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PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *HORDEUM VULGARE* LINN SEED

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ABSTRACT
Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. *Hordeum vulgare* Linn seeds (*H. vulgare,* Commonly known as barley, Family: *Poaceae*), is an erect annual herb, 50 to 100 cm high, cultivated in the plains as well as in the hilly region of Himalaya, up to an altitude of 4000 m, in Indo- Gangetic area and Madhya Pradesh. It is locally called Jav and its seed used by traditional medical practitioners in the treatment of many diseases including liver diseases. Seeds are useful in vitiated conditions of kapha and pitta, asthma, fever, bronchiotitis, urocystitis, urethritis, gastric disorders, ulcers and anemia etc. The objective of this study was to investigate pharmacognostical, phytochemical features and antioxidant activity of hydroalcoholic extracts of *H. vulgare* seed by using DPPH assay method. The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. The *in vitro* antioxidant activity of hydroalcoholic extract of the seed was assessed against DPPH method using standard protocols. Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, steroids, saponins and carbohydrate. The total phenolic and flavonoids content of *H. vulgare* seed of hydroalcoholic extract was 0.693 and 0.497mg/100mg respectively. The activities of hydroalcoholic leaves extract against DPPH assay method were concentration dependent with IC 50 values of ascorbic acid and extracts 27.82 and 76.92µg/ml respectively. These studies provided information for correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *Hordeum vulgare* Linn, *Poaceae,* Pharmacognostical, Phytochemical, Antioxidant.

1. INTRODUCTION
Plant products have been part of phytomedicine since time immemorial. These can be derived from any part of the plant like leaves, flowers, bark roots, fruits, and seeds [1]. Herbal medicines have become more popular in the treatment of any diseases due to the popular belief that green medicine is safe, easily available and with fewer side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine and there is a lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use [2]. The medicinal properties of some plants have been documented by some researchers [3-5]. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products [6]. Extraction and characterization of several phytocompounds of these green factories have given birth to some high activity profile drugs [7]. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today

face either extinction or less of genetic diversity [8]. Knowledge of the chemical constituents of the plant is desirable because such information will be valuable for the synthesis of complex chemical substances. Reactive oxygen species (ROS) or oxygen free radicals can cause damage to cells and tissues during infections and various degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases, like Alzheimer's disease, mutations and cancer [9, 10]. The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) have been restricted because of serious concerns about their carcinogenic potential [10, 11]. Natural antioxidants, especially phenolics and flavonoids, are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [12]. Numerous studies were carried out on plants with antioxidant properties [11-13]. However, there is still great interest in finding new antioxidants from natural sources. *H. vulgare* (barley) is one of three species of genus Hordeum belonging to the grass family Poaceae, is an annual crop used throughout ancient civilizations as an important food source. The genus Hordeum consists of 32 species and 45 taxa. All varieties of HV have hollow stems in the forms of cane, produced from the fibrous roots. There is a spike-shaped arrangement of seed at the end of every stem [14]. Barley is a one of the widely consumed cereal, because of its dietary and technological properties. In fact, barley meals and fractions are now gaining renewed interest as ingredients for the production of nutritious foods (pastas, baked products), due to their concentration of bioactive constituents, such as β-glucans and tocols [15].The consumption of barley support the body's own self healing mechanisms. The components of barley aid the body in maintaining cells in a healthy condition and work to rectify abnormalities. Barley has been used as an aid in the treatment of a variety of conditions such as diabetes, skin abnormalities, arthritis, digestive diseases, weight loss, detoxifying and cancer etc [16]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* antioxidant activity of seeds of *H. vulgare* in Bhopal region of Madhya Pradesh.

2. MATERIAL AND METHODS

2.1. Plant material

The seeds of plant *H. vulgare* were collected in the month of August 2019 from the local market of Sagar, MP. Herbarium file of plant part was prepared and authenticated by Dr. Pradeep Tiwari (Professor), Department of Botany, Dr. HS Gour University Sagar, (M.P.) and the specimen voucher no. assigned was BOT/H/09/23/517. After that Herbarium file was submitted in Department. Seeds were pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 22 to maintain uniformity and packed into airtight container and stored in cool and dry place. The material was used for the further study.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

2.3. Defatting of plant material

Powdered seeds of *H. vulgare* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

2.4. Extraction by soxhletion method

300 gm of dried plant material were exhaustively extracted with hydroalcoholic mixture (500 ml, 50:50 v/v mthanol: water) at 60°-70°C for 24 h using soxhletion method. The extract was evaporated above their boiling points. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis [17].

2.5. Macroscopical evaluation

Macroscopical study is the morphological description of the seed which can be seen by naked eyes and it was performed by following the standard methods to determine the taste, size, color and odor of the seeds of *H. vulgare* [18].

