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NON-ENZYMATIC ASSAY BASED IN-VITRO ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF FREEZE DRIED WHEAT (*TRITICUM AESTIVUM* L.) SEEDLINGS JUICE POWDER: NATURE'S FINEST MEDICINE-PART-II

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Keywords:

Triticum aestivum (L.); Wheat Seedling Juice Powder (WSJP); Freeze Dried; *In-vitro* Antioxidant Activity; 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity; Non-Enzymatic Assay.

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ABSTRACT: Free radical scavenging potential and phytochemical analysis of the two different extracts of lyophilized wheat seedling juice powder (WSJP) *Triticum aestivum* L.(Gramineae),"Nature's Finest Medicine" was evaluated *in vitro* by using diphenyl-picryl-hydrazyl (DPPH) assay and preliminary phytochemical analysis. In this method the antioxidants present in WSJP extracts reacted with DPPH, which is a stable free radical and converted it to 1,1-diphenyl-1,2-picryl, hydrazine. The scavenging effect of plant extracts and standard (L-ascorbic acid) on the DPPH radical decreased in the following order: L-ascorbic acid>Methanolic extract (I) > Water extract (II) and it was found to be 97.3%, 58.6% and 35.1% at concentration of 100µg/mL, respectively. The results were expressed as IC_{50}. Ascorbic acid which was used as a standard showed an IC_{50} of 19.2µg/mL, whereas, the crude methanolic (I) and water (II) extracts of lyophilized wheat seedlings juice powder showed antioxidant activity with IC_{50} values of 81.4 and131.3, respectively at 1-100µg/mL concentration. None of the extracts were found to be more active than the standard i.e. ascorbic acid. But still, free radical scavenging activity of methanolic (I) and water (II) extracts were confirmed in the present investigation. In the present study, different extracts of lyophilized wheat grass juice powder showed concentration dependent free radical scavenging activity. Significant results were recorded first time in the given estimated parameters. Therefore, this is a pioneer attempt towards the prevention and treatment of various ailments and especially antiaging properties and better understanding of therapeutic potential of WSJP not described earlier.

INTRODUCTION: Young seedlings of wheat (*Triticum aestivum* L.) of family Gramineae also known as Gehun, Kanak, Godhuma, Gandham [1], popularly called as wheat grass/wheat seedings are being used for extraction of crude juice for herbal medicine. The common wheat or bread wheat widely cultivated in almost all parts of the world, is native to South-West Asia and the Mediterranean region. The wheat plant is believed to have multifarious pharmacological activities in addition to its numerous nutritional values.

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Wheat seedlings stimulates metabolism, restores alkalinity of blood and is also a de-toxificant which helps to restore healthy cells [2]. The use of wheatgrass juice for therapeutic purposes was developed and popularized by Dr. Ann Wig more (1909-1996), as a part of herbal therapeutic nutritional approach [3].

The therapeutic qualities of wheatgrass juice have been attributed to its nutritional content, including chlorophyll, enzymes, Vitamins (A, C and E), bioflavonoids, substantial amount of micronutrients andamino acids [4]. During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts, with the maximum antioxidant potential [5]. The concentration of Vitamins such as C, E, β-carotene, in addition to ferulic acid and vanilic acid increases

with the germination period [6]. The wheat seedlings also contain chlorophyll, which was found to be responsible for inhibiting the metabolic activation of carcinogens [7, 8]. It is also reported that 50 pounds of wheatgrass is equivalent to 450 pounds of garden vegetables [9]. In Asia and Europe, wheatgrass based products are consumed in the form of juices, powders and extracts for healthy growth of human body. Wheat grass has been identified as a complete food providing all the nutrients. Wheatgrass juice provides manifold health and wellness benefits by curing problems related with digestive system and anaemia. Wheat seedlings act as a blood purifier and strengthen the immune system. Anti-carcinogenetic activity of wheatgrass juice has already been reported [10].

It is well known and reported widely that, wheat grass possesses anti-mutagenic and antioxidant activity because of the redox enzymes including catalase, peroxidase and other antioxidant compounds such as phenolic acids, alkyresorcinols and amino phenols [5, 11]. The products of wheatgrass are also reported to cure many dreadful diseases such as thalassemia and distal ulcerative colitis [12, 13].

The research work by Bar-Sela and co-workers reported that the breast cancer patients who took wheatgrass juice daily showed a decreased need for blood and bone marrow building medications during chemotherapy, without diminishing the effects of the therapy [14]. Cytotoxic effects of commercial wheatgrass and fibre towards human acute promyelocytic leukemia cells were also reported recently[15]. Wheatgrass has been recommended as a supplement for the last four to five decades as a treatment for various diseases, but till today very little clinical data exists to support its therapeutic uses. Research scientists and physicians from all over world are conducting extensive studies on animal models and human systems to develop herbal medicines, dietary supplements as alternative or complimentary therapy for the treatment of chronic diseases. Epidemiological studies have shown that wheatgrass products are protective against cardiovascular disease, diabetes and cancer [16-19].

