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Research Article

FLAVONOIDS AND PHENOLIC CONTENT IN WHEAT GRASS PLANT (*TRITICUM AESTIVUM*)

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ABSTRACT

The study was mainly designed to screen various solvent extracts of the leaves of wheatgrass (*Triticum aestivum*) to show the potent antioxidant activity in order to find possible sources for novel antioxidants in food and pharmaceutical supplements. A detailed study was performed on the antioxidant activity of the methanol extract and chloroform extract of *T. aestivum*. The fractions were initially screened for the phenolics and flavonoids and then estimated for the total polyphenols and the flavonoids. Further, the fractions were used for showing the potential antioxidant activity. The antioxidant activity was displayed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and H_2O_2 scavenging activity. The plant responded positive for both the phytochemicals screened. The total phenolic content was found to be more for methanol fraction (44%) when compared with the chloroform fraction (12%). The total flavonoids were more for the chloroform fraction (40.420 ± 0.13 µg/mL) when compared with the methanol fraction (22.873 ± 0.05). Methanol fraction was shown to contain more polyphenols (44%), than the methanol fraction (12%). Methanol fraction displayed strong DPPH scavenging activity (IC_{50} 4.258 ± 0.54 minute) in reference to the positive control ascorbic acid exhibited 3.3266 ± 0.38 minute. The scavenging activities of the chloroform fraction were (IC_{50} 5.217 ± 0.52 minute). The scavenging activity for hydrogen peroxide of various solvent extracts from Wheatgrass was in the order of positive control > methanol > chloroform respectively. The data from H_2O_2 activity showed that the activity was dose-dependent. The H_2O_2 activity at a concentration of 200 µg/mL was found to 57.11%, 71.13%, and 86.54% for the chloroform, methanol, and positive control fractions, respectively.

Keywords: *Triticum aestivum*, Total phenolics, 2,2-diphenyl-1-picrylhydrazyl, Antioxidant, H_2O_2 scavenging activity.

INTRODUCTION

Since ages, herbal medications have been used for relieving the symptoms of many of the diseases [1]. Despite the advanced technology observed in modern medicine in recent decades, plants still make an important contribution to the field of medicine. Many of the plants have been investigated for their medicinal properties and especially the antioxidant properties. Natural antioxidants extracted in their crude form contain many chemical constituents, which are very effective to prevent the destructive processes caused by oxidative stress [2]. Though the plants screened for their medicinal properties are not checked for their toxicity effects, still they are enormously used and are generally accepted that medicines derived from plant products are safer [3,4].

Many of the papers so far published have clearly stated the key roles for reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases. This evidence has brought the attention of many biologists to work on the antioxidants for prevention and treatment of diseases, and maintenance of human health [5]. Human body has a magnificent and inherent anti-oxidative mechanism and the anticancer, anti-aging mechanisms originate from this property [6,7]. Antioxidants help in deactivating the free radicals, which attack the biological cells [8]. Owing to this, consumption of food that are rich in naturally occurring antioxidants has considerably increased [9,10].

Oxygen free radicals also called as ROS includes non-radical species such as hydrogen peroxide. These are highly reactive chemical species released in all the tissues during normal aerobic cellular metabolism and cause damage to the various intracellular components such as nucleic acids, lipids and proteins [11]. Free radicals released as a result of endogenous metabolic reactions are involved in a number of diseases like tumors, shock, inflammation, diabetes, infertility, brain dysfunction and cancer. Free radicals and ROS are controlled in biological systems by some enzymes possessing antioxidant activities such as superoxide dismutase and peroxidase [12-14]. An imbalance between ROS and the inherent antioxidant capacity of the body recommends the body to go for dietary and/or medicinal supplements during the disease attack [15].

The antioxidant contents of medicinal plants may contribute to the protection they offer from disease [15]. Liver diseases are still a major health problem. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation, which leads to the tissue injury. Antioxidant agents of natural origin have drawn special interest because of their free radical scavenging capacity [16]. The use of medicinal plants with a high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages [17]. The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds. Phenolic antioxidants are believed to quench oxygen-derived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical [18]. Many of the antioxidant activities of phenolics claimed so far has indicated that they were as active as buthylated hydroxyaniscle or buthylated hudroxytoluane [19].

Polyphenol antioxidants have protective effects against different diseases, including cardiovascular, inflammatory and neurological diseases, as well as cancers [13]. Moreover, most of the polyphenols reported so far, have been proved of their effectiveness in free radical scavenging capacities.

