Invitro anti-oxidant Activity of *Hordeumvulgare* Leaf

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ABSTRACT

Objective: The present study aimed to evaluate the in-vitro antioxidant activity of *Hordeum vulgare* belonging to family Poaceae. **Methods:** The shade-dried stem part of *H. vulgare* (1kg) was powdered and extracted by chloroform, petroleum ether, ethanol, and aqueous extraction methods using soxhlation. The extracts were concentrated using a rotary evaporator under decreased pressure at 40 °C until they were free of solvents. Thereby crude extracts were provided and employed for further studies. The antioxidant activity of *Hordeum vulgare* leaf using DPPH* radical scavenging model and to assess the antioxidant activity of *Hordeum vulgare* leaf and stem using Nitric oxide free radical (NO*) scavenging model and to assess the antioxidant activity of *Hordeum vulgare* leaf using superoxide free radical (SO*) scavenging model and to assess the antioxidant activity of *Hordeum vulgare* leafusing hydroxide free radical (OH*) scavenging model.**Results:** The graph was extrapolated between different concentrations of the plant extracts and the inhibition percentage to find out the half-maximal inhibition concentration. The extracts exhibited dose-dependent neutralization of DPPH*, NO*, SO*, and OH* free radicals and their activity was compared with standard curcumin. The IC50 was calculated for 310 µg, 620 µg, and more than 640 µg/ml of the ethanolic extract of *Hordeum vulgare* stem against DPPH*, NO*, SO*, and OH* free radicals, respectively. This indicated that the ethanolic extract of *Hordeum vulgare* leaf exhibited antioxidant activity.**Conclusion:**The antioxidant activity was exhibited due to the presence of tannins, flavonoids, and phenolic compounds, which were present in the methanolic extract of Hordeum vulgare.

Key Words: *Hordeum vulgare, antioxidant, flavonoids, tannins.*

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INTRODUCTION

Oxidative stress due to the toxic effects of free radicals in the tissue is noticeable in the pathogenesis of various pathological conditions such as aging process, anemia, arthritis, asthma, inflammation, ischemia, mongolism, neurodegeneration, Parkinson's disease, and perhaps dementia [1, 2]. Antioxidants are molecules that inhibit the oxidation of other molecules and are radical scavengers that protect the human body against free radicals. Free radicals induce liver damage. Likewise, the metabolism of certain drugs like paracetamol produces free radicals, which cause liver damage. Antioxidants can protect against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and other mechanisms, thereby helping to prevent the free radical-induced diseases [3-5].

The largest exocrine gland of our body, the liver, plays key roles in the body's homeostasis. The liver takes care of catabolic and anabolic pathways of the nutrients we consume and detoxifies the ingested food-based chemicals [6]. A variety of ingested chemicals induces liver injury mostly by causing oxidative stress in hepatic tissue and leads to numerous diseases, including cancer [7].

Due tothe casual and widespread dysfunction of the liver including over-the-counter medications, prescription, and environmental toxins, which lead to hepatitis, cirrhosis, and liver disease, further research on the use of antioxidants is needed to prevent and/or improve hepatic

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injury. This improvement process is usually referred to as "chemoprevention" and there is ample evidence from many experiments to support its efficacy [8].

Hordeum vulgare [9]is a grass that belongs to family *Poaceae*, tribe *Triticeae* and genus Hordeum. It is one of the first cultivated grains, especially in Eurasia since 10000 years ago. *Hordeum vulgare* is widely considered as a food crop in various parts of the world including some Asian countries (North Korea and China), many countries of South America (Chile and Peru), highlands of Ethiopia, Tibet, and Nepal, the Himalayas, Middle East (Syria, Iraq, Iran, and Saudi Arabia), and the semiarid regions of Africa (Tunisia, Libya, Algeria, and Morocco).

PHYTOCHEMICAL ANALYSIS:

Barley contains an assortment of phytochemicals in different concentrations usually determined by genotypic or environmental factors or the interaction of both factors. Phytochemicals contained in barley can exist in bound, conjugated, or free forms and are categorized into several main classes including folates, phytosterols, phenolic acids, lignans, and flavonoids.

