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RESEARCH ARTICLES

Nutritional composition, *in vitro* prebiotic potential, and antimicrobial efficacy of Wheatgrass (*Triticum aestivum*)

Renu Tripathi[1] · Neetu Mishra[2] · Dhananjay Pandey[3] · Kamal Shah[4] · Nagendra Singh Chauhan[5]

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Abstract

The current research prospect deals with the *in-vitro* nutritional analysis, prebiotic potency, antimicrobial activity and HPLC of the wheatgrass extract.The antimicrobial activity was assessed by agar well diffusion method. The proximate analysis revealed the presence of carbohydrates, protein, fiber, fat moisture and ash along with calcium, phosphorous and iron in the wheatgrass powder. Furthermore, the prebiotic potential of wheatgrass fiber was assessed against the probiotic strains viz., *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* procured from MTCC, Chandigarh, India. The results revealed that, maximum growth of *L. acidophilus* was recorded in MRS broth containing 0.5% (w/v) wheatgrass fiber, whereas the maximum growth of *L. rhamnosus* was observed in MRS broth containing 0.25% (w/v) wheatgrass fiber.Successively, the wheatgrass ethanolic extract at different concentrations was evaluated for their antibacterial activity for Gram-positive bacteria viz., *Bacillus cereus*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, Gram-negative bacteria as *Salmonella typhi* and fungi as *Aspergillus brasiliensis*. The findings showed that, *S. pneumoniae* exhibited maximum zone of inhibition (21.31 ± 0.74 mm) against 20 µg/ml and *S. aureus* showed the highest zone of inhibition (20.33 ± 0.42 mm) at 20 µg/mlconcentration of wheatgrass ethanolic extract. Finally, the HPLC of the extract revealed the presence of abscisic acid, chlorophyll a, chlorophyll b, chlorophyllin, apigenin, and rutin conferring prebiotic potency and antimicrobial activity to wheatgrass. However, further efforts to explore several pharmacological potentialities of wheatgrass *in-vivo* will be the future direction of this research.

Keywords Nutritional analysis · Prebiotic · Antimicrobial · HPLC · Bioactives · Wheatgrass

Introduction

Microgreens are the natural boon to mankind, owing to their tremendous health benefits. Over the past decades, microgreen have emerged as a topic of global concern making a noteworthy impact on human health as therapeutics (Mishra et al. 2025).Wheat is the world's largest common edible cereal crop from the family Poaceae (Fig. 1). Young seedlings of wheat are also known as the wheatgrass (Shakya et al. 2014).Wheatgrass is a promising microgreen also called as the powerhouse of varied bioactive compounds conferring health promoting benefits for the human body (Hattarki and Bogar 2017; Tullo and Abera 2023).Wheatgrass is endowed with enormous proteins, total dietary fiber, carbohydrates, vitamins A, C, E, niacin, riboflavin, and folic acid along with several minerals like iron, calcium, phosphorous, magnesium, selenium (Devi et al. 2019). Wheatgrass possesses several pharmacological properties viz., anti-aging, anti-cancer, anti-ulcer, anti-diabetic,

✉ Neetu Mishra
neetum1976@gmail.com

1 Department of Home Science, Govt. Kamladevi Rathi Mahila P.G. Mahavidyalaya, Rajnandgaon, Chhattisgarh 491441, India

2 Department of Family and Community Sciences, University of Allahabad, Prayagraj, Uttar Pradesh 211002, India

3 Department of Botany, Govt. Naveen Girls College, Surajpur, Chhattisgarh 497229, India

4 Institute of Pharmaceutical Research, GLA University, Mathura 281406, India

5 Drugs Testing Laboratory Avam Anusandhan Kendra, Raipur, Chhattisgarh 492010, India

Fig. 1 Pictorial presentation of wheatgrass

anti-allergic, anti-microbial, antioxidant, anti-inflammatory, anti-arthritic and prebiotic activity (Choudhary et al. 2021; Minocha et al. 2022). The therapeutic activity of wheatgrass is conferred by the presence of its bioactives(Khan et al. 2015; Thakur et al. 2020). The wheatgrass is a magnificent source of prebiotics which are mostly fibers that are non-digestible food ingredients and beneficially affect the host's health by selectively stimulating the growth and activity of some genera of microorganisms in the colon (Dwivedi et al. 2014). Moreover, wheatgrass is an excellent source of polyphenols, flavonoids, and tannins which confer antimicrobial efficacy to wheatgrass (Pehlivanogluet al. 2015). The literature review reveals that very confined scientific investigation and research studies have been documented on the pharmacological efficacy of wheatgrass, so furtherefforts to conduct extensive research on the efficacy of wheatgrass is the need of the hour.