There are reports on the antimutagenic effect of oxidative DNA damage towards benzo(a)pyrene induced mutagenicity [11]. Falcioniand co-workers have demonstrated the inhibition effect of wheatgrass on oxidative DNA damage [10]. It has been shown that wheatgrass extracts contain significant amounts of phenolic compounds including flavonoids [20]. Phenolic compounds of plant products are mainly responsible for the antioxidant activity which reverse the effect of ROS mechanism by various pathways, and also reduce incidence of cancer [21]. Reactive oxygen species (ROS) are produced as a by-product of various metabolic processes, in living organisms mainly during respiration.

Normal physiological concentrations of ROS usually have a role of regulation of cell activities, whereas higher concentrations cause oxidative damage. Oxidative stress has been implicated in the pathophysiology of many diseases including cancers. The generation of ROS is prevented by ROS interacting enzymes (catalase, superoxide dismutase) and additional antioxidants containing medicinal plants (green tea, wheat). Recently, investigators have focused on the antioxidant potential of plant tissues. There are some reports on inhibition of *in vitro* metabolic activation of carcinogens by wheat sprout extracts [7, 8] and the antimutagenic effect of oxidative DNA damage towards benzo(a)pyrene induced mutagenicity [11].

We have developed organic wheat seedling juice powder, which has been used for analysis various *in-vitro* and *in-vivo* bioactivities. In the present research, attempts have been used and approved non-enzymatic *in-vitro* antioxidant activity analysis on different extracts of wheat seedling juice powder (*T. aestivum* L.). This is a pioneer attempt towards the prevention and treatment of various ailments especially antiaging properties of this herb for better understanding of therapeutic potential of this medicinal grass which was previously not described on freeze dried wheatgrass juice powder.

A very little information has been available on the antioxidant profile of the wheatgrass and its different products such as wheatgrass powder, wheatgrass juice powder, etc. Therefore, the present study was designed to estimate the antioxidant activity of lyophilized Wheat Seedling Juice Powder (WSJP). We know that, wheatgrass is not significantly popular due to little convincing

research on its different indoor or outdoor growing conditions, low characteristic features corresponding to its taste, aroma, colour, poor shelf-life and its cost. Our main objective for future study is to improve the different indoor or outdoor growing conditions for wheat grass and also to optimize its taste, aroma and shelf-life of wheat grass juice powder for supreme recognition worldwide without modification in its originality.

MATERIALS AND METHODS:

Plant Material:

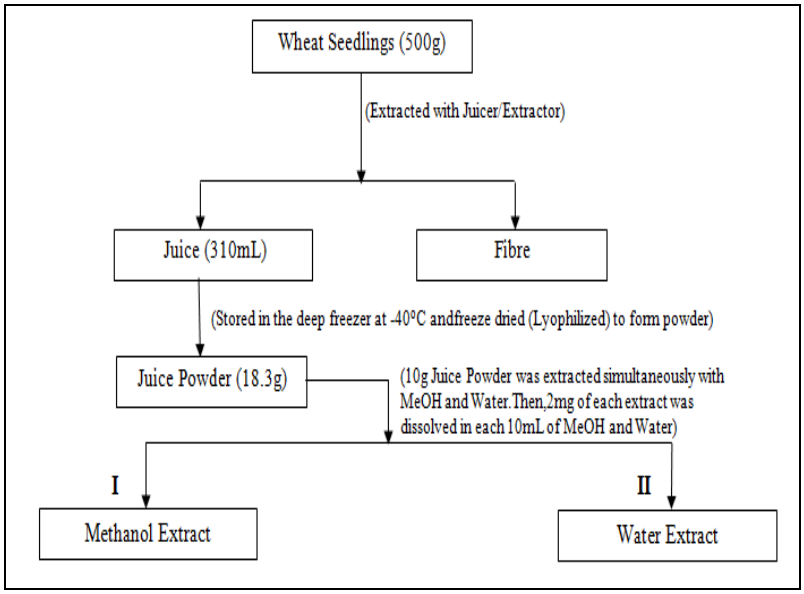
The plant material comprises of (*Triticum aestivum* L.), lyophilized Wheat Seedling Juice Powder (WSJP), was taken for determination of antioxidant activity. The wheat seedlings were grown in the green house and prepared in the laboratory of Akal College of Agriculture, Eternal University, Himachal Pradesh.

Which comes under the foot hills of North-Indian Himalayan region (at the altitude range of 1400m) on the banks of the famous Giri River. Plants specimens were identified and were deposited in the Herbarium, with accession number (V. Sharma-20129).

Preparation of Plant Extracts:

The 7-10 days old (500g) wheat seedlings were washed thoroughly with running tap water, followed by washing with deionised autoclaved water to remove the dust particles, possible parasites and then used for extraction of juice with the help of a manual extractor/juicer (MJ-445, Miracle Company, Taiwan). 310mL juice was collected from 500g seedlings and stored in the deep freezer for pre-freezing at -40^{o}C for 1h. The stored juice was then freeze dried (lyophilized) to form powder (18.3g). The freeze dried powdered sample was then stored in closed air tight bottles for further experimentation. 10g of freeze dried juice powder was extracted simultaneously with methanol and water.

