Journal of Oilseed Brassica, 11 (2) : 110-115, July 2020

Phenolic acid content and antioxidative capacity of methanolic extracts from quality Indian mustard genotypes

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(Received: 11 September 2019; Revised: 05 January 2020; Accepted: 03 April 2020)

Abstract

Brassica is one of the foremost oilseed crop cultivated in several parts of the world. Its oil is consumable and defatted meal is a good element for use in aquaculture, animal and human diets, which acquires a copious amount of health-enhancing phytochemicals and anticarcinogenic compounds such as glucosinolates and antioxidative principles such as phenolics, flavonoids, vitamin- C & E. The present investigation emphasized on the phenolic content and antioxidative activity in 30 germplasm of *Brassica juncea*. It is catechizing that methanolic extracts of a defatted meal of PRQ-2005-11(BE) (24.14 mg/ml) exhibit paramount *in-vitro* antioxidative property and F3-F4(3) (6.48 mg/g) possessed utmost total phenolic content. Ortho dihidroxyphenol content differ from 0.070 mg/ml (LG 2005-36-1) to 0.323 mg/ml (F3-F4(2)). The reducing power activity was observed to be highest in PLGM-2002-2-7(1.722) followed by PRQ-2005-11-BE (1.674 mg/ml), DCF4-F5-3 (1.672 mg/ml) in a dose-dependent manner. The predominant free radical-scavenging activity was observed in DCF4-F5(3) (84.27 mg/ml) at the concentration of 800 µg/ml. Whereas the heightened chelating activity was found in PRQ-2005-4(BL) (71.81 mg/ml) followed by PRQ-2004-9 (71.54 mg/ml) and PDLM 2003-9-6 (70.23 mg/ml) at the concentration of 800 µg/ml. Thus the defatted meal of 30 Indian mustard genotypes was found to possess rich antioxidative capacity which further utilized in the preparation of functional foods as a viable food ingredient.

Key words: *Antioxidative, Indian mustard, phenolics, quality*

Introduction

Brassica juncea commonly known as an Indian mustard, is a crucifer vegetable belongs to the family Brassicaceae comprising 3,709 species and 338 genera, is one of the ten most economically important plant families and besides excellent source of oil, vegetables, mustard condiments and fodder (Warwick *et al*., 2009). The defatted meal is a by-product of the oil removal process and is underutilized and mainly used as fodder and fertilizer in spite of having eminent nutritional quality (Hashmi *et al.,* 2010). Mustard meal is already known to possess a broad range of therapeutic effects, such as antioxidant, anti-inflammatory, antitumor, and anticancer properties but the mustard meal which was previously overlooked is now being reported as a rich source of antioxidants (Papola *et al.,* 2017). The oilcake used as animal feed and manure whereas green leaves are used for vegetable and green fodder. The meal was found to be rich in different bioactive principles with health promoting properties which are rich in anti-oxidative factors and good source of health promoting dietary minerals (Punetha *et al.,* 2015).The seed meal is an important component of human nutrition and animal feed due to its desirable protein and amino acid contentand low antinutrient constituents (Punetha *et al.,* 2018). Its antioxidant potential mainly corresponds to high phenolic content which acts as reducing agents, hydrogen donors, and singlet oxygen quenchers and other compounds such as ascorbic acid and flavonoids (Szydowska Czerniak *et al.,* 2010). The seed extract of *Brassica juncea* is an efficient antimicrobial and antifungal agent against pathogenic microbes (Ogidi *et al.,* 2019).

Therefore it is imperative to study phenolic content and antioxidative capacity of defatted meal of 30 Indian mustard for its utilization in preparation of functional food and for determining their quality traits and nutritional status.

Materials and Methods

Plant material

The present investigation was carried out on 30 genotypes of Indian mustard obtained from the Department of Genetics and Plant Breeding, GBPUA&T, Pantnagar, India.

Preparation for defatted meal

The collected seeds were dried at 50°C in hot air oven till

constant weight was achieved. The finely crushed seeds in n-hexane (1g/40ml) were defatted in Soxhlet's apparatus for 6 hr. Again residue was elicited with fresh hexane for 2 hr. The defatted seed remnant was extracted with 80% methanol (1 g/10 ml) in a shaker at room temperature for 4 hr and the residue was encored with methanol for 2 hr. Methanolic extracts were filtered, centrifuged at 5,000 g for 5 min which is concentrated in a vacuum evaporator and stored at -20°C for further use.