Materials and methods

Cultivation of wheatgrass

The experiment entailed growing wheatgrass indoors. Wheat grains (Sharbati Cultivar) were procured from the local market in Prayagraj, India. The wheat grains after soaking for 12 h in waterwere strained and knotted in wet cloth and kept for sprouting which were spreaded over the soil. A small amount of water was sprinkled and 3–4 h of indirect sunlight were permitted each day to promote grass growth and prevent excessive nutrient loss from direct sunlight exposure. The sown grains began to develop, and the grass was harvested after 7–9 days. At this point, wheatgrass is at its nutritious peak (Tripathi et al. 2017).

Nutritional analysis

Drying at 105 °C of wheatgrass powder was employed to determine the moisture level. The protein levels were determined by using micro Kjeldhal method. The fat was estimated by the soxhlet apparatus. Fiber was estimated by the acid-alkaline digestion. Iron level was determined at 480 nm by UV-visible spectrophotometer (Shimadzu, UV-160 A model). The carbohydrate and ash were estimated by AOAC, (2005). The phosphorous content was determined at 650 nm in a UV-visible spectrophotometer. The calcium content was estimated by Ranganna (2005). The amount of nutrients was expressed as mg/100 g.

In-vitro screening of prebiotic potential

The growth of probiotic bacteria was investigated utilizing wheatgrass fiber as a prebiotic supplement. To extract fiber, the aqueous solution of wheatgrass powder (1:100 w/v) was heated for 1 h at 100 °C with continuous stirring. The 80% ethanol (1:1 v/v) was used for precipitation at room temperature for 24 h. Centrifugation was employed to separate precipitate. The precipitate fiber was washed with distilled water, and then again with 80% ethanol. Finally, the extracted fiber was lyophilized and used in further experiments (Modhukumar and Muralikrisma 2010).

The prebiotic potential was assessed by adding wheatgrass fiber to MRS broth at various concentrations such as 0.1%, 0.25%, and 0.5% w/v. The probiotic strains are selected based on their beneficial impact on human health, which included *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* were procured from MTCC, Chandigarh, India and were inoculated to the MRS broth. Commercial inulin (Sigma-Aldrich Chemicals Pvt. Ltd., St. Louis, Missouri, USA) was utilized as a positive control, and MRS broth served as a negative control. The samples were incubated for 24 h at 37 °C under anaerobic conditions. Samples were obtained after 24 h to estimate probiotic growth. The prebiotic potency of wheatgrass was determined by the growth of probiotics measured as absorbance (Abs) by spectrophotometer at 600 nm.(Silva et al. 2007; Macedo et al. 2008; Olson and Aryana 2012; Teixeira et al. 2016).

Table 1 Specification for HPLC analysis

HPLC instrument	Systronics
Column	HiQ Sil C18-HS
Pump	Isocratic type
Column size	4.6 mm × 250 mm × 5 µm
Injection volume	20 µl
Run time	20 min
Concentration of extract	10 µl/ml (dissolved in ethanol)
Column temperature	25 °C
Determinant	Chlorophyllin, Chlorophyll a, Chlorophyll b, Rutin, Apigenin, Abscisic acid

Table 2 Mobile phase, flow rate and wavelength of standards used in HPLC analysis

Standards	Mobile phase	Flow rate (ml/min)	Wavelength (nm)
Chlorophyllin	Methanol	1.0	423
Chlorophyll a	Acetonitrile: water (50:50)	1.5	438
Chlorophyll b	Acetonitrile 2%v/v acetic acid (40:60)	1.3	436
Rutin	Methanol −0.4% H_3PO_4	1.0	220
Apigenin	Methanol: acetonitrile	1.0	337
Abscisic acid	Methanol: DW (50 :50)	1.0	254

Assessment of anti-microbial activity

Wheatgrass extract was tested for its antimicrobial efficacy against pathogenic microorganisms comprising of Gram-positive bacteria viz., *Bacillus cereus*, *Streptococcus pneumoniae*,and *Staphylococcus aureus*, one Gram-negative bacteria as *Salmonella typhi*, and one fungi as *Aspergillus brasiliensis*. The strains were procured from MTCC, Chandigarh, India. The antimicrobial efficacy was assessed with ethanolic extract of wheatgrass powder. Wheatgrass powder was prepared by the heat treatment of fresh leaves and then cooling them.After drying at 40 °C for 24 h, the leaves were grinded (3/8 mesh) and stored at 4 °C (Durairaj et al. 2014). Ratio of wheatgrass powder to ethanol was 1:10 (Merck). The resulting suspension was vigorously shaken for 10 min before centrifugation at 4000 rpm (RV10; Staufen, Germany). The suspension was filtered and dried in a water bath at 40 °C. Stock solution of dried extract and ethanol was prepared at concentrations of 10 µg/ml, 15 µg/ml, and 20 µg/ml. The resultant extracts were then stored at 5 °C.

