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Research article

Phytochemical analysis, GC-MS characterization and antioxidant activity of *Hordeum vulgare* seed extracts

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ABSTRACT

Barley scientifically known as *Hordeum vulgare (HV)* is a major grain crop. Over the course of time, great interest has been developed in the usage of barley, because of its various pharmacological activities. Current study is designed to determine the chemical constituents of *Hordeum vulgare (HV)* seed extract by GC-MS technique, and *Invitro* antioxidant assays i.e. 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) and 2-azino-bis(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS) methods. GC-MS identified 16 non-polar compounds in the hexane extract of *HV* plant, which includes carboxylic acid (6.25%), fatty acid (37.5%), carboxylic acid amide derivative of fatty acid (6.25%), triterpinoids (18.75%), fat soluble vitamin (6.25%), phytosterol (6.25%), stigmastanes (6.25%), beta diketones (6.25%), and cycloartenol (6.25%) respectively. The major compound includes Hexadecanoic acid, methyl ester (6.84%), n-Hexadecanoic acid (8.58%), 9,12-Octadecanoic acid (Z,Z)-, Methyl Ester (8.04%), 9,12-Octadecadienoic acid (Z,Z) (57.01%), Lup-20(29)-en-3-one (3.57%), γ-Sitosterol (3.31%). Some constituents such as Lup-20(29)-en-3-one, campesterol and squalene were observed and were not previously reported. Total phenolic and total flavonoid content were determined using spectrophotometric technique and calculated as gallic acid equivalents GAE/g dry weight and rutin equivalent RE/g of dry weight respectively. The highest phenolic content exhibited by the acetone extract of *HV* seedsi.e. 0.0597 mg GAE/g while the highest flavonoid content exhibited by dichloromethane extract i.e. 0.09 mg RE/g and 0.25 mg QE/g of dry weight respectively. All the extracts showed significant antioxidant activity in DPPH and ABTS cation decolorization assays. Methanol and dichloromethane extract showed the highest DPPH radical scavenging activity i.e. 52.41% and 42.07% at the concentration of 100 mg/ml respectively. Moreover, the IC_{50} has been determined by the acetone and methanol extract of *HV* seeds. The high antioxidant activity of its seed extracts has made this plant pharmacologically important. Conclusively, there is a vast scope to further explore the active principals of barley so that more of its pharmacological properties can be identified.

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1. Introduction

Barley is one of the prime cereal crops. Scientifically it is known as *Hordeum vulgare* and has been used since ages to meet the need of the food worldwide [1]. Globally, barley is widely cultivated and occupies the fourth position both in manufacturing and agriculture field amongst cereal crops. Majority of the barley products (≤70%) are utilized for cattle feed while the remaining 21% is utilized for fermenting, malting and refining ventures, leaving around 6% of the products for human consumption [2]. Owing to its dietary characteristics and possible medical advantages, it has been speculated that barley is an important component of human dietary regime. Over the years, great interest has been developed regarding the usage of barley as food for humans because of its various health benefits [3].

Hordeum vulgare (HV) plant belongs to the Poaceae family. The plant family is of considerable economic significance as it contains crops like rice, maize, oats, wheat, etc [4]. They easily grow in nearly every habitat available for the flowering plants apart from sea bed [5].

Barley consists of significant percentages of roughages, minerals and bioactive mixtures, for example, iron (6 mg per 100 g crude matter) [6,7], zinc (3.3 mg per 100 g crude matter) [6,7], and calcium (50 mg per 100 g crude matter) [6,7]. Barley consists of (10–20%) protein [8] contrasted with (9.5%)corn [8], (14%) wheat [6] and, (10–16%) rice grain [9]. Contrasted with different oats,