Table 1: Phytochemical studies

S. No	Group of Phytoconstituents	Extracts			
		Petroleum ether	Chloroform	Ethanol	Aqueous
1	Carbohydrates	-	+	+	+
2	Amino acids		-	-	-
3	Proteins	+	-	-	-
4	Fats and oils	+	-	-	-
5	Alkaloids	-	-	+	-
6	Terpenoids	-		+	-
7	Flavonoids	-		+	-
8	Cardiac glycosides	-	+	-	
9	Saponin	-	+	-	-
10	Tannins and Phenolic compounds	-	-	+	-

EVALUATION OF ANTIOXIDANT ACTIVITY:

1. DPPH* free radical scavenging activity.

Procedure

3 ml of a DPPH-methanol solution (40µg/ml) was mixed with 1 ml of each concentration and kept for 20 min for the reaction to occur. Then the absorbance was determined at 517 nm and the inhibitions percentage was calculated using the following equation [11]:

% inhibition = [1- (Ab. of Sample / Ab. of control)] × 100.

Table 2: DPPH* free radical scavenging activity.

S:NO	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of DPPH* free radical neutralization						
	Curcumin	20.5±1.2	29.11±1.1	30.8±2.2	42.07±2.4	50.3±2.5	69.8±2.1	***76.3±2.3
2	Pet. Ether	1.2±0.02	2.2±0.2	3.4±0.3	4.2±0.8	6.2±0.2	7.3±1.1	**10.3±1.7
3	Chloroform	10.2±0.6	10.2±1.9	22.1±0.1	29.2±0.9	39.4±0.3	39.3±0.9	**52.3±2.5
4	Ethanolic	19.1±2.3	23.5±0.2	31.6±1.3	37.7±1.4	41.8±1.6	52.1±1.2	**61.2±2.8
5	Aqueous	2.8±1.4	10.2±0.87	12.3±1.8	18.5±2.2	29.2±2.4	33.2±1.2	29.3±0.3

Results are mean±SD for 6 animals, significant at ***p<0.001 and **p<0.05 compared to control.

Effect of *Hordeumvulgare*leaf extracts on DPPH* free radicals.

