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Evaluation of Antioxidant Activity of *Triticum aestivum* Methanolic extracts Using Fenton reaction

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ABSTRACT

The study was mainly extracts of the leaves of wheatgrass (Triticum aestivum) to show the antioxidant activity in food and pharmaceutical supplements. A detailed study was performed on the antioxidant activity of the methanol extract of Triticum aestivum (Potted, Hydroponic & Field). In the present study the methanolic extract Triticum aestivum was evaluated for antioxidant activity by the OH free radical scavenging activity using Fenton reaction. This study was conducted to investigate the effect of Triticum aestivum extract using Fenton reaction. The dried leaf of Triticum aestivum was extracted with methanol using a Soxhlet extractor. The total phenolics content of bark as determined by Fenton reaction and was found to be good antioxidant activity as dose depended manner. The antioxidant activity of plant extract was carried put with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer. In this plant Triticum aestivum extract, there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. The result suggests that the Triticum aestivum extract can be used as food antioxidant together with the improvement of food palatability. Further studies are in processing of analyzing the synergic association of extract with synthetic antioxidant and to identify compounds with antioxidant activity in cinnamon extracts. In conclusions the present study indicated that Triticum aestivum extract may be a potential source of a natural antioxidants.

Keywords: *Triticum aestiyum; hydroponic; extraction; methanolic extracts; phenolic content; flavonoid content; antioxidant activity.*

INTRODUCTION

Natural products especially those of wild origin, always have been an important source of therapeutic agents. Natural products are frequently based an ethanol botanical information as of now many of the drugs used are developed from medicinal plants (Heinrich, et. al., 2003). The herbal medications have been used for relieving the symptoms of many of the diseases (Maqsood, et al., 2010). Natural antioxidants extracted in their crude form contain many chemical constituents, which are very effective to prevent the destructive processes caused by oxidative stress (Zengin, et al., 2011). Wheat grass is believed to have many unexplained natural healing qualities. Many of the phytonutrients (plant nutrients) contained in cereal grasses have yet to be identified and it is not completely known how they provide such great benefits to our health. Wheat Grass is one of the most alkaline green leafy vegetables known and part of the cereal grass family. Wheat grass is a affordable way to get your daily life servings of fruits and vegetables. Each serving wheatgrass is packed full of vitamins, minerals, enzymes, amino acids, and phytonutrients and carotenoids to promote optimal Health. The antioxidant contents of medicinal plants may contribute to the protection (Harman D., 1998). Antioxidant agents of natural origin of their free radical scavenging capacity (Osawa., et al., 1990). The use of medicinal plants with a high level of antioxidant

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constituents has been proposed as an effective therapeutic approach for hepatic damages (Govind, 2011). Polyphenol antioxidants have protective effects against different diseases, including cardiovascular, inflammatory and neurological diseases, as well as cancers (Bandoniene D. & Murkovic M., 2002). The most of the polyphenols have been proved of their effectiveness in free radical scavenging capacities Antioxidants are chemical compounds that can bind to free oxygen radicals thus preventing these radicals from damaging healthy cells which could lead to cancer. The young grass of the common wheat plant, *Triticum aestivum* is known as wheatgrass. Wheatgrass is known to be a rich source of vitamins, antioxidants and minerals. It also contains Vitamin A, B1, C and E, many minerals including calcium, iodine, selenium and zinc. *Triticum aestivum* is known to contain antioxidant enzymes superoxide dismutase and cytochrome oxidase that have the potential to convert reactive oxygen species to a hydrogen peroxide and an oxygen molecule. Chlorophyll, one of the primary components in the wheatgrass extract, was found to augment blood formation then the immune system through inhibition of metabolic activation of carcinogens.