2.6. Physicochemical parameters

2.6.1. Loss on drying

Ten gm of the powdered drug was accurately weighed in a tarred petridish. It was dried at 105°C for 1 hour in hot

air oven and then reweighed. Loss on drying was determined by calculating the initial and final weight.

2.6.2. Total ash value

Five gm of powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The percentage w/w of ash with reference to the air-dried drug was calculated.

2.6.3. Alcohol soluble extractive value

Five gm of coarsely powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hour, shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in tared flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

2.6.4. Water soluble extractive value

Five gm of coarsely powdered air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precautions against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in tared flat-bottomed dish dried at 105°C and weighed.

2.6.5. Foaming index

One gm coarse powder was weighted and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minute on water bath. It was cooled and filtered in to a 100 ml volumetric flask. Volume was diluted by adding sufficient amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index [19, 20].

2.7. Phytochemical screening of the extract

The extract of *H. vulgare* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [21, 22].

2.8. Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al.* [23]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

2.9. Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [23]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

2.10. Antioxidant activity

2.10.1. DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method [23]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC_{50}), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher is the antioxidant activity.

3. RESULTS AND DISCUSSIONS

The crude extracts so obtained after the soxhletion extraction process was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *H. vulgare* hydroalcoholic extracts was 5.9 %w/w. Morphological characteristics of *H. vulgare* seeds are summarized in table 1. It is an erect annual herb, 50 to 100 cm high, cultivated in the plains as well as in the hilly region of Himalaya, up to an altitude of 4000 m. Their seed colour has Yellowish brown, characteristics odour and taste and having length = 8-12mm; width = 2-4 mm; thick= 2-3mm. *H. vulgare* seeds were shade dried and turned to powder for various physiochemical parameters like loss on drying, total ash value, alcohol soluble extractive, water soluble extractive and foaming index which are summarized in table 2.

Table 1: Morphological characteristic of *H. vulgare* seeds

Characters	Observations
Colour	Yellowish brown
Odour	Characteristics
Taste	Characteristics
Size	Length = 8-12mm; width = 2-4 mm; thick=2-3mm
Shape	Round

Table 2: Physiochemical analysis of powder of *H. vulgare* seeds

Parameters	Observations
Loss on drying	3.9 % w/w
Total Ash value	12.93 % w/w
Alcohol soluble extractive	10.36 %
Water soluble extractive	12.35 %
Foaming index	21 (ml)

The results of qualitative phytochemical analysis of the crude powder of seed of *H. vulgare* were shown in Table 3. Hydroalcoholic extracts of *H. vulgare* showed the presence of alkaloids, glycosides, flavonoids, steroids, saponins and carbohydrate. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.015x - 0.001$, $R^2 = 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $y = 0.035x + 0.009$, $R^2=0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. The total phenolic and flavonoids estimation of hydroalcoholic extracts of leaves of *A. scholaris* showed the content values of 0.693 and 0.497 respectively Table 4. DPPH radical scavenging assay measured hydrogen donating nature of extracts [18]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *H. vulgare* hydroalcoholic extract was found to be 76.92µg/ml as compared to that of ascorbic acid (27.82µg/ml). A dose dependent activity with respect to concentration was observed Table 5 & Figure 1.

Table 3: Phytochemical screening of *H. vulgare* seeds extracts

S. No.	Tests	Hydro alcoholic extract
	Carbohydrates	
1	i) Molisch's Test	(+)
	ii) Benedict's test	(+)
	Tannins	
2	i) with 5%ferric chloride solution	(–)
	ii)with 10% aqueous Potassium dichromate solution	(–)
	iii) with 10% lead acetate solution	(–)
	Alkaloids	
3	i) Dragendorff'sTest	(+)
	ii) Mayer's Test	(+)
	Glycosides	
4	i) Legal Test	(+)
	ii) Baljet Test	(+)
	Flavonoids	
5	i) Shinoda's Test	(+)
	ii) Alkaline reagent test	(+)
	Steroids and Sterols	
6	i) Libermann-Burchard Test	(+)
	ii) Salkowski Test	(+)
	Proteins and Amino Acids	
7	i) Biuret Test	(+)
	Saponins	
8	i) By shaking the extract in test tube	(–)

Table 4: Results of total phenol and flavonoids content

Extract	Total phenol content	Total flavonoids content
	mg/100mg	
Hydroalcoholic	0.693	0.497

Table 5: % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

Concentration (µg/ml)	% Inhibition	
	Ascorbic acid	Hydroalcoholic extract
10	41.5	23.2
20	47.7	24.4
40	52.9	34.2
60	67.4	39.6
80	75.8	55.7
100	89.6	58.4
IC 50	**27.82**	**76.92**

