Meyer A S, Wasterhouse A L. Phenolic composition and antioxidant activity of prunes and prune juice (Prunus domestica). J. Agri. Food Chem. 1998, 46:1247-1252.
25. Fernandes E R, Borges M F, Silva F A, Carvalho F D, Basos M L. Evaluation of superoxide scavenging activity of cinnamic acid derivatives. 2nd International Electronic Conference on Synthetic Organic Chemistry (ecsoc-2). 1998.
26. Lyon G D, Mcgill R M. Inhibition of growth of Erwinia caratovora in vitro by phenolics. Pot. Res. 1988, 31:461-467.
27. Mehrotra R S. 'Plant Pathology'. Pub. Tata-Mcgraw Hill Publishing Co. Ltd. New Delhi. 1997, pp.544.
28. Troszynska A, Ciska E. Phenolic compounds of seed coats of white and coloured varieties of pea (Pisum sativum L.) and their total antioxidant activity. Czech J. Food Sci. 2002, 20: 15-22.
29. Wang X, Warkentim T D, Briggs C J, Oomah B D, Campbell C G, Woods S. Total phenolics and condensed tannins in field pea (Pisum sativum) and grass pea (Lathyrus sativum). Euphytica, 1998, 101: 97-102.
30. Cherit M, Arfaoui A, Rhaiem A. Phenolic compounds and their role in bio-control an resistance of chickpea to fungal attacks. T. J. Pl. Prot. 2007, 2:7-21.
31. Dilis V, Trichopoulon A. Nutritional and health properties of pulses. Mediterr. J. Nutr. Metab. 2009, 1:149-157.

Author Information

Preeti Tiwari, M.Sc., PhD
V.B.S. Purvanchal University Jaunpur

Amitabh Singh, M.Sc. (Ag.), Ph.D.
N-8/236, R-22, Ganesh Dham Colony, Newada, Sunderpur Varanasi-221005

UP Singh, M.Sc. (Ag.), Ph.D.
N-8/236, R-22, Ganesh Dham Colony, Newada, Sunderpur Varanasi-221005

S. Maurya, M.Sc. (Ag.), Ph.D.
Department of Mycology and plant Pathology Institute of Agricultural Sciences

Mandavi Singh, MS
Department of Anatomy Institute of Medical Sciences Banaras Hindu University Varanasi-21005, India