Wheatgrass (*Triticum aestivum*) is a member of the family poaceae, which includes a wide variety of wheat-like grasses. There have been almost no clinical studies in humans to support claims made for wheatgrass or wheatgrass diet programs. The above-ground parts, roots, and rhizome are used to make medicine. Wheatgrass is primarily used as a concentrated source of nutrients. It contains ample load of vitamin A, vitamin C, and vitamin E, iron, calcium, magnesium, and amino acids [14]. Wheatgrass is used for increasing production of hemoglobin; preventing tooth decay; improving wound healing; and preventing bacterial infections. It is also used for removing deposits of drugs, heavy metals, and cancer-causing agents from the body, and for removing toxins from the liver and blood [20].

It is also used to treat many conditions, but so far there is no published scientific evidence to support effectiveness for any of these uses. Wheatgrass is also found to use as an ailment to treat a number of conditions including the common cold, coughs, bronchitis, fevers, infections, and inflammation of the mouth and throat [21]. In folk medicine, practitioners used wheatgrass to treat cystitis, gout, rheumatic pain, chronic skin disorders, and even constipation [22]. Although most people use wheatgrass juice as a dietary supplement or as a serving of vegetables, some proponents claim that a dietary program commonly called "the wheatgrass diet" can cause cancer to regress or "shrink" and can extend the lives of people with cancer [23]. They believe that the wheatgrass diet strengthens the immune system, kills harmful bacteria in the digestive system, and rids the body of toxins and waste matter [14].

There is no research has been reported about the antioxidant activities of wheat grass. The main objective of this study was to investigate the total phenolics and the antioxidant activities of the wheatgrass.

MATERIALS AND METHODS

Extraction and plant extract preparations

Adequate quantity of unpolished wheat grain was soaked overnight in water in a container. The soaked wheat-grain were spread on the surface of the soil filled in plastic trays. The tray was covered with newspaper to provide darkness, which helps the sprouting. The plantlets after 9 days, which grew to a height of about 15-16 cm were selected for the extraction procedure.

The wheat grass blades were cut and homogenized in a mortar and pestle and about 5 g of the homogenized paste was dissolved in 20 mL of (methanol and chloroform). The samples were then in an orbital shaker for overnight incubation. The solvents are then filtered and supernatants containing the components are concentrated in a hot air oven at 55°C. The concentrated samples are then dissolved in 10% dimethyl sulfoxide. The crude samples are later stored at 4°C for further use. The three fractions (methanol, chloroform and petroleum ether) were used for further screening experiments.

Screening for phytochemicals

The three fractions were then screened for the phytochemicals especially, the flavonoids and Phenols.

Flavonoid test

The extracts were screened for flavonoids by the protocol described elsewhere [24]. Briefly to 0.2 mL of each extract, 1 mL of ammonia solution was added and mixed properly. To this mixture 1 ml of concentrated sulfuric acid was added. Development of yellow color was indicated as a positive for flavonoids.

Phenolics test

The extracts were screened for phenolic by the protocol described elsewhere [25]. Briefly to 0.2 mL of extract, 1-2 drops of ferric chloride (0.5%) was added. Development of green color indicates positive for phenolics.

Analysis of flavonoid content

Flavonoid content of the extracts was quantified using the aluminum chloride assay method. The C-4 keto group and the hydroxyl group of either the C-3 or C-5 of flavonoids react with the aluminum chloride to form an acid-stable complex.

In brief 500 µL of plant extract was dissolved in 1.5 mL of ethanol (95%) and 0.1 mL of 10% aluminum chloride. To this 0.1 mL of 1 M sodium acetate are added. The volume is made up to 5 mL with distilled water. Absorbance of the yellow-green complex was measured at 415 nm after 30 minutes.

Quercetin was used as a standard. Flavonoid content of the plant extracts was expressed as mg quercetin equivalents per gram dried weight (mg QE/g dried weight). All the experiments were carried out in triplicates.

Analysis of phenolic content

The extracts collected were then analyzed for the total phenol content. The Phenolic content was assayed using the Folin–Ciocalteu assay (Singleton, 1965). Phenolic compounds, at basic pH reduce the phosphomolybdic and phosphotungstic acid reagent, forming a blue complex. In brief, 0.5 ml of extract was mixed with 0.5 mL of Folin–Ciocalteu reagent and incubated for 5 minutes at room temperature. This was followed by the addition of 0.5 mL sodium carbonate. The mixture was incubated in the dark at room temperature for about 2 hrs.

After the blue color had been developed, absorbance was taken at 765 nm. Gallic acid was used as a standard. The concentration of phenolic content was expressed as milligram gallic acid equivalents per gram of dried weight (mg GAE/g dried weight). All the experiments were carried in triplicates.

2,20- diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

The free radical scavenging activity of the fractions was measured *in vitro* by DPPH assay according to the method described earlier [26,27]. A stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 1.018 ± 0.02 at 517 nm using a spectrophotometer.

