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Antioxidant and Free Radical Scavenging Activity of Methanolic Extract of (*Hordeum vulgare*) Barley

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ABSTRACT

Aim: The aim of the study is to investigate the antioxidant activity of (HORDEUM VULGARE) Barley.

Materials and Methods

Thebarley(*Hordeumvulgare*)leaves16-28cminheightwerewashedwith water, cutintopieces, and dried by a hot air dryer at 70ºC for 24 hour and methanolic extract was prepared. Sample were dissolved in parts of 80% methanol in 1:3 ratios and placed in hot water bath shaker for 16 to 18 hours followed by filtration using muslin cloth. The filtrate was stored and the residue was re-extracted by same procedure. Reaction mixture was added to the methanolic extract and incubated at room temperature for 5minutes.Theabsorbance of the mixture at 560 nm was measured with aspectrophotometer.

Results

Methanol is likewise an excellent solvent, because of its high polarity. Methanol was observed simpler to penetrate the cell membrane to extract the intracellular elements from the plant material. In PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by way of PMS-NADH coupling response reduces NBT. Methanolic extract of barley in concentration of 200μg/mL shows 85% inhibition of superoxide radical. Whereas the methanolic extract in concentration of 30–120 μg/mL inhibit hydroxyl free radical generation in concentration dependentmanner.

Conclusion

The present study revealed that barley extracts has potent antioxidant activity, achieved by free radical scavenging and reducing power assays. Methanolic extracts of extracts were investigated for anti-free radical activity by superoxide and hydroxyl assay.

Keywords: Antioxidant, HordeumVulgare, Superoxide radicals, Hydroxyl radicals, Methanol ex- tract

INTRODUCTION

Antioxidants may play an important role in the prevention of chronic diseases namely Alzheimer's disease, cardiovascular diseases, rheumatism, diabetesmellitus, cerebrovascular diseases, and cancer by arresting oxidative damage (Saurabh and Komal). Cereals and their derivatives are the most essential foods in the Mediterranean weight loss program especially because of the energy that they offer, because of their excessive carbohydrate content material. however, in latest years, researchers have also begun to examine their antioxidant profiles (Bonoli M et al., 2004). Barley is a broadly fed on cereal, because of its nutritional and technological houses. In reality, barley meals and fractions are now gaining renewed interest as

ingredients for the manufacturing of functional ingredients pastas, baked merchandise (Marconi, E et al., 2000), (Marconi E et al., 2003), (Idehen E et al., 2017), due to their concentration of bioactive compounds, such as â-glucans and tocols. Representative phe- nolic compounds in barley are benzoic acid, cinnamic acid, p-coumaric acid, and ferulic acid and corresponding derivatives in unfastened and certain forms withester-linkages to the cell wall (Peterson D &M. Barley 1994), (Jadhav SJ et al., 1998), (OH S et al., 2014). The critical part of phytochemicals with low molecular weight present in barley grain is group of antioxidants which include tocopherols, lignans, flavonoids and phenolic acids. higher concentra- tions of those compounds are determined inside the outer layers of the kernel which represent the bran. Phenolic compounds have attracted the eye of meals and scientific scientists because of their strong in vitro and in vivo antioxidant activities and their capability to scavenge unfastened radicals, spoil radical chain reactions and scavenging metals. The plentiful content material of phenolic compounds in barley (Hordeumvulgare L.) reveals that it is able to function a superb nutritional supply of herbal antioxidants with antiradical and anti-proliferative potentials for disease prevention and fitness advertising (Lahouar L et al., 2014). Plant phenols have many biochemical properties such as antioxidant, anti-inflammatory, anti-cancer and anti-microbial action. Barley (Hordeumvulgare L.), a member of the grass family, Poaceae, is one of the world's main cereal crops. The present research work was aimed appraising the efficacy methanol in the extraction of potent antioxidants from the bar- ley (Anwar F et al., 2010). free radicals are controlled by enzymes along with medicinal flora, end result, greens and seeds and may represent a critical source of antioxidants and they may be used to reduce oxidative damage and tissue injury (Mahmoudi T et al., 2015). Juvenile barley contains mineral components such as calcium, cop- per, iron, magnesium, potassium, zinc, and vitamins (B1, B2, B3, B6, B7, C, E, K), in addition to chlorophyll, proteins, enzymes, carotenoids, and antioxidants. Various compounds influencing the health-promoting characteristics of barley grass have been identified, which include, among others, 3-O-feruloylquinic acid, isoorientin-7-O-rutinose, luteolin-6, C-arabinoside-8-C-glucoside, ferulic acid, isovitexin-7-O-glucoside, apigenin-6-C-arabinoside-8-C-glucoside, saponarin, isoorientin-7-O-[6-feruloyl]-glucoside-40-O-glucoside, isovitexin-7-O-rutinose, isoscoparin-7-O-glucoside, and isoorientin-7-O-[6-feruloyl]-glucoside (Cisowska JK et al., 2020). The antioxidant movement of methanolic extracts of scarcely seeds can be utilized to secure vegetable oils fromoxidation (Sinha A et al., 2012).