Antimicrobial efficacy was performed using the method of agar well diffusion. Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) was prepared for bacteria and fungi respectively which was then autoclaved before pouring 25 ml of each into a sterile petri plate. The microbial broth (100 µl) was spreaded over the solidified petri plate. The cork borer was used to puncture the wells on the petri plates, and 20 µl of wheatgrass ethanol extract solutions (10 µg, 15 µg, and 20 µg) were poured into each well. The positive control for bacteria was 1000 ppm oxy-tetracycline, while for fungi it was 5000 ppm terbinafine. Whereas, the ethanol was used as a negative control in one well. All petri plate comprised of five wells: one for the positive control, one for the negative control, and three for the various wheatgrass extract concentrations. The plates were left for 15–20 min in laminar air flow (LAF) followed by sealing and incubating at 37 °C for 12–14 h for bacteria and 27 °C for 24–48 h for fungi. After incubation, the zone of inhibition, were measured in mm (Parihar and Bohar 2006).

Identification of bioactive compounds of wheatgrass by HPLC

Wheatgrass extract's bioactive compounds were analyzed using reversed phase (C18) high-performance liquid chromatography (Systronics), C-18 column (HiQ Sil C18-HS 4.6 mm × 250 mm × 5 µm). The HPLC system has a manual sample injection valve, a 20 µl loop, and a column thermostat (Table 1). Wheatgrass ethanol extract was prepared using standard solutions and filtered by 0.22 µm Milli-pore (Billerica, USA).

Mobile phase, flow rate, and wavelength were all set to standard (Table 2). 20 µl of sample was injected into the column. The bioactive compounds present in wheatgrass extract was estimated by comparing the peak retention time and area of the wheatgrass extract chromatographic peak with that of standard solutions.

Statistical analysis

The statistical analysis by One-way ANOVA with Tukey's multiple comparison test was performed. All the values are indicated as mean ± SD and all the P-values < 0.05 were statistically significant.

Results and discussion

Nutritional analysis of wheatgrass powder

The nutritional analysis of wheatgrass powder was evaluated for moisture, protein, fiber, fat, ash, carbohydrate, calcium, iron and phosphorousas shown in Table 3. Moisture content is one of the most essential characteristics that affectsshelf life of foods and save them from microbial attack, particularly when dried. Wheatgrass powder was found to have a moisture content of 6.78 g/100 g. In the food sector, the

Table 3 Nutritional analysis of wheatgrass powder

Parameters	Nutritive values
Moisture	6.78 g/100 g
Protein	28.23 g/100 g
Fat	0.65 g/100 g
Ash	5.5 g/100 g
Fiber	23.94 g/100 g
Carbohydrate	41.85 g/100 g
Calcium	188.4 mg/100 g
Phosphorous	46.74 mg/100 g
Iron	30.96 mg/100 g

moisture level of dried flours was anticipated as 3% to 10% (Li et al. 2015). Above findings suggested that the moisture content of wheatgrass powder was within the specified range. Wheatgrass has a protein content of 28.23 g/100 g. Our findings are corroborated with the comparable data of 27.26% protein in wheatgrass reported by Qamar et al. (2012) and Jain and Jain (2014), reported 25.5% protein in wheatgrass powder. Chaturvedi et al. (2013) reported 20.2 g protein. Ghumman et al. (2017) reported a somewhat lower protein content ranging from 22.01 to 25.77%. Wheatgrass has a fat content of 0.65 g/100 g. On the other hand, wheatgrass powder possessed a higher amount of fat. Jain and Jain (2014) reported 0.90 g fat per 100 g, which was slightly higher than the findings of this study.