To 1.8 mL of DPPH solution 0.2 mL of the extract was added. The reaction mixture was shaken well and incubated in the dark. The decrease in the absorbance was recorded at 517 nm after 0 minute, 5 minute, 10 minute, 20 minute and 30 minute. The control was prepared as above without any sample. The experiment was done in triplicates with vitamin C as a positive control.

The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation: % inhibition = Absorbance (control) at 517 nm – Absorbance (sample) at 517 nm/Absorbance (control) at 517 nm * 100.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was performed for the extracts according to the protocol described elsewhere [28]. 0.1 mL of extract was added to a test tube and the volume is made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). The contents are mixed properly and added with 0.6 mL of hydrogen peroxide solution (2 mM) prepared in 50 mM phosphate buffer (pH 7.4). The contents were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 minute, against a blank. Ascorbic acid is sued as a positive control. The activity was tested at different concentrations of the crude extracts. Hydrogen peroxide scavenging activity was calculated using the following equation: Scavenging activity = 1-Absorbance of sample/Absorbance of sample *100.

RESULTS AND DISCUSSION

Phytochemical screening

The three fractions were then screened for the phytochemicals especially, the flavonoids and phenols. The extracts showed a positive response for both the flavonoids and phenolics test. Abundant quantity of flavonoids was found in the case of chloroform extract, and more quantity of phenolics was found in the case of methanol extract.

Analysis of flavonoid content

Flavonoid content of the wheat plant extracts was expressed as mg QE/g dried weight. The concentration of the flavonoids found for the aqueous extraction was found to be 127.093 µg/mL of QEs (Table 1). The calibration equation for quercetin was $y = 0.006x–0.002$ ($R^2 = 0.991$), where x is the quercetin concentration in mg/L and y is the absorbance reading at 415 nm.

The chloroform extract was found to show more flavonoid content than the organic extracts. All the experiments were carried out in triplicates. The concentration of the quercetin used was 0.5 mg/mL. The flavonoid

content was found to be more (40.420 ± 0.13 µg/mL) for the chloroform extraction phase than the methanolic phase (22.873 ± 0.05) (Table 2, Fig. 1).

Estimation of polyphenols

The absorbance of the blue color that developed was read at 765 nm using a spectrophotometer. The concentration of total phenolic content was expressed as GAEs by reference to the gallic acid standard calibration curve. The calibration equation for gallic acid was y = 0.0736x−0.007 (R^2 = 0.993), where x is the Gallic acid concentration in mg/L and y is the absorbance reading at 765 nm. The concentration of total Phenolic compounds in the extract was determined by using the formula: T = CV/M; Where, T = Total phenolic content mg/g of plant extract in GAE, C = concentration of gallic acid from the calibration curve, V = volume of the extract in mL, M = wt of the pure plant methanol extract.

The results of Folin–Ciocalteu total phenols photometric assay are reported in Table 1. Methanol fraction was shown to contain more polyphenols (44%), than the methanol fraction (12%).

DPPH radical-scavenging activity

The free radical scavenging activity of the fractions was measured *in vitro* by DPPH assay according to the method described earlier [26,27]. Negative control was with an absorbance of about 1.018 ± 0.02 at 517 nm using a spectrophotometer. The control was prepared as above without any sample. The experiment was done in triplicates with vitamin C as a positive control. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation: % inhibition = Absorbance (control) at 517 nm – Absorbance (sample) at 517 nm/Absorbance (control) at 517 nm *100.

A two-way ANOVA between the fractions (chloroform, methanol and positive control) and % of inhibition was done to evaluate the effectiveness of the Wheatgrass. All effects were statistically significant at the 0.05 significance level. There was a significant effect of fractions and the % of inhibition (DPPH scavenging activity) remembered at the $p<0.05$ level. The fractions (chloroform, methanol and positive control) showed a significance to % of inhibition ($F(2,8)= 23.6464$, $p=0.000438$) and to the time of incubation ($F(4,8)= 57.91271$, $p=0.00651$).

Methanol fraction displayed strong DPPH scavenging activity (IC_{50} 4.258 ± 0.54 minute) in reference to the positive control ascorbic acid exhibited 3.3266 ± 0.38 minute. The scavenging activities of the chloroform fraction were (IC_{50} 5.217 ± 0.52 minute).

The scavenging activity for hydrogen peroxide of various solvent extracts from Wheatgrass was in the order of positive control > methanol > chloroform respectively (Fig. 1).