MATERIALS AND METHODS

Barley seeds was collected from the local market and grown in herbal garden. When the leaves achieve appropriate height, then used for research purpose. The sample was authenticated by Department of Pharmacognosy in University College of Pharmacy, Guru Kashi University, India.

EXTRACT PREPARATION

The collected leaves having size 16-28 cm were first washed with distilled water separately to removed unwanted foreign material like soil and dust then dried in hot air oven at 40ºC-70ºCfollowed by grinding. Sample were dissolved in parts of 80% methanol in 1:3 ratios and placed in hot water bath shaker for 16 to 18 hours followed by filtration using muslin cloth. The filtrate was stored and the residue was re-extracted by same procedure. The extracts obtained were pooled and filtered. The combined methanol specimen was evaporated to dryness using a vacuum rotary evaporator at 65ºC (Nepal P et al., 2018).

SUPEROXIDE SCAVENGING ACTIVITY

Superoxide radical is generated in phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) systems by oxidation of NADH and assayed by the reduction of Nitro blue tetrazolium (NBT). In this test, the superoxide radical was produced in 3 mL of Tris– HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM), 1 mL of NADH (78 μM) and sample solutions of extract in water. The reaction was started adding 1 mL of 10 M PMS to the mixture. The reaction mixture was incubated at 25◦C for 5 min, and the absorbance was recorded at 560 nm against blank samples. Ascorbic acid was used as a control. The decrease in the reaction mixture absorbance was the indication of an increase in the scavenging activity of superoxide anion. The percentage inhibition of superoxide anion radical generation was calculated using the followingformula:

$$\%inhibition = \frac{\left[\left(Abs_{(control)} - Abs_{(Sample)}\right)\right]}{Abs_{(control)}} \times 100$$

Where, $Abs_{control}$ is absorbance of the SO+methanol (reaction mixture without the test sample) and Abs_{sample} is absorbance of reaction mixture with the test sample (Table 1 and Figure1) (Bora KS & Sharma A 2011).

Hydroxyl scavenging activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction. This method was recommended by Yu and colleagues. Reaction mixture contained 60 μl of 1.0 mM $FeCl_2$, 90 μl of 1 mM 1,10-phenanthroline, 2.4

ml of 0.2 M phosphate buffer (pH 7.8), 150 μL of 0.17 M H_2O_2, and 1.5 ml of extract at various concentrations. Adding H_2O_2started the reaction. After incubation at room temperature for 5 minutes, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated (Table 2 and Figure 2). The percentage inhibition of hydroxyl anion was calculated by using same formula in superoxide scavenging activity (Sharma SK & Singh AP 2012).

RESULTS

The results represent significant antioxidant activity of Methanolic extract of Barley (*Hordeumvulgare L.*) plant products. However, there are certain phytochemical that are not soluble in water such as condensed tannins, flavones, coumarin etc. Methanol is likewise an excellent solvent, because of its high polarity. Methanol was observed simpler to penetrate the cell membrane to extract the intracellular elements from the plant material. In PMS-NADH-NBT system, superoxide an ion derived from dissolved oxygen by way of PMS-NADH coupling response reduces NBT. The decrease of absorb anceat 560 nm with antioxidants indicates the consumption of super oxideanion in there action mixture could be seen in (Figure 1 and Table 1). The result shows the percentage inhibition of superoxide radical generation at different concentrations (50-200µg/mL) as compared with Ascorbic Acid (standard). Methanolic extract of barley in concentration of 200 µg / mL shows 85% inhibition of superoxide radical. The hydroxyl radical scavenging activity in a concentration-dependent manner in the range of 30–120 µg/mL in the reaction mixture with 83% scavenging at a concentration of 120 µg/mL. Consumption of hydroxyl anion in reaction indication antioxidant activity of methanolic extract. (Figure 2 and Table 2)