In the past research in nutrition and food science has focused on plant products with potential antioxidant activities. Such products are also rich in fibre, have no cholesterol and contain antioxidants such as carotenoids and flavonoids. The compounds which mainly responsible for the antioxidant effect are a class of phenolic compounds including flavonoids and their derivatives besides carotenoids and tocopherols (Nocole et al., 1996). The search is on for plant products with high antioxidant activities. Germination and sprouting causes extensive changes in the seeds. During this stage, the synthesis of useful compounds such as vitamins and phenolics occurs. Wheat *(Triticum aestivum)* germinated over a It is presumed that the wheatgrass is a rich source of vitamins, antioxidants and minerals in a bioavailable form. Wheatgrass contains vitamins C and E, β-carotene ferulic acid and vanillic acid whose concentration increases with the germination period and reaches mum on day 7 of growth (Hanninen et al., 1999). Wheatgrass extracts also possess superoxide scavenging and ferric reducing power (Peryt et al., 1992). Their ability to inhibit oxidative DNA damage was also demonstrated.

The antioxidant activity of *Triticum aestivum* at various levels of protection. The present study, the Antioxidant potential of *Triticum aestivum*, at different type of germination under different growth conditions. Methanolic extracts of wheatgrass, prepared from fresh wheat grass collected at various stages of growth. Samples were collected on 8-10,days after germination (Tap water and soil in pot & tap water).The antioxidant activities of Methanolic extracts were determined. The antioxidant activities of wheatgrass were estimated using different assays for its Evaluation of Antioxidant Activity of *Triticum aestivum* (field, tap water and soil in pot & tap water) Extract using Fenton Reaction Hydrogen peroxide Scavenging activity of the wheatgrass extracts was checked.

MATERIALS AND METHODS

Plant Material – *Triticum aestivem* (wheatgrass), Field (outdoor) at, Dhamtari and potted and tap water without soil (Hydroponic, Indoor), Raipur (Chhattisgarh), India.

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 a pot for the potted wheatgrass sample. The wheat grain soaked in cloth and covered by the cotton clothes and

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spray water in cloth within gap of 2 to 3 hrs which helps the sprouting. The plants after 9 to 11 days, increase in a height about 15-16 cm, it's for the hydroponic sample, and field wheatgrass (outdoor) were selected for the extraction procedure.

The wheat grass blades were cut and prepare juice with help of the mixture grinder. Juicefiltered in a cotton clothes and then evaporated juice the access amount of water by using hot plate& then pore sample in large size Petrie plates and dry in hot air oven for 4-5 hrs. In 55°C after dry scratch the Petri plates help of the spatula and collect a crystal form of the sample and this is the final Extraction.

Extraction process - The important part the process extraction process elements of West part and collect the active component use for the checked in Antioxidant Activity. Took.5gm of the crystal form of the sample and make a Thimble help of the Whatmman filter paper. Methanolic solvent prepare 1:1 and add flask and thimble kept the extraction chamber and provide a heat set temperature 64.7°C. The final extract kept on the air tight box.

Chemicals and Reagent samples - The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbance's were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Deoxyribose assay to assess OH -radical scavenging activity –
The OH- radical scavenging activity of *Triticum aestivum* (potted, field, & tap water) extract (10–100 ug/ml) was determined according to the deoxyribose method reported of (Halliwell et al.,1987). In the protocol the presence of 100 l MEDTA. FeCl3, H2O, and ascorbic acid were prepared in degassed H2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L ascorbic acid, 100 lM FeCl3, H2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbance's were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The result of lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs:532 nm Control Abs.532 nm sample Abs.} \times 100}{\text{532 nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC50, the concentration necessary for 50% inhibition concentration of TBARS.

RESULTS

The result of the effect of the examined *Triticum aestivum* extracts as well ascontrol solution on OH- radical production. They show all extract of *Triticum aestivum* extract and control solution asa Ascorbic acid inhibited the production of OH- radicals. The free radical scavenging activity of hydromethanolic extract of Triticumaestivum presented reducing power, the free radicals OH- scavenging activity of the extract increase with increasing the concentration. Extent of hydroxyl radical scavenged was determined the increasing in

