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# Nutritional & Anti-Nutritional and Anti-Oxidative Profiling of Globally Utilized Diverse Seed Coat Color Mustards

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**ABSTRACT: Mustard is widely utilized in diet by people all over the world for its taste and spice based use. However, certain anti-nutritional factors are evidently present in it which limits its use and consumption in both human and animal based diet. *Sinapis alba* (White seeds), *Brassica nigra* (Black seeds) and *Brassica juncea* (Brown seeds) are three genotypes being considered herein for research analysis in their seeds, aimed at screening of anti-nutritional factors like sinapine content, total glucosinolates, phytic acid along with potent anti-oxidative properties like total antioxidant content, ferrous ion chelating activity, reducing activity and DPPH scavenging activity. So the beneficial and non-beneficial aspects of three globally used mustard genotypes are presented hereby which elucidate presence of important fundamental phyto-chemicals and their distribution in different coat color mustard genotypes. The results obtained have deciphered that *Brassica juncea* is having highest amount of total glucosinolates, methionine and total antioxidative capacity which verifies the fact that methionine is metabolic precursor of glucosinolates and due to which total anti-oxidative capacity is highest in it among three genotypes. The *S. alba* widely used in European region interestingly carries intermediate values of phytochemicals determined here among black and brown seed mustard genotypes. While, Beta- carotene possesses anti-oxidative properties, its content is found highest in *Brassica nigra* suggesting its role in scavenging free radicals at higher rates. Meanwhile, the compounds like phytic acid and sinapine esters which are known for generation of free radicals are present contrastingly in highest amounts in the *B. nigra*. Phytic acid and Sinapine esters also hinders the bioavailability of important nutrients and proteins, while being present overall in varying concentrations in all mustard genotypes limiting their large scale use in fish feed, poultry feed, cattle feed and human based diet. To enhance contribution of mustard in fish feed and poultry feed from 10% and 30% to 70 % and 60%, respectively is the major future challenges need to be addressed and for which primary step is evaluation of key biochemicals. Moreover, the consumption of mustard is also done by humans for which, an evaluation of nutritional and anti-nutritional factors becomes essential.**

**Keywords:** Anti-nutrients, Brassicaceae, Indian mustard, Phytate, Biochemicals.

## INTRODUCTION

Overgrowing population puts up pressure on agricultural needs. World's impoverished countries holds account for 80% of total population having native plant resources as their primary source of healthcare, according to the World Health Organization (WHO). One important angiospermic family of plants having therapeutic, scientific and commercial significance is the Brassicaceae family, also known by its traditional name, Cruciferae. Commonly referred to as 'the mustard family', it includes about 3,700 species spread across about 338 genera (Biondi *et al.,* 2021). Of this economically significant family, a plant, Indian mustard (*Brassica juncea*) has long been valued in India by name of 'spice king', as well as used over the world, accounting for possession of therapeutic and nutritional in diet potential. Each and every constituent of the plant, including the root, stems, leaves, and seeds has been adapted for use as food. Indian mustard also known as Oriental mustard, is the most frequently cultivated species because of its adaptability, yielding capacity, and relatively higher tolerance to key diseases, pests and stresses that typically impact other *Brassica* species (Bora *et al.,* 2021). Mustard seeds being small in size (1 to 2 mm) and round in shape, varies from yellow to black in color (Salehi *et al.,* 2021). Indian mustard is also a rich source of protein (32-41%) and oil (37-49%), (Favela-González *et al.,* 2020). The anti-microbial virtue represented by brassica could lead to usage of it as packaging material by seed

released compound allyl and benzyl isothiocyanates (Bahmid et al., 2020). Of the greatest interest is the development of its seed usage as feed for both ruminants and non-ruminants (Gacek *et al.,* 2018). Health promoting effects of mustard reveal their special role in human diet (Frazie *et al.,* 2017) and as well as in protecting from carcinogenic agents (Ahmed *et al.,* 2020).

Certain bioactive secondary metabolites like glucosides carotenoids (zeaxanthin, lutein, and β-carotene), alkaloids, phytosterols, terpenoids polyphenols, flavonoids and even phenols remain in the defatted meal obtained so following the oil extraction (Gupta *et al.,* 2019; Haug and Lantzsch 1983; Kolodziejczyk *et al.,* 1999; Kwon *et al.,* 2020). To quote for glucosinolates is the fact that their presence primarily in plants belonging to Brassicaceae family limits the usage and marketing of these mustard meals in cattle, fishes and poultry (Li *et al.,* 2018). Glucosinolate breakdown products are specific to their environment, as their functional group changes with change in variable levels of pH and these compounds exhibit anti-tumor and anti-microbial action which is highly significant in therapeutic studies (Abdel-Massih *et al.,* 2023).