Then 2mg each extract was dissolved in 10mL of methanol and water to form two separate extracts. Extracts were then filtered with Whatman's filter paper No.1. The filtrate was collected and stored in air tight tubes under 4^{o}C till further analysis (Flow chart 1). Finally, different concentrations of each extract were prepared for the free radical scavenging activity.



FLOW CHART 1: EXTRACTION PROCEDURE APPLIED ON WHEAT SEEDLINGS JUICE POWDER (*T.AESTIVUM* L.).

Preparation of Chemical Solutions:

For antioxidant activity, chemical solution of L-ascorbic acid as a control was used and prepared by dissolving 2mg of L-ascorbic acid in 10mL of methanol. For Diphenyl-picryl-hydrazyl assay, 0.1mM stock solution of DPPH in methanol was prepared. All the chemicals, including solvents, were of analytical grade from E. Merck, India and Sigma (New Delhi, India).

Reaction Time:

In the original method a reaction time of 30min was recommended [22]. There were so many different views for reaction time, given by workers all over

the world. Shorter time had also been used for the reaction, such as 10min [23]. However, in view of the fact that the rate of reaction varied widely among substrates, the best practice seemed to be to follow the reaction until it had gone to completion. In present attempt of research work, 30min reaction time was followed, to check the antioxidant activity.

Absorbance Measurements - Wavelength and Instrument Used:

During evaluation of antioxidant activity for these two extracts (I, II), we followed the methodology given by Blois in 1958 [24]. Absorbance was measured at 517nm by using Labtronics (LT-2900) Double Beam (UV-Vis) spectrophotometer. The working wavelength of maximum absorbance, to be used for the absorbance measurements were given by various workers such as 515nm by Brand-Williams, 516nm by Schwarz, 517nm by Blois and 518nm by Leit[25].

Free Radical Scavenging Activity (Diphenyl-picryl-hydrazyl Assay):

The free-radical scavenging capacity of the freeze dried juice powder (2mg each) dissolved with 10mL of methanol and water extracts were evaluated with the DPPH stable radical following the methodology described by Blois [24]. Briefly, 0.1mM alcoholic solution of DPPH in methanol was prepared and 2mL of this solution was added to 0.3mL of different extract concentrations (1-100µg/mL) and allowed to react at room temperature. After 30min, the absorbance values were measured at 517nm against the blank, which did not contain the extract. One synthetic antioxidant, L-ascorbic acid was used as control.

The radical scavenging activity (percent(%) inhibition) was expressed as percentage of DPPH radical elimination calculated according to the following equation:

$$\text{Percentage Inhibition (\%)} = (A_{control}\text{-}A_{sample}/A_{control}) \times 100$$

Where $A_{control}$ is the absorbance of the control (L-ascorbic acid) and A_{sample} is the absorbance of reaction mixture (in the presence of sample). All tests were run in triplicates (n = 3), and the average values were calculated.

IC_{50} Value:

One parameter (IC_{50}) inhibition concentration was introduced for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It was the amount of sample necessary to decrease the absorbance of DPPH by 50%. This parameter was introduced by Brand-Williams and his colleagues [26].

Phytochemical Screening:

Preliminary qualitative phytochemical screening was carried out on two different freeze dried juice powdered extracts of Wheat Seedling Juice Powder (*T. aestivum* L.) with the following methods to find out the exact relationship between secondary metabolites and antioxidant activity. These two methanolic and water extracts were tested for the presence of active principles such as: phenols, tannins, flavonoids, alkaloids, steroids, sterols, quinones, saponins, proteins, **c**arbohydrate, coumarins, sugars, polysaccharides, organic acid and fatty acid. Following standard procedures were used.

Test for Phenols:

Ferric chloride Test:

Take 1mL for the solution in a test tube, add acetic acid and then a few drops of 1% ferric chloride reagent. If it turns brownish green or blue black, then it may contain phenolic composition or tannin.

Test for Tannins:

Braymer's Test:

5mL of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution[27].

Test for Flavonoids:

Ferric chloride Test:

Test solution when treated with few drops of ferric chloride solution would result in the formation of blackish red colour indicating the presence of flavonoids.

Alkaline reagent Test:

Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which would become colourless on addition

of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

NaOH Test:
A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

H_2SO_4 Test:
A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

Test for Alkaloids:
Hager's Test:
Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Wagner's Test:
A fraction of extract was treated with Wagner's test reagent (1.27g of iodine and 2g of potassium iodide in 100 mL of water) and observed for the formation of reddish brown colour precipitate [28].

Test for Steroids:
Steroids:
1ml of the extract was dissolved in 10mL of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [29].

Test for Sterols:
H_2SO_4 Test:
The fraction of extract was treated with ethanol and H_2SO_4 and observed for the formation of violet blue or green colour.

Test for Quinones:
A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Saponins:
Foam Test: Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result[30].

Bubble Test:
5mL aqueous extract was shaken vigorously for 2 min. The appearance of foam that persisted for at least 15 min or the forming of an emulsion when olive oil was added confirmed the presence of saponins.

