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CONTENTS

Comparative phytochemical analysis in high-yielding *Brassica juncea* varieties

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ABSTRACT: The present investigation deals in deciphering the biochemical analysis of two high yielding Indian mustard varieties, PDZ-1 and Kranti. The anti-nutritive and nutritive content in seeds of both these varieties was analyzed. The parameters revealing the anti-nutritive potential were deciphered as phytic acid, total glucosinolates and sinapine content. The methionine, tryptophan, β-carotene, total carbohydrate, fat, fiber, crude protein and oil were estimated as nutritive factors with proximate analysis. The results of experimentation revealed higher levels of all anti-nutritional factors in Kranti as compared to PDZ-1. Phytic acid (1.83±0.13), total glucosinolates (91.25±0.19) and sinapine content (2.38±0.16) were altogether higher in Kranti than PDZ-1 in which values were 1.02±0.08 for phytic acid, 14.22±0.21 for total glucosinolates and 1.34±0.06 for sinapine content. The nutritive contents were comparable in both the mustard varieties. However, PDZ-1 possessed higher values of oil (43.52±0.59) and crude protein (35.61±0.67) than Kranti, oil (37.29±0.78) and crude protein (29.93±0.45). An assessment of anti-oxidative potential, total phenols, ortho-dihydric phenols and total flavonoids was also done in this research investigation. Indian mustard is utilized in almost every household as oil for cooking purposes, extracted from its seeds. Besides their higher protein content, the seeds are not used in large proportions in diet due to the involvement of anti-nutritional factors. In future, more emphasis should be laid on lowering the content of the anti-nutritional factors so that the consumption of mustard seeds in diet could be enhanced for gaining the nutritional benefits.

Key words: Anti-nutritional, Indian mustard, mustard seeds, nutritional, phytochemistry

Mustard has been cultivated by human civilization from a long period of time and it originated from India and China and then spread to other parts of the world due to its popular pungent taste as well as several beneficial body ailment healing roles (Kang *et al.,* 2021). The taxonomists have divided mustard into various genera among which *Brassica juncea* is popularly known as Indian mustard or brown mustard or oriental mustard (Patel *et al.,* 2022). The phytochemistry of mustard is very interesting and hot topic in present scenario as it contains both nutritive and anti-nutritive factors (Rahman *et al.,* 2024). The nutritive factors like crude protein, fat, carbohydrate, tryptophan, methionine and β-carotene are as abundant as any other cereal crop and encompasses even most of them (Frazie *et al.,* 2017; Das *et al.,* 2022) but here comes the action of anti-nutritive factors like total glucosinolates, phytic acid and sinapine content which altogether limits the consumption of mustard seeds as food in large proportion in diet (Sarwar Gilani *et al.,* 2012; Salim *et al.,* 2023; Duraiswamy *et al.,* 2023). However, the mustard oil extracted from its seeds is utilized worldwide (Mhatre *et al.,* 2020; Sharma *et al.,* 2023). The oil contains a prominent amount of saturated and un-saturated fatty acids but the oil extraction is a process with various levels of purification and processing (Batool *et al.,* 2024; Stojanović *et al.,* 2023). Moreover, double zero 'canola' having low erucic acid and low total glucosinolate content is more extensively used for the oil extraction purpose (So and Duncan, 2021). As is clear from the involvement of anti-nutritive factors which actually limit down the consumption of mustard seeds, their role in nature is much more diverse when it comes to bio-control of insects, as various reports are available which suggest their bio-fumigation potential in management of various insect pests, especially glucosinolates and their breakdown products (Aguilar-Marcelino *et al.,* 2022; Garg *et al.,* 2024). Despite these functional advantages and technical limitations in use of mustard seeds in human food, these are given as feed to poultry animals, cattle and fishes in limited proportions accounted by the same anti-nutritional factors (Maiga *et al.,* 2011; Nath *et al.,* 2018).

The present investigation deals in deciphering the phytochemical yet comparative assessment of two *Brassica juncea* varieties, PDZ-1 and Kranti. The objectives of the study indicate the phytochemical parameters of study which largely decide the proportionate use of mustard seed and seed meal especially, phytic acid, glucosinolates and sinapine content.

MATERIALS AND METHODS

Procurement of Seed

The seeds of PDZ-1 and Kranti were procured from Norman E. Borlaug Crop Research Center, GBPUA&T, Pantnagar, Uttarakhand.

Chemicals and Glassware

All chemicals and glassware used for research analysis were assessed from 107 A, Metabolite Research Laboratory, Department of Biochemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Quantitative Phytochemical Analysis

Determination of Nutritional Metabolites

Methionine

Methionine quantification was conducted according to the procedure by Horn *et al.* (1946), with absorbance measured at 520 nm. A standard curve for methionine (99.98 % pure) was created using concentrations from 0.5 to 3.0 mg.