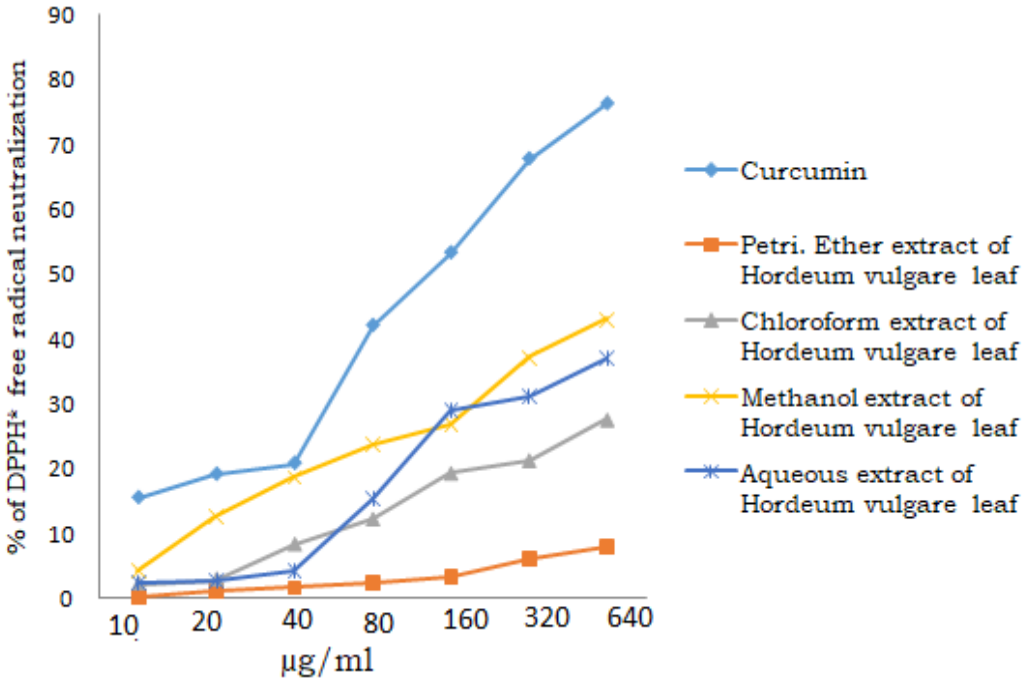

Figure 1: Effect of Hordeumvulgareleaf extracts on DPPH* free radicals.

NO* free radical scavenging activity:

Procedure:

One ml of each concentration of the test sample was added to 1ml of sodium nitroprusside solution and incubated at 37°C for 3hr. 0.3 ml of Griess reagent was added to 1 ml of the incubated solution. The absorbance was measured at 570 nm using UV spectrophotometry[10].

The radical inhibition percentage of the samples was calculated using the following formula:

% inhibition = [1- (Ab. of Sample / Ab. of control)] × 100.

RESULTS

Table 3: Effect of *Hordeumvulgare*leafextracts on NO* free radicals.

S.No	Con (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of NO* free radicals neutralization						
1	Curcumin	15.5±2.2	19.11±0.1	20.8±1.2	42.07±2.4	53.3±1.5	67.8±1.1	***76.3±1.3
2	Pet. Ether	0.2±0.02	1.1±0.02	1.6±0.03	2.3±0.01	3.2±0.02	6.1±1.01	7.9±0.7
3	Chloroform	2.2±0.3	2.9±0.2	8.3±0.4	12.2±0.1	19.3±0.1	21.3±0.7	*27.6±0.4
4	Methanol	4.1±0.1	12.5±0.2	18.6±0.1	23.7±0.3	26.8±0.6	37.1±0.1	**43.1±0.2
5	Aqueous	2.3±0.2	2.7±0.2	4.3±0.2	15.4±0.9	29±0.2	31.2±0.2	37.1±0.2

Results are as mean ± SD for 6 animals; Significant at *** P< 0.001, **P<0.01, and *P<0.05 compared to control

Effect of *Hibiscus plantifolius* stem extracts on NO* free radicals.