Test for Proteins:
Ninhydrin Test (aqueous):
The extract was treated with aqueous ninhydrin, purple colour indicates the presence of protein.

Test for Carbohydrate:
Fehling's Test:
Each extract was dissolved in distilled water and filtered. The filtrate was heated with 5mL of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

Test for Coumarin:
1mL of ethanol extract was stirred and placed separately in a test tube. The result of the solution was seen in the following experiments.

Opened loop - closed loop response:
In the test tube, add 2 drops of 1% sodium hydroxide solution, and heat in boiling water for 3 min to get clear solution. Also add 4 drops of 2% hydrochloric acid to the solution. If the solution becomes cloudy, that means it contains coumarins [31, 32].

Test for Sugar and Polysaccharides:
The extracts were taken to do the following experiments separately.

Fehling Reaction:
4mL of the aqueous extract was placed in test tube and 5 ml mixture of equal volumes of Fehling's solutions A and B was added and boiled in a water bath for 5 min. It resulted in brick- red cuprous oxide precipitate that contains sugar. The solution will remove sediment filtration. To the filtrate, add 1 ml and 10% hydrochloric acid in boiling water bath for home heating hydrolysis for few minutes. Let it cool. Then drop 10% sodium hydroxide

solution adjusted to neutral pH, and repeats the Fehling's reaction. If still produces the brick-red cuprous oxide precipitates, then it contains polysaccharides.

Iodine Test:
1mL of the aqueous extract was taken in a test tube and 1 drop mixture of iodine solution was added. The change in colour denotes the presence of polysaccharide.

Test for Organic acid:
Take the water extract and do the following experiment separately.

pH Test Strip Reaction:
If the solution is put in a wide range of pH test paper, and found to be acidic, then it may contain organic acids or phenolics.

Fatty Acids:
0.5mL of extract was mixed with 5mL of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids [33].

RESULTS AND DISCUSSION:
The wheat seedling juice powder is believed to have manifold pharmacological properties in addition to its important nutraceutical uses. The present work includes screening of antioxidant properties of two different extracts of lyophilized wheat seedling juice powder along with their preliminary phytochemical evaluations. It is well known fact that, a diet rich in vegetables and fruits provides protection against cardiovascular and other chronic diseases originating from oxidative stress. Therefore, suitable antioxidant therapies to control oxidative damage have already attracted the worldwide attention in recent years.

It has been already reported that phytochemicals used to study in cell culture system and animal models have provided a wealth of information on the mechanism by which such nutraceuticals show their beneficial effects [34, 35]. The preliminary phytochemical studies indicated the presence or absence of phenols, flavonoids, tannins, alkaloids, steroids, sterols, saponins, coumarines, sugars, **c**arbohydrate, polysaccharides, proteins, organic acids and fatty acid in one or both the extracts (**Table 1**).

TABLE 1: PRELIMINARY QUALITATIVE PHYTOCHEMICAL SCREENING OF TWO DIFFERENT FREEZE DRIED JUICE POWDERED EXTRACTS OF *T. AESTIVUM* (L.).

S. No.	Name of Secondary Metabolites	Extract-I (Methanol)	Extract-II (Water)
1.	Phenols	+	+
2.	Tannins	+	+
3.	Flavonoids	+	+
4.	Alkaloids	+	-
5.	Steroids	+	-
6.	Sterols	+	-
7.	Quinones	+	-
8.	Saponins	+	+
9.	Proteins	+	-
10.	Carbohydrates	-	+
11.	Coumarin	+	+
12.	Sugars	-	+
13.	Polysaccharides	-	+
14.	Organic Acid	+	-
15.	Fatty Acid	+	-

Like other medicinal plants, it is hypothesized that different extracts of wheat seedling juice powder showed antioxidative effect, which can be attributed to the presence of phenolic compounds such as alkaloids, flavonoids, and tannins in the seedlings of *T. aestivum* L. These phenolic components possess many hydroxyl groups including *o*-dihydroxy group which have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine.

The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in methanol solution at 517 nm.

The scavenging effect of plant extracts and standards on the DPPH radical decreases in descending order: L-ascorbic acid >Methanolic extract (I) > Water extract (II). These were 97.3%, 58.6% and 35.1% at concentration of 100μg/mL, respectively (**Table 2**).

TABLE 2: MEASUREMENT OF ABSORBANCE AT 517NM AND INHIBITION EFFECT (%) OF DIFFERENT EXTRACTS WITH STANDARD L-ASCORBIC ACID FROM CONCENTRATION RANGE OF 1-100 µg/mL.

Concentration of Extract/ Standard (µg/mL)	Absorbance of Ascorbic Acid (Standard)	Inhibition Effect (%)	Absorbance of Methanol Extract (I)	Inhibition Effect (%)	Absorbance of Water Extract (II)	Inhibition Effect (%)
100	0.030	97.3	0.465	58.6	0.731	35.1
90	0.031	97.2	0.512	54.5	0.712	36.7
80	0.031	97.2	0.569	49.4	0.701	37.6
70	0.032	97.1	0.614	45.4	0.813	27.7
60	0.043	96.1	0.701	37.7	0.842	25.2
50	0.056	95.1	0.713	36.6	0.816	27.4
40	0.144	87.2	0.805	28.4	0.857	23.8
30	0.365	67.5	0.836	25.7	0.918	18.4
20	0.579	48.5	0.917	18.5	0.936	16.8
10	0.794	29.4	0.937	16.7	1.016	9.7
1	0.956	15.1	1.007	10.5	1.102	2.1

(Given values are average of triplicate i.e. *n*=3; DPPH= 1.125).