Ash content indicated total minerals present in wheatgrass. Present analysis revealed a 5.5% ash content, which is equivalent to those reported by Ghumman et al. 2017 and Jain and Jain (2014). Wheatgrass powder was found to be an excellent source of dietary fiber. Dietary fibers contribute significantly to human health and well-being. The fiber content of wheatgrass powder was found 23.94 g/100 g, as also reported by Qamar et al.(2012) . Jain and Jain (2014) reported that wheatgrass powder had a higher crude fiber content (30%). The carbohydrate content of wheatgrass was 41.85 g/100 g. The carbohydrate content of wheatgrass powder ranged from 40.60 to 43.10 g/100 g. The results obtained are equivalent to those published by Jain and Jain (2014) who reported lower carbohydrate content (33 g/100 g). The recoded observations in this study are in line with the values given by Shirude 2011.

Wheatgrass is known as a complete nutrient package since it contains critical minerals including iron, calcium, phosphorus, magnesium, zinc, selenium, and many more. The major mineral analysis shows that wheatgrass powder is enriched with minerals that are necessary for healthy and normal body functions. Calcium aids in bone growth and development, acts as a cofactor in enzymatic reactions and maintains mineral homeostasis (Pravina et al. 2013). In current investigation, calcium level of wheatgrass power was 188.4 mg/100 g. The result of present investigation was in line with the findings of Chouhan (2014) reported

Table 4 Growth of probiotic strains at different concentrations of wheatgrass

Prebiotic samples	Concentration (%)	*L. acidophilus* (Abs)	*L. rahmnosus* (Abs)	*L. plantarum* (Abs)
Inulin	0.1%	$2.30^{c} \pm 0.014$	$1.43^{d} \pm 0.54$	$1.92^{e} \pm 0.23$
Inulin	0.25%	$2.48^{d} \pm 0.10$	$1.62^{c} \pm 0.48$	$1.53^{d} \pm 0.18$
Inulin	0.5%	$2.56^{b} \pm 0.12$	$1.76^{b} \pm 0.47$	$1.64^{c} \pm 0.14$
Wheatgrass	0.1%	$2.53^{d} \pm 0.13$	$1.83^{d} \pm 0.75$	$1.78^{f} \pm 0.65$
Wheatgrass	0.25%	$2.64^{e} \pm 0.14$	$2.44^{f} \pm 0.62$	$1.86^{c} \pm 0.45$
Wheatgrass	0.5%	$2.93^{f} \pm 0.12$	$1.96^{b} \pm 0.79$	$1.82^{b} \pm 0.12$
Negative control	Without carbon source	$0.63^{a} \pm 0.06$	$0.54^{a} \pm 0.07$	$0.52^{a} \pm 0.09$

Means with the different letters are statistically significant by Tukey's test ($p < 0.05$)

SD, standard deviation

186.6 ± 15.27 mg/100 g calcium content in wheatgrass powder. In contrast, Chaturvedi et al. (2013) reported that the wheatgrass powder had more calcium content. Iron acts as a cofactor in a variety of enzymes such as peroxidases, cytochromes, and xanthine oxidases (Mamatha et al. 2004). Wheatgrass powder has an iron content of 30.96 mg/100 g. The results are comparable to the lower values published by Kulkarni et al. (2006a) and the higher values reported by Jain and Jain (2014), which are 52 mg/100 g. This study sample contained 46.74 mg/100 g of phosphorus. Weather, edaphic factors, and agronomic practices all have an impact on mineral content. Qamar et al. (2012) found almost identical results, with a phosphorus content of 36.66 mg/100 g wheatgrass.

In-vitro screening of prebiotic potential of wheatgrass

Wheatgrass possess polyphenols, flavonoids, proteins, complex carbohydrates, fibers, vitamins, and minerals, which confer several potential health benefits. Current research was envisaged to demonstrate in-vitro prebiotic potential of wheatgrass fiber against probiotic strains. Three *Lactobacillus* strains viz., *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* were chosen as probiotics, with wheatgrass fiber serving as a plant prebiotic. All strains examined exhibited growth when supplemented with crude fiber derived from wheatgrass powder. The effects of wheatgrass fiber on the growth of *Lactobacillus* species were depicted in Table 4. The maximum growth of *L. acidophilus* was found in MRS broth containing 0.5% (w/v) wheatgrass fiber, whereas the maximum growth of *L. rhamnosus* was observed in MRS broth containing 0.25% (w/v) wheatgrass fiber, and the lower concentration of 0.1% of wheatgrass fiber was efficacious for the growth of *L.*

plantarum, as compared to other used wheatgrass fiberconcentrations (0.25% and 0.5%).