Additionally, it has been reported that mustard show presence of stronger antioxidants than fruits, cereals, and nuts (Ogidi *et al.,* 2019). Studies have suggested that preventive or putative therapeutic properties of mustard seeds can be associated with this antioxidant property, because oxidative damage of DNA and proteins and free radical-mediated peroxidation of membrane lipids are believed to be associated with a variety of chronic pathological repercussions *viz.,* cancer, atherosclerosis, neurological disorders, and ageing (Dua *et al.,* 2014; Nguyen *et al.,* 2020). Mustard seeds also exhibit broad range of physiological effects including anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-coagulant, anti-mutagenic, anti-fertility, anti-bacterial, anti-diabetic, antiulcer, antifungal, antiviral, anti-protozoal, anti-venom, anti-fibrotic, hypotensive and hypocholesteremic activities (Ogidi *et al.,* 2019; Papola *et al.,* 2015; Prieto *et al.,* 1999; Punetha and Adhikari 2020; Ramirez *et al.,* 1998; Rasera *et al.,* 2019). Allylisothiocyanate (AITC) is chiefly responsible for anti-cancerous activity of mustard, being produced from sinigrin and 80% of volatile fraction in seeds contain AITC. Apart from these effects, AITC is a potent hindering agent for potential bio-absorption of proteins and minerals in feed diet (Tarar, *et al.,* 2022). Additionally, mustard seeds can be used in culinary flavouring, animal feed as well as for treatment for inflammatory illnesses including rheumatism and arthritis (Melrose, 2019).

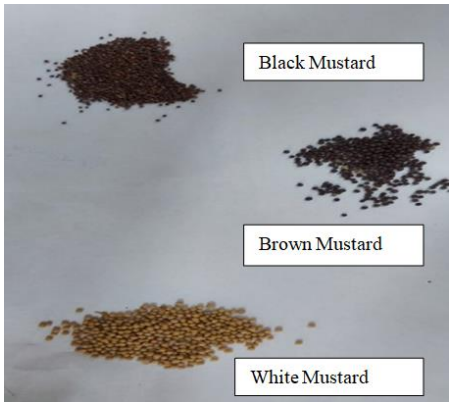
Cruciferins (accounting for 80% of total proteins) and napins (9-20%) are major protein families present in mustard defatted seed meals which wholly account for 35-40% of total crude protein. Cruciferins have their role in nutritional aspects while napins are accounted for other biological properties like solubility in biological systems and allergenic attributes (Leitzow 2021). This is the presence of phytic acid that actually limits the feeding biological absorption of mustard proteins and hence is responsible for bio non-availability of major protein fractions (Bopitiya, 2022). Irrespective of proteins bio-availability increase in mustard seed, the quality enhancement of mustard oil from seed, through addition of poly halite has led to the attainment of nutritional enrichment as it contains added amounts of potassium, sulphur, magnesium and calcium (Pramanick *et al.,* 2023). To further increase the nutritive potential of mustard, foliar spray of nitrogen and boron is sought to have accompanied the increase in oil quality, yield and nutritional attributes (Dhaliwal *et al.,* 2022).

Further investigation of this plant as a possible source of pharmacologically standardized phyto-therapeutic is warranted given the broad spectrum of beneficial effects that have been perceived with this plant. The use of genetic engineering in developing mustard plant has an impeccable role by targeting specific nutritional and anti-nutritional factors (Thakur *et al.,* 2020). Thus, in order to advance their use for the welfare of humans and animals, the present study was conducted in an effort to assess the nutritional & anti-nutritional metabolic profiles and proximate chemical composition of quality Indian mustard seeds.

## MATERIALS AND METHODS

*A. Collection of Plant Material*

Seeds of three mustard genotypes (Figure 1) viz., *Brasssica juncea* (Brown mustard), *Sinapis alba* (White mustard) and *Brassica nigra* (Black mustard) were obtained from Norman E. Borlaug Crop Research Center, GBPUA&T, Pantnagar, Uttarakhand, India.




Seeds of Black, Brown and White Mustard

**Fig. 1.** Three color coat mustard seeds.

*B. Quantitative Phytochemical Analysis*

***Estimation of Total Antioxidant Content.*** The phosphomolybdenum method given by (Prieto *et al.,* 1999) was used to estimate the total antioxidant content where absorbance was measured at 695 nm against a reagent blank. Ascorbic acid solution of 1mg/mL concentration was used for standard curve.