The potential of L-ascorbic acid to scavenge DPPH radical became almost stable after 70 or 80 µg/mL (**Fig.1**).

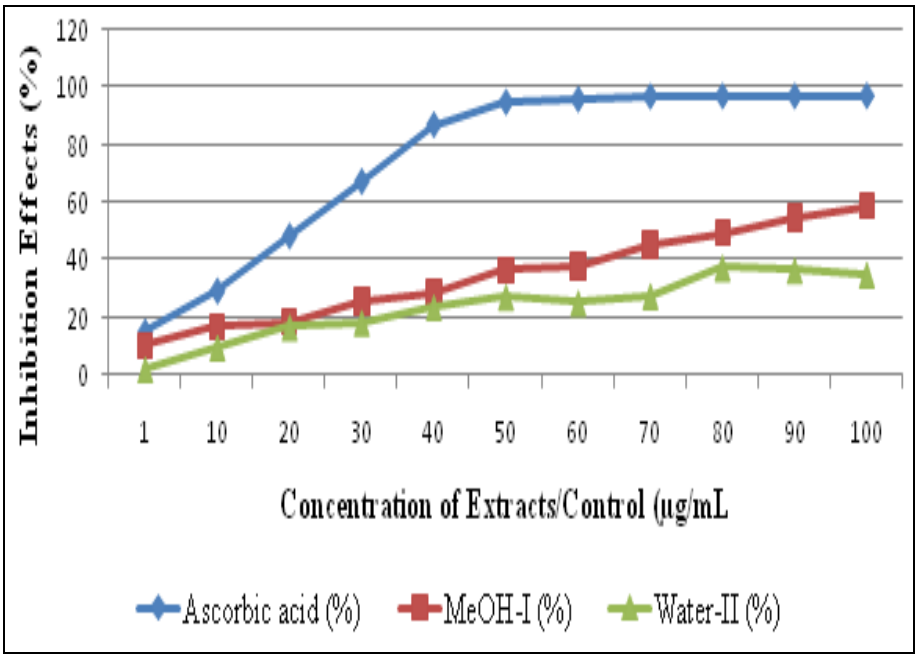


FIG.1: FREE RADICAL SCAVENGING ACTIVITY OF DIFFERENT EXTRACTS OF WHEAT SEEDLING JUICE POWDER (*T. AESTIVUM* L.) AND L-ASCORBIC ACID (CONTROL) WAS SPECTROPHOTOMETRICALLY MEASURED AT 517 nm USING DPPH ASSAY.

(Given values are average of triplicate i.e. n=3; DPPH= 1.125).

The experimental data which obtained from all two extracts, it is clear that methanolic extract (I) showed maximum percentage of inhibitory effect i.e. 58.6% at 100µg/mL of concentration. Among these two extracts and standard tested for *in vitro* antioxidant activity, using the DPPH method, the L-ascorbic acid, crude methanolic (I) and water (II) extracts of lyophilized wheatgrass juice powder (*T. aestivum* L.) showed antioxidant activity with IC_{50} values of 19.2, 81.4 and 131.3 respectively at 1-100 µg/mL concentration (**Tables 3-5**). However, none of the extracts were found to be more active than the standard i.e. (L-ascorbic acid with IC_{50} value of 19.2 at 1-100 µg/mL) since their IC_{50} value found to be higher. Free radical scavenging activity of methanolic (I) and water (II) extracts was confirmed in the present investigation (**Fig. 2-4**).

TABLE 3: *IN VITRO* DPPH ANTIOXIDANT ACTIVITY SHOWING IC_{50} VALUES OF STANDARD L-ASCORBIC ACID.

Extracts/ Standard	Antioxidant activity		
	DPPH Scavenging effect (%) at 1-100 µg/mL conc.	Inhibition Effect (%)	DPPH (IC_{50}) (µg/mL)
L-ascorbic acid	1	15.1	19.2
	10	29.4	
	20	48.5	
	30	67.5	
	40	87.2	
	50	95.1	
	60	96.1	
	70	97.1	
	80	97.2	
	90	97.2	
	100	97.3	

TABLE 4: *IN VITRO* DPPH ANTIOXIDANT ACTIVITY SHOWING IC50 VALUES FOR METHANOLIC EXTRACT OF WHEAT SEEDLING JUICE POWDER (*T. AESTIVUM* L.).

