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Broccoli seed germination enhances their nutritional composition, antioxidant, anticancer, and antimicrobial activities, and *in vitro* nutrient digestibility

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The present study aimed to investigate the effect of germination on the nutritional composition, antioxidant, antidiabetic, anticancer, and antimicrobial activities of broccoli seeds, as well as their *in vitro* nutrient digestibility. The five-day germination process resulted in a significant increase in the levels of crude fibre (p<0.05), methionine (p<0.01), calcium (p<0.01), beta-carotene (p<0.01), vitamin C (p<0.01), and free amino acids (p<0.01). However, it decreased (p<0.01) protein levels in sprouts compared to seeds. A notable improvement (p<0.01) was observed in the DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and metal chelating activity in broccoli sprouts compared to seeds. The levels of total polyphenol (p<0.01) and flavonoid (p<0.05) were significantly higher in sprouts compared to seeds. Broccoli sprouts demonstrated increased levels of the anticancer agent glucosinolate (p<0.05) compared to seeds. Broccoli sprouts exhibited improved antidiabetic activity by significantly inhibiting α-glucosidase activity (p<0.05) compared to broccoli seeds. When compared to seeds, broccoli sprouts showed considerably higher (p<0.05) antibacterial capabilities against Gram-negative bacteria, including *Salmonella typhimurium* and *Escherichia coli*, while no effect was observed against Gram-positive bacteria. Germinated broccoli seeds showed significantly higher *in vitro* protein digestibility (p<0.01) as well as *in vitro* starch digestibility (p<0.01). Our findings indicate that the germination of broccoli seeds increases its nutritional and phytochemical contents and, therefore, can be considered a potent functional food with enhanced disease-prevention properties.

Keywords: Antidiabetic activity, Antioxidant, Broccoli sprouts, Glucosinolate, *In vitro* digestion, Phytochemical

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Introduction

Plant foods have been gaining interest in recent times not only for nutrition but also because they contain several bioactive constituents that have been shown to possess health-promoting properties[1]. The Brassicaceae family, known as cruciferous vegetables, is rich in nutritional composition, including carbohydrates, proteins, various carotenoids, phenolic substances, anthocyanins, coumarins, therapeutic antioxidant enzymes, terpenes, vitamins C, E, K, and folate, and minerals and also a good source of dietary fibre[2]. Cruciferous vegetables contain chemicals known as glucosinolates, which are secondary metabolites that are economically valuable and have potential health benefits, particularly in cancer prevention [3].

In recent years, studies on Broccoli (*Brassica oleracea* L. var. *italica*), one of the most important vegetables of the Brassicaceae family, have been increasing due to its nutritional value and chemoprotective potential[4,5]. The biological activity of this vegetable is mostly related to its sulfur compounds like glucosinolates and polyphenol content[6]. Glucosinolates are sulfur and nitrogen-containing glucosides that are found almost exclusively in cruciferous vegetables. When the plant tissues and cells are damaged, glucosinolates are hydrolysed by the enzyme myrosinase, resulting in several pungent degradation products, including isothiocyanates (the most nutritionally and economically important products) and nitriles[7]. Sulforaphane (4-methylsulfinylbutyl isothiocyanates) is a type of sulfur-containing isothiocyanate, which is receiving increasing attention due to its anticarcinogenic function. Phenolic compounds attract interest due to their antioxidant capacity and high free radical scavenging activity, which contribute to positive health effects. Flavonoids are associated with a reduced risk of Diabetes Mellitus, cancer, and infectious and inflammatory diseases[8]. High levels of phenolic compounds show antibacterial activities against

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foodborne pathogens, including Gram-negative and Gram-positive bacteria[4].

Recently, one study has shown that the sprouting of seeds results in improved nutritional quality and elevated levels of phytochemicals, thereby producing a positive health impact[9]. A few recent studies have reported anti-inflammatory, antidiabetic, anticarcinogenic, and antioxidant properties of broccoli sprouts, thereby preventing various inflammatory diseases[10,11]. These properties may be attributed to their rich presence of bioactive compounds, including glucosinolates and their degradation product isothiocyanates, polyphenols, carotenoids, minerals, and vitamins, with concentrations higher than the adult plant[12]. These cruciferous sprouts contain an inducing activity that is 10–100 times more potent than mature vegetables[13].

Broccoli has been well-reported in terms of nutritional composition, but few studies have focused on determining the nutritional value of broccoli sprouts and broccoli seeds. Studies that report the effect of germination on antioxidative properties and antibacterial activity are contradictory. No studies have reported the effect of *in vitro* digestion on broccoli sprouts. Therefore, the purpose of this study was to determine the nutritional, biochemical composition, and phytochemicals present in broccoli seeds and sprouts. This was accomplished by identifying the variations in proximate composition and amino acid content. *In vitro* protein digestibility and starch digestibility were performed. Additionally, antioxidative, antidiabetic, antinutritional, and antimicrobial activity were analysed.