***β-Carotene Content Determination.*** The β-Carotene was quantified using water-saturated butanol (8:2) as per the method (Sies and Stahl 1995) reported. Absorbance measured at 440 nm. Solution of β-

Carotene dissolved in water-saturated butanol was used for standard curve formation.

***Determination of Total phenol Content.*** The Folin-Ciocalteu colorimetric method developed by (Sharma *et al.,* 2019) was modified to evaluate total phenol content in aqua-methanol plant extracts. Absorbance measured at 765 nm. Gallic acid was used for standard curve in various concentrations ranging from 20-100 µg/mL.

***Determination of Ortho-dihydric Phenols.*** Ortho-dihydric Phenol (ODP) was determined using Arnow's technique as proposed by (Sharma *et al.,* 2019). Absorbance was measured at 515 nm against a reagent blank. For standard solution, 10 mg of catechol was dissolved in 10 mL of distilled water yielding a concentration of 1 mg/mL.

***Total Flavonoid Content Estimation.*** Method proposed by (Chang *et al.,* 2002) was applied for estimation the total flavonoid content and was modified as necessary. Absorbance was measured at 415 nm. Quercetin was used as a standard which was prepared at different concentrations (20-100 µg/mL) in methanol.

***Determination of Sinapine Content.*** The estimation of sinapine determination in the sample was carried out using the procedure outlined by (Kolodziejczyk *et al.,* 2019). Absorbance was read at 330 nm taking methanol as a blank. The following formula was used to calculate the sinapine content present in the meal:

$$\% \text{ sinapine} = \frac{2.184 \times \text{Absorbance} \times 10}{\text{weight of sample taken(g)}}$$

***Quantification of Essential Amino Acids***

**Methionine.** Methionine present in the samples was quantified using the procedure outlined by (Horn *et al.,* 1946). Absorbance of the color in the resulting mixture was measured with a spectrophotometer set at 520 nm against blank. Standard curve for methionine was generated with quantities ranging from 0.5-3.0 mg of methionine.

**Tryptophan.** To quantify tryptophan in the test samples, method proposed by (Spies and Chambers 1949) was applied. The absorbance was measured at 454 nm against reagent blank. Standard graph was formed using tryptophan concentrations ranging from 40–200 µg/mL.

**Determination of Total Glucosinolates.** The method developed by (Mawlong *et al.,* 2017) was used to perform spectrophotometric estimation of methanolic extracts by using sodium tetrachloropalladate. Absorbance was measured at 425 nm against a blank set with same procedure without the extract.

**Determination of Phytic Acid Content.** The method established by (Haug and Lantzsch 1983) was used for estimation of phytic acid in freshly ground seed samples. Absorbance was measured at 519 nm taking 0.2 N HCl as a blank. For the standard, sodium phytate solutions containing 20–100 µg of phytic acid were taken.

***Determination of Potent Anti-oxidant Activities***

**Ferrous Ion Chelating Activity.** The method established by (Pavithra and Vadivukkarasi 2015) used for ion chelating activity and standard used was EDTA.

**DPPH Scavenging Activity.** The method established by (Huang *et al.,* 2012) used for DPPH Scavenging activity and standard used was ascorbic acid.

**Reducing Activity.** The method established by (Yen 2000) used for reducing activity and standard used was gallic acid.

*C. Proximate Analysis*

Using the standard methods of the Association of Official Analytical Chemists (AOAC) reported moisture, ash, crude fat, crude protein and crude fiber contents were analyzed in powdered Indian mustard seed samples. Based on the net difference between the other nutrients and the overall percentage composition, the total carbohydrate content was calculated (Wang *et al.,* 2016).

## RESULTS & DISCUSSION

The biochemical elucidation of three color differentiated genotypes of Indian mustard seeds was done in the current study. The study revealed the presence of nutritional phytochemical metabolites (Table 1), the presence of anti-nutrient compounds (Table 2) like phytic acid, sinapine and glucosinolates, proximate composition (Table 3) and potent anti-oxidative activities (Table 4).

