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Pharmacological Evaluation of Antioxidant Potential of Hydro-Alcoholic Extract of *Triticum aestivum* (Wheat Grass)

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KEYWORDS

ABSTRACT:
The present study was based on the pharmacological evaluation of antioxidant potential of hydro-alcoholic leaves extract of Triticum aestivum (Wheat grass). T. aestivum was grown under indoor conditions in Bareilly. The plant was authenticated by Dr. Alok Srivastava (Associate Professor) Plant Science. The authentication letter reference no. RU/PS/19/03 as issued from department of plant science MJP Rohilkhand University, Bareilly. Dried powdered leaves (50gm) and root (50gm) were extracted separately with Soxhlet apparatus using 500 ml of hydro-alcoholic solution (1:1) ethanol: water for 24 hrs. All the animals were divided into 4 groups (n=6) i.e., group I (control) rats treated with vehicle only (0.5% carboxymethylcellulose sodium, p. o.). Group II, III, and IV rats administered leaves extract of Triticum aestivum (100, 200, and 400mg/kg, p. o.), respectively, for 7 days. Blood samples of the experimental animals was collected by retro orbital artery bleeding after 16 hr of the last dose. Blood samples were centrifuged for 10 minutes at 2000 rpm to separate the serum. Triticum aestivum leaf extract evaluated for GSH, CAT, LPO, and SOD. In results, upon calculation, the % yield for Triticum aestivum leaves and root extract was obtained as 54% and 18%, respectively. (Triticum aestivum leaf extract) the % antioxidant activity was observed as 24.56*%, 25.21*%, 39.10*% and 64.85*% in the concentration of 50µI, 100µI, 150 µI and 200 µI, respectively. SOD level was observed as 3.84±1.41 U/mg protein, 4.07±0.13 U/mg protein and 4.10±0.61 U/mg protein in group 2, 3 and 4, respectively which exhibited the significant decrease in SOD level. In conclusion, a potent antioxidant and modulation effects in CAT, GSH, and TBARS by the hydroalcoholic leaves extract of T. aestivum. In future aspect, it suggests to determine the effectiveness and safety of T. aestivum hydroalcoholic leaves extract as a possible natural antioxidant source.

INTRODUCTION

Triticum aestivum (wheatgrass) of the Kingdom plantae, family Poaceae (Gramineae) and genus Triticum L. has been a major nutritional source since the history of man. Wheatgrass is generally known for its high chlorophyll content which represents 70% of its substance constituents. Wheatgrass has been utilized as conventional homegrown medication and is profoundly esteemed for its remedial and healthful properties [1]. The viability of the plant removes is for the most part because of the nearness of bioactive constituents like phenolics, flavonoids and different substance. During germination, nutrients, minerals, and phenolic mixes incorporating flavonoids are integrated in wheat sprouts, and wheat grows arrive at the most extreme cancer prevention agent potential [2]**.** Flavonoids produce a wide scope of organic impacts in a few mammalian cell frameworks and this was demonstrated by numerous individuals in vitro and in vivo tries. Rutin additionally called rutoside, quercitin-3-rutinoside and sophorin widely found in numerous plants, including Triticum aestivum. This flavonoid has a wide scope of organic exercises antimicrobial, calming, cancer prevention agent, neuroprotective, antiviral, and antiulcerogenic. The anticarcinogenic efficacy of rutin has not yet been completely investigated [3][4]. Wheatgrass juice is a rich wellspring of Vitamins A, C, E and B complex including B12. It contains a huge number of minerals, for example, calcium, phosphorus, magnesium, soluble earth metals, potassium, zinc, boron and molybdenum [5]. The different proteins answerable for its pharmacological activities are protease, amylase,

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lipase, cytochrome oxidase, transhydrogenase and super oxide dismutase [6].

