Original Article

Characterization of nutritional content and *in vitro* - antioxidant properties of *Plantago ovata* seeds

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Abstract

Introduction: The present investigation was carried out to evaluate *Plantago ovata* seeds for its nutrients including water- and fat-soluble vitamins, minerals, oligosaccharides, free sugars, fatty acid profile, polyphenols, and *in vitro*-antioxidant properties.

Materials and Methods: The vitamins, sugar profile, and oligosaccharides were analyzed by high-performance liquid chromatography, and the fatty acid profile was evaluated by gas chromatography coupled with flame-ionization detector. Phenolic components and antioxidant activity were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), metal chelating activity, and reducing power assays.

Results: The results revealed that *P. ovata* seed flour is the rich source of protein (17.70%) and dietary fiber (24.77%). Essential minerals including Fe, Cu, Mn, Zn, and K, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, α-tocotrienol, and δ-tocotrienol were detected in varying concentrations. Total phenolic content and flavonoid content were found to be 8.72 mg GAE/g and 2.11 mg CE/g, respectively. Antioxidant property analyzed by different methods was reported as DPPH radical scavenging activity (67.9%), ABTS scavenging activity (65.89%), FRAP assay (1.68 μmol Fe (II) equiv/g), metal chelating activity (63.20%), and reducing power (78.40 μmol AAE/g).

Conclusion: There are no available reports on the vitamin composition of *Psyllium* seeds. *Psyllium* seed is rich in nutrients and biological active compounds that may be utilized in the development of nutraceutical or functional foods.

Keywords: Antioxidant properties, carbohydrate, fatty acid, minerals, polyphenols, *Psyllium* seeds, vitamins

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INTRODUCTION

Demand of functional food is rising across the globe due to significant rise in noncommunicable diseases including cardiovascular disease (CVD), cancer, diabetes, and hypertension. Therefore, novel functional ingredients are being tested for their potential and suitability to serve in the development of functional foods. In this connection, *Psyllium* (*Plantago ovata*) seed, which is rich in soluble and insoluble fiber,[1] macronutrients, and micronutrients, is expected to have high levels of phytochemicals and considered as a health-promoting agent which may be utilized as functional food ingredient during food processing.

Psyllium belongs to genus *Plantago* comprising more than 200 species. Among them, *P. ovata* is known for its versatile uses which

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is commonly cultivated[2] due to its high yielding capacity and elevated levels of husk. *Psyllium* seed serves as a potential source of bioactive compounds, including polyphenols and flavonoids, which have immense health benefits.[3] These polyphenols are considered to be very effective to cure different types of neurodegenerative diseases and CVDs.[4] Apart from polyphenols and phytonutrients, *Psyllium* polysaccharide is well known for its health benefits which also has antioxidant and gel-forming properties due to high levels of branched acidic arabinoxylan.[5] The consumption of dietary fiber has been shown to have inverse association with type-2 diabetes, cancer, and CVD.[6]

Psyllium husk has been predominantly used in several food products and was studied for its usage as food supplements and in bakery products.[1,7] However, flour from *Psyllium* seeds has not been explored for its nutraceutical potential in any food product probably due to lack of appropriate information of its nutritional and nutraceutical potential. However, few reports are available on the characterization of *Psyllium* husk. Therefore, the present research was carried out to study the carbohydrate composition, fatty acid profile, vitamin composition, polyphenolic compounds, and antioxidant properties of whole seeds of *Psyllium* in a comprehensive manner.

MATERIALS AND METHODS

Materials

Whole *Psyllium* seeds were purchased from the local market in Hyderabad, Telangana, India. Sample was milled to fine powder (particle size <250 μm) and stored in an air-tight container for further use. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ), ferrozine, and catechin were procured from Sigma-Aldrich Co. St. Louis, USA. All other chemicals used were of analytical grade, and each test was performed in triplicates.

Determination of proximate composition and color characteristics

Sample was evaluated for protein, fat, dietary fiber (total, soluble, and insoluble), ash, and total carbohydrate contents using the methods.[8] The color measurement (CIE color system, L*, a*, b*) was carried out using a Hunter Colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA). The L* value indicates the lightness, 0–100 representing dark to light. The a* value gives the degree of the red-green color, with a higher positive a* value indicating more redness. The b* value indicates the degree of yellow-blue color, with higher positive b* value indicating more yellow.