Antioxidant activity

Total phenolic content

Detection of total phenolic content was based on method described by Singleton & Rossi, 1965. 0.5 mL of the sample extract was transferred into a test tube and mixed 0.2 mL of 50 % (v/v) FCR. After 3 min, 0.5 mL of saturated Na_2CO_3 was added and the volume was made up to 10 mL by adding distilled water in the reaction mixture. The absorbance was measured at 765 nm.

Estimation of ortho-dihyroxy phenols

The ortho-dihydroxy phenols estimated by using Arnow's method (1937). Respective methanolic extract (1 mL) was mixed with 1 mL of 0.5N HCl and Arnow's reagent. 2 mL of 1 N NaOH was added to the reaction mixture. Arnow's reagent undergoes a reaction with ODP and produces a pink-colored complex which can be measured colorimetrically at 515 nm.

Estimation of total antioxidant content

The total antioxidant content in samples was estimated by method described earlier (Prieto *et al.,*1999). The absorbance of the sample was spectrophotometrically measured at 695 nm against a blank.

Flavonoid content

The total flavonoid content determined by the method of Choi *et al.* (2006). The absorbance of color developed at 510 nm was determined. Total flavonoid contents were calculated as quercetin (mg/g) from a calibration curve.

Reducing power activity

The method developed by Yen *et al.* (1993) is utilized for the quantification of reducing power of the methanolic extracts. Absorbance at 700 nm is measured spectrophotometrically against the different amount of methanolic extracts.

2, 2'-Diphenyl picrylhydrazyl (DPPH) free radical scavenging activity

DPPH free radical scavenging activity were determined by, following the procedure described by Yen *et al*. (1993). Different concentrations of prepared methanolic extracts (200 µg/ml to 1000 µg/ml) were mixed with 5 mL of 0.004 % methanolic solution of DPPH and then the mixture was placed for 30 minute in the dark and the absorbance of the samples were read at 517 nm using UV-spectrophotometer. The percentage of DPPH Radical scavenging activity was calculated by the following equation.

$$\text{DPPH Radical scavenging activity (\%)} = 1 - A_t/A_0 * 100$$

Where A_t is the absorbance of the sample and A_0 is the absorbance of the control at 517 nm.

Ferrous ion chelating activity

Measuring Fe^{2+}chelating ability of antioxidant was determined by the Fe^{2+}-ferrozine complex formed at 562 nm (Hsu *et al*., 2003). The different concentrations of methanolic extracts were added 0.1 mL of 2 mM $FeCl_2.4H_2O$, 0.2 mL of 5 mM ferrozine and methanol to make up the volume to 5 mL. The solutions were mixed and allowed to react for 10 min. The absorbance at 562 nm was measured. The Chelating activity was calculated by the following equation:

$$\text{Chelating activity (\%)} = 1 - A_t/A_0 * 100$$

Where A_t is the absorbance of the sample and A_0 is the absorbance of the control at 562 nm.

Results and Discussion

Phenolic content

Phenolic compounds are the pivotal groups among all the phytochemicals, possessing antioxidant capacity. In this study, the maximum phenol content observed in F3-F4(3) (6.48 mg/g) whereas least total phenolic content in PRQ2002-4 (YE) (2.73 mg/g) (Table 1). The total phenolic content varied significantly. Phenolics are prominent for human as well as for animal diets because they are the rich secondary metabolites of plants and these secondary metabolites prevent cancer and coronary heart disease (Hertog *et al*., 1995).

The orthodihydroxy phenol (ODP) varies from 0.070 mg/g (LG 2005-36-1) to 0.323 mg/g (F3-F4 (2) (Table 1). They are crucial group of plant polyphenolics and liable for different bioactive properties of various plant extracts. For antimicrobial properties both 3,4-dihydroxy phenylethanol glucoside and benzamide are prominent (Pradhan *et al.,* 1999). The total antioxidant content of quality germplasm varied from 15.835 mg/g (PRQ2004-8) to 24.14 mg/g (PRQ-2005-11(BE) (Table 1). The promising genotypes having high antioxidant level were PRQ-2005-

Table 1: Total phenol, ortho dihydroxyphenol, antioxidant and flavonoids content in Indian mustard