Fig 1. *Triticum aestivum* (Wheat Grass)

The other outstanding component of wheatgrass is its high extent of amino acids, for example, aspartic corrosive, glutamic corrosive, arginine, alanine and serine. For more than 8000 years, wheat has played a vital role in human diets throughout Europe, Anatolia, west Asia, and northern Africa [7]. As one of the "huge three" cereal crops, wheat is the primary food crop grown worldwide, with about 600 million tons harvested annually. One important source of plant sterols in the diet of humans is bread wheat. One of the major wheat-producing countries in the world is Turkey. Turkey produced over 21.03 million tons of wheat between 2011 and 2016 [8]. It was traditionally utilized in asthma, atherosclerosis, Parkinson's disease, joint pains, constipation, hypertension, diabetes, insomnia, bronchitis, sterility, hemorrhage and obesity [9].

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Poales
Family	:	Poaceae
Genus	:	Triticum
Species	:	*aestivum*

It likewise has a high substance of bioflavonoids like apigenin, quercetin and luteolin. These compounds add to its cancer prevention agent movement. Different mixes present, which make this grass remedially successful, are the indole mixes, choline and laetrile (amygdalin) [10]**.** The present study was aimed to investigate chemical constituents and to evaluate antioxidant activity of wheat grass leaves and roots in hydro-alcoholic extract.

MATERIALS AND METHODS

Cultivation and harvesting and of plant material

First of all, Wheat grass (T. aestivum) was grown under indoor conditions in Bareilly. Overnight soaked wheat grass seeds were used to cultivate. Little quantities of water were sprinkled evenly over soil and 3-4 hours of indirect sunlight projection was allowed daily for growth of grass, on tenth day wheat grass was harvested and used for further study

Authentication of plant material

After complete process of dryness, prepared herbarium file of the plant and submitted for authentication in Department of Plant Science, MJP Rohilkhand University Bareilly. The plant was authenticated by Dr. Alok Srivastava (Associate Professor) Plant Science. The authentication letter reference no. RU/PS/19/03 as issued from department of plant science MJP Rohilkhand University, Bareilly.

Air drying of plant material

The root and leaves of Triticum aestivum were collected separately and dried in shade at room temperature for air drying.

Size reduction of plant material

After air drying root and leaves of Triticum aestivum were chopped with the help of knife and then powdered with a mechanical grinder. The powder will be passed through sieve no.40 and stored in a labeled air tight container.

Extraction of Plant

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Dried powdered leaves (50gm) and root (50gm) were extracted separately with Soxhlet apparatus using 500 ml of hydro-alcoholic solution (1:1) ethanol:water for 24 hrs. The solvent (500ml) added to each round bottom flask, which was attached to a Soxhlet extractor and condenser. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When, the Soxhlet chamber is gradually fill the level of solvent reaches the siphon it pours back into the distillation flask. This cycle was allowed to repeat many times for 24 hours then, the solution of extract placed on water bath for a few days at a 40-50 °C for the removal of solvents and obtained drug extract [11].

The % yield was calculated as % Yield= Weight of extract (g) / Weight of dry powder (g) ×100

Pharmacological screening

A. In-Vitro Antioxidant Activity

DPPH Radical Scavenging Assay: Free radical scavenging activity of different extracts of leaves of wheat grass plant was measured by 2,2-diphenyl-1-picryl hydrazyl (DPPH). In brief, 0.1 M solution of DPPH in methanol was prepared. This solution (1 ml) was added to 3 ml of different extracts in methanol at different concentration (10, 20, 30 µg/ml). Here, only those extracts are used which are soluble in methanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp for 30 min then absorbance was measured at 517 nm by using UV Spectroscopy. Reference standard compound being used was ascorbic acid. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity [20][21].