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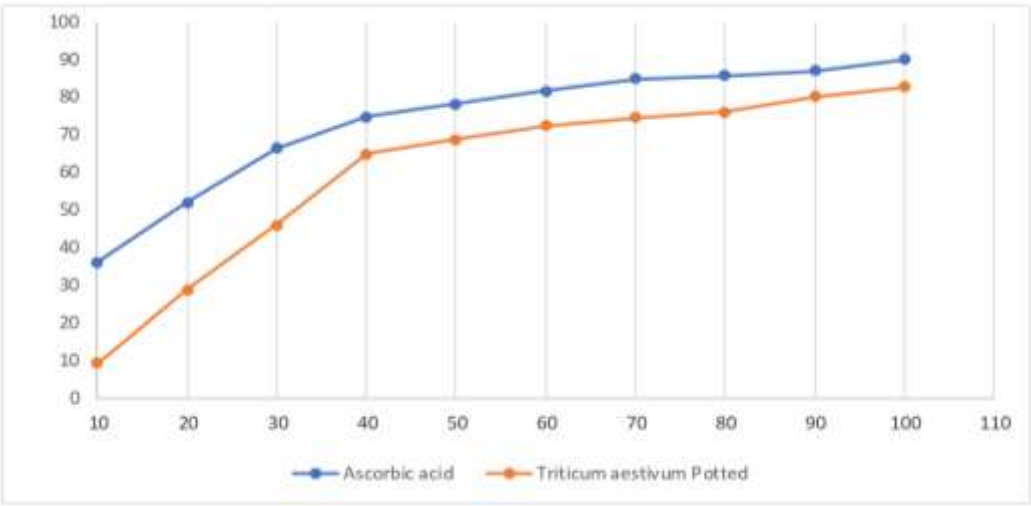
intensity of light-yellow coloured, which was determined at 532 nm. According to this result the potted & field of *Triticum aestivum* has shown better results than hydroponic of *Triticum aestivum*. The plant extract showed strong antioxidant capacity in vitro and thus the extract can be considered as a good source of natural antioxidant. We observed the result and found that the potted *Triticum aestivum* was performed well for OH- radical inhibition compare then field *Triticum aestivum*& hydroponic *Triticum aestivum* & the second opinion we found that the field *Triticum aestivum* performed better in for removing OH- radicals compare than hydroponic *Triticum aestivum.* In overall observation we found that the potted *Triticum aestivum* was best in performance and the hydroponic *Triticum aestivum* was low in performance.

Table 1: Antioxidant activity of Ascorbic acid and methanolic extract of Potted *Triticum aestivum*

Concentration (µg/µl)	Ascorbic acid (Mean±SE)	Triticumaestivum Potted (Mean±SE)
10	36.08±0.23	9.21 ±0.39
20	51.82±0.12	28.72±0.19
30	66.34±0.10	45.86±0.18
40	74.72±0.09	64.72± 0.34
50	78.15±0.07	68.72±0.16
60	81.46±0.05	72.36±0.13
70	84.77±0.17	74.46 ±0.22
80	85.60±0.30	75.88 ±0.16
90	86.77±0.11	80 ±0.28
100	89.97±0.35	82.62 ±0.14

IC50 Values

SI	GROUP	IC50 Value
1.	Ascorbic acid	66.90(µl/ml)
2.	Triticum aestivum	84.86(µl/ml)



Graph 1: Antioxidant activities of ascorbic acid & potted Triticum aestivum using Fenton reaction.