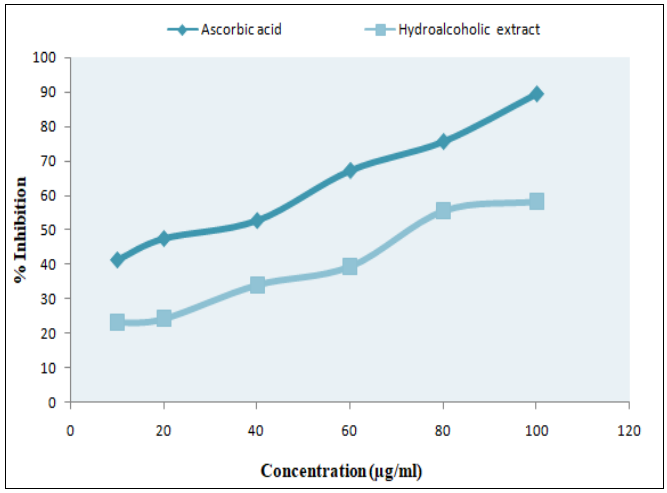

Fig. 1: % Inhibition of ascorbic acid and hydroalcoholic extract

4. CONCLUSION

Phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The total phenolic and flavonoid content in hydroalcoholic seeds extract is further proved by in vitro antioxidant studies. Potential antioxidant activity has good correlations with the therapeutic use in the treatment of cardiovascular disorders. Further research to isolate individual compounds, their *in-vivo* antioxidant activities with different mechanism is needed.

Conflict of interest
None declared

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5. REFERENCES

1. Cragg GM, Newman DJ. *Pharm Biol.,* 2001; **39(sup1)**:8-17.
2. Sofowora E. Hibueze U, Akubugwo E. *Agric Biol J North America.,* 2011; **2**:1437-1444.
3. Chibueze U, Akubugwo E. *Agric Biol J North America.,* 2011; **2(12)**:1437-1444.
4. Idu M, Obaruyi G, Erhabor J. *Ethnobot Leafl.,* 2008; **2009(4)**:9-18.
5. Banso A, Adeyemo S. *Afr J Biotechnol.,* 2007; **6(15)**:1785-1787.
6. Ivanova D, Gerova D, Chervenkov T, Yankova T. *J Ethnopharmacol.,* 2005; **96(1-2)**:145-150.
7. Mandal V, Mohan Y, Hemalatha S. *Pharmacogn Rev.,* 2007; **1(1)**:7-18.
8. Misra A. *J Med Plants Res.,* 2009; **3(13)**:1140-1146.
9. Lee SE, Hwang HJ, Ha JS, Jeog HS, Kim JH. *Life Sci.,* 2003; **73**: 167-179.
10. Tadhani MB, Patel VH, Subhash R. *J Food Comp Anal.,* 2007; **20**: 323-329.
11. Baydar NG, Ozkan G, Yasar S. *Food Cont.,* 2007; **18**:1131-1136.
12. Kumaran A, Karunakaran J. *LWT-Food Sci Tech.,* 2007; **40**:344-352.
13. Prakash D, Suri S, Upadhyay M, Singh BN. *Int J Food Sci Nutr.,* 2007; **58**:18-28.
14. Behall KM et al. *Am J Clin Nutr.,* 2004; **80**:1185-1193.
15. Sharifi et al., *World Sci J.,* 2006; **5**:88-94.
16. Mokhtar et al., *American J Biochem Biotechnol.,* 2006; **2(1)**:1-8.
17. Mukherjee PK. Quality control of herbal drugs. 2nd Ed. Business Horizons; 2007.
18. Naik RA, Harisha CR, Acharya RA. *Int J Pharm Pharm Sci.,* 2018; **10**:61.
19. Ahmad T, Singh SB, Pandey S. *Int J Pharm Res Rev.,* 2013; **2**:53-60.
20. Patel NM. *Asian J Pharm Res.,* 2011; **1**:49-52.
21. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. *Pharmacologyonline,* 2019; **1**:27-33.
22. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. *Pharmacologyonline*, 2019; **1**:21-26.
23. Parkhe G, Jain P, Jain DK. *Pharmacologyonline*, 2018; **2**:227-233.
24. Hudson BJ. Food antioxidants. In: Gordon MH, editor. The Mechanism of Antioxidant Action in Vitro. London: Elsevier Applied Science; 1990.
25. Karole S, Jain DK, Abbas S, Abbas N, Kare P. *Indian J Pharm Pharmacol.,* 2022; **9(1)**:67-74.