B. In-Vivo Antioxidant Activity

Experimental Animals: Albino rats (130–160g) of either sex were obtained from CPCSEA approved breeder/Animal house. They will be randomly housed at an ambient temperature of 25 ± 1°C and 45–55% relative humidity, in polypropylene cages with 12 h light: 12 h dark cycle. The animals were allowed free access to standard food pellets and water ad libitum. All the experimental protocols were conducted before the prior permission of Institutional Animal Ethics Committee of the Institute (IFTM University, Lodhipur Rajpoot, Moradabad. Registration No 837/PO/ReBiBt/S/04/CPCSEA) for the purpose of control and supervision of experiments on the animal [22].

Group design

All the animals were divided into 4 groups (n=6) as follows:

- Group I animals treated with vehicle only (0.5% carboxymethylcellulose sodium) and served as control group.
- Group II, III, and IV animals administered orally with 100, 200, and 400mg/kg of leaves extract of Triticum aestivum (LETA), respectively, for 7 days.

Table 1. Group design

	Number of animals in each group			
	Experimental Groups			
Control Group Group 1	**Group 2**	**Group 3**	**Group 4**	**Total Animals required**
Vehicle only	100 mg/kg LETA extract	200 mg/kg LETA extract	400 mg/kg LETA extract	
06	06	06	06	24

Blood collection

Blood samples of the experimental animals was collected by retro orbital artery bleeding after 16 hr of the last dose. Blood samples were centrifuged for 10 minutes at 2000 rpm to separate the serum. The rats were sacrificed by diethyl-ether anaesthesia on day 8 and liver was excised, rinsed in 0.25 M sucrose solution and 10% w/v homogenate was prepared in 0.15M KCl, centrifuged at 1000 rpm for 10 min followed by centrifugation of the supernatant at 12000 rpm for 15 min. The supernatant obtained was used for estimation of various oxidative enzymes.

Estimation of Serum Antioxidant Enzymes: Various enzymes involved in the oxidative damage to the tissues due to toxicity were estimated as per the prescribed methods.

1. Estimation of Superoxide Dismutase:

Procedure: Equal quantity (0.5ml) of plasma and ice-cold water was mixed thoroughly to make total volume of 1 ml. About 2.5 ml ethanol and 1.5ml chloroform (chilled reagent) was added to the above mixture and shaken for 60 seconds before its centrifugation at 4˚C. The enzyme activity in the supernatant was determined

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as follows. Assay mixture contained 0.1ml of sample, 0.2ml NADH, 0.1ml of Phenazine methosulphate (186µM), 1.2ml of sodium pyrophosphate buffer (pH 8.3, 0.052M) and 0.3ml of 300µM nitrobluetetrazolium. The mixture was incubated at 30˚C for 90 seconds followed by stirring with 4 ml of n-butanol. Mixture was permitted to stand for 10 min before its centrifugation and butanol layer was separated. Colour intensity of the chromogen in the butanol layer was measured at 560 nm spectrophotometrically and concentration of SOD was expressed as units/mg protein. One unit of enzyme activity was defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute [23].

2. Estimation of Catalase:
Procedure: To 0.1ml of liver homogenate supernatant, 1ml of 0.01 M phosphate buffer (pH 7.0) was added. One ml of freshly prepared 30mM $H_2O_2$was added to start the reaction and as soon as the reaction is completed, 2ml of dichromate-acetic acid reagent (5% $K_2Cr_2O_7$ in glacial acetic acid) was added to discontinue the reaction. Spectrophotometric change in the absorbance at 620nm was observed to evaluate the rate of decomposition of H_2O_2. Activity of catalase was expressed as units/mg protein [24].

3. Assay of Lipid peroxidase:
Procedure: The thiobarbituric acid reactive substances (TBARS) assay was performed by standard method [Ohkawa et al., 1979] using MDA equivalents derived from tetraethoxypropane. The method involved heating of biological samples with TBA reagent (20% Trichloroacetic acid, 0.5% TBA and 2.5 N HCl) for 20 min in a boiling water bath. After cooling, the solution was centrifuged at 2000 rpm for 10 min and the precipitate obtained was removed. The absorbance of supernatant was determined at 532 nm against a blank that contained all the reagents except biological sample. The level of lipid peroxides was expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient $1.56x10^5M^{-1}cm^{-1}$[24].