Vitamins analysis

Analysis of water-soluble vitamins was carried out using high-performance liquid chromatography (HPLC, Dionex Ultimate 3000 RSLC, USA) and C18 column (250 mm × 4.6 mm, 3 μm) (Hypersil™, BDS, Thermo Fischer Scientific) which was set at 40°C for riboflavin, niacin, and pantothenic acid and 35°C for Vitamin B6. Fluorescence detector was used for riboflavin, and niacin, whereas photodiode array (PDA) detector was used for pantothenic acid and total folates analysis.[9]

Tocopherols and tocotrienols were analyzed according to the previously reported method.[9] Extraction was carried out after saponification in the presence of alcoholic potassium hydroxide using n-hexane. Analysis was done on normal-phase HPLC system (Dionex Ultimate 3000 RSLC, USA) using C18 column (100 mm × 4.6 mm, 3 μm) (Spherisorb, Waters) maintained at 25°C and PDA detector.

Caroteniods were analyzed according to the method previously explained.[9]

Fatty acid profiling

Fatty acids in the sample were analyzed as fatty acid methyl ester (FAME) by gas chromatography (GC) coupled with flame-ionization detector (FID) (Agilent Series, 7890 Series, USA).[10]

Carbohydrate profiling

Monosaccharides and oligosaccharides profile was analyzed by previously reported method[11] using the HPLC system (Dionex Ultimate 3000 RSLC, USA) with Supelcosil LC-NH_2 (25 cm × 4.6 mm, 5 μm) column and ELSD detector.

Determination of mineral composition

Mineral elements were evaluated after wet digestion method previously reported.[9]

Total phenolic content

Total phenolic content (TPC) was determined by folin–Ciocalteu reagent method.[12] The results were reported as milligram gallic acid equivalents per gram (mg GAE/g) of the sample.

Total flavonoid content

Total flavonoid content (TFC) was determined by aluminum chloride colorimetric method.[12] The results were reported as milligram catechin equivalents per gram (mg CE/g) of the sample.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl-radical scavenging activity

Antioxidant activity was measured using a modified version of the reported method.[13] Sample (100 mg) was extracted with 1 ml of methanol and reacted with 3.9 ml of a 6×10^{-5} mol/L of DPPH solution. Absorbance (Abs) at 515 nm was read at 0 and 30 min.

$$\text{Antioxidant activity (\%)} = (1 - [\text{Abs}_{\text{sample, t}} = 30/\text{Abs}_{\text{control, t}} = 0]) \times 100$$

Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid$^{\bullet+}$-radical scavenging assay

Free radical scavenging activity of the sample was determined by $ABTS^{\bullet+}$ radical cation decolorization assay.[14] 100 μl of the sample extract was mixed with 3.8 mL $ABTS^{\bullet+}$ working solution; sample extracts were diluted if required. The absorbance of the mixture was measured at 734 nm after 6 min of incubation (SPECORD S600, Analytik Jena, Germany) at room temperature, and the percent of inhibition was calculated using the formula.

$$ABTS^{\bullet+} \text{ scavenging effect (\%)} = ([AB - AA]/AB) \times 100,$$

where AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard.

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was carried out based on the procedure described in the literature.[15] The absorbance of the mixture was measured at 593 nm after 4 min (SPECORD S600, Analytik Jena, Germany). The standard curve was constructed using $FeSO_4$ solution, and the results were expressed as μmol Fe (II)/g.

Metal chelating (Fe^{+2}) activity

Metal chelating activity was carried out as discussed by the method.[13] The chelating activity of the extract for Fe^{+2} was calculated as follows:

Iron (Fe^{+2}) chelating activity (%) = (1 – [absorbance of sample at 562 nm/absorbance of control at 562 nm]) ×100.

Reducing power

Reducing power analysis was carried out by the method previously reported.[13] The absorbance of the mixture was measured at 700 nm using spectrophotometer (SPECORD S600, Analytik Jena, Germany). The results were reported as micromole ascorbic acid equivalents per gram (μmol AAE/g) of the sample.

RESULTS AND DISCUSSION

Proximate composition and color characteristics

Protein, crude fat, and ash content were found to be 17.7, 3.75, and 1.00 g/100 g, respectively [Table 1]. Total dietary fiber (TDF) and insoluble dietary fiber were recorded with 24.77 and 19.56 g/100 g, respectively, while 21% of TDF was observed as soluble dietary fiber (SDF). Similar to the present investigation, Romero-Baranzini *et al*.[16] reported protein content (17%), dietary fiber (25%), and total carbohydrates (49%) in *Psyllium* seeds, however higher content of ash (3%) and fat (6.7%). On the contrary to the present investigation, another study reported high content carbohydrate (87.4%) and lower content of protein (2.93%) and fat (0.3%) similar dietary fiber content.[17] The variability in the results may be attributed to the difference in varieties, environmental factors, and soil composition where they were grown.