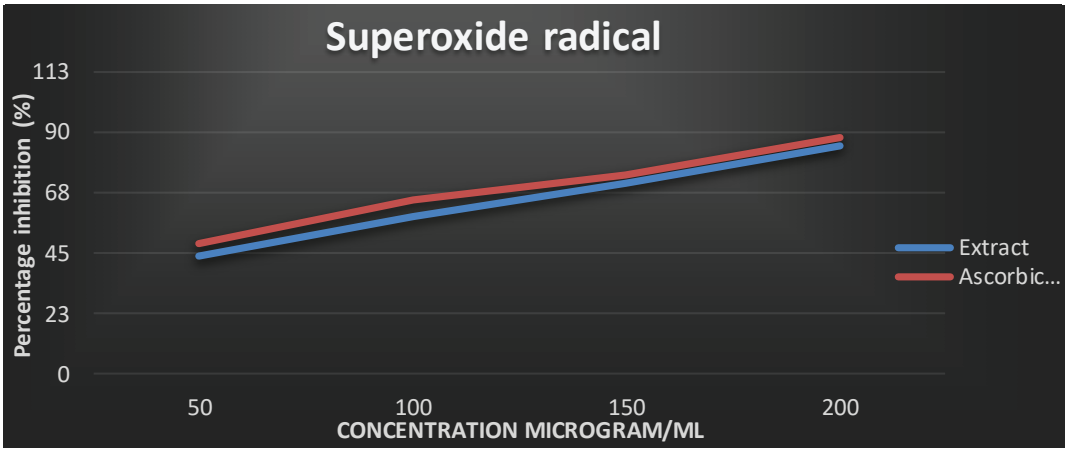

Figure 1: - The superoxide radicals scavenging activities at different concentration

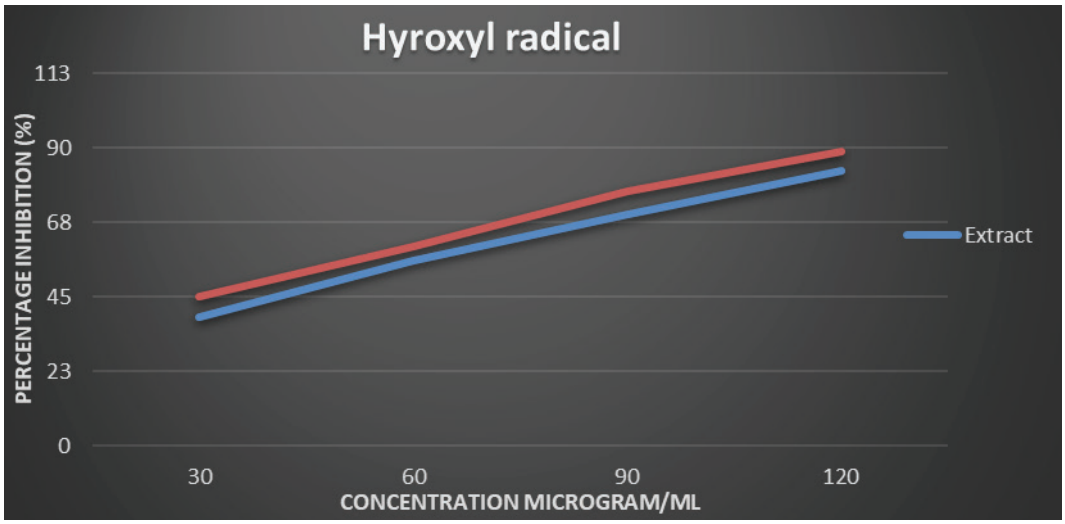

Figure 2: - The hydroxyl radicals scavenging activities at different concentration

Table 1: - Superoxide radical scavenging activity

			Percentage scavenging activity	
S. No.	**Concentration (µg/mL)**	**Absorbance**	**Extract (test)**	**Ascorbic acid (std.)**
1	50	0.53	44±0.13	49±0.23
2	100	0.38	59±0.17	65±0.09
3	150	0.27	71±0.16	74±0.10
4	200	0.14	85±0.32	88±1.10