4. Assay of Reduced Glutathione (GSH):
Procedure: To measure the GSH level, the tissue homogenate in 0.1 M phosphate buffer (pH 7.4) was taken. Equal volumes of 20% trichloroacetic acid (containing 1 mM EDTA) and homogenate was mixed to precipitate the tissue proteins. The mixture was centrifuged for 10 min at 200 rpm and the supernatant (200 µl) was then transferred to a new set of test tubes. Simultaneously Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) will be prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution and was added (1.8 ml) to the test tube. Then all volume of the test tubes was made up to 2 ml. The solutions were measured at 412 nm against blank after completion of the total reaction. Absorbance values were evaluated with a standard, obtained from standard curve of known GSH [25].

RESULTS AND DISCUSSION

Percentage yield
Upon calculation, the % yield for Triticum aestivum leaves and root extract was obtained as 54% and 18%, respectively as shown in below tables-

Pharmacological Screening
Triticum aestivum Leaves leaf extract was tested for the antioxidant activity. In sample (Triticum aestivum leaf extract) the % antioxidant activity was observed as 24.56*%, 25.21*%, 39.10*% and 64.85*% in the concentration of 50µI, 100µI, 150 µI and 200 µI, respectively. However, the standard (ascorbic acid) demonstrated the antioxidant activity as 68.66**% at the concentration of 200 µI. Thus, percent inhibition of the herbal extract was found almost similar to the standard group at its highest dose. The free radical scavenging property was determined in the dose-dependent manner.

A. In Vitro Screening Methods of Antioxidant Activity: DPPH Free Radical Scavenging Activity

Table 2: DPPH radical scavenging activity of Triticum aestivum L. leaf extract

Concentration	OD 517 nm		% antioxidant Activity	
	Sample	Standard	Sample	Standard
50µl	1.061	2µl 1.022	24.56*	28.36*
100µl	0.753	4µI 0.798	25.21*	35.72*
150 µl	0.899	6µI 0.928	39.10*	61.82**
200 µl	0.403	8µI 0.449	64.85*	68.66**

Control OD at 517 nm 1.433, *P<0.05, **P<0.01, and P< 0.001

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(vs. control), n=3.

DPPH scavenging effect (%) or Percent inhibition = $A_{Std} – A_{Test} / A_{Std} \times 100$.

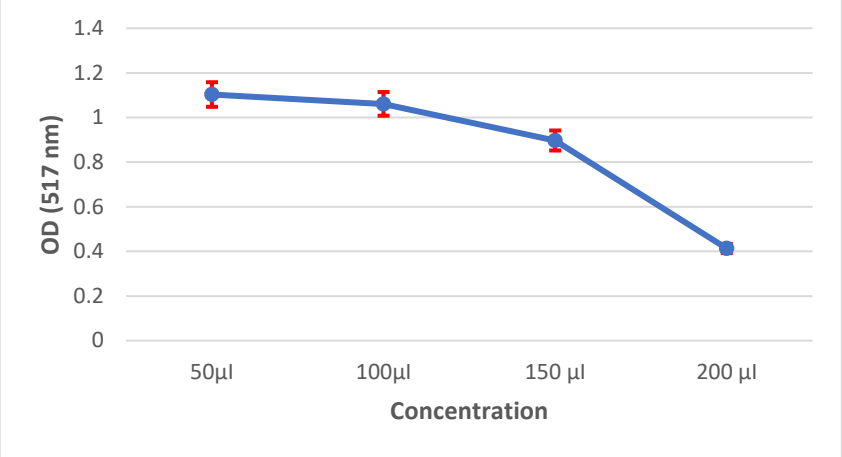

Fig 2. Concentration and absorption of sample at 517 nm

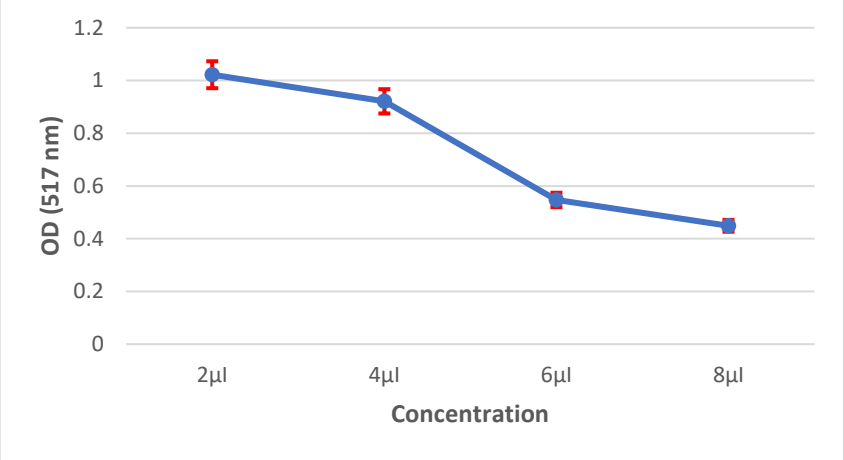

Fig 3. Concentration and absorption of standard at 517 nm

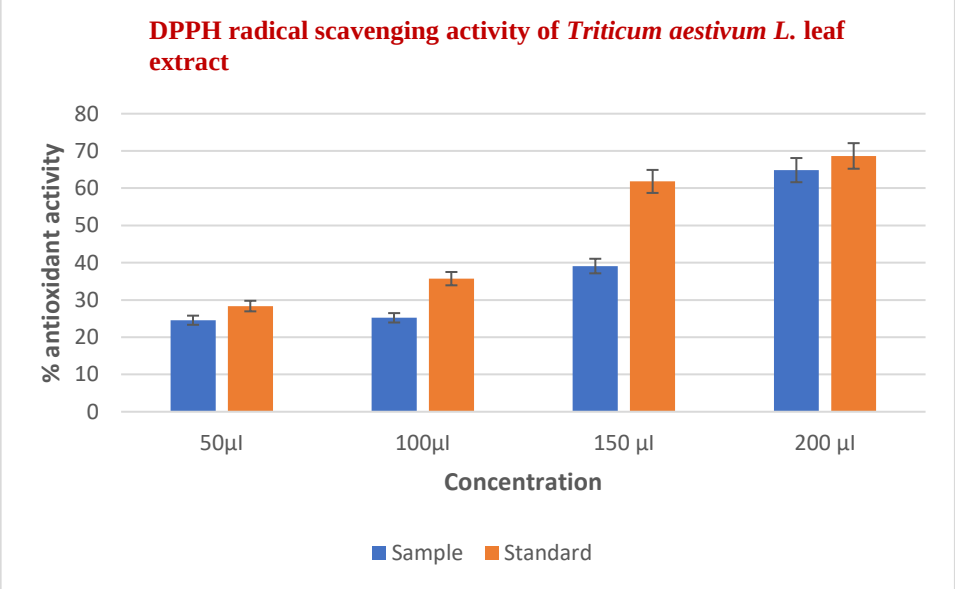

Fig 4. DPPH radical scavenging activity of Triticum aestivum L. leaf extract

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B. Biological Parameters

Estimation of Serum Antioxidant Enzymes:

Triticum aestivum L. leaf extract exhibited a modulatory response on the serum enzyme levels when tested for the assay of Reduced Glutathione (GSH), Estimation of Catalase (CAT), Assay of Lipid Peroxidase (LPO), and Estimation of Superoxide Dismutase (SOD).