Materials and Methods

Chemicals and standards

The broccoli seeds were procured by ordering from the Indian Agricultural Research Institute (IARI), Regional Station, Katrain (Kullu Valley), Himachal Pradesh. Trolox, DPPH, Rutin, Sodium tetrachloropalladate (ll) and Ferrozine were purchased from Sigma-Aldrich Company. α-amylase from human saliva Type IX-A, Pepsin from porcine gastric mucosa and pancreatin from porcine pancreas were purchased from Sigma. Folin and Ciocalteus Phenol (FCP), Ferrous ammonium sulphate, Ferric Chloride Anhydrous, Bromocresol Green Indicator, Sodium Acetate Trihydrate and 3,5-Dinitrosalicylic acid were purchased from Sisco Research Laboratories Pvt. Ltd. Nutrient agar, nutrient broth, ferrous sulphate heptahydrate and gallic acid monohydrate were obtained from HiMedia.

Determination of hydration capacity and index of broccoli seeds

About 10 g of seeds were counted and transferred to a measuring cylinder. Water (50 mL) was added to the cylinder, which was then covered with aluminium foil and left overnight at room temperature. The seeds were drained, and superfluous water was removed with filter paper. The swollen seeds were reweighed. Hydration capacity and hydration index were calculated using the following formula,

$$\text{Hydration capacity (g/100 grains)} = \frac{\text{weight of grains after soaking (g)} - \text{weight of grains before soaking (g)}}{\text{total number of grains (N)}}$$

The hydration index was calculated using the following formula,

$$\text{Hydration index} = \frac{\text{hydration capacity per 100 grains}}{\text{weight of total number of grains}}$$

Determination of swelling capacity and index of broccoli seeds

10 g of seeds were weighed, counted and transferred to a measuring cylinder, and the total volume was recorded. These seeds were then soaked in 50 mL of water. The cylinder was covered with aluminium foil and left overnight at room temperature. The water was drained, and the volume of soaked seeds was noted in a graduated cylinder. Swelling capacity and the swelling index were calculated using the following formula.

$$\text{Swelling capacity} = \frac{\text{volume of seeds after soaking (mL)} - \text{volume of seeds before soaking (mL)}}{\text{Total number of seeds (N)}}$$

The swelling index was calculated by using the formula,

$$\text{Swelling index} = \frac{\text{Swelling capacity per 100 seed}}{\text{Volume of 100 seeds}}$$

Determination of bulk density and tapped density

The bulk density (pB) was measured by pouring the sample into a graduated measuring cylinder of defined volume (vB) and weighing the cylinder and its contents to the nearest 0.1g ($m2$), then subtracting the weight of the empty cylinder ($m1$). Then the bulk density was calculated using the equation ($pB = (m2 - m1) \div vB$). Tapped density was estimated by tapping the measuring cylinder 100 times with 1 gm of sample.

Seed germination and sample preparation

The broccoli seeds (*B. oleracea* L. var. *italica*) were procured from the Indian Agricultural Research Institute

(IARI), Regional Station, Katrain (Kullu Valley), Himachal Pradesh. About 100 g of broccoli seeds, divided into two sets, were cleaned, washed and soaked for 6 hours. After 6 hours, the water was discarded, and the seeds were allowed to germinate for 5 days at normal room temperature. The beaker was kept in an inverted position, allowing excess water to drain out. After 5 hours of interval, the developed sprouts were rinsed and again kept in the inverted position to avoid fungal contamination. After the germination period was completed, the samples were allowed to dry in a hot air oven at 60°C for 8 hours. The samples were ground into fine powder using a mixer, homogenised, and sieved with a 5 mm sieve. The prepared samples for analysis were packed with polyethylene plastic bags and stored in the fridge at -20°C.

Determination of proximate composition

The ash content was determined by keeping the seeds and sprouts in a muffle furnace at 600°C for 6 hours. The moisture content of the seeds and sprouts was determined by drying them to a constant weight at 70°C. The crude fibre was determined through acid and subsequent alkali treatment. The total protein content was measured using the Kjeldahl method.