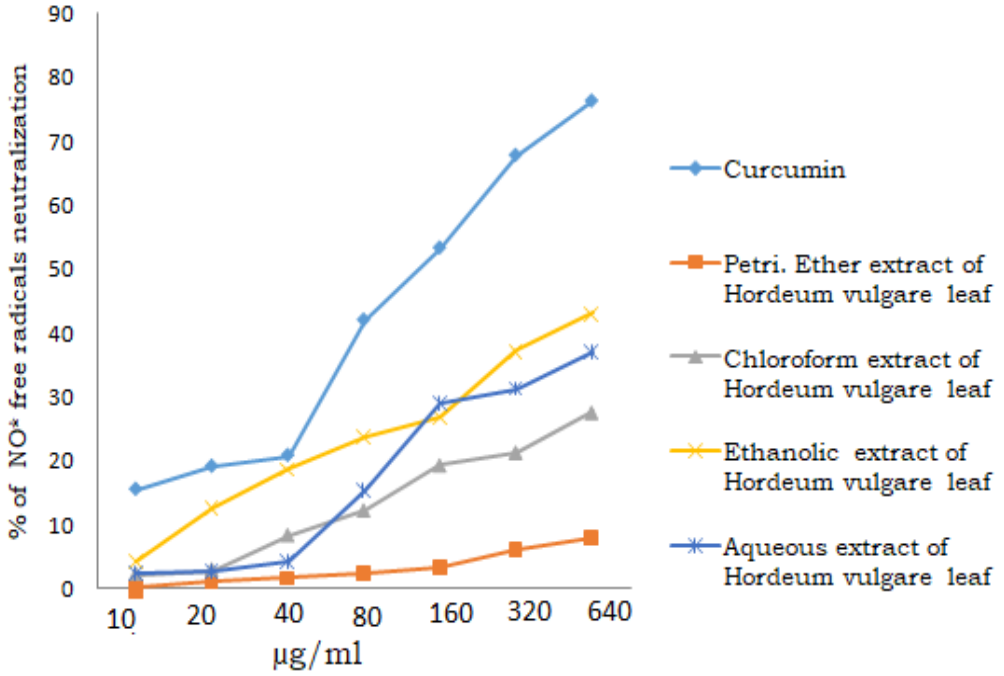

Figure 2: Effect of *Hibiscus plantifolius* stem extracts on NO* free radicals.

SuperoxideAnion (SO*) free radical scavengingactivity

Procedure:

Superoxide radicals are produced in PMS-NADH systems through the oxidation of NADH. In this study, they were assayed by the reduction of nitro blue tetrazolium (NBT). So that, they were generated as mixes of 3ml Tris-HCI buffer (16 mM, pH 8.0) containing 1ml NADH (78 mM), 1ml NBT (50 mM), and the sample solution of different concentrations of MEHP in water. The reaction began by the addition of 1ml phenazine methosulphate (PMS)(10 mM) to the mixture.

The reaction mixture was kept at 25°C for 5min and the absorbance was measured at 560 nm by a spectrophotometer against blank samples. Curcumin was used as the control [12].

The decrease in the reaction mixture absorbance was the indication of an increase in the scavenging activity of superoxide anion. The following formula was used to calculate the inhibition percentage of superoxide anion.

% inhibition = [1- (Ab. of Sample / Ab. of control)] × 100.

Table 4: SuperoxideAnion (SO*) free radical scavengingactivity

S:no	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of SO* free radicals neutralization						
1	Curcumin	1.5 ±0.2	4.5± 0.3	10±0.2	15.8±0.95	29.5±0.45	40.9±0.67	***64.7±0.93
2	Pet. Ether	1.2±0.02	1.6±0.3	4.6±0.3	5.4±0.1	13.2±0.2	19.1±0.3	22.9±1.8
3	Chloroform	1.7 ±0.01	1.9±0.3	12.7±1.7	21.2±0.3	31.3±0.3	34.1±0.7	**39.6±0.1
4	Ethanolic	1.1±0.02	18.5±0.1	21.6±0.4	29.7±0.3	37.8±1.6	41.1±0.2	**52.1±0.2
5	Aqueous	1.8±0.3	2.1±0.3	9.3±0.4	19±0.3	27 ±0.1	34.1±0.2	39.3±0.1

Results are as mean±SD for 6 animals; Significant at *** P< 0.001 **P<0.01 compared to control

Effect of *Hordeumvulgare*leafextracts on SO* free radicals.