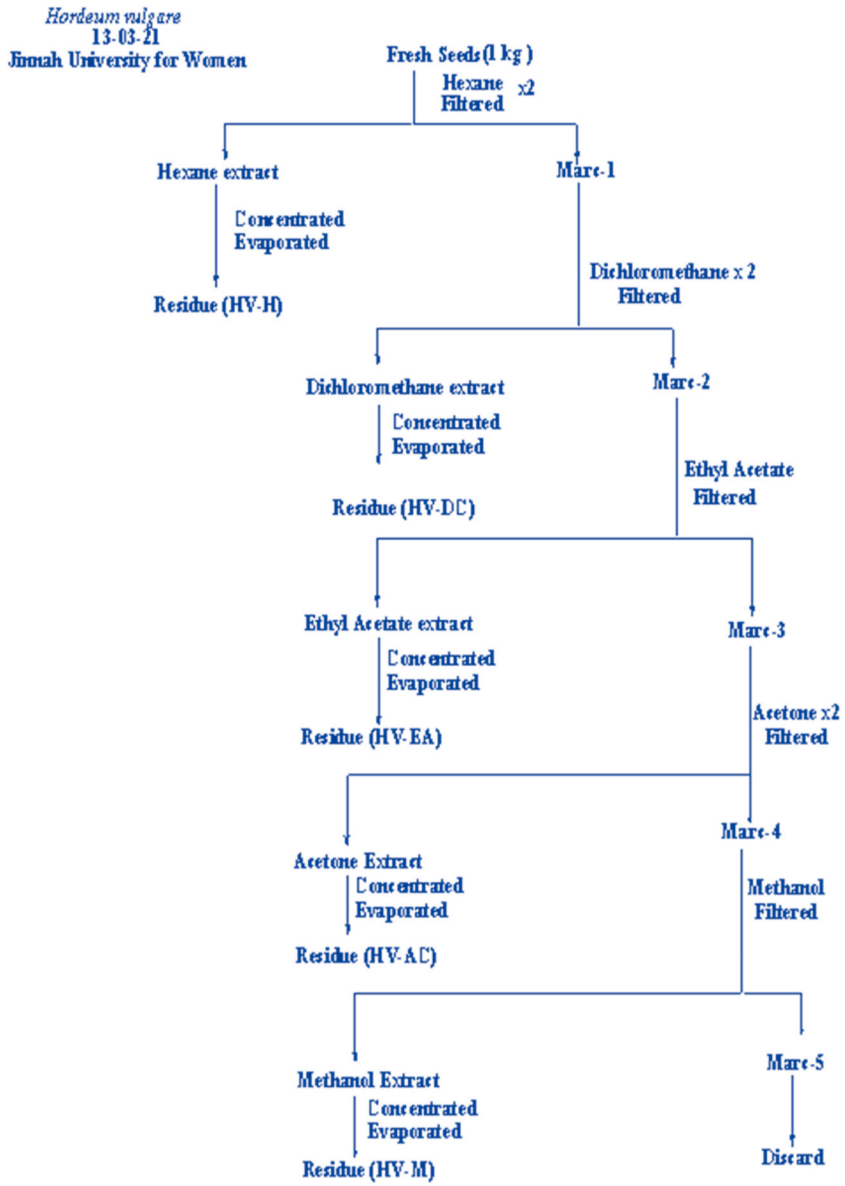


Fig. 1. Scheme for Extraction of *Hordeum vulgare* seeds from non-polar to polar solvents.

barley contains the most elevated levels of β-glucan, trailed by rye, wheat, and oat [10,11]. Barley is additionally viewed as a significant dietary source of antioxidant [12], because of its higher content of Vitamin E, which is highly contrasted with different cereals [13]. Barley is the solitary grain harvested due to the presence of eight vitamin E isomers [9]. It is frequently employed for treatment of a number of diseases especially skin related diseases. Barley being a extraordinary contributor of vitamins, carbohydrates, polyphenols and proteins is under consideration of various food and nutritional industries.

A number of chemical constituents have been reported for this plant [14]. According to a research, the composition of green barley includes flavones C-glycosides [15], 2-*O*-glycosyl isovitexin [15], lutonarin [15], saponarin [16], catalase [16], superoxide dismutase [15], carotenoids [15], peroxidase [16], chlorophyll [15], Vitamin C [15], flavonoids [17] and Vitamin E [18].

Due to its diverse applications, the *Hordeum vulgare (HV)* hold holds substantial potential as a promising source for the development of novel antioxidants. The current study, aimed to determine the phytochemical composition of barley seeds specifically in hexane extract. A very limited data is available that explores the hexane extract of *Hordeum vulgare (HV)*. The current study report, fatty acids and triterpenoids reported in higher percentages in the hexane extract as compared to previous reported studies [19].

The reported pharmacological activities of *HV* plant includes, antidiabetic [14], antihypertensive [20], anti-inflammatory [21], antiurolithiatic [22], antiglycation [23], anticancer [24], antitumor [24], antiobesity [25], hypolipidemic [26], antistress [14] and antiulcer effects [27].

The goal of the present study is to confirm the antioxidant activity of hexane, dichloromethane, ethyl acetate, acetone, and methanol extracts of *HV* using *invitro* assays. Additionally, it also aims to investigate the phytochemical analyses of *HV*.

2. Material and methods

2.1. Chemicals and reagents

All chemicals of analytical grade purchased from well-known international chemical suppliers were used for the current study. Chemicals like n-Hexane 95%, dichloromethane, acetone, methanol for gradient, gallic Acid (extra pure), aluminium chloride-anhydrous were purchased from Duskan Reagents and Duskam Pure Chemicals. Ethyl acetate, sodium carbonate, anhydrous, potassium acetate (extra pure) were procured from Daejung Chemicals & Metals Co., Ltd. DPPH (2,2-diphenyl-1-picrylhydrazyl), sulphuric Acid, ferric chloride-6-hydrate, trolox (±)-6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid were purchased from Sigma Aldrich. Potassium persulfate, Millon's reagent, ninhydrin, L-ascorbic acid was bought from BDH Laboratory Supplies. Folin ciocalteus phenol reagent was procured from Sisco Research Laboratories Pvt Ltd. ABTS (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), (+)-Rutin Trihydrate was obtained from Alfa Aesar. Fehling Solution A and B were bought from General Purpose Reagent (GPR) while hydrochloric acid was purchased from Merck.

2.2. Plant materials

The seeds of *HV* plant were purchased from the local market of Karachi, Pakistan in the month of March'2021. A voucher specimen (HG NO 78626) was submitted to the department of Pharmacognosy, Jinnah University for women and was verified by Dr. Aiman. The total quantity of *HV* seeds utilized in the sequential extraction during the experimentation was 1 Kg. The *HV* seeds were washed, air dried, and sequentially extracted with hