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HPTLC Profile of Quercetin Content of Common Bean (Uttarakhand) Landraces Growing in Uttarakhand

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ABSTRACT

Methanol extracts of seeds of 20 Landraces of *Phaseolus vulgaris* L. grown in different regions of Uttarakhand, were analyzed for the quercetin content using High Performance Thin Layer Chromatograpy (HPTLC). Total quercetin content varied from 50-410 mg kg^{-1} dry seed weight. Highest amount of quercetin has been reported in small red kidney beans, (Pur-3) growing in purola region, whereas extract of six landraces showed negligible or undetectable amount of quercetin. Quercetin is an important functional compound and results obtained from this study suggest that some beans may be good source of quercetin.

Key words: HPTLC, quercetin, nutraceutical, common bean

INTRODUCTION

Common bean is one of the most important legumes for direct human consumption. Common bean had been recognized since a long time for its protein content. Recent studies have also enlightened its neutraceutical potential. Seed coat of beans comprises of many polyphenolic components (Oomah *et al*., 2005) Studies carried out by Beninger *et al*. (1998) have shown that pigment responsible for seed coat color in *Phaseolus vulgaris* are flavonoids. Many of the flavonoids also impart positive health benefits as antioxidants (Amic *et al*., 2003; Hertog *et al*., 1993; Hertog *et al*., 1992). All the seeds of common beans taken in this study are cultivated in hilly regions in Uttarakhand using traditional methods and preventing them from pathogen attack and usage of any chemical treatment.

Our previous study (Mishra *et al*., 2010) covering polyphenolic content and antioxidative activities of these common beans cultivated in Uttarakhand have shown that few landraces are enriched in these polyphenolic components. Hence to further obtain the precise picture of flavonoids content in these landraces, HPTLC technique has been carried out. Since there is no literature available regarding flavonoid content of common bean landraces of Uttarakhand region.

Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a bioflavonoid, frequently found in consumed foods including apples, berries, onion, tea and vegetables (Formica and Regelson, 1995). Quercetin has many beneficial effects on human health, including cardiovascular protection, anticancer activity, cataract prevention, antiviral activity and anti-inflammatory effects (Nagata *et al*., 1999).

Glycosidic forms of quercetin and kaempferol present in seeds of *P. vulgaris* has been identified to have positive health benefits on human (Beninger *et al*., 1998; Beninger and Hosfield, 1999;

Clifford, 1996; Romani *et al.*, 2004). Common dry beans contain a wide range of flavonoids, including flavonols, their glycosides, anthocyanins, proanthocyanidins and isoflavones, as well as some phenolics acids (Aparicio-Fernandez *et al.*, 2006).

Recent study conducted by Lin *et al.* (2008) based on phenolics profile of 24 common bean samples belonging to 10 commercial market classes of US obtained by HPLC-DAD-ESI/MS, have shown that flavonoid components were significantly different allowing the beans to be classified into six groups. They have also reported the first detection of Quercetin-3-O-pentosylhexoside and flavonoid glucoside malonates and the first detailed detection of hydroxycinnamates in common beans.

Another study conducted on Italian beans (*Phaseolus vulgaris* L.) ecotypes for determination of flavonol content has been carried out, where 23 accessions were analyzed (Dinelli *et al.*, 2006). Total flavonol content varied from 0.19-0.84 g kg^{-1} of seed fresh weight, where kaempferol and its conjugated forms are mainly detected.

There is no literature present regarding quercetin content present in landraces of *Phaseolus vulgaris* L. growing in Uttarakhand. As quercetin is an important functional food, screening of these landraces on nutraceutical basis can be very fruitful for the local farmers as well as for the research purpose.

MATERIALS AND METHODS

Plant material: The present study included 20 landraces of common bean collected from different places of Uttarakhand. The place from where the seeds were collected and altitude of the place above mean sea level were recorded (Table 1). All the bean samples were manually cleaned and finely powdered to pass through a 20 mm sieve prior to extraction.

Sample extraction: Dry beans (*Phaseolus vulgaris* L.) were grinded into fine powder by the grinder. Bean flour (2 g) was extracted with 2% HCl in 80% Methanol (20 mL) and kept on shaker at 90 rpm for 24 h. Samples were centrifuged at 9000 rpm for 10 min and the supernatant was recovered and stored in the dark at -20°C until analysis.

Standard and chemicals: Quercetin hydrate (95+% purity) was purchased from Sigma-Aldrich, HPTLC silica gel 60 F_{254} (20×10 cm) were purchased from Merck. Methanol was purchased from Rankem (India).

HPTLC instrumentation: A Camag HPTLC system equipped with an Automatic TLC sampler (Linomat 5), TLC scanner 3 (WINCATS version 1.3.4) with UV cabinet and twin trough glass tank (20×10 cm) was used for the analysis. The samples were applied using automated TLC sampler in 6.0 mm bands at 8.0 mm from the bottom, both sides and 12.1 mm space between the bands.

Standard solution and calibration curves: Standard solution containing 1 mg mL^{-1} of quercetin hydrate was prepared by dissolving 10 mg of quercetin hydrate in 10 mL of 80% methanol. Standard solution of 0.1, 0.2, 0.5, 1.0, 2.0 μL were applied to the HPTLC plates for preparing five point linear calibration curve. The experimental parameters were identical for all the above analysis.