Table 2: - Hydroxyl Scavenging activity

			Percentage scavenging activity	
S. No.	**Concentration (µg/mL)**	**Absorbance**	**Extract (test)**	**Ascorbic acid (std.)**
1	30	0.55	39±0.14	45±0.12
2	60	0.42	56±0.13	60±0.13
3	90	0.25	70±0.20	77±0.12
4	120	0.12	83±0.07	89±0.19

DISCUSSION

The investigations on antiradical and antioxidant activeties of phenolic compounds, inclusive of flavonoids and phenolic acids have been reported (Manian R et al., 2008). research has shown that the polyphenols discovered in dietary and medicinal flora could inhibit oxidative stress with the aid of antioxidant mechanisms (Manach C et al., 2004). Hydroxyl radical scavenging is an essential antioxidant activity due to very excessive reactivity of the hydroxyl radical which allows it to react with a wide variety of molecules discovered in dwelling cells along with sugars, amino acids, lipids and nucleotides. Even though OH formation can arise in several approaches, by using some distance the most important mechanism in vivo is the Fenton reaction, in which a transition metallic is worried as a pro-oxidant within the catalyzed decomposition of superoxide and hydrogen peroxide (Stohs SJ and Bagchi D 1995). The antioxidant activity changed into exhibited due to the presence of phenolic compounds, tannins, and flavonoids that were present in the ethanolic extract of Hordeumvulgare leaf (Sowjanya K et al., 2019). The phenolic compounds determined by way of us have been suggested in barley through other authors with the exception of ellagic acid. But the extraction strategies used are one of a kind, as a result it's far difficult to evaluate the results. The bad correlation among overall polyphenol content in barley grain and oil can be defined with the aid of dominance of various compounds in ethanol extracts and grain oil due to the fact exclusive extraction solvents have been used (Legzdina L et al., 2018). The superoxide anion radical scavenging activity of Barley extract assayed by the PMS-NADH system is shown in Table 1. The superoxide scavenging activity of barley was increased markedly with the increase in concentrations. Barley also exhibited concentration dependent scavenging activity against hydroxyl radical generated Table 2. The present investigation said that Hordeumvulgare leaf extracts, the methanolic extract exhibited extensive neutralization of superoxide and hydroxyl loose radicals in addition to pastime as compared to standard Ascorbic Acid. The literature statistics have established that it consists of maximum awareness of antioxidants.

CONCLUSION:

The present study revealed that barley extracts has potent antioxidant activity, achieved by free radical scavenging and reducing power assays. Methanolic extracts of extracts were investigated for anti-free radical activity by superoxide and hydroxyl assay. Thus, the antioxidant activity of Barley (Hordeumvulgare L.) may be attributed to the presence of this compounds as confirmed by qualitative phytochemical analysis. Hence these results support the view that some traditionally used Indian medicinal plants are promising source of potential antioxidants. Further, study on determination of toxicity of the Barley (Hordeumvulgare L.) extracts should be carried on in order to use the plant extracts as antioxidant and dietary supplement.

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Statement of Ethics

The experimental techniques and protocol used in this study have been not include any human and animal.

Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

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Author Contribution

Rohit Mittal, Devinder Kumar, Harmel Singh Chahal; designed carried out the experiments, and drafted, data analysis, image process the manuscript. All the authors state that they approve the final version of the manuscript.

REFERENCE

Anwar, F., Qayyum, H. M., Hussain, A.I., and Shahid, I (2010). Antioxidant activity of 100% and 80% methanol extracts from barley seeds (Hordeumvulgare L.):

stabilization of sunflower oil. Grasas Y Aceites 2010, 61 (3), 237-243.

Bonoli, M., Verardo, V., Marconi, E and Caroni, F., (2004). Antioxidant Phenols in Barley (Hordeumvulgare L.) Flour: Comparative Spectrophotometric Study among Extraction Methods of Free and Bound Phenolic Compounds; J. Agric. Food Chem. 2004, 52, 5195-5200.

Bora, K.S., and Sharma, A. (2011). Evaluation of antioxidant and free-radical scavenging potential of Artemisia absinthium; Pharmaceutical Biology, 49:12, 1216-1223.