Determination of calcium

Sample of ash solution was taken as 3 and 1.5 mL in test tubes; 2 drops of bromocresol green indicator were added with the addition of saturated sodium acetate until a blue colour developed; by adding 4% oxalic acid, calcium was precipitated as calcium oxalate till the solutions changed to yellowish green. The precipitates were heated in a sand bath at 70°C for 45 minutes and allowed to stand for 24 hours. The next day, the supernatant was discarded, and the precipitates were washed 3 times with 3 mL of 2% ammonia to remove excess oxalic acid. The mixture was centrifuged, and the supernatant was discarded. The tubes were placed in an inverted position to drain out all ammonia. 3 mL of 1 N H_2SO_4 was added and heated for 5 min at 50-70°C in a water bath; the obtained content was titrated against 0.01 N $KMnO_4$. The calcium content was calculated using the formula:

$$\text{Calcium (mg \%)} = \frac{\text{titre value (blank} - \text{sample)}}{1} \times \frac{\text{Ca content of KMnO 4}}{\text{aliquot taken}} \times \frac{\text{volume made up}}{\text{sample taken}} \times 100$$

Determination of vitamin C

Estimation of vitamin C by 2, 6- Dichlorophenol Indophenol visual titration method. The sample was prepared by crushing it in a mortar and pestle with 3% HPO_3. The extracted sample was titrated with standard dye to a pink endpoint, which should persist for at least 15 sec.

Determination of methionine

The sample extract of seeds and sprouts was treated with 10% sodium hydroxide and 10% sodium nitroprusside. After incubating it for ten minutes, 3% glycine was added, followed by incubation for 10 min and the addition of 2 mL orthophosphoric acid. The absorbance of the reddish-brown colour was read spectrophotometrically at 520 nm. The result is expressed as mg/mL.

Sample extraction for phenolic content and antioxidant determination

The seed and sprout powder of broccoli was used for extraction to determine phenolic content and antioxidant activity. The 2.0 g of seed and sprout samples were extracted thrice in 80% aqueous methanol (pH 2.0) by shaking at room temperature for 90 min. The supernatants were centrifuged and filtered, and the volume of each sample was made up to 50 mL with the solvent using a volumetric flask. The extracts were stored at -20°C to estimate the total phenolic content and total antioxidant capacity. The seed and sprout samples were extracted in duplicate batches.

Determination of total phenol

Briefly, 0.1 mL of the sample extract was mixed with 0.5 mL of Folin reagent (1:1 H_2O), followed by 10 mL of 7.5 gm% Na_2CO_3. After incubating for 1 h at 37ºC, the absorbance of mixed samples was measured at 750 nm[14]. Gallic acid was used as a standard, and the results were expressed in milligrams of gallic acid equivalent per 100 g sample (mg GAE/100 g).

Determination of total flavonoids

The methanolic extracts (0.25 mL) of the seeds and sprouts of broccoli were mixed with distilled water, 0.3 mL of $NaNO_3$ and 0.6mL of 10% $AlCl_3$ and mixed properly. After 6 min of incubation, 2 mL of 1N NaOH was added. The absorbance of the mixed sample produced a pink colour, which was determined at 510 nm using a spectrophotometer against blank[14]. The comparison was made using Rutin as standard, and the results were expressed as

mg of Rutin equivalent per 100 g sample (mg RE/100 g).

Antioxidant capacity

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the methanolic extract of broccoli seeds and sprouts was determined on the basis of the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. The absorbance was read at 517 nm[15]. Percentage inhibition was calculated, and the results were expressed as mg Trolox equivalent (TE) per 100 g sample.

Determination of Ferric Reducing Antioxidant Power (FRAP)

The sample extract was treated with FRAP reagent, and the blue-coloured complex was read at 593 nm[16]. A comparison was made using the water-soluble vitamin E analogue Trolox standard. The results were expressed as mg Trolox Equivalent per 100 g of sample (mg TE/100 g).

Determination of Metal Chelating Activity

The extracted samples (0.2 mL) were added to 0.2 mL of 0.1 mM FeSO4 and 0.4 mL of 0.25 mM ferrozine. After 10 min of incubation at room temperature, the absorbance of the mixture was read at 562 nm[17]. The results were expressed as mg EDTAE/g extract.

Determination of Glucosinolate

The methanolic extract was prepared by homogenising a 0.1 g defatted sample in a 2 mL vial with 80% methanol. After incubating overnight, this homogenate was centrifuged at 3000 rpm for 4 min, the supernatant was collected, and volume made up to 2 mL with 80% methanol. From the supernatant, 100 µL of extract was used for estimation by adding 0.3 mL double distilled water and 3 mL of 2 mM sodium tetrachloropalladate (58.8 mg Sodium tetrachloropalladate + 170 µL concentrated HCl +100 mL double distilled water). A blank was set without the extract, and after 1 hour of incubation at room temperature, absorbance was read at 425 nm using a spectrophotometer[18]. Total glucosinolates were calculated by putting the OD into the predicted formula.