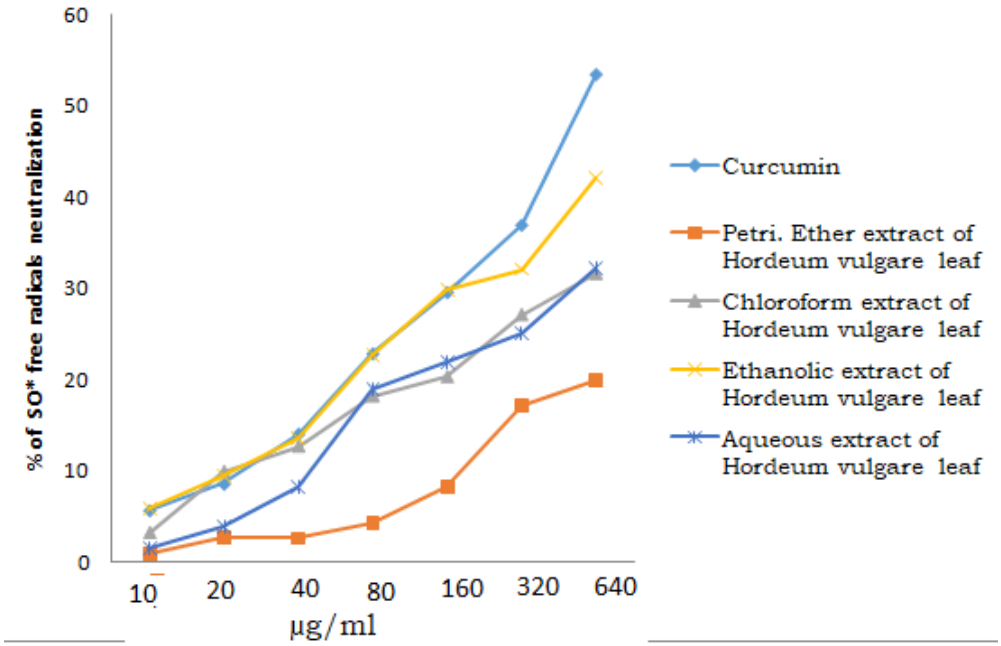

Figure 3: Effect of *Hordeumvulgare*leafextracts on SO* free radicals.

HYDROGEN PEROXIDE (OH*) FREE RADICAL SCAVENGING ACTIVITY:

Different concentrations of the extract were dissolved in 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 600 µl of hydrogen peroxide (43 mM, 30%). The absorbance value at 230 runs of the reaction mixture was recorded at 10-min intervals between 0 and 40min for each concentration.

% inhibition = [1- (Ab. of Sample / Ab. of control)] × 100.

Table 5: Hydrogen peroxide (OH*) free radical scavenging activity

S:no	Con. (µg/ml)	10	20	40	80	160	320	640
	Extracts	% of OH* free radicals neutralization						
1	Curcumin	5.6±0.5	8.5±0.6	14±0.2	22.8±0.2	29.5±0.5	36.9±0.7	**53.4±09
2	Pet. Ether	0.9±0.09	2.7±0.5	2.6±0.3	4.3±0.3	8.2±0.2	17.1±0.9	19.9±0.3
3	Chloroform	3.2±0.1	9.9±1.5	12.7±1.4	18.2±0.3	20.3±0.9	27.1±0.2	*31.6±1.2
4	Ethanolic	5.9±0.02	9.5±0.01	13.6±0.4	22.7±0.3	29.8±1.6	32.1±0.2	*42.1±0.2
5	Aqueous	1.5±0.3	3.9±0.3	8.3±1.4	19±0.3	22 ±1.4	25.1±0.1	32.3±0.2

Results are as mean ± SD for 6 animals; Significant at *** P< 0.001 **P<0.01, *P<0.05 compared to control

Effect of *Hordeum vulgare* leaf extractson OH* free radicals.