Sl. No.	Samples	Total phenols (mg/g)	Ortho dihydric phenols(mg/g)	Total antioxidant(mg/g)	Flavonoids(mg/g)
1	F4-F5(1)	4.39 ± 0.189	0.224 ± 0.025	19.07±0.067	0.890 ± 0.025
2	F4-F5 (2)	3.90 ± 0.110	0.247 ± 0.0028	20.10±0.100	1.548 ± 0.035
3	F4-F5 (4)	4.88 ± 0.03	0.199 ± 0.0901	23.09±0.16	2.021 ± 0.045
4	F4-F5 (5)	2.88 ± 0.105	0.249 ± 0.05	22.20±0.200	2.031 ± 0.025
5	F3-F4(1)	4.38 ± 0.085	0.148 ± 0.0017	18.98±0.076	1.540 ± 0.013
6	BC3-BC4(1)	4.051 ± 0.01	0.225 ± 0.0028	17.05±0.086	1.080 ± 0.0251
7	BC3-BC4(2)	3.62 ± 0.100	0.221 ± 0.025	16.06±0.103	1.436 ± 0.295
8	DCF4-F5(1)	3.86 ± 0.02	0.244 ± 0.025	20.43±0.381	1.426 ± 0.01
9	DCF4-F5(3)	4.01 ± 0.100	0.265 ± 0.0138	20.22±0.386	1.286 ± 0.0538
10	DL-2005-3-47(Y)-7	4.448 ± 0.079	0.186 ± 0.0017	16.05±0.122	1.515 ± 0.099
11	PRQ-2005-11(BE)	3.246 ± 0.075	0.098 ± 0.0011	24.14±0.130	1.156 ± 0.06
12	LG2005-6-8(B)-6	5.07 ± 0.12	0.325 ± 0.0011	20.73±0.180	1.136 ± 0.019
13	PRQ2002-4 (YE)	2.73 ± 0.005	0.157 ± 0.0144	21.31±0.272	2.120 ± 0.075
14	PRQ2004-8	3.12 ± 0.07	0.216 ± 0.0144	15.835±0.143	1.289 ± 0.035
15	PRQ2004-9(B)	2.77 ± 0.060	0.098 ± 0.0017	22.28±0.196	1.156 ± 0.06
16	NUDH-YJ-3-3	4.02 ± 0.011	0.149 ± 0.0250	18.08±0.076	1.331 ± 0.035
17	PRQ 2005-4(BL)	4.29 ±0.175	0.105±0.008	18.11±0.287	1.271±0.055
18	PLGM-2002-2-7	6.11 ±0.153	0.137±0.002	18.32±0.277	1.48±0.065
19	F4-F5(7)	5.38 ±0.158	0.202±0.020	23.02±0.034	1.445±0.1
20	F4-F5(6)	5.21 ±0.081	0.074±0.0003	17.27±0.235	1.396±0.055
21	LG BC 2005-6-8	2.93 ±0.061	0.090±0.007	20.16±0.14	1.141±0.025
22	PDLM-2002-4-14	5.06±0.162	0.086±0.002	17.42±0.484	1.335±0.009
23	PDLM 2003-9-6	5.74±0.052	0.154±0.005	17.55±0.132	1.56±0.115
24	PRQ 2005-1	4.11 ±0.079	0.104±0.006	16.20±0.208	1.635±0.099
25	PLGM-2002-2-1	4.71 ±0.045	0.199±0.025	17.06±0.103	1.525±0.11
26	BC3-BC4(3)	5.87±0.162	0.174±0.025	16.25±0.072	1.944±0.03
27	F3-F4(3)	6.48 ±0.163	0.149±0.05	20.19±0.112	1.719±0.035
28	DCF4-F5(2)	5.06 ±0.118	0.236±0.087	21.22±0.068	1.471±0.035
29	F3-F4(2)	4.62 ±0.159	0.323±0.049	20.72±0.131	1.505±0.035
30	LG 2005-36-1	5.91 ±0.020	0.070±0.002	22.08±0.230	1.266±0.01
	CD 1%	0.2339642	0.6658819	0.4374516	0.1167523

11(BE) (24.14 mg/g), F4-F5(7) (23.02 mg/g), F4-F5 (4) (23.09 mg/g), F4-F5 (5) (22.20 mg/g), PRQ2002-4 (YE) (21.31 mg/g) and PRQ2004-9 (B) (22.28 mg/g). It has been reported that total antioxidant capacity depends on total phenolics namely phenolic acids, flavonoid fraction, anthocyanins, glucosinolates and vitamin C (De Nicola *et al.,* 2013).