Color analysis of the *P. ovata* seeds revealed that the lightness value (L*) was found to be 69, whereas a* (redness) and b* (yellowness) values were found to be 5.4 and 14.56, respectively. There are no previous reported data on the color characterization of *P. ovata* seeds; however, a study on the cookies prepared with the incorporation of *Psyllium* husk up to 12% level has been reported for L* a* b* value of up to 63, 7.5, 19, respectively.[18]

Vitamins content

Among the water-soluble vitamins analyzed [Table 1], niacin (4.18 mg/100 g) was found to be the highest followed by pantothenic acid (0.87 mg/100 g), Vitamin B6 (0.25 mg/100 g), riboflavin (0.13 mg/100 g), and total folates (32.19 μg/100 g). Among the isomers of the Vitamin-E analyzed, α-tocotrienol and δ-tocotrienol content were found to be 6.0 and 5.0 μg/100 g, respectively, and the total carotenoids content was found to be 183.86 μg/100 mg, whereas the carotenoids such as zeaxanthin, lutein, and lycopene were detected in trace amounts. To the best of our authors' knowledge, there are no previously reported data on vitamin composition of *P. ovata* whole seeds. However, Longvah *et al*.[19] reported riboflavin, niacin, pantothenic acid, and total folates content of 0.14, 1.19, 0.27 and 51.11 mg/100 g in fenugreek seeds.

Fatty acids content

The total monounsaturated fatty acids content was 38.43% FAME with the predominance of oleic acid (38.09%) followed by eicosenoic acid (0.34%). Total polyunsaturated fatty acid content was 45.92% which comprised linoleic acid (42.22%) followed by linolenic acid (3.69%). The total saturated fatty acid content was found to be 15.65% with the highest proportion of palmitic acid (11.76%) followed by stearic acid (3.56%) and arachidonic acid (0.33%) [Figure 1]. Patel *et al*.[20] reported fatty acid profile of *Psyllium* seed, wherein the range of fatty acids (C14–C24) was reported with predominance of linoleic acid (64%), together with alpha-linolenic acid (12%), *cis*-11-eicosenoic acid (7%), palmitic acid (6.5%), and stearic acid (6%). Romero-Baranzini *et al*.[16] have reported similar fatty acid profile for *Psyllium* seed sample, except for linolenic acid which was reported with slightly higher content (6.9%) in comparison with the present investigation.

Carbohydrate composition

Psyllium (*P. ovata*) polysaccharide contains a backbone chain of β-(1 → 4)-linked D-xylopyranosyl residues. Side chains (trisaccharide branches) are commonly present at position 3 (however, sometime, a xylopyranosyl side chain is observed at position 2). The trisaccharide branches comprised of sequence L-Ara*f*-α-(1 → 3)-D-Xyl*p*-β-(1 → 3)-L-Ara*f*.[21] Analysis of carbohydrate profile in *P. ovata* seeds is depicted in Table 2. The monosaccharides and oligosaccharides analyzed were glucose, fructose, sucrose, raffinose, stachyose, and verbascose, respectively.

The total of the free sugars and oligosaccharide content was found to be 1.036 g/100 g. Among the free sugars, glucose content was found to be the highest with 0.189 g/100 g followed by sucrose (0.132 g/100 g) and fructose (0.087 g/100 g). However, among the oligosaccharides, only raffinose was detected with 0.628 g/100 g, and stachyose and verbascose were not detected. Several studies have a significant difference in the monosaccharide composition of polysaccharides extracted from leaves or seeds of *Psyllium*.[22]

Mineral composition

Mineral composition of *P. ovata* seeds is given in Table 3. Among the minerals analyzed, potassium was found to be the highest with 687 mg/100 g and the selenium was the least detected element with 18 μg/100 g. Iron, zinc, copper, and manganese content were found to be 6.75, 3.15, 2.39 and 1.06 mg/100 g, respectively. In a similar study by Bukhsh *et al*.[23] reported Fe, Zn, Cu, Mn, and K of 21.7, 99.4, 59, 6.0, and 1000 μg/g, respectively, whereas Ghani *et al*.[24] reported mineral composition of *Psyllium* seeds as mg/kg for Fe (0.175), Zn (0.063), Cu (0.021), Mn (0.231), and K (0.105). However, Guo *et al*.[5] reported potassium content of 8 mg/g in *Psyllium* husk which was almost similar to the present study. The variation in minerals content of *Psyllium* seeds was probably due to the difference in growing conditions and genotypic differences. Heavy metals analyzed in the study, *viz*., lithium, cobalt, arsenic, and molybdenum, were found to be 12.65, 31.2, 22.53, and 27.14 μg/100 g, respectively. Evaluation of heavy metals is very important in the medicinal plants and plant

foods since these plants are mostly used for drug formulation and/or consumed directly to treat some diseases.