Simulated *in vitro* gastrointestinal digestion

In vitro starch digestibility

100 mg sample was suspended in 9 mL acetate buffer (0.2 M, pH 6, containing 200 mM $CaCl_2$ and 0.5 mM $MgCl_2$). The sample was then equilibrated at 37°C. 1 mL of mixed enzyme solution with the activity of 10 U/mL α-amylase and 18 U/mL of amyloglucosidase was added. 100 µL of 95% ethanol (v/v) was added to 100 µL of aliquots of the above solution at 0, 20, 120, and 180 min to inactivate enzymes. The unreacted starch residues were separated after centrifugation at 2000 gm for 5 min[19]. Glucose concentration was measured with a GOD/POD Assay Kit. The result is expressed as mg % of glucose concentration.

In vitro protein digestibility

The static *in vitro* gastrointestinal digestion for protein was carried out according to a standardised protocol as described[20] with minor modifications. Briefly, the protein digestibility was done in three phases: (1) Oral Phase: 5 g of sample was mixed with 3.5 mL of Simulated Salivary Fluid (SSF) electrolyte stock solution and mixed together with 0.5 mL of 1500 U mL^{-1} salivary α-amylase solution made up in SSF (α-amylase from human saliva Type IX-A, 1000-3000 U/mg protein, Sigma), followed by addition of 25 µL of 0.3 M $CaCl_2$, and 975 µL of water and thoroughly mixed. (2) Gastric Phase: 10 mL of oral bolus was mixed with 7.5 mL of Simulated Gastric Fluid (SGF) electrolyte stock solution, followed by 1.6 mL Porcine pepsin stock solution of 25000 U/mL made up in SGF (pepsin from porcine gastric mucosa 3200-4500 U/mg protein, Sigma), and to this added 5 µL of 0.3M $CaCl_2$, 0.2 mL. of 1 M HCl to reach pH 3.0 and 0.695 µL of water. (3) Intestinal phase: 20 mLof gastric chyme was mixed in 11 mL of Simulated Intestinal Fluid (SIF) electrolyte stock solution, 5.0 mL pancreatin solution of an 800 U/mL made up in SIF based on trypsin activity (pancreatin from porcine pancreas, Sigma), followed by 40 µL of 0.3 M CaCl, 0.15 mL of 1 M NaOH to reach pH 7.0 and 1.31 mL of water. pH in the gastric and intestinal phases was verified every 30 minutes. The supernatants at each phase were separated, collected and used in further analysis. Three sample sets were labelled as follows: A = Original samples or samples before digestion, B = samples after gastric digestion, and C = samples after intestinal digestion.

Determination of total soluble nitrogen

The Kjeldahl method was performed for the estimation of the total nitrogen of the samples (A, B, and C) according to method 981.10 of the

AOAC International using Kjeldahl (KELPLUS) Apparatus, Pelican. The Kjeldahl method involves a three-step approach for the quantification of protein: digestion, distillation, and titration[21].

$$\text{Nitrogen (\%)} = \frac{14.01*(\text{sample titre} - \text{blank titre})*\text{normality of HCl}*14*1000}{\text{sample weight (g)}*100}$$

Determination of protein content

The protein content in samples (A, B, and C) was estimated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor set at 6.25. Hence, the per cent protein is calculated as follows:

Protein (%): 6.25 X % Nitrogen

Determination of free amino acids (FAA)

80% ethanolic extract of the sample was added to citrate buffer, and the volume was made up to 1 mL, followed by the addition of 1 mL of ninhydrin reagent. The content was mixed by vortex and boiled in a water bath for 15 min. After cooling in ice, 5mL of diluent solvent was added and mixed well. The absorbance was read at 570 nm using a colourimeter.

Determination of protein digestibility

The *in vitro* digestibility was expressed as the percentage difference in protein content in the supernatant of the sample before digestion compared to that in the sample during digestion and after digestion. The degree of protein digestibility was calculated using the following formula:

$$\text{Digestibility (\%)} = \frac{(\text{Initial protein content} - \text{Protein content after simulated } \textit{in vitro} \text{ digestion})}{\text{Initial protein content}} \times 100\%$$

Estimation of antinutritional factor phytic acid

0.5 mL of sample extract was added to 1 mL of ferric solution and 4.5 mL of distilled water, then heated in a boiling water bath for 30 min. It was then cooled in ice water for 15 minutes. After adjusting to room temperature, 2 mL of 2-2dipyridal solution was added and mixed properly. Absorbance was measured at 519 nm exactly after 1 min[22]. A standard graph was prepared by plotting optical density vs. phytate concentration. Results are expressed as phytic acid mg %.

Antidiabetic activity

Anti-α amylase activity

Briefly, in plasma tubes, 0.2 mL of sample was added to 0.4 mL of Sodium phosphate buffer and 0.2 mL of (porcine pancreatic α amylase) PPA enzyme. The sample solution was incubated at 37°C for 10 minutes. 0.2 mL of starch solution was added to it and incubated for 40 minutes at 37°C. 0.2 mL of HCL and 0.2 mL of potassium iodide were added to each plasma tube. The absorbance was read at 620 nm[23]. The results are expressed as the percentage inhibition of the 80% methanolic extracts.