Extracts/ Standard	Antioxidant activity		
	DPPH Scavenging effect (%) at 1-100 µg/mL conc.	Inhibition Effect (%)	DPPH (IC_{50}) (µg/mL)
Methanolic Extract	1	10.5	81.4
	10	16.7	
	20	18.5	
	30	25.7	
	40	28.4	
	50	36.6	
	60	37.7	
	70	45.4	
	80	49.4	
	90	54.5	
	100	58.6	

TABLE 5: *IN VITRO* DPPH ANTIOXIDANT ACTIVITY SHOWING IC_{50} VALUES FOR WATER EXTRACT OF WHEAT SEEDLING JUICE POWDER (*T. AESTIVUM* L.).

Extract/ Standard	Antioxidant activity		
	DPPH Scavenging effect (%) at 1-100 µg/mL conc.	Inhibition Effect (%)	DPPH (IC_{50}) (µg/mL)
Water Extract	1	2.1	131.3
	10	9.7	
	20	16.8	
	30	18.4	
	40	23.8	
	50	27.4	
	60	25.2	
	70	27.7	
	80	37.6	
	90	36.7	
	100	35.1	

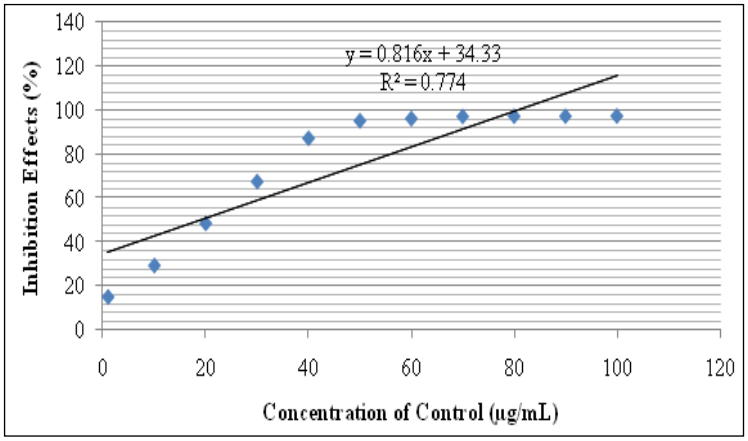


FIG. 2: *IN VITRO* DPPH ANTIOXIDANT ACTIVITY SHOWING GRAPH FOR IC_{50} VALUES OF STANDARD L-ASCORBIC ACID.

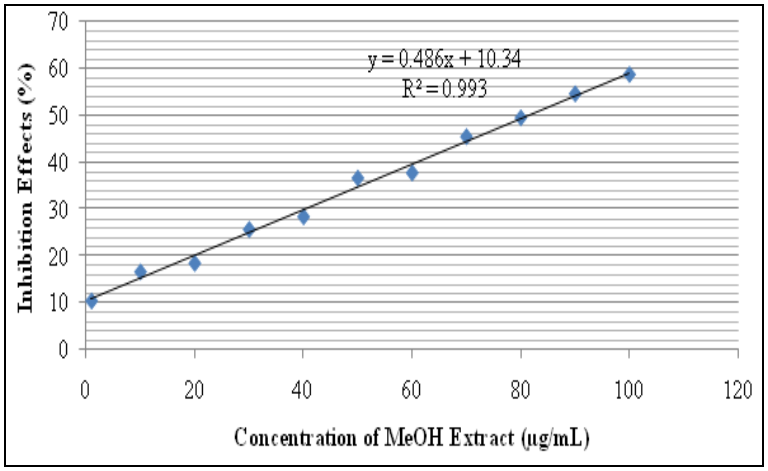


FIG. 3: *IN VITRO* DPPH ANTIOXIDANT ACTIVITY SHOWING GRAPH OF IC_{50} VALUES FOR METHANOLIC EXTRACT OF WHEAT SEEDLING JUICE POWDER (*T. AESTIVUM* L.).

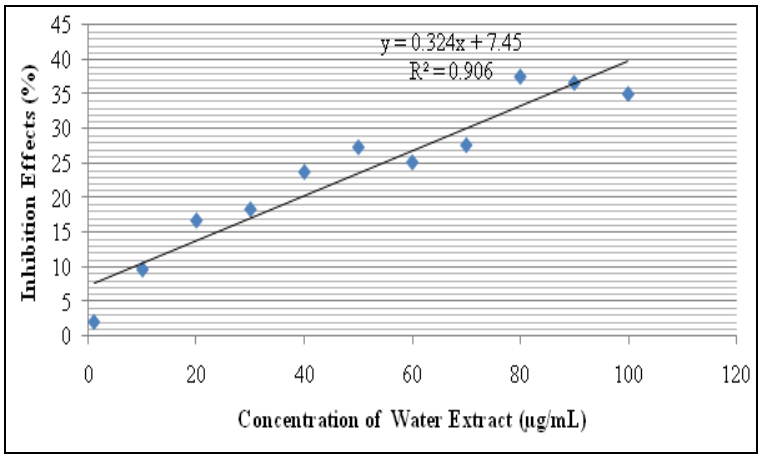


FIG.4: *IN VITRO* DPPH ANTIOXIDANT ACTIVITY SHOWING GRAPH OF IC50 VALUES FOR WATER EXTRACT OF WHEAT SEEDLING JUICE POWDER (*T. AESTIVUM* L.).