SOD level was observed as 3.84±1.41 U/mg protein, 4.07±0.13 U/mg protein and 4.10±0.61 U/mg protein in group 2, 3 and 4, respectively which exhibited the significant decrease in the Superoxide Dismutase (SOD) level. While, control group showed SOD level as 4.02±0.04 U/mg protein. Group 2 demonstrated the highest decrease in SOD level. Group 2 treated with leaves extract of Triticum aestivum(100mg/kg) exhibited the highest decrease in catalase level as 17.12±1.07 U/mg protein, in comparison to group 3 (200mg/kg) and group 4 (400mg/kg). Thus, Triticum aestivum herbal extract showed protective behavior at all the dose levels when compared to control group.

Similarly, highest inhibition of TBARS was observed as 186.26±7.63 nM/mg of protein in Group 2 which was administered leaves extract of Triticum aestivum (100mg/kg). The effect was observed in descending order with increase in the dose. Minimum TBARS level was estimated in control group.

Table 3: Estimation of effect of *Triticum aestivum* L. leaf extract on Serum Antioxidant Enzymes

Parameter	Group 1	Group 2	Group 3	Group 4
SOD	4.02±0.04	3.84±1.41	4.07±0.13	4.10±0.61
CAT	26.32±2.42	17.12±1.07	25.21±2.31	23.19±0.61
TBARS	141.07±3.25	186.26±7.63	172.32±4.23	175.47±9.08
GSH	10.32±0.62	5.91±5.26	8.98±5.27	8.55±0.35

Values are expressed mean ± S.D for six rats in each group. 1 As compared with control, 2 represents P<0.001, * represents P<0.01. GSH activity (µg/mg protein), CAT (U/mg protein), SOD(units/mg protein) & TBARS (nM/mg of protein).

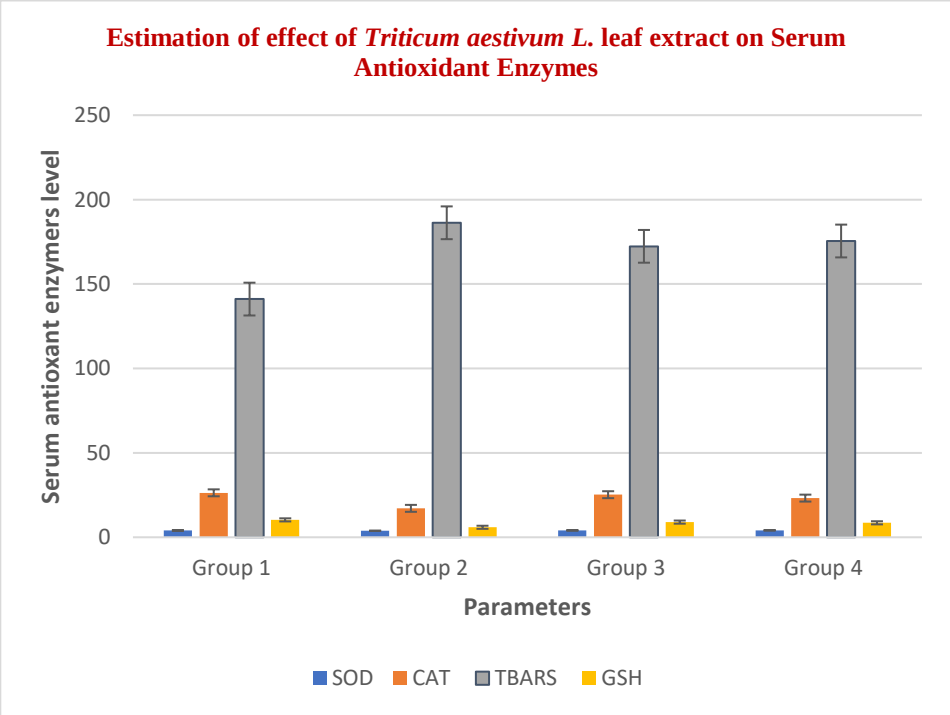

Fig 5. Estimation of effect of Triticum aestivum L. leaf extract on Serum Antioxidant Enzymes