The study's findings demonstrate that the growth rate of all studied probiotic strains increases with wheatgrass powder supplementation, but no set concentration was observed to growth enhancement of probiotics; different probiotics have different growth rate at different concentrations. The utilization of wheatgrass fiber as a carbon source boosted optical density. This study concluded that wheatgrass fiber was utilized as a carbon source added to the bacteria, resulting in an increase in bacterium growth (Azmi et al. 2012). This may provide proof that prebiotics derived from wheatgrass fiber significantly enhanced the growth of standard probiotics.The similar condition was found in the research undertaken by Wichienchotet al. 2010.

Antimicrobial activity of wheatgrass

Wheatgrass ethanolic extract was tested at various concentrations for antibacterial efficacy against Gram-positive bacteria viz., *Bacillus cereus*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, one Gram-negative bacteria as *Salmonella typhi*, and one fungi as *Aspergillus brasiliensis*. Table 5 summarizes the antibacterial efficacy of wheatgrass ethanolic extract against several bacterial strains. The results showed that extract of wheatgrass was efficacious in inhibiting the growth of bacteria. *S. pneumoniae* exhibited maximum zone of inhibition (21.31 ± 0.74 mm) against a 20 µg/ml concentration of ethanolic extract of wheatgrass.*S. aureus* showed the highest zone of inhibition (20.33 ± 0.42 mm) at 20 µg/ml and (18.43 ± 0.64 mm) at 15 µg/ml concentrations, with the lowest zone of inhibition (15.38 ± 0.75 mm) at 10 µg/ml concentration. *B. cereus* demonstrated highest zone of inhibition (19.16 ± 1.45 mm) at 20 µg/ml and the lowest zone of inhibition (16.20 ± 0.48 mm) at 10 µg/ml. At 10 µg/ml, *S. typhi* has demonstratedzone of inhibition of (11.24 ± 0.56 mm), succeeded by (13.39 ± 0.46 mm) at 15 µg/ml and (15.48 ± 0.37 mm) at 20 µg/ml. At 20 µg/ml, *A. brasiliensis* exhibited a maximal inhibition zone of 16 mm. Commercial antibiotics Oxy-tetracycline (1000 ppm) and Terbinafine (5000 ppm) were employed as positive controls for bacterial and fungi respectively, they reduced bacterial growth and increased zone of inhibition significantly when compared to wheatgrass ethanolic extract with all tested bacterial and fungal strains at different concentrations. Wheatgrass ethanolic extract of was recorded to be efficient against all the strains of microorganisms examined, with a varying degree of antibacterial efficacy on different bacteria (Pallavi et al. 2011; Das et al. 2012) this is because plant extracts often contain polyphenols, flavonoids, alkaloids, and tannins, which are well known for their antibacterial activity (Rauha et al. 2000) and ethanolic extract of wheatgrass contains a considerable amount of polyphenols and flavonoids (Das et al. 2011).The flavonoids present in wheatgrass viz., apigenin and rutin kill bacteria primarily by disrupting the bacterial cell membrane, leading to its loss of integrity and function. Additionally, apigenin also increase reactive oxygen species (ROS) and nitric oxide (NO) production, contributing to bacterial cell death, and can inhibit bacterial ATP production (Allemailem et al. 2024; Mane et al. 2024).The study found that higher concentrations (20 µg/ml) had much stronger antibacterial activity than lower concentrations (10 µg/ml). Concentrations ranging from 10 to 20 µg/ml increase the zone of inhibition for all strains. This data implies that all tested strains have a dose-dependent response to wheatgrass ethanolic extract. A higher concentration (20 µg/ml) of wheatgrass extract showed highest antibacterial efficacy for all tested microbial strains compared to the lower dose (10 µg/ml). The results of this investigation are in line with the observations of Bhalodia and Shukla 2011noted that a direct correlation was recorded between the antimicrobial efficacy and the extracts concentrations.

The above data showed that Gram-positive bacteria have a larger zone of inhibition than Gram-negative bacteria. In good support of experimental results, several studies have also reported that Gram-positive bacteria are more sensitive to antibiotics due to their thick porous peptidoglycan cell wall which allows antibiotics to easily penetrate the cell and reach their targets, whereas Gram-negative bacteria are more resistant because their thin peptidoglycan layer is protected by an additional, complex outer membrane made up of lipopolysaccharides (LPS) with pores called porins, which acts as a significant protective barrier, preventing antibiotics to penetrate and reach their intracellular targets within the cell (Nikolaidis et al. 2014; Datta and Gupta 2019; Nostro et al. 2000). Figure 2 shows that wheatgrass extract had varying degrees of antibacterial activity against