Total phenolic content, total flavonoid content, and antioxidant properties

TPC and TFC of *Psyllium* seeds analyzed were found to be 8.72 mg/g GAE and 2.11 mg/g CE, respectively [Table 4]. The antioxidant activity in the seeds of *P. ovata* was evaluated using various methods such as DPPH radical scavenging activity assay, ABTS radical scavenging activity assay, FRAP antioxidant activity, metal chelating activity, and reducing power activity.

DPPH radical scavenging activity of the *Psyllium* seed was found to be 67.9%. Patel *et al*.[20] reported for the DPPH scavenging activity of the *Psyllium* plant wherein they have reported maximum activity in the seed extract, followed by leaf and husk extracts. ABTS antioxidant activity is expressed as the percent inhibition of ABTS+, and it was found to be 65.89% for *Psyllium* seeds. Patel *et al*.[20] also reported the similar results in *Psyllium*, wherein the highest ABTS activity was observed in the seed extract, followed by leaf and husk extracts; it was found that 150 μg seed extract showed 94% inhibition, whereas the same amount of inhibition was observed with 200 μg leaf extract followed by husk extract which had 90% inhibition with 300 μg extract. In another study on *P. ovata*, the percent inhibition of ABTS free radicals for polysaccharides extracted from seed fractions was reported, which ranged between 59.95% and 65.63%, Similarly, they observed the percent inhibition in husk fractions ranging from 50.62% to 66.44%.[22] The FRAP activity was found to be 1.68 μmol equivalent $FeSO_4$/g. There are no previous reported available on the FRAP activity of *Psyllium* seeds. The reducing power of a compound is directly correlated to the antioxidant activity. The metal chelating activity and reducing power of *Psyllium* seeds were found to be 63.20% and 78.4 μmol AAE/g, respectively. It has been speculated that polyphenols and flavonoids present in the *Psyllium* seeds has antioxidant potential due to its electron donor ability.[25]

Table 1: Proximate composition, color characteristic, and vitamins in *Plantago ovata* seed

Parameter	Content	Unit
Moisture	1.91±0.25	g/100 g
Total carbohydrates	50.87±1.94	g/100 g
Protein	17.70±0.78	g/100 g
Total fat	3.75±0.42	g/100 g
Ash	1.00±0.16	g/100 g
Total dietary fiber	24.77±0.30	g/100 g
(i) Insoluble fiber	19.56±0.61	g/100 g
(ii) Soluble fiber	5.18±0.95	g/100 g
Riboflavin	0.13±0.40	mg/100 g
Niacin	4.18±0.37	mg/100 g
Pantothenic acid	0.87±0.87	mg/100 g
Total vitamin B6	0.25±0.90	mg/100 g
Total folates	32.19±0.70	μg/100g
α-Tocotrienol	6.00±0.002	μg/100 g
δ-Tocotrienol	5.00 ±0.003	μg/100 g
Total carotenoids	183.86±2.40	μg/100 mg
Color characteristics		
Lightness	69.00±5.3	L*
Redness	5.40±0.9	a*
Yellowness	14.56±3.2	b*

CONCLUSION

The present investigation, *Psyllium* seeds, was evaluated for their comprehensive nutritional profile and antioxidant potential. *Psyllium* seeds were found to be a good source of antioxidants which was confirmed by the higher DPPH and ABTS radical scavenging activity. The higher content of polyphenols including flavonoids could be responsible for the higher antioxidant potential of *Psyllium* seeds. The vital micronutrients including Fe, Zn, niacin, and total folate were also found in elevated level which may be helpful to improve the health status of the population by incorporating the *Psyllium* seeds in the food products. Besides, *Psyllium* seeds are good source of SDF which is known for its numerous health benefits. The nutritional profile of the *Psyllium* seeds suggested its wide application as food ingredient to improve the dietary fiber content and antioxidant potential and may be helpful in the development of novel functional and nutraceutical foods.