In *Brassica* crops quercetin, kaempferol and isorhamnetin are the main flavonols and most commonly found as *O*-glycosides (Crozier *et al.,* 2008). In this study the four genotypes having high flavonoid content were PRQ2002-4(YE) (2.120 mg/g), F4-F5(5) (2.031 mg/g), F4-F5(4) (2.021 mg/g), BC3-BC4 (3) (1.944 mg/g) (Table 1). An inverse relationship between flavonoid intake and coronary heart disease was identified in clinical studies (Nijveldt *et al.,* 2001).

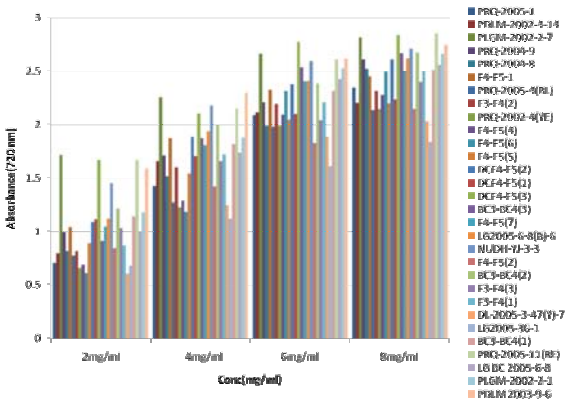

Figure 1: Reducing power activity of different genotypes of Indian mustard at different concentrations

Reducing power activity

The reducing capacity of the extracts Fe^{3+}/ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity (Yildrim *et al.,* 2000). The dose-dependent reducing power activity curve in Indian mustard genotypes depicted in fig.1. At 2mg/ml methanolic extracts PLGM-2002-2-7 showed maximum absorbance followed by PRQ-2005-11-BE (1.674 mg/ml), DCF4-F5-3 (1.672 mg/ml), whereas DL-2005-3-47(Y)-7 (0.612 mg/ml) gave lowest absorbance. As the dose increases then the reducing activity also enhanced.

DPPH free radical-scavenging activity

DPPH free radical scavenging activity is widely used to evaluate antioxidant activity in a relatively short time compared with other methods. The decrease in absorbance occurs when the DPPH radical accepts an electron or hydrogen from an antioxidant. It is well accepted that antioxidant capacity was proportional to the polyphenols since Canola seed cakes possessed the highest recovery of polyphenols and antioxidant capacity (Teh *et al.,* 2014).

Methanolic extracts of PLGM-2002-2-7, DCF4-F5(3), PDLM 2003-9-6 exhibited maximum DPPH scavenging activity (47.36, 43.43, 41.03 mg/ml respectively) at the dose level of 200 µg/mL of the extract per 5 mL methanol containing 40 ppm of DPPH and minimum radical scavenging activity was observed in F4-F5(6) (12.40 mg/ml) in comparison to the standard (Fig. 2). As the concentration of the sample was increased the percentage of radical scavenging was also increased. The present findings justify by earlier findings (Punetha *et al.,* 2015). The antioxidant properties of edible portions of *Brassica* species are mainly due to their polyphenolic components (Ayaz *et al.,* 2008).

Ferrous ion chelating activity

Iron, a transition metal, is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases (Halliwell *et al.,* 1990). Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation by decomposing lipid hydroperoxides into peroxyland alkoxyl (Halliwell *et al.,* 1986).