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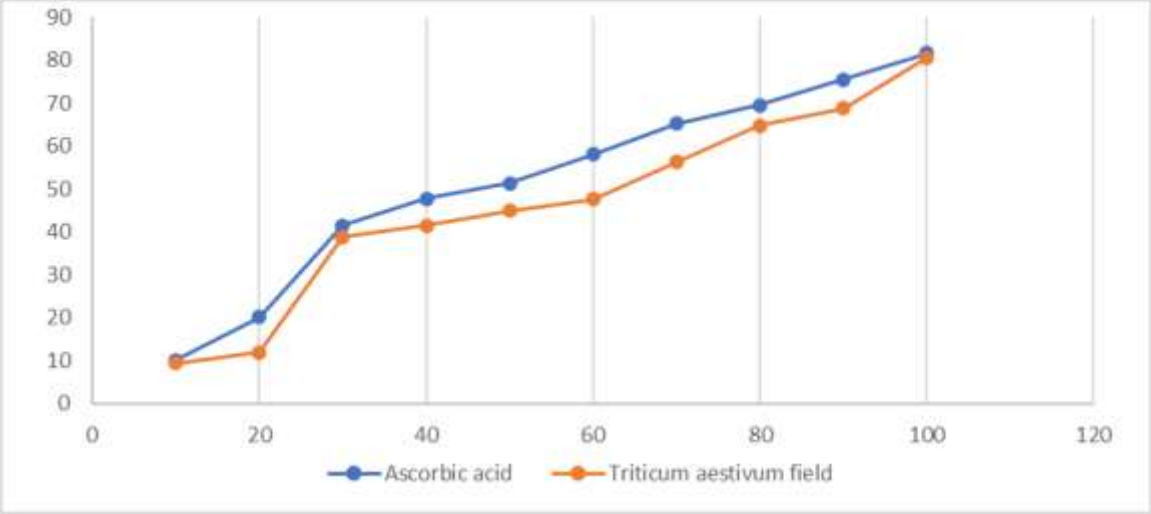
Table 2: Antioxidant activity of Ascorbic acid and methanolic extract of field *Triticum aestivum.*

Concentration (µg/µl)	Ascorbic acid (Mean±SE)	Triticum aestivum hydroponic (Mean±SE)
10	10.06±1.21	9.38 ±0.87
20	20.16±1.08	11.85±0.79
30	41.49±1.05	38.75±0.33
40	47.69±0.73	41.42±0.61
50	51.30±0.86	44.87 ±0.93
60	58.00±0.68	47.57±0.70
70	65.23±0.63	56.25±0.06
80	69.58±0.76	64.74±0.27
90	75.43±0.61	68.75±0.16
100	81.65±1.10	80.58 ±0.23

IC50 Value

SI	GROUP	IC50 Value
1.	Ascorbic acid	47.35(µl/ml)
2.	*Triticumaestivum*	49.35(µl/ml)



Graph 2: Antioxidant activities of ascorbic acid & field Triticum aestivum using Fenton reaction

Table 3: Antioxidant activity of Ascorbic acid and methanolic extract of hydroponic *Triticum aestivum*

Concentration (µg/µl)	Ascorbic acid (Mean±SE)	*Triticum aestivum* field (Mean±SE)
10	11.76±1.46	6.96 ±0.83
20	20.02±1.27	12.34 ±1.23
30	42.48±1.07	19.3 ±2.12
40	49.02±0.92	21.73 ±1.51
50	53.42±2.53	75.12 ±1.00
60	61.89±2.71	40.37±0.53
70	67.67±1.95	47.78±1.49

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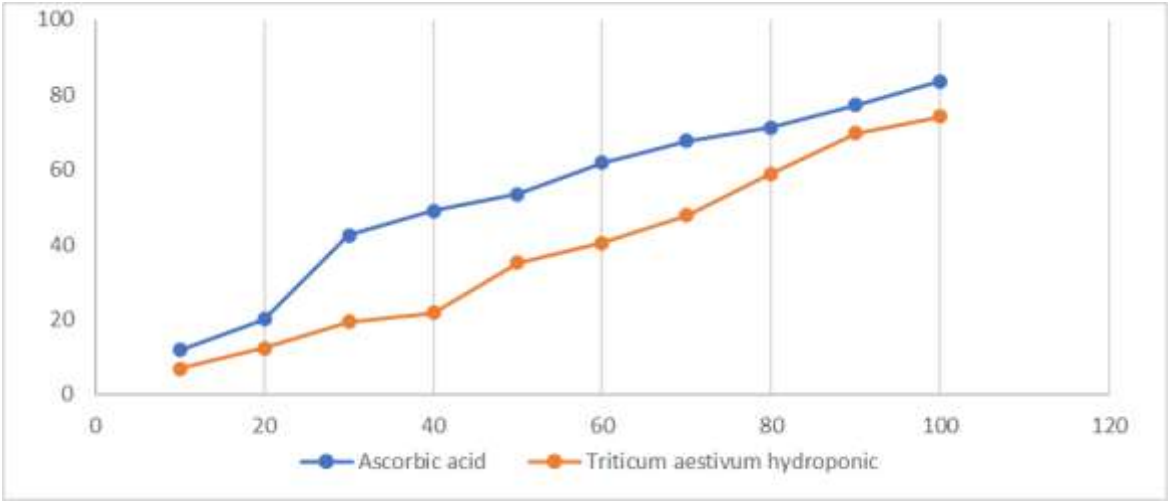
80	71.28±1.17	58.86±0.82
90	77.30±0.75	69.62±0.61
100	83.63±0.73	74.22 ±0.52