In the previous study, it was determined whether T. aestivum's methanolic extract inhibited the generation of nitric oxide. T. aestivum prevented the formation of nitric oxide radicals from sodium nitroprusside at physiological pH. It was discovered that as their concentrations grew, so did the nitric oxide scavenging activity of the T. aestivum methanolic extract. The extracts exhibit a fair percentage of inhibition that is similar to that of ascorbic acid. The extract's ferric reducing ability ranged from 0.06 to 0.15 at 200µg–1000µg, while conventional ascorbic acid has a range of 0.07 to 0.16. Compared to ascorbic acid, the FRAP

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values in the Triticum aestivum methanolic extract were substantially lower. By reducing the oxidized intermediates of the lipid peroxidation process, the antioxidant compounds can function as primary and secondary antioxidants, as indicated by their reducing power attribute, which also shows that they are electron donors. Every lab and researcher interested in oxidative stress and its consequences can benefit from the FRAP tests, which provide a possible index of antioxidant defense that is both technologically accessible and useful. Figure 2.7 illustrates an increase in absorbance from 0.06 at 200 µg/ml to 0.15 at 1000 µg/ml, indicating that the ferric reducing capacity of T. aestivum's methanolic extract was comparable to that of synthetic antioxidants.

Due to the high concentration of polyphenols and flavonoids found in these herbs, there is a cumulative effect that results in enhanced anti-inflammatory properties of both herbs. The antioxidant activity of wheatgrass was found to have potent antioxidant

properties at different concentrations according to their seed developmental stages [26]. Ascorbic acid in coconut water is reported to inhibit lipid peroxidation in rats in animal research by Mat et al., while L-arginine, another ingredient in coconut water, lowers the production of free radicals [27].

Numerous studies have shown that the coconut's ability to scavenge free radicals is also responsible for its enhanced antioxidant properties [28][29]. Vitamins that are abundant in wheatgrass can scavenge free radicals and are crucial parts of antioxidant defense systems. This may help control how much hydrogen peroxide is released from the cells. Wheatgrass has stronger anti-inflammatory properties and helps remove toxins from the body in addition to its high protein and amino acid content and antioxidant activity. Wheatgrass has been shown in a study by Dasari et al. to have an anti-inflammatory effect on formalin-induced rat paw edema [30].

The high concentration of readily absorbed vitamins, enzymes, and other trace minerals in both phytomedicines may also contribute to the gel's overall increased effect. It has also been highlighted that these phytochemicals' antioxidant activity contributes to chemoprevention by lowering the oxidative stress that leads to the genesis of cancer [31].

In addition, group 4 treated with leaves extract of Triticum aestivum (400mg/kg) exhibited the maximum Reduced Glutathione Level as 8.55±0.35 µg/mg protein. However, group 2 and 3 showed less significant effect in contrast to control group. Therefore, in all the enzyme parameters Triticum aestivum was found to be an effective anti-oxidant herbal extract that might be due to reducing the cellular oxidation and release of ROS.

CONCLUSION

The T. aestivum leaves extract in this investigation demonstrated the highest levels of antioxidant activity both in vitro and in vivo, which may have been caused by the high flavonoid content of these two extracts. The evaluation of T. aestivum's in vitro antioxidant status against a wide range of free radical scavengers offers compelling evidence that the plant may function as a natural source of antioxidant defense. It was shown that nearly every antioxidant had a notable effect on the free radical scavenging activity. A dose-dependent increase was seen in the scavenging of DPPH, SOD, CAT, Reduced Glutathione (GSH), and TBARS by the hydroalcoholic leaves extract of T. aestivum.

In future prospectives, it suggests to determine the effectiveness and safety of T. aestivum hydroalcoholic leaves extract as a possible natural antioxidant source. Its biologically active constituent would be incorporated in suitable dosage form for better bioavailability and pharmacological response.

FUNDING

Nil.

CONFLICT OF INTEREST

Authors have declared for none ‘conflict of interest’.

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