Ferrozine forms complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is inhibited, resulting in a decrease in the color of the complex. Measurement of color reduction is proportional to the metal chelating activity of the coexisting chelator (Yamaguchi *et al.,* 2000).The chelating activity on Fe^{2+} of methanolic extracts of thirty Indian mustard genotypes was tested using various amount of extracts in the presence of $FeCl_2$ and ferrozine in a dose-dependent manner. The chelating activity was found maximum in BC3-

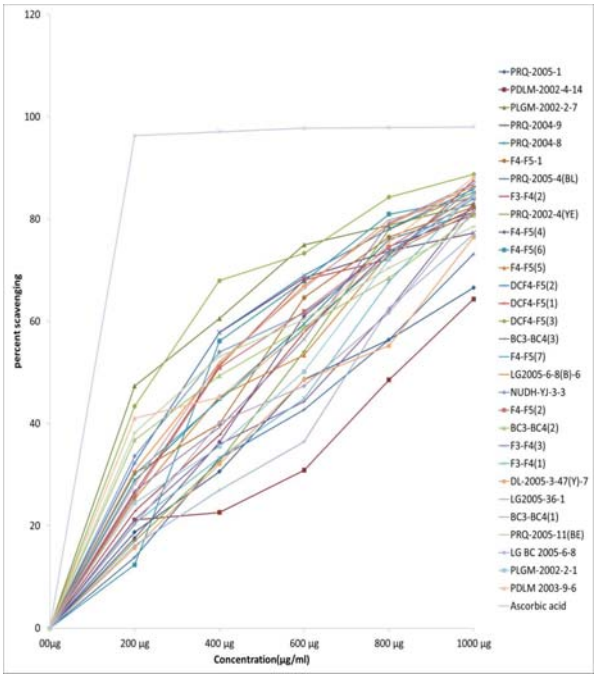

Figure 2: DPPH radical scavenging activity in defatted meal of quality germplasm of Indian mustard

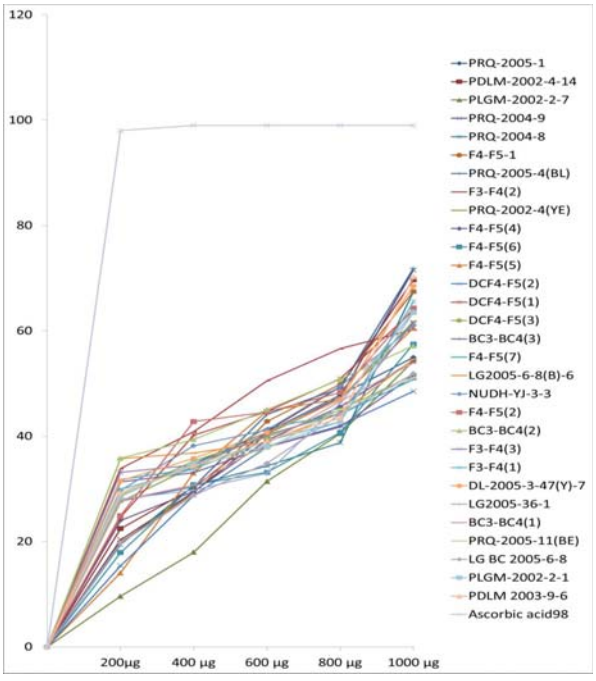

Figure 3: Metal chelating activity in defatted meal of quality germplasm of Indian mustard

BC4(2) (35.80 mg/ml) followed by LG2005-6-8(B)-6 (35.75 mg/ml), F3-F4 (2) (33.84 mg/ml), F3-F4(3) (33.14 mg/ml) and the minimum was found in PLGM-2002-2-7 (9.6 mg/ml) at 200µg/ml (Fig. 3).

Conclusion

Indian mustard represents a major part of the human diet all over the world providing nutritionally significant constituents. The defatted meal contains promising dietary constituents suchphenolics, antioxidative compounds, flavonoids responsible for prominent antioxidative activity which may provide protection against free radical damage & lipid peroxidation, preventing DNA damage, cardiovascular diseases and cancer. The antioxidant properties of phenolic compounds are based on their scavenging ability, primarily due to their hydroxyl groups. The bioactivity of these compounds may be related to their ability to chelate metals and inhibit lipoxygenase. In present study anti-oxidative activities of defatted meal were determined by DPPH radical scavenging activity, reducing power activity and ferrous ion chelating activity. The anti-oxidative activities of defatted meal were determined by DPPH radical scavenging activity, reducing power activity and ferrous ion chelating activity. These assays provide important information about the total anti-oxidative capacity of defatted meal which further be utilized for determining the nutritional status of Indian mustard germplasm. The information is also useful to ascertain their potential towards development of new and better cultivars.

Acknowledgement

Authors are thankful to DST (SERB) for providing research grant and Plant Breeder, GBPUAT, Pantnagar for providing the seed material for execution of present research work

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