Anti-α glucosidase activity

Briefly, 0.2 mL of sample was mixed with 0.5 mL of sodium phosphate buffer in plasma tubes. 0.1 mL of α-glucosidase enzyme was added to it and incubated at 37°C for 10 minutes. 0.2 mL of p-NPG (p-Nitrophenyl-α –D-glucopryanoside) was added to the previous solution and incubated for 30 minutes at 37°C. 0.5 mL of sodium carbonate was added to each tube. The absorbance was read at 405 nm. Sodium phosphate buffer was used as blank[24]. The sample blanks containing the test sample, substrate, and buffer without α-glucosidase were also assayed.

Antimicrobial activity.

Nutrient Agar plates were overlaid with growth cultures of test pathogens, namely Gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) in nutrient broth. Wells of 5mm diameter were made on the agar plates and filled with 25 µL of 80% methanol extract. Plates were incubated aerobically at 37°C for 24 hours. The antimicrobial effect of the supernatant was evaluated by measurement of the clear zone.

Statistical analysis

All the parameters are reported as mean±SD. An independent t-test was used to compare the mean values of the individual parameters between broccoli seeds and broccoli sprouts. Statistical significance was considered at $p < 0.05$. All the statistical analysis was carried out using SPSS version 20.0.

Results and Discussion

Hydration capacity and hydration index of broccoli seeds and sprouts

In the present study, the hydration capacity and hydration index of broccoli seeds were 0.0036 g/100 grain and 0.003, respectively. The swelling capacity and swelling index of broccoli seeds were found to be 0.0042 mL/100g and 0.00014, respectively.

Bulk and tapped density of broccoli of broccoli seeds and sprouts

The bulk density of broccoli sprouts was found to be significantly higher than broccoli seeds (Seed: 0.40±0.018 g/cm³; Sprout: 0.44±0.01 g/cm³; $p<0.05$). The tapped density in raw seeds was 0.400 g/cm³, while in broccoli sprouts, the tapped density is higher at 0.462 g/cm³. A significant increase ($p<0.05$) was observed in broccoli sprouts than in seeds. Flours of boiled sesame seed and sprout at 8 h and 10 h have the highest bulk density of (0.87 g/mL) and the lowest value of (0.71 g/mL) of the 8 h soaked sample[25].

Proximate composition

The results of the Parameters of the basic proximate composition of seeds and sprouts of broccoli are listed in Table 1.

The ash ($p<0.05$) and moisture ($p<0.01$) content were significantly higher in the broccoli sprouts compared to raw seeds. A similar result was observed[26], which also reported higher ash content (7.21 g/100 g) in broccoli sprouts than in raw seeds (4.45 g/100 g). Another study[27], found that the value of ash in the broccoli sprouts was 7.95 g 100 g-1 of dry matter, which is higher than in broccoli seeds.

The moisture content of sprouts is significantly ($p<0.01$) influenced by sprouting time, and after 120 hours, it was increased to 62.67% compared to un-sprouted seeds at 9.75%[28]. Studies also report that 96 hours of sprouting significantly increased the moisture content in mung and kabuli chickpea sprouts as compared to their seeds[29]. The increase in water uptake of a seed with time depends on the number of cells within the seed that need to be hydrated.

In the present study, we observed similar crude fibre content in both broccoli seeds and sprouts. One study reported a significant increase ($p<0.05$) in soluble and insoluble fibre in germinated 4 varieties of legumes compared to their seeds[30]. Increased fibre content may be due to newly synthesised polysaccharides during germination. In contrast, crude fibre levels in broccoli sprouts 10.35 g/100 g of dry matter are lower when compared to broccoli seeds (15.47 g/100 g of dry matter)[25].

The protein content was lower ($p<0.01$), whereas the free amino acid content was higher ($p<0.01$) in broccoli sprouts than raw seeds. A similar study reported that the content of free amino acid increased by 10-fold during germination (broccoli seeds: 0.25 g/100g; sprout: 2.33 g/100g)[31]. A very recent study also reported increased amino acid levels in germinated seeds. This is because the germination process deactivates the protease inhibitors and also increases proteolytic activity. During germination, the storage proteins break down by proteases to produce free amino acids and small peptides.

Nutritional composition

Table 2 shows the nutritional composition of broccoli seeds and sprouts.

In the present study, the levels of calcium were found to be significantly higher in broccoli sprouts as compared to broccoli seeds ($p<0.01$). The cruciferous sprouts contained higher amounts of calcium, approximately 12% more than the seeds[32]. A recent study also reported that germination significantly increases the calcium content in Quinoa. This increase may be because germination increases the phytase activity and decreases the phytic acid content[33].