Table 1: The values of flavonoids and total polyphenols of the chloroform and methanol extracts. All the values are done in triplicates. The concentration is expressed in µg/mL and in value±SD

	Wheat grass		
	Polyphenols		Flavonoids
	Concentration	% Phenols	Concentration
Chloroform	0.3070±0.09	12	40.420±0.13
Methanol	1.0966±0.09	44	22.873±0.05

SD: Standard deviation

Table 2: The screening test for flavonoids and phenolics of chloroform and methanol extracts

Serial number	Test	Chloroform	Methanol
1	Flavonoids	++	–
2	Phenolics	–	+++

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was performed for the extracts according to the protocol described elsewhere [28]. Ascorbic acid issued as a positive control. Hydrogen peroxide scavenging activity was calculated using the following equation: Scavenging activity = 1-Absorbance of sample/Absorbance of sample *100. The data showed that the activity was dose dependent. The H_2O_2 activity at a concentration of 200 µg/mL was found to 57.11%, 71.13%, and 86.54% for the chloroform, methanol and positive control fractions, respectively (Fig. 2).

DISCUSSION

The wheat grass leaves were screened for the flavonoids and phenolics, and the extracts showed a positive response for both the flavonoids and phenolics test. Abundant asquantity of flavonoids was found in the case of chloroform extract, and more quantity of phenolics was found in the case of Methanol extract.

The chloroform extract was found to show more flavonoid content than the organic extracts. The flavonoid content was found to be more (40.420 ± 0.13 µg/mL) for the chloroform extraction phase than the methanolic phase (22.873 ± 0.05). The results of Folin–Ciocalteu total phenols photometric assay are reported in Table 1. Methanol fraction was shown to contain more polyphenols (44%), than the methanol fraction (12%).

Methanol fraction displayed strong DPPH scavenging activity (IC_{50} 4.258 ± 0.54 minute) in reference to the positive control ascorbic acid exhibited 3.3266 ± 0.38 minute. The scavenging activities of the chloroform fraction were (IC_{50} 5.217 ± 0.52 minute). The scavenging activity for DPPH scavenging activity of various solvent extracts from Wheat grass was in the order of positive control > methanol >

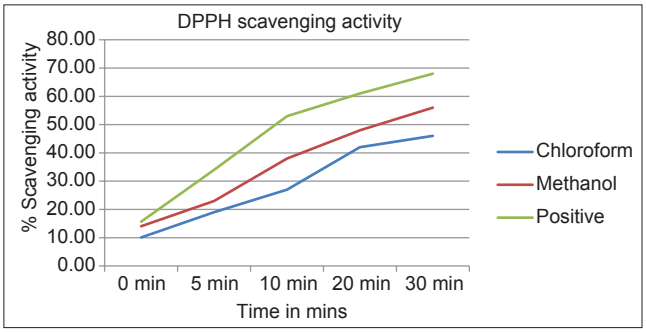

Fig. 1: Antioxidant activities of leaves of wheatgrass by different solvents (chloroform, methanol). Each value represents a mean ± standard deviation: All the values are average of triplicates. Positive control used is ascorbic acid

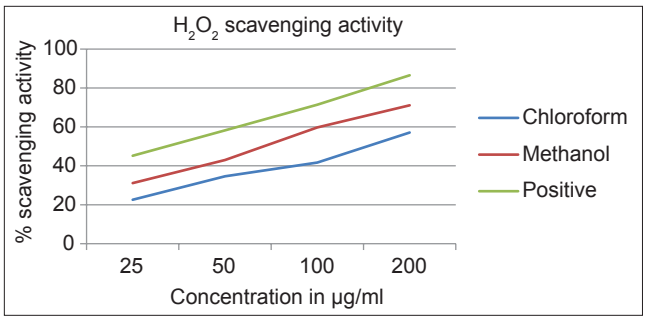

Fig. 2: Hydrogen peroxide scavenging activities of leaves of wheat grass by different solvents (chloroform, methanol). Each value represents a mean ± standard deviation: All the values are average of triplicates. Positive control used is ascorbic acid. Concentration of the samples are expressed in µg/mL

chloroform respectively. The data from hydrogen peroxide activity showed that the activity was dose dependent. The H_2O_2 activity at a concentration of 200 µg/mL was found to 57.11%, 71.13%, and 86.54% for the chloroform, methanol and positive control fractions, respectively. Methanol fraction showed more activity when compared with the chloroform fractions.

CONCLUSION

Wheat grass gas has been used by many of the ayurvedic practitioners since ages. They have been used in many of the ailments such as anemia, liver disorders, digestion problems and cancer related diseases. They are mostly rich in antioxidant properties. However, so far very little work has been done on the activity of these plant extracts. The study showed a high level of flavonoids and phenolics. They also exhibited a high level of antioxidant properties. Keeping these in view, the plant extracts rich in phenolics and flavonoids can be used in treating the diseases such as cancer, stomach ailments, anemia and other blood related diseases that result mainly due to the free radicals generated in the body. Further, we plan to study the components of the phenols present in the sample using the gas chromatography-mass spectrometry.

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