The methanolic and water extracts have been chosen because of its expected flavonoid contents that were reported to have antioxidant activity in many other plants [36]. The activity of phenolics having antioxidant activity further confirmed this view [37]. The phytochemical analysis of wheatgrass showed the presence of tannins, flavonoids, saponins, and sterols. The antioxidant activity of the phenolic, tannins, and flavonoid compounds are attributed to its redox properties which can act as reducing agents, hydrogen donators, and singlet oxygen quenchers[38]. Polyphenolic shaving hydroxyl groups are very important plant constituents which can protect body from oxidative stress [39].

The major clinical utility of wheat grass in diseased conditions might be due to the presence of biologically active compounds and minerals in it and due to its antioxidant potential which is derived from its high content of bioflavonoids. Furthermore, few indole compounds present in it might be also responsible for its therapeutic potential. The presence of chlorophyll, which is almost chemically identical to haemoglobin, in wheat grass makes it more useful in various clinical conditions involving haemoglobin deficiency and other chronic disorders [40].

Phytochemical studies are in progress to evaluate the therapeutic efficacy of wheat grass, grown through the concept of organic farming and free from pesticides, herbicides, insecticides, heavy metals and other harmful chemicals and toxins, in various disease conditions and the patients are being benefitted by the multitude potential of wheat grass.

Considering the facts that very little scientific and clinical studies have been done on the use of wheat seedlings in various diseases, efforts are needed to conduct extensive studies on the wheat seedlings

both in experimental models and human subjects to develop wheat seedling therapy. Since the production of organic wheat grass is cheap and it does not require the involvement of pharmaceutical industry, wheat grass therapy could be easily developed as an alternative or complimentary therapy for the benefit of large number of people suffering from chronic diseases in developing countries.

CONCLUSION: In the present research, attempts have been made to provide the scientifically and clinically approved studies on the freeze dried wheatgrass juice powder (*T. Aestivum* L.) for better understanding of therapeutic potential of this medicinal grass. The present study clearly concluded that methanol extract of wheatgrass possesses the ability to control oxidative damage and acts as antioxidant. Its free radical scavenging property has potential to prevent many complications. Our current investigation supports the traditional use of wheatgrass in the treatment of various diseases.

Wheatgrass naturally rich in essential vitamins, minerals, enzymes, amino acids, dietary fibres and world's best antioxidant "chlorophyll" may be useful for all age groups. Thus wheatgrass therapy may serve as a preventive and curative measure for such health problems which may lead to serious diseases like diabetes mellitus, anaemia, ulcers, arthritis, cancer, thalassemia and others.Thus it is clear that polyphenolic antioxidants in wheatgrass juice powder play an important role as bioactive principles. However, polyphenolic constituents present in these extracts which are responsible for this activity need to be investigated in detail.

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REFERENCES:

1. Duke JA: *Triticumaestivum*L. Handbook of energy crops. 1983.
2. Fahey JW, Stephenson KK, Dinkova-Kostova AT, Egner PA, Kensler TW and Talalay P: Chlorophyll, chlorophyllin and related tetrapyrroles are significant inducers of mammalian phase 2 cytoprotective genes. Carcinogenesis 2005; 26(7):1247-1255.
3. Wigmore A. The wheat grass Book, New York. Avery Publishing group 1986.
4. Walters R: The Alternative Cancer Therapy Book. New York Avery Publishing Group, 299-308, 1992.
5. Kulkarni SD, Tilak JC, Acharya R, Nilima RS, Devasagayam TPA and Reddy AVR: Evaluation of the antioxidant activity of wheatgrass (*Triticumaestivum* L.) as a function of growth under different conditions. Phytother Res 2006; 20(3): 218-227.
6. Hänninen O, Rauma AL, Kaartinen K and Nenonen M: Vegan diet in physiological health promotion. ActaPhysiol Hung 1999; 86: 171-80.
7. Lai CN, Dabney BJ and Shaw CR: Inhibition of in vitro metabolic activation of carcinogens by wheat sprout extracts. Nutr Cancer 1978; 1: 27-30.
8. Lai CN: Chlorophyll: the active factor in wheat sprout extracts inhibiting the metabolic activation of carcinogens *in vitro*. Nutr Cancer 1979; 1: 19-21.
9. Meyerowitz S: "Nutrition in Grass" Wheatgrass Nature's Finest Medicine: The complete guide to using grass foods & Juices to revitalize your health 6thed. Great Barrington (Massachusetts): Sproutman Publications, 1992; 53.
10. Falcioni G, Alzuola I, Marsili V and Gianfranceshi GL: Antioxidant activity of wheat sprout extracts in vitro inhibition of DNA oxidative damage. Journal of food Science 2002; 67: 2918-2922.
11. Peryt B, Szymezy KT and Lesca P. Mechanism of antimutagenecity of wheat sprout extracts. Mutation Research 1992; 269: 201-205.
12. Marwaha RK, Bansal D, Kaur S and Trehan A: Wheatgrass juice reduces the transfusion requirement in patients with thalassemia major: a pilot study. Indian Pediatrics 2004; 41: 716-720.
13. Ben-arye E, Goldin E, Wengrower D, Stamper A, Kohn R and Berry E: Wheatgrass juice in the treatment of active distal ulcerative colitis a random double blind placebo-controlled trial. Scandinevian Journal of Gastroenterology 2002; 37: 444-449.
14. Bar-Sela G, Tsalic M, Fried G and Goldberg H: Wheatgrass juice may improve haematological toxicity related to chemotherapy in breast cancer patients: A pilot study. Nutrition and Cancer 2007; 58: 43-48.
15. Alitheen NB, Oon CL, Keong YS, Chuan TK, Li HK and Yong HW: Cytotoxic effects of commercial wheatgrass and fibre towards human acute promyelocytic leukaemia cells (HL60).*Pak J Pharm Sci* 2011; 24(3): 243-250.
16. Thompson LU: Antioxidants and hormone-mediated health benefits of whole grains. Critv Food SciNutr 1994; 34: 473-97.
17. Jacobs DR, Jr Andersen LF and Blomhoff R: Wholegrain consumption is associated with a reduced risk of non-cardiovascular, non-cancer death attributed to inflammatory diseases in the Iowa Women's Health Study. Am J ClinNutr 1998; 68: 248-57.
18. Meyer KA, Kushi LH, Jacobs DR Jr, Slavin J, Sellers TA and Folsom AR. Carbohydrates, dietary fiber, and incident type2 diabetes in older women. Am J ClinNutr 2000; 71: 921-30.