IC50 Value

SI	GROUP	IC50 Value
1.	Ascorbic acid	49.01(µl/ml)
2.	*Triticumaestivum*	50.66(µl/ml)



Graph 3: Antioxidant activities of ascorbic acid & hydroponics Triticum aestivum using Fenton reaction

DISCUSSION

The Indian medicinal system remains the most ancient yet living traditional with sound philosophical and experimental basis. It is a science of life with a holistic approach to health and personalized medicine. It is known to be complete medical system that comprisedphysical, physiological, philosophical, ethical and spiritual health. In countries beyond India, Ayurveda therapies and practices have been integrated in general wellness applications and in some cases in medical use (Semwal. et al., 2015).

The results of the examined *Triticum aestivum* extract as well as control solutions on OH- radical production. They show that all extract of *Triticum aestivum* extract and control solution as a Ascorbic acid inhibited the production of OH- radicals. The free radical scavenging activity of hydro methanolic extract of Triticum *aestivum* presented reducing power, the free radicals OH- scavenging activity of the extract increase with increasing the concentration. Extent of hydroxyl radical scavenged was determined the increasing in intensity of light yellow coloured, which was determined at 532nm. The oxidant activity was compared with ascorbic acid as a positive control. It was observed that the free radical OH- scavenging activity increases with the increase concentration of the extract. The oxidant activity was compared with ascorbic acid as a positive control. According to this result the potted & field of *Triticum aestivum* has shown better results than hydroponic of *Triticum aestivum*. The plant extract showed strong antioxidant capacity in vitro and thus the extract can be considered as a good source of natural antioxidant.

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CONCLUSION

The work done so far indicate the importance of wheatgrass in term antioxidant properties. From the result obtained in this study, crude methanolic extract proved show highest free radical scavenging activity. They show that all extract of *Triticum aestivum* extract and control solution as a Ascorbic acid inhibited the production of OH- radicals. The free radical scavenging activity of hydro methanolic extract of *Triticum aestivum* presented reducing power, the free radicals OH- scavenging activity of the extract increase with increasing the concentration. Extent of hydroxyl radical scavenged was determined the increasing in intensity of light yellow coloured, which was determined at 532nm. The oxidant activity was compared with ascorbic acid as a positive control. It was observed that the free radical OH-scavenging activity increases with the increase concentration of the extract. The oxidant activity was compared with ascorbic acid as a positive control. According to this result the potted & field of *Triticum aestivum* has shown better results than hydroponic of *Triticum aestivum*. The plant extract showed strong antioxidant capacity in vitro and thus the extract can be considered as a good source of natural antioxidant.

FUTURE PROSPECTS

The future of Triticum aestivum processing may lie in the plant itself; plans for more resilient and faster growing plants are being made. This may mean that a yield of juicer ready plants can be grown in half the time that we grow wheatgrass now. More wheatgrass consumption, more health benefits. Another thing we can all look forward to in the future is better quality prepared wheatgrass juice. Powdered wheatgrass has always been the other option if you cannot get or make fresh wheatgrass juice. But in future, when better packaging solutions that seal in the natural enzymes and vitamins of wheatgrass juice are found, we may expect close to fresh packaged wheatgrass juice readily available in supermarkets and stores.

Whatever your lifestyle preferences may be, adding wheatgrass to your diet will boost your health and energy levels greatly. Wheatgrass juicing will be a common occurrence in the homes of the future, but it is never too early to start getting yourself acquainted with the benefits of wheatgrass juicing.

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