Table 5 Assessment of antimicrobial activity of wheatgrass

Species name	Zone of inhibition (mm)				
	10 µg/ml	15 µg/ml	20 µg/ml	Positive control	Negative control
Streptococcus pneumoniae	16.32 ± 0.69^{a}	19.46 ± 0.85^{b}	21.31 ± 0.74^{c}	27.38 ± 0.56^{d}	Nil
Staphylococcus aureus	15.38 ± 0.75^{a}	18.43 ± 0.64^{b}	20.33 ± 0.42^{c}	26.31 ± 0.56^{d}	Nil
Bacillus cereus	16.20 ± 0.48^{a}	17.31 ± 0.58^{b}	19.16 ± 1.45^{c}	24.34 ± 0.78^{d}	Nil
Salmonella typhi	$11.24 \pm 0.56_{a}$	13.39 ± 0.46^{b}	15.48 ± 0.37^{c}	20.33 ± 0.78^{d}	Nil
Aspergillus brasiliensis	12.29 ± 0.47^{a}	15.54 ± 0.26^{b}	16.44 ± 1.12^{b}	23.29 ± 0.78^{c}	Nil

Means with the different letters are statistically significant by Tukey's test ($p < 0.05$)

SD, standard deviation

different microorganisms. Wheatgrass extract has a significant antibacterial efficacy and it effectively inhibits the growth of Gram-positive bacteria as that of Gram-negative bacteria and fungi. *S. pneumoniae* was shown to be the most sensitive, whereas *S. typhi* was demonstrated to be more resistant. Similar findings were also reported by Taguri et al. 2004; Santas et al. 2010; Pandey and Gupta 2022.

Elucidation of bioactives of wheatgrass by HPLC

Chromatographic analysis of the bioactive compounds found in wheatgrass was carried out using HPLC. In this research, six different phenolic standards were compared to the chromatograms produced by wheatgrass extract. A number of studies have revealed that polyphenolic compounds are key plant elements with a variety of pharmacological and physiological benefits. Phenolic compounds exhibit a wide spectrum of antioxidant properties due to their scavenging ability via their hydroxyl groups. Aside from that, polyphenols help prevent several ailments like Alzheimer's disease, cancer, and cardiovascular disease (Uddin et al. 2014). Results of HPLC estimation demonstrated several bioactives in wheatgrass extract. Similarly, the bioactives from herbs were also documented (Pandey and Gupta 2019). It includes abscisic acid, chlorophyll a, chlorophyll b, chlorophyllin, apigenin, and rutin with varying retention times, which contributes to it enhance antioxidant activity.

Standard abscisic acid was measured, with a typical peak at 2.258. The wheatgrass sample was then analyzed at 254 nm wavelength. It contained 18 characteristic peaks, with largest area percentage of 2.258. Standard chlorophyll a was determined, with a characteristic peak at 3.825. subsequently, the wheatgrass sample was analyzed at 438 nm. It had 14 characteristic peaks and characteristic peak of chlorophyll a was observed at 3.825. Standard chlorophyll b was determined, with a characteristic peak at 6.225. Subsequently, the wheatgrass sample was analyzed at 436 nm. It had 31 characteristic peaks and characteristic peak of chlorophyll b was observed at 6.225. Standard chlorophyll in was determined, with a characteristic peak at 4.992. Two characteristic peaks were seen at 423 nm in a chlorophyllin sample, with 4.992 having the highest area percentage. Standard apigenin was identified, with a characteristic peak at 2.517. In the study of wheatgrass samples at 337 nm, seven characteristic peaks were identified, with largest area percentage of 2.517. The standard rutin was determined, with characteristic peak at 5.325. Subsequently, the wheatgrass sample was analyzed at 220 nm. Eleven characteristic peaks