Table 1 — Proximate composition of broccoli seeds and sprouts (g/ 100 g of dry matter)

Proximate composition	Broccoli seeds	Broccoli sprouts
Ash (%)	4.16±0.115	7.18±0.242*
Moisture (%)	0.8±0.062	88.78±0.149**
Crude Fibre (%)	9.65±1.15	10.73±1.53
Protein (%)	51.59±0.24	48.46±1.32**
Free amino acid (mg %)	6784.85±34.90	7761.6±34.9**

Values are expressed as Mean±SD;**$p<0.01$ and *$p<0.05$ as compared to broccoli seeds

Table 2 — Nutritional composition of broccoli seeds and sprouts (g/ 100 g of dry matter)

Nutritional composition	Broccoli seeds	Broccoli sprouts
Calcium (mg %)	17.825±2.27	39.22±3.19**
β- carotenoid (µg%)	1134.13±106.92	16082.99±2237.2**
Vitamin C (mg %)	15.875±0.59	71.53±4.21**
Methionine (mg/mL)	435.60±14.50	1882.02±14.012**
Free Amino Acids (gm%)	6784.85±34.90	7761.6±34.9**

Values are expressed as Mean±SD;**$p<0.01$ as compared to broccoli seeds

In the present study, we observed that the β- carotenoid levels in sprouted broccoli seeds were significantly higher (p<0.01) than in the raw seeds. The total carotenoid (β-carotene) concentration of broccoli sprouts was significantly higher, ranging from 118 to 221 mg per 100 g of dry weight as compared to broccoli seeds[12]. Beta-carotene, which has antioxidant capacities, is derived from lycopene by the action of lycopene beta cyclase. Germination may activate this enzyme.

In the present study, the vitamin C content in broccoli sprouts was higher (p<0.01) as compared to raw seeds. The vitamin C content of the broccoli sprouts (3.62 mg /g) was higher than that of broccoli seeds (3.39 mg /g)[34]. The vitamin C content was absent in un-sprouted seeds and significantly increased its content during the sprouting/ germination process up to 28 to 54 mg/100 g[35]. The increased vitamin C during germination is attributed to the reactivation of the enzyme involved in the conversion of L-galactono-1,4-lactone to ascorbic acid.

In the current study, we observed higher methionine content (p<0.01) in broccoli sprouts as compared to raw seeds. The methionine content of broccoli sprouts was 4.96 mg/g of dry matter, significantly higher than broccoli seeds at 4.22 mg/g of dry matter[25].

Total antioxidant and antioxidant capacity

Table 3 shows the total antioxidant and antioxidant capacity of broccoli seeds and sprouts.

In the present study, the level of total phenol (p<0.01) and total flavonoids (p<0.05) was significantly higher in broccoli sprouts compared to broccoli seeds. The present study agrees with the study done on six varieties of broccoli sprouts, which reports 1.12 to 3.58 times higher total phenol and total flavonoid levels in broccoli sprouts than in seeds after 3 days of germination[36]. Broccoli sprouts, after five and eight days of germination, contained the highest levels of total phenol and total flavonoids[4]. Another study[25] found a significant increase (p<0.05) in polyphenols and flavonoid content between seed and 3, 5, 8, and 11 days of broccoli sprouts, with the highest concentration observed in 11-day old sprouts.

DPPH assays are used to evaluate free radical scavenging properties. We observed a higher DPPH radical scavenging capacity in broccoli sprouts as compared to broccoli seeds (p<0.01). These results show a progressive increase (p<0.05) in DPPH radical scavenging capacity in broccoli sprouts (683.88 µmol TE/g) as compared to broccoli seeds (174.04 µmol TE/g). Similar behaviour of DPPH in broccoli seeds and broccoli sprouts was obtained, which quantified total phenol and total flavonoids[25]. Highest DPPH radical scavenging capacity[4] in three-day sprouts, followed by five-day and eight-day broccoli sprouts (70% methanolic extract).

The present study reports that the levels of FRAP were significantly higher in the broccoli sprouts as compared to broccoli seeds (p<0.01). Our result is supported by another study, which reported significantly higher FRAP value in broccoli sprouts compared to seeds[36]. A continuous increase in FRAP levels was observed, ranging from 5.81 to 17.09 mg trolox/g dry weight with germination time[37].

In the present study, the metal chelating activity of broccoli sprouts was significantly higher as compared to broccoli seeds (p<0.01). A study on chelating activity reported that the highest metal chelating activity after 48 hours of germination was determined with 90% ethanol (1,744 mg EDTAE/100 gm) and 90% acetone (1,145 mg EDTAE/100 g) extraction[38]. Furthermore, another study reported the chelating activity improved during germination[39].