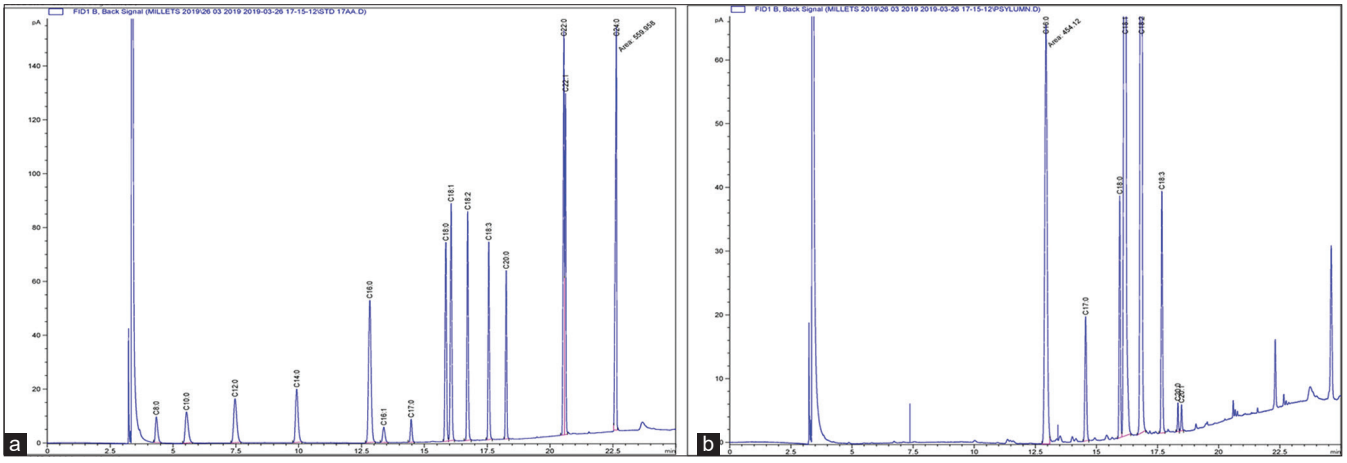

Figure 1: Fatty acids profile analysis by gas chromatography. (a) Gas chromatogram of fatty acids standards profile; C8:0 (caprylic acid), C10:0 (capric acid), C12:0 (lauric acid), C14:0 (myristic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C17:0 (heptadecanoic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (α-linolenic acid), C20:0 (arachidic acid), C22:0 (behenic acid), C22:1 (erucic acid), C24:0 (lignoceric acid). (b) Gas chromatogram of fatty acids profile of *Psyllium* seeds

Table 2: Free sugars and oligosaccharides composition in *Plantago ovata* seed

Carbohydrate composition	Content	Unit
Fructose	0.087±0.021	g/100 g
Glucose	0.189±0.561	g/100 g
Sucrose	0.132±0.004	g/100 g
Raffinose	0.628±0.051	g/100 g
Stachyose	ND	
Verbascose	ND	

ND: Not detected

Table 3: Mineral content in *Plantago ovata* seed

Minerals	Content
Iron	6.75±0.9 mg/100 g
Manganese	1.06±1.5 mg/100 g
Zinc	3.15±2.8 mg/100 g
Copper	2.39±1.3 mg/100 g
Potassium	687±5.1 mg/100 g
Lithium	12.65±1.6 μg/100 g
Cobalt	31.20±1.2 μg/100 g
Arsenic	22.53±0.6 μg/100 g
Selenium	18.06±1.6 μg/100 g
Molybdenum	27.14±3.4 μg/100 g

ND: Not detected

Table 4: Total phenolic content, flavonoid content and antioxidant properties of *Plantago ovata* seed

Parameter	Content
Total phenolic content	8.72±0.17 mg GAE/g
Total flavonoid content	2.11±1.50 mg CE/g
DPPH radical scavenging activity	67.90±2.90%
ABTS radical scavenging activity	65.89±1.01%
Metal chelating activity	63.20±0.60%
FRAP assay	1.68±5.60 μmol Fe(II) eq/g
Reducing power	78.40±1.40 μmol AAE/g

*GAE: Gallic acid equivalent, CE: Catechin equivalent, AAE: Ascorbic acid equivalent; eq: Equivalent, FRAP: Ferric reducing antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power

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Conflicts of interest

There are no conflicts of interest.

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