19. Nicodemus KK, Jr Jacobs DR and Folsom AR: Whole and refined grain intake and risk of incident postmenopausal breast cancer (United States). Cancer Causes Control 2001; 12: 917-25.
20. Calzuola I, Marsili V and Gianfranceschi GL: Synthesis of antioxidants in wheat sprouts. J. Agric Food Chem 2004; 52: 5201-6.
21. Blokhina O, Virolainen E and Fagerstedt K: Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 2003; 91: 179-94.
22. Kim JK, Noh JH, Lee S, Choi JS, Suh H, Chung HY, Song YO and Choi WC: The first total synthesis of 2, 3, 6-tribromo-4, 5- dihydroxybenzyl methyl ether (TDB) and its antioxidant activity. Bull Korean Chem Soc 2002; 23(5): 661-662.
23. Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Huynh-Ba T, Lambelet P, Mc Phail D, Skibsted LH and Tijburg L: Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. Eur Food Res Technol2001; 212: 319-328.
24. Blois MS: Antioxidant determinations by the use of a stable free radical. *Nature* 1958; 181: 1199-1200.
25. Leita o GG, Leit a o SG and Vilegas W: Quick preparative separation of natural naphthoquinones with antioxidant activity by high-speed counter-current chromatography. J Naturforsch 2002; 57c:1051-1055.
26. Brand-Williams W, Cuvelier ME and Berset C: Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 1995; 28: 25-30.
27. Treare, GE and Evans WC: Pharmacognosy, 17 edn, Bahive Tinal, London, 1985: 149.
28. Wagner HXS, Bladt Z and Gain EM: Plant drug analysis. Springer Veralag, Berlin, Germany. 1996; 360.
29. Gibbs RD: Chemotaxonomy of Flowering Plants.Vol.1, McGill Queen's University Press, Montreal and London 1974.
30. Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N and Krishnan MRV: Phytochemical investigation on a tropical plant. Pak J Nutri 2009; 8: 83-85.
31. Rizk AM: Constituents of plants growing in Qatar. Fitoterapia 1982; 52: 35-42.
32. Liu AL: *In vitro* anti-influenza viral activities of constituents from *Caesalpiniasappan*, Planta Med 2009; 75: 337-9.
33. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO: Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Trop J Pharm Res 2008; 7:1019-1024.
34. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J ClinNutr 1999; 70: 475S-90S.
35. Marx JL: Oxygen free radicals linked to many diseases. Science 1985; 235: 529-31.
36. Gupta RC, Sharma V, Sharma N, Kumar N and Singh B: *In vitro* Antioxidant Activity from Leaves of *Oroxylum indicum*(L.) Vent. -A North Indian Highly Threatened and Vulnerable Medicinal Plant. Journal of Pharmacy Research 2008; 1: 66-72.
37. Imran M, Khan H, Shah M, Khan R and Khan F: Chemical composition and antioxidant activity of certain Morus species. Journal of Zhejiang University 2010; 11(12): 973–980.
38. Gulcin I, Elmastas M and Aboul-Enein HY: Determination of antioxidant and radical scavenging activity of basil (*Ocimumbasilicum* L. Family Lamiaceae) assayed by different methodologies. Phytotherapy Research 2007; 21(4): 354–361.
39. Jing LJ, Mohamed M, Rahmat A and Bakar MFA: Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda, Boesenbergiapulchella* varattenuata and *Boesenbergiaarmeniaca*). Journal of Medicinal Plant Research 2010; 4(1): 27–32.
40. Singh N, Verma P, Pandey BR: Therapeutic Potential of Organic *Triticum aestivum* Linn. (Wheat Grass) in Prevention and Treatment of Chronic Diseases: An Overview. International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(1): 10-14.

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