Glucosinolate levels in broccoli seeds and sprouts

Glucosinolates are a unique and important class of thioglucosides, which are considered key components

Table 3 — Total polyphenols, total flavonoids content, DPPH radical scavenging capacity and metal chelating activity of broccoli seeds and sprouts

Parameters	Broccoli seeds	Broccoli sprouts
Total Polyphenol (mg GAE/100 gm)	246.52±4.31	505.62±10.05**
total flavonoids (mg RE/100 gm)	704.47±12.36	812.42±9.53*
DPPH activity (mg TE/100 gm)	27.63±0.357	35.70±0.335**
FRAP (mg TE/100 gm)	858.05±25.85	2520.0±46.19**
Metal chelating activity (mg EDTAE/g extract)	8.05±0.337	10.45±0.281**

Values are expressed as Mean±SD;**p<0.01 and *p<0.05 as compared to broccoli seeds

of an active chemical defence found in Brassicas. In the present study, the value of total glucosinolate is significantly higher in broccoli sprouts as compared to broccoli seeds (Seed: 12.39±0.36; Sprouts: 27.16±0.65 (µmol/g); $p<0.05$).

The total glucosinolate levels were strongly influenced by germination time, and a significant ($p<0.001$) increase in glucosinolate concentration was observed at day 3 of germination compared to seeds but decreased significantly after 12 days of germination[40]. Another study reports that the value of total glucosinolate content accounted for over 50% of the glucoraphanin in the broccoli sprouts, which was quantified in the range of 605 to 1172 mg per 100 g of fresh weight (FW)[41]. In contrast, another study showed that the value of total glucosinolate content was significantly lower in the broccoli sprouts at 496.6 (µmol/g) as compared to broccoli seeds at 884.7 (µmol/g)[21].

Phytic acid content of broccoli seeds and sprouts

In the present study, the phytic acid content was significantly higher in broccoli sprouts as compared to broccoli seeds (Seeds: 43576.6±287.8; Sprouts: 106941.75±538.05 (mg %); $p<0.01$). In contrast to our result, studies on the influence of germination on Phytic Acid Content and Phytase Activity in Quinoa[33], rice, maise, millet, sorghum and wheat seeds[42] significantly increased the phytase activity in all varieties and a decrease in phytic acid content. The dissimilarity observed in our study may be due to the application of different extraction and analytical techniques.

***In vitro* starch digestibility (IVSD) of broccoli seeds and broccoli sprouts**

The mean value for *in vitro* starch digestibility of broccoli seeds and broccoli sprouts is listed in Table 4.

Germination conditions had a significant ($p<0.01$) positive influence on the IVSD broccoli sprouts compared to seeds. The released glucose levels in broccoli sprouts at 0, 20, 120, and 180 min of digestion were significantly higher ($p<0.01$) than those in broccoli seeds. A study showed a similar result and reported that germination significantly ($p<0.05$) increased the *in vitro* starch digestibility in pigeon pea sprouts (24.50 to 39.13%; 25–35°C; 12–48 hours) compared to unsprouted pigeon peas (19.60%)[43].

***In vitro* protein digestibility of broccoli seeds and broccoli sprouts**

This is the first study which studied the effect of germination on protein digestibility in broccoli seeds. The simulated gastrointestinal *in vitro* protein digestibility of broccoli influenced by germination conditions is presented in Table 5.

It has been observed that 5 days of germination significantly increases the percentage of protein digestibility ($p<0.01$) in broccoli sprouts as compared to seeds. The free amino acid percentage was also increased in broccoli sprouts compared to raw seeds ($p<0.01$) after gastrointestinal digestion. However, the total soluble nitrogen ($p<0.05$) and protein content ($p<0.01$) were found to be significantly lower in broccoli sprouts compared to raw seeds after gastrointestinal digestion.

Table 4 — *In vitro* starch digestibility of broccoli seeds and broccoli sprouts at 0, 20, 120 and 180 minutes

Time (minutes)	Broccoli seeds (mg %)	Broccoli sprouts (mg %)
0	38.25±0.21	42.95±0.69**
20	46.73±0.77	50.60±0.74**
120	55.09±0.88	67.65±0.90**
180	66.78±0.61	115.77±0.91**

Values are expressed as Mean±SD;**$p<0.01$ and *$p<0.05$ as compared to broccoli seeds

Table 5 — Total soluble nitrogen and protein percentage content in broccoli seeds and broccoli sprouts

Digestion phases	Nitrogen (%)		Protein (%)		Free amino acid (gm %)		*In vitro* protein digestibility (%)	
	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts	Seeds	Sprouts
Before digestion	8.76±0.41	07.72±0.29**	51.59±0.24	48.46±1.32**	511.56±0.71	812.82±1.13**	-	-
Gastric phase	5.58±0.16	3.36±0.07**	34.76±0.29	20.91±0.53**	2667.71±2.29	4116.7±8.03**	32.61±0.81	56.80±2.01**
Intestinal phase	3.32±0.02	1.26±0.02*	20.74±0.63	8.06±0.08**	4430.9±7.89	5272.73±2.76**	61.71±4.59	83.35±0.37**