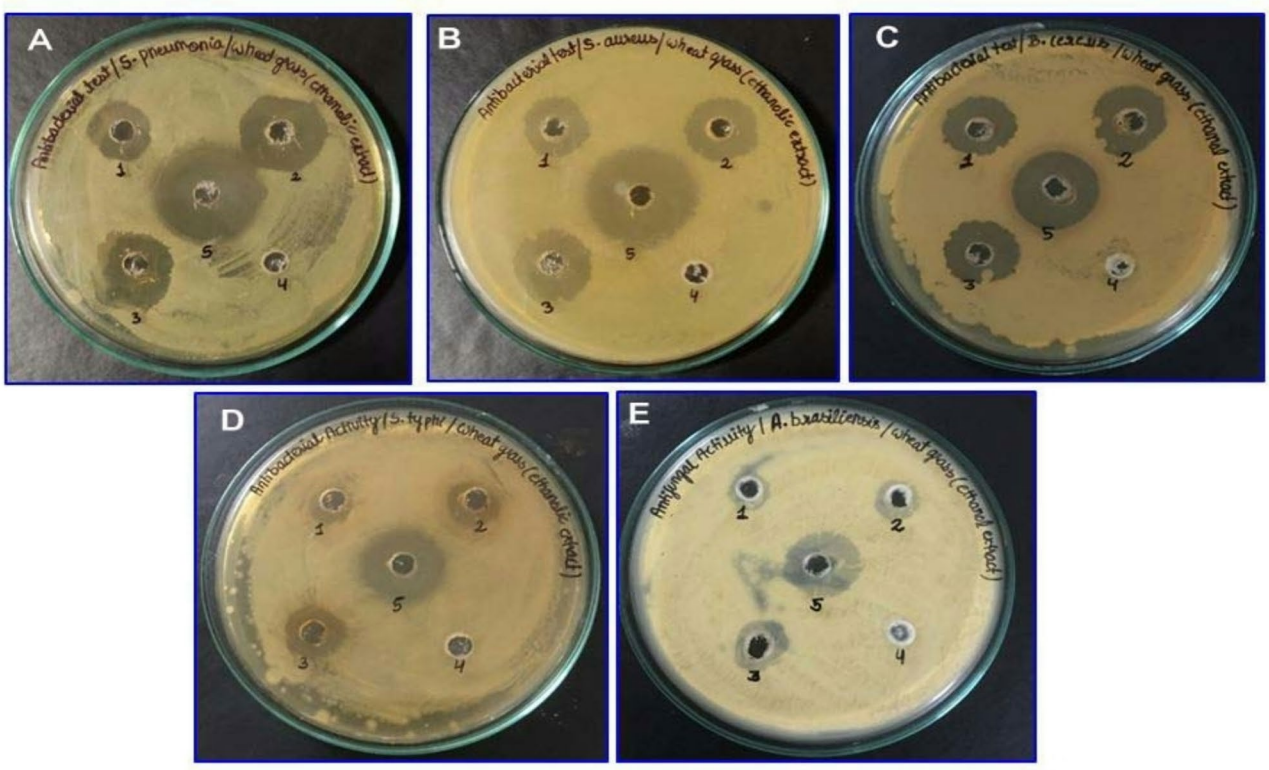

Fig. 2 Zone of inhibition with ethanolic extracts of wheatgrass against **A** *S. pneumonia* **B** *S. aureus* **C** *B. cereus* **D** *S. typhis* **E** *A. brasiliensis*. Wells are loaded with different concentration of wheatgrass ethanolic extract (well 1 = 10 µg/ml, well 2 = 15 µg/ml, well 3 = 20 µg/ml, well 4 = negative control and well 5 = positive control)