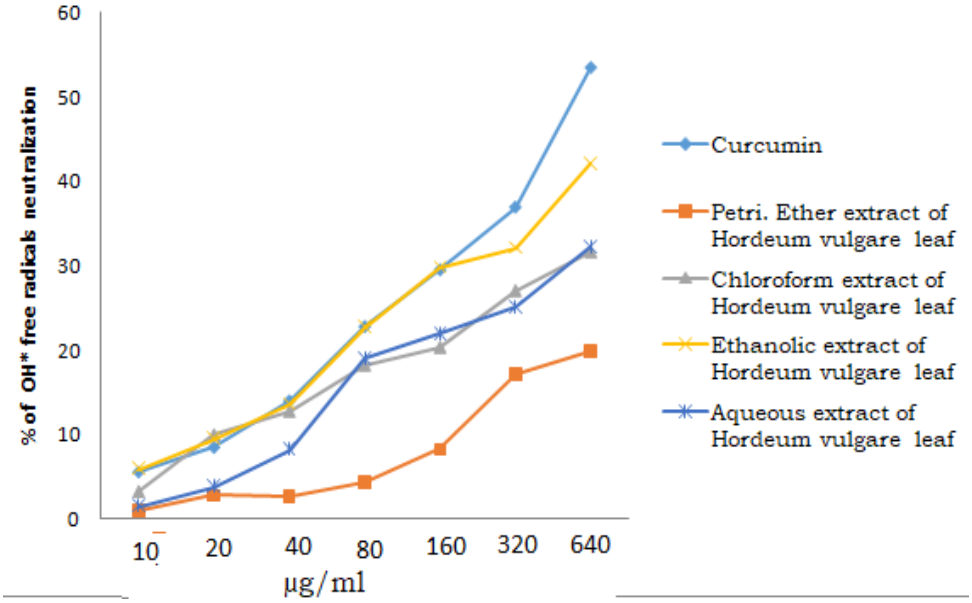

Figure 4: Effect of *Hordeum vulgare* leaf extractson OH* free radicals.

Calculation of 50% inhibition concentration

The graph was extrapolated between concentrations of the plant extracts and % of inhibition to find out the 50% inhibition concentration. The extracts exhibited the dose-dependent neutralization of DPPH*, NO*, SO*, and OH* free radicals and the activity was compared with standard curcumin (as shown in the resulted Tables)

The IC50 was calculated for 310 µg/ml, 620 µg/ml, and more than 640 µg/ml of the ethanolic extract of *Hordeum vulgare* stem against of DPPH*, NO*, SO* and OH* free radicals, respectively. This indicated that the ethanolic extract of *Hordeum vulgare* leaf had more antioxidant activity compared to other extracts [13].

DISCUSSION:

Many scientific studies have revealed that the antioxidative activity of herbal plants is due to the phytochemicals in it, for example, saponins and flavonoid. The present investigation reported that among different *Hordeum vulgare* leafextracts,the ethanolic extract exhibited greater neutralization of DPPH*, NO*, SO*, and OH* free radicals as well as activity compared to standard curcumin. The antioxidant activity was exhibited due to the presence of phenolic compounds, tannins, and flavonoids that were present in the ethanolic extract of *Hordeum vulgare* leaf.

REFERENCES

[1] Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences. 1993 Sep 1;90(17):7915-22.

[2] Dean RT, Gieseg S, Davies MJ. Reactive species and their accumulation on radical-damaged proteins. Trends in biochemical sciences. 1993 Nov 1;18(11):437-41.

[3] Ceruti PA. Oxy-radicals and tumour promotion. Science. 1994;344:862-3.

[4] Lee J, Koo N, Min DB. Reactive oxygen species, aging, and antioxidative nutraceuticals. Comprehensive reviews in food science and food safety. 2004 Jan;3(1):21-33.

[5] Kumar GS, Jayaveera KN. A Textbook of Pharmacognosy and Phytochemistry. S. Chand Publishing; 2014.

[6] Rajan S, Gokila M, Jency P, Brindha P, Sujatha RK. Antioxidant and phytochemical properties of Aegle marmelos fruit pulp. Int J Curr Pharm Res. 2011;3(2):65-70.

[7] Ardestani A, Yazdanparast R. Antioxidant and free radical scavenging potential of Achillea santolina extracts. Food chemistry. 2007 Jan 1;104(1):21-9.

[8] Blois MS. Antioxidant determinations by the use of a stable free radical. nature. 1958 Apr 26;181(4617):1199-200.

[9] Ananth A, Rajan S. Antibacterial and phytochemical investigation of datura stramonium leaf extract against clinical wound samples. European J Biomed and Pharmaceutical Sciences, 2015; 2(2): 281-294.

[10] Jonathan Y, Aus J Basic and Appl Sci, 2009, 3(4), 3975-3979.

[11] Blazovics A, Lugasi A, Szentmihalyi K, Acta Biol Szegediensis,2003, 47,99- 102.

[12] Gulcin I, Oktay M, Kirecci E, Kufrevioglu I, Food Chem, 2003, 83,371- 382.

[13] Riceevans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB, Free Rad Re, 1995, 22,375-383.