Values are expressed as Mean±SD;**$p<0.01$ and *$p<0.05$ as compared to broccoli seeds

Table 6 — Antibacterial activity of broccoli seeds and sprouts

Microorganism	Diameter of the Inhibition Zones (mm)	
	Broccoli seeds	Broccoli sprouts
Gram-positive		
Staphylococcus aureus	5.9±0.03	5.61±0.06
Bacillus cereus	12.22±0.09	11±0.05
Gram-negative		
Escherichia coli	10.08±0.09	15.13±0.10*
Salmonella typhimurium	6.41±0.01	13.46±0.17*

Values are expressed as Mean±SD;*p<0.05 as compared to broccoli seeds

A very recent review article demonstrated that germination causes various changes in the protein characteristics of legume seeds. These include the breakdown of storage protein into smaller peptides and free amino acids. Thereby, germination increases protein digestibility, protease activity, smaller peptides and free amino acids. Germination decreases soluble protein content and protease inhibitors. This can explain the higher protein digestibility, higher free amino acids and lower protein levels in broccoli sprouts compared to seeds[44].

α-glucosidase and α-amylase inhibitory activity

α-glucosidase is a hydrolysing enzyme that catalyses the conversion of disaccharides and oligosaccharides to absorbable sugars, resulting in increased blood glucose levels. Therefore, inhibition of this enzyme is important in controlling diabetes. In the present study, percentage (%) inhibition of α-glucosidase activity by the broccoli sprouts was found to be significantly higher than that in broccoli seeds (Seeds: 54.06±3.36%; Sprouts: 62.2±1.71%; p<0.05). Similar observation reports that germinating broccoli seeds led to increased α-glucosidase inhibition by 16% (Seed: 75.33±3.11%; Sprout: 91.32±2.43%)[9]. The increase in α-glucosidase inhibitory activity may be due to increased levels of flavonoids in broccoli sprouts, which act as competitive inhibitors for α-glucosidase. In addition, the higher glucosinolate and phenolic compounds in sprouts also may inhibit alpha-glucosidase enzymes through the activation of the Nrf2 pathway[45].

In the present study, percentage (%) inhibition of α-amylase activity by the broccoli sprouts was found to be significantly lower compared to broccoli seeds (Seeds: 90.25±0.33%; Sprouts: 76.05±1.64%; p<0.01). The highest activity of α-amylase inhibitors was found in raw lentil seed (161.27 αAI/g f.m.), and the activity decreased after 2 days of germination (24.49 αAI/g f.m.)[46].

Antibacterial activity of broccoli seeds and sprouts

The results of the antibacterial activities of broccoli seeds and sprouts are presented in Table 6. The antibacterial activities were assessed by the presence or absence of inhibition zone diameters (IZD). Broccoli sprouts showed significant antibacterial activities against the tested Gram-negative bacteria (p<0.05) compared to broccoli seeds. No significant difference in antibacterial activities against the tested Gram-positive bacteria was observed between seeds and sprouts.

A similar study reported that significant antibacterial activities of broccoli sprouts extract were observed against both Gram-positive (IZD: 17.84–21.03 mm) and Gram-negative bacteria (IZD: 18.36–26.44 mm)[4]. The presence of bioactive compounds, such as phenolics, flavonoids, and glucosinolates, can inhibit the growth and activity of various microorganisms by destabilising the plasma membrane or inhibiting the extracellular enzymes.

Conclusion

The current investigation provides novel insights into the potential consequences of the germination of broccoli seeds on antioxidant activity, antidiabetic activity, antimicrobial capacity, *in vitro* nutrient digestibility, antinutritional factors, and nutritional composition. The analysis of proximate and other nutrient composition revealed that the broccoli sprouts are a better source of crude fibre, free amino acids, methionine, calcium, beta-carotene, and vitamin C than the seeds. Germination of broccoli seeds for five days significantly improved the levels of total polyphenol and flavonoid. The improved DPPH, FRAP, and metal chelating activity revealed that the broccoli sprout extract is more potent in reducing free radical damage, thereby causing degenerative diseases. The analysis of the antidiabetic potential of the extracts indicated that sprout extracts were more effective as α-glucosidase inhibitors than the raw seeds. Broccoli seed extract demonstrated significant antibacterial activity against tested foodborne bacteria. The germination process in broccoli seeds also improved nutrient digestibility. Therefore, based on the above findings, broccoli sprouts could be utilised as a functional food source

with improved nutritional composition, better nutrient digestibility, and antidiabetic and antibacterial activity.

Conflict of interest statement

The authors declare that they have no conflict of interest to disclose.

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