**Table 1: Nutritional Phytochemical Metabolites.**

| Nutritional Phytochemical Metabolites | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sr. No.** | **Mustard** | **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. | Black mustard | 1.92±0.12 | 1.07±0.81 | 6.08±0.23 | 5.90±0.05 | 0.763±0.08 | 0.754±0.76 |
| 2. | White mustard | 1.88±0.08 | 1.01±0.67 | 4.76±0.12 | 4.22±0.16 | 0.695±0.06 | 0.689±0.54 |
| 3. | Brown Mustard | 1.97±0.17 | 1.12±0.14 | 4.08±0.18 | 5.83±0.18 | 0.812±0.13 | 0.817±0.32 |

**Table 2: Anti-nutritional Compounds Profile.**

| Anti-nutritional Compounds | | | | |
|---|---|---|---|---|
| **Sr. No.** | **Mustard** | **Phytic acid Content (mg/100g)** | **Glucosinolate Content (µmol/g)** | **Sinapine Content (%)** |
| 1. | Black mustard | 1.22 ±0.12 | 52.32 ± 0.25 | 3.12±0.03 |
| 2. | White mustard | 1.43 ±0.21 | 47.63±0.56 | 2.14±0.07 |
| 3. | Brown Mustard | 1.47±0.32 | 85.81±0.33 | 2.18±0.11 |

**Table 3: Proximate Composition of Different Color Coated Mustard.**

| Proximate Composition | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sr. No.** | **Mustard** | **Moisture (%)** | **Ash (%)** | **Dry matter (%)** | **Oil Content (%)** | **Crude Protein (%)** | **Crude Fiber (%)** | **Total carbohydrate (%)** |
| 1. | Black mustard | 2.02±0.8 | 3.98±0.18 | 96.94±0.12 | 37.02±0.06 | 33.96±0.15 | 7.89±0.07 | 22.19±0.15 |
| 2. | White mustard | 2.50±0.09 | 4.12±0.19 | 98.76±0.11 | 41.17±0.13 | 31.91±0.04 | 9.65±0.04 | 26.22±0.14 |
| 3. | Brown Mustard | 2.17±0.17 | 3.80±0.19 | 97.13±0.01 | 38.87±0.03 | 32.86±0.03 | 9.08±0.08 | 26.49±0.06 |

**Table 4: Estimation of Potent Anti-Oxidant Activities.**

| Potent Anti-oxidative Activities | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Sr. No.** | **Mustard** | **Total Antioxidant Activity** | **Ferrous Ion Chelating Activity** | | **DPPH Scavenging Activity** | | **Reducing Activity** | |
| | | **Methanolic Extract** | **Methanolic Extract** | **Hexane Extract** | **Methanolic Extract** | **Hexane Extract** | **Methanolic Extract** | **Hexane Extract** |
| 1. | Black mustard | 18.84±0.16 | 12±0.16 | 19±0.11 | 89±0.14 | 113±0.04 | 14±0.13 | 13±0.31 |
| 2. | White mustard | 18.66±0.17 | 10±0.22 | 13±0.09 | 85±0.19 | 112±0.05 | 16±0.15 | 17±0.19 |
| 3. | Brown Mustard | 19.86± 0.07 | 14±0.13 | 11±0.21 | 81±0.08 | 109±0.04 | 17±0.04 | 12±0.16 |

The elucidation of phytochemicals in mustard types analyzed here falls in accordance to the pertaining research (Janhavi *et al.,* 2022; Chaudhary *et al.,* 2016; Pichhi *et al.,* 2020). The three globally utilized mustard genotypes undertaken here for research consideration supports their medicinal, anti-inflammatory, nutritional but potentially limited use in diet (due to presence of certain anti-nutritional factors) via the biochemical elucidation here. The higher oil, crude fiber and dry matter content in *S. alba* (White mustard) justifies its comparatively larger morphological seed size than other two mustard genotypes included in this study. Among the anti-nutritional compounds elucidated here, *B. juncea* holds highest values for phytic acid and total glucosinoilate content while, sinapine content was found to be highest in *B. nigra*. Focusing on key biochemicals here, in mustard types, we have put forwarded the selection criteria for which the feed have to undergo for its utilization to the animals. Nonetheless, due to the implication of mustard in human diet, it also becomes a necessary concern to be raised for its nutritive and anti-nutritive factors. The ultimate answer to all these queries is the phytochemcial estimation of plant parts used for consumption like, seed here.

## CONCLUSION

The white mustard, *S. alba* holds intermediate values for glucosinolate and sinapine content among the three globally utilized mustard genotypes, analyzed in here for research. The phytic acid content was slightly lower in *S. alba* in respect to that of *B. juncea*, while *B. nigra* was found out to be with least phytic acid content.

## FUTURE SCOPE

The phytochemicals present in three mustard genotypes provides an essence of their importance in human diet. However, its potential as feed for animals can be exploited by deciphering the presence of nutritional and anti-nutritional factors.

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**Conflict of Interest.** None.

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