Tryptophan

The Spies and Chambers (1949) method was employed for tryptophan quantification. Absorbance was recorded at 454 nm, with a standard curve constructed using tryptophan (97.84 % pure) concentrations from 40-200 µg/mL.

β-Carotene content determination

β-Carotene was quantified according to the protocol by Sies and Stahl (1995), utilizing water-saturated butanol (8: 2). Absorbance was measured at 440 nm. The standard curve was prepared using a solution of β-carotene (95.66 % pure) dissolved in water-saturated butanol.

Determination of total phenol content

A modified Folin-Ciocalteu colorimetric assay (Sharma *et al.,* 2019) was employed to estimate total phenol content in plant extracts prepared in aqua-methanol. The absorbance was recorded at 765 nm, with gallic acid (98.23 % pure) used to create a standard curve at concentrations of 20-100 µg/mL.

Determination of ortho-dihydric phenols

Ortho-dihydric phenols were quantified using Arnow's method as described by Sharma *et al.* (2019). Absorbance was measured at 515 nm against a reagent blank, with catechol (95.67 % pure) dissolved in distilled water (1 mg/mL) serving as the standard solution.

Total flavonoid content estimation

The total flavonoid content was determined following a modified method proposed by Chang *et al.* (2002). Absorbance was measured at 415 nm, with quercetin (98.77 % pure) prepared in methanol at concentrations ranging from 20-100 µg/mL used for the standard curve.

Quantification of Anti-Nutritional Compounds

Determination of total glucosinolates

Total glucosinolates were quantified using the spectrophotometric method developed by Mawlong *et al.* (2017), utilizing sodium tetrachloropalladate (99.99 % pure). Absorbance was measured at 425 nm, with a blank prepared following the same method without extract.

Determination of phytic acid content

Phytic acid content was determined based on the method established by Haug and Lantzsch (1983). Absorbance was recorded at 519 nm using 0.2 N HCl as a blank. Sodium phytate solutions (20-100 µg of phytic acid, 99.99 % pure) were used as standards.

Determination of sinapine content

Sinapine content was calculated using the method outlined by Kolodziejczyk *et al.* (1999). Absorbance was read at 330 nm, using methanol as a blank. The

sinapine percentage was calculated with the following formula:
% sinapine = (2.184 × Absorbance × 10) / (sample weight in grams)

Proximate analysis
Moisture, ash, crude fat, crude protein and crude fiber in powdered Indian mustard seed samples were determined following the Association of Official Analytical Chemists (AOAC) standard methods. The total carbohydrate content was calculated by subtracting the values of other nutrients from the overall composition (Wang *et al.,* 2016).

Determination of Antioxidant Activities
Ferrous ion chelating activity
Ion chelating activity was determined using the method of Pavithra and Vadivukkarasi (2015), with EDTA (99.99 % pure) used as the standard.

DPPH scavenging activity
The DPPH scavenging activity was measured using the method by Huang *et al.* (2012) with ascorbic acid (98.78 % pure) as the standard.

Reducing activity
Reducing activity was assessed using the method by Yen (2000) with gallic acid (98.23 % pure) as the standard.

Estimation of total antioxidant content
The total antioxidant content was determined following the phosphomolybdenum method described by Prieto *et al.* (1999). Absorbance was recorded at 695 nm using a reagent blank as a reference. A 1 mg/mL ascorbic acid solution served as the standard for the calibration curve.

Statistical Analysis
Every phytochemical test was done in 3 replications and results were explicated as mean ± standard deviation. The statistical analysis of experimental analysis used ANOVA at $p<0.05$.

RESULTS AND DISCUSSION

The nutritional phytochemical metabolites of two mustard varieties, PDZ-1 and Kranti, were analyzed (Table 1). PDZ-1 showed a higher methionine content (2.23 g/100g protein) and tryptophan content (1.89 g/100g protein) compared to Kranti (1.25 g/100g protein and 1.32 g/100g protein, respectively). PDZ-1 also had higher β-carotene content (4.76 ppm), total phenol content (6.12 mg GAE/g), ortho-dihydric phenol (ODP) content (0.954 mg/g) and flavonoid content (0.875 mg QE/g dry weight) compared to Kranti, which had lower values across all these metrics.

The anti-nutritional compound analysis (Table 2) revealed that Kranti had significantly higher levels of phytic acid (1.83 mg/100g), glucosinolates (91.25 µmol/g) and sinapine (2.38%) compared to PDZ-1, which showed lower values for phytic acid (1.02 mg/100g), glucosinolates (14.22 µmol/g) and sinapine (1.34%).