Table 1: Determination of quercetin content in 20 landraces of common bean grown in Uttarakhand by HPTLC

Sample location		Market class	Quercetin content (mg kg^{-1})	Standard error
Majkhali	Maj-1	Black Mottled	92^{e}	0.02
Purola	Pur-1	Light red kidney	61^{f}	0.02
Purola	Pur-2	Cranberry	111^{e}	0.06
Mori	Mor-1	Large red kidney	127^{d}	0.08
Purola	Pur-3	Small red	410^{a}	0.29
Dhankot	Dhan-1	Pinto	104^{e}	0.04
Tapovan	Tap-1	Red pinto	90^{e}	0.05
Tapovan	Tap-2	Navy	ND	-
Munsiyari	Mun-1	Dark garbanzo	105^{e}	0.03
Dunagiri	Dun-1	Red pinto	98^{e}	0.05
Dwarahaat	Dwara-1	Pink	110^{e}	0.03
Lohaghaat	Loha-1	Light red kidney	Nd	-
Harsil	Har-1	Pinto	50	0.02
Joshimath	Josh-1	Small red kidney	180^{b}	0.06
Chamba	Cham-3	Small white	Nd	-
Purola	Pur-4	Pinto	Nd	-
Tapovan	Tap-3	Cenela	140^{c}	0.06
Chakrata	Chak-2	Pinto	53^{f}	0.03
Munsiyari	Mun-2	Cranberry	Nd	-
Ramgarh	Ram-1	Light brown	Nd	-

Different letters shows significant differences at $p<0.05$ using Duncan's multiple range test

Development of the chromatogram: The TLC plate was developed in a camag twin-trough glass tank which was pre-saturated with mobile phase (n-hexane: Ethyl acetate: Formic acid 6:4:0.1 v/v/v). The composition of the mobile phase was optimized using varying polarity of solvents. The plate was developed to a height of about 8 mm from the base of application. After development, the plate was air dried and spots were visualized under UV light at 262 nm. Quantitative evaluation of the plate was performed in the remission/absorption mode at 262 nm, with the following conditions slit width 5.00×0.45 mm, micro scanning speed 200 mm s^{-1} and data resolution 100 μm/step.

RESULTS

HPTLC analysis of bean extract for the quantitative analysis of quercetin was carried out among 20 bean samples collected from different locations of Uttarakhand (North India). HPTLC analysis was carried out on TLC precoated aluminum plates with silica gel 60 F_{254} as stationary phase using mobile phase (n-hexane: Ethyl acetate: Formic acid 6:4:0.1 v/v/v) with Rf value of 0.78 (Fig. 1). Quantitative analysis was carried out in the absorbance at 262 nm.

The identification of band of quercetin in the bean extract was confirmed by comparing the UV-Vis absorption spectra with the standard. The five point linear calibration curves of quercetin was found to be linear over the range of 100-2000 ng.

Quercetin was detectable in 14 out of 20 common bean extracts. Quercetin content ranged from 50 to 410 mg kg^{-1} (Table 1). Higher concentration of quercetin was detected in Pur-3 (410±0.29 mg kg^{-1}) dark red small kidney beans whereas minimum in Har-1 (50±0.02 mg kg^{-1}) pinto beans.

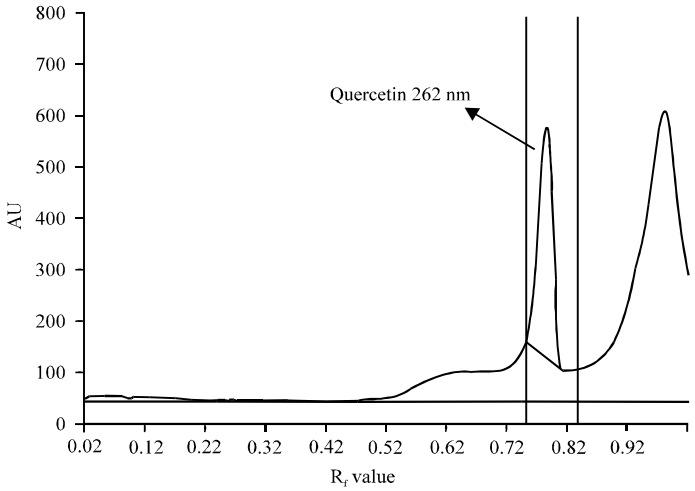

Fig. 1: Chromatogram of standard quercetin

DISCUSSION

Many studies has been conducted to assess the quercetin content in various fruits and vegetable including *Phaseolus vulgaris* L. To our knowledge this is the first report on comparative quantitative estimation of quercetin content in *Phaseolus vulgaris* (L.) landraces growing in Uttarakhand. Quercetin found as glucosides and its malonyl derivatives in light red kidney beans whereas dark red kidney beans contain diglycosides of quercetin (Lin *et al*., 2008). In another study by Miean and Mohamed (2001), 114.5 mg kg^{-1} of quercetin was reported in *Phaseolus vulgaris* (L.) seeds.

In general, Phaseolus genus flavonols and other phenolic compounds are usually stored in seed tegument due to their anti-pathogenic and anti-feeding activities. Thier localization in tegument seed assures the best protection of seeds from external attacks (pathogen, insects) (Dinelli *et al*., 2006).

Several studies have extensively reported the beneficial effects of flavonoids intake for human health. As reported from researches fruits and vegetables are imported sources of these compounds. Moreover, it has been determined that beans (*P. vulgaris*) are sources of these compounds especially Quercetin. Quercetin and its glycoside derivatives are increasingly receiving interest as new generation of anticancer molecules (Kothan *et al*., 2004; Dechsupa *et al*., 2007; Galati and O'Brien, 2004).

Today dry beans are receiving increasing attention as a functional food (Cardador-Martinez *et al*., 2002). The results of the present study can promote the safeguard, by farm conservation, of these landraces and reveal the economic potential to the farmers.

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