Cisowska, JK et al., (2020). Cultivars in Yield, Antioxidant Potential, and Cholinesterase Inhibitory Activity, Sustainability 2020, 12, 1938.

Idehen, E., Tang, Y., and Sang, S., (2017), Bioactive phytochemicals in barley. Journal of Food and Drug Analysis (2017); 25(1):148-161.

Jadhav, S.J., Lutz, S.E., Ghorpade, V.M., and Salunkhe, D.K., (1998), Chemistry and value-added processing. Crit. ReV. Food Sci. 1998, 38, 123-171.

Lahouar L et al., (2014). Phytochemical content and antioxidant properties of diverse varieties. Food Chemistry 145 (2014) 578–583.

Legzdina, L., et al., (2018). Effect of genotype and crop management systems on the content of antioxidants in hulless and covered spring barley. Zemdirbyste-Agriculture, 105 (4): 315–322.

Mahmoudi T et al., (2015). Antioxidant activity of Iranian barley grain cultivars and their malts. Afr. J. Food Sci. 2015, 9(11): 534-539.

Manach, C.,Scalbert, A., Morand, C., Remesy, C and Jimenez, L., (2004), Polyphenols: Food Sources and Bioavailability; American Journal of Clinical Nutrition, 2004; 79(5): 727-747.

Manian, R.,Anusuya, N., Sidduraja, P., and Manian, S., (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of Camellia sinensis (L.) O. Kuntz, Ficusbengalensis L. and Ficusracemosa L; Food Chemistry 107 (2008) 1000–1007.

Marconi, E., Panfili, G., Ferrante, M., Raponi, F., Falasca, L., and Cubadda, R., (2003), Functional pastas and bakery products from barley flour enriched in bioactive compounds. Proceedings of the Second International Workshop "Durum Wheat and Pasta Quality: Recent AchieVements and New Trends", Roma, November 19-20, 2003, pp 229-232.

Marconi, E.,Graziano, M., and Cubadda, R., (2000). Composition and utilization of barley pearling byproducts for making functional pastas rich in dietary fiber and â-glucans. Cereal Chem. 2000, 77, 133-139.

Nepal, P., Singh, M., Baniya, A., Singh, S., Sainju, H.K., and Shrestha, R (2018). Comparative Antioxidant, Antimicrobial and Phytochemical Assesments of Leaves of Desmostachyabipinnata L. Stapf, Hordeumvulgare L. and Drepanostachyumfalcatum (Nees) Keng f. Nepal Journal of Biotechnology. Dec. 2018; 6(1):1-10.

OH S et al., (2014). Evaluation of In vitro Antioxidant Properties of Roasted Hulled Barley (Hordeumvulgare L.); Food Sci. Biotechnol. 2014; 23(4): 1073-1079. (DOI 10.1007/s10068-014-0147-8).

Peterson, D., and Barley, M., (1994), tocols: effects of milling, malting and mashing. Cereal Chem. 1994, 71, 42-44.

Saurabh, G., andKomal, S., (2017). Comparative Characterization for Antimicrobial Activity and Bioactive Compounds Present in Leaf Extract of Ocimum sanctum. J Food IndMicrobio 2017, 3:1. DOI: 10.4172/2572-4134.1000120.

Sharma, S.K., and Singh, A.P. (2012). In Vitro Antioxidant and Free Radical Scavenging Activity of Nardostachysjatamansi DC. J Acupunct Meridian Stud 2012;5(3):112e118.

Sinha, A.,Meena, A.K., Panda, P., Srivastaval, B., Gupta, M.D., and Padhi, M.M., (2012). Phytochemical, Pharmacological and Therapeutic Potential of Hordeumvulgare Linn. - A Review; Asian J. Research Chem; 2012; 5(10):1303-1308.

Sowjanya, K.,Pravallika, B., Poojitha, J., and Kumar, A., (2019). Invitro anti-oxidant Activity of Hordeumvulgare Leaf. eIJPPR 2019; 9(5):136-141.

Stohs, S.J., andBagchi, D., (1995). Invitro anti-oxidant Activity of Hordeumvulgare Leaf. Oxidative Mechanisms in the toxicity of metal ions. Free Radical Biology & Medicine, 1995; 18(2): 321-336, 1995.