Table 1: Nutritional phytochemical metabolites of *B. juncea* varieties

		Nutritional Phytochemical Metabolites					
Sr. No.	Name of the sample	Methionine Content(g/100g protein)	Tryptophan Content (g/100g protein)	β-carotene content (ppm)	Total Phenol content (mg GAE/g)	ODP content (mg/g)	Flavonoid content (mg QE/g dry weight)
1.	PDZ-1	2.23±0.19	1.89±0.17	4.76±0.12	6.12±0.16	0.954±0.03	0.875±0.12
2.	Kranti	1.25±0.11	1.32±0.14	3.08±0.15	5.64±0.11	0.749±0.05	0.823±0.18

Table 2: Anti-nutritional compounds profile of *B. juncea* varieties

		Anti-nutritional Compounds		
Sr. No.	Name of the sample	Phytic acid Content (mg/100g)	Glucosinolate Content (µmol/g)	Sinapine Content(%)
1.	PDZ-1	1.02±0.08	14.22±0.21	1.34±0.06
2.	Kranti	1.83±0.13	91.25±0.19	2.38±0.16

Table3: Proximate Composition of Different Color Coated Mustard

		Proximate Composition						
Sr. No.	Name of the sample	Moisture (%)	Ash (%) (%)	Dry matter (%)	Oil Content (%)	Crude Protein (%)	Crude Fiber	Total carbohydrate
1.	PDZ-1	1.33±0.09	3.58±0.08	94.23±0.11	43.52±0.59	35.61±0.67	10.06±0.21	28.63±0.85
2.	Brown Mustard	2.78±0.04	3.19±0.12	97.72±0.01	37.29±0.78	29.93±0.45	9.81±0.35	24.51±0.72

Table 4: Estimation of Potent Anti-Oxidant Activities

		Potent Anti-oxidative Activities						
Sr. No.	Name of the sample	Total Antioxidant Activity		Ferrous Ion Chelating Activity		DPPH Scavenging Activity Activity		Reducing Activity
		Methanolic Extract	Methanolic Extract	Hexane Extract	Methanolic Extract	Hexane Extract	Methanolic Extract	Hexane Extract
1.	PDZ-1	11.66±0.34	9.15±0.42	8.16±0.11	80.13.±0.22	109.35±0.19	12.73±0.09	14.11±0.37
2.	Kranti	18.24± 0.26	12.79±0.37	10.21±0.23	84.53±0.31	104.65±0.24	15.86±0.25	18.94±0.21

The proximate composition analysis (Table 3) showed that PDZ-1 had lower moisture content (1.33%) compared to brown mustard (2.78%). PDZ-1 also had higher oil content (43.52%), crude protein (35.61%) and crude fiber (10.06%) than brown mustard, which had values of 37.29%, 29.93% and 9.81%, respectively. In contrast, brown mustard had a higher dry matter content (97.72%) and total carbohydrate content (24.51%) compared to PDZ-1, which had 94.23% dry matter and 28.63% total carbohydrates.

The potent antioxidative activities of PDZ-1 and Kranti were compared (Table 4). Kranti exhibited higher total antioxidant activity (18.24) and ferrous ion chelating activity in both methanolic (12.79) and hexane extracts (10.21) compared to PDZ-1, which had values of 11.66, 9.15 and 8.16, respectively. For DPPH scavenging activity, PDZ-1 showed higher activity in hexane extract (109.35) while Kranti had a slightly higher methanolic extract value (84.53). In reducing activity, Kranti outperformed PDZ-1 in both methanolic (15.86) and hexane extracts (18.94), while PDZ-1 had values of 12.73 and 14.11, respectively.

The elucidation of phytochemicals in two high yielding *B. juncea* varieties, PDZ-1 and Kranti analyzed here falls in accordance to the previous research (Janhavi *et al.,* 2022; Chaudhary *et al.,* 2016; Pichhi *et al.,* 2020; Punetha and Adhikari, 2020; Garg *et al.,* 2023). *B. juncea* is a globally utilized mustard for its medicinal, anti-inflammatory, nutritional but potentially limited use in diet by virtue of certain anti-nutritional factors present, shown and supported by biochemical elucidation here.

CONCLUSION

The phytochemical profiling has revealed that PDZ-1 contains relatively less content of anti-nutritional factors (phytic acid, total glucosinolates and sinapine) and possesses higher content of nutritive factors (protein, carbohydrates, tryptophan, methionine, β-carotene and oil content) than are present in Kranti. However, the anti-oxidative potential of Kranti is more as compared to PDZ-1 because the latter one contains lower amount of total glucosinlates which comprehensively accounts for their anti-oxidative effects. Nonetheless, the nutritive value of PDZ-1 is far much superior to that of Kranti and is more ideal for feed purposes for animals.

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