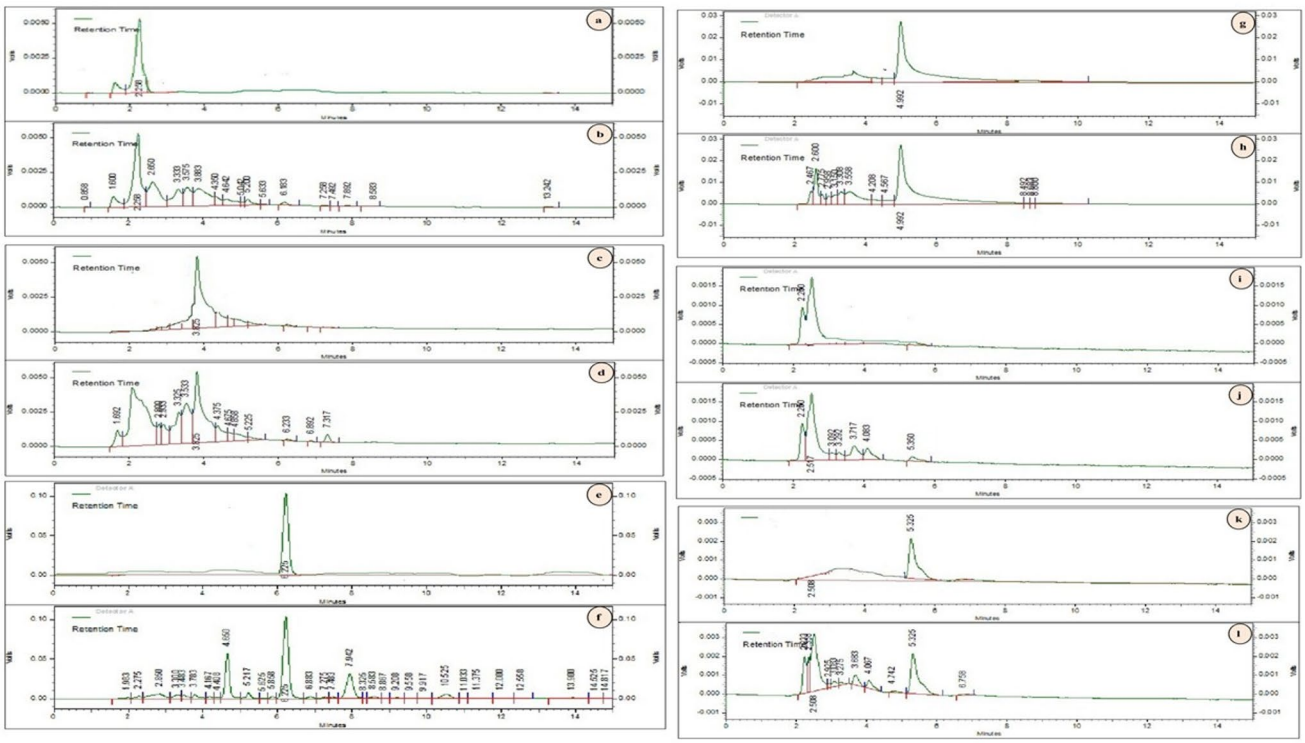

Fig. 3 High performance liquid chromatography of ethanolic extract of wheatgrass **A** standard abscisic acid (254 nm) **B** wheatgrass extract (Area % = 2.258) **C** standard chlorphyll a (438 nm) **D** wheatgrass extract (Area % = 3.825) **E** standard chlorophyll b (436 nm) **F** wheatgrass extract (Area %=6.225) **G** standard chlorophyllin (423 nm) **H** wheatgrass extract (Area % = 4.992) **I** standard apigenin (337 nm) **J** wheatgrass extract (Area % = 2.517) **K** standard rutin (220 nm) **L** wheatgrass extract (Area % = 5.325)

were identified, with largest area percentage of 5.325. The HPLC chromatogram of standards and wheatgrass extracts are presented in Fig. 3.

HPLC has been used to perform both quantitative and qualitative analyses on a variety of biological and non-biological components. For chromatographic analysis, samples must pass through both mobile and stationary phases. Each component has its specific interaction with the mobile and stationary phases. Components having stronger interactions with the stationary phase travel slower than those with weaker or no interactions, which move faster. Thus, HPLC separations are determined by the strength of the components' interactions with the phases. These interactions between components and phases impact the duration that analytes travel inside the column as well as the peak broadening (Lozano-Sanchez et al. 2018).

Conclusion

The present investigation deals with the nutritional analysis, prebiotic potentiality, antimicrobial efficacy and HPLC analysis of bioactive compounds from wheatgrass. The results revealed that wheatgrass powder contain good amount of carbohydrates, protein, fiber, fat moisture and ash along with mineral content such as calcium, phosphorous and iron. Furthermore, the in-vitro prebiotic potential of wheatgrass fiber was assessed against the probiotic strains, which demonstrated the growth of all tested strains with wheatgrass fiber. However, the maximum growth of *L. acidophilus* was found in MRS broth containing 0.5% (w/v) wheatgrass fiber, whereas the maximum growth of *L. rhamnosus* was observed in MRS broth containing 0.25% (w/v) wheatgrass fiber.Successively, the wheatgrass ethanolic extract at different concentrations was investigated to evaluate antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi. Antimicrobial assessment showed maximum zone of inhibition in Gram-positive bacteria and fungi in comparison to Gram-negative bacteria which shows that the wheatgrass ethanolic extract is more effective to inhibit Gram-positive bacteria in comparison to Gram-negative bacteria under investigation. The results revealed that *S. pneumoniae* exhibited maximum zone of inhibition (21.31 ± 0.74 mm) against 20 µg/ml and *S. aureus* showed the highest zone of inhibition (20.33 ± 0.42 mm) at 20 µg/mlconcentration of wheatgrass ethanolic extract. Finally, the wheatgrass ethanolic extract showed the presence of abscisic acid, chlorophyll a, chlorophyll b, chlorophyllin, apigenin, and rutin by the high-performance liquid chromatography analysis.

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Declarations

Conflict of interest The authors declared no conflict of interest.

Data availability All data analyzed during the study are available in the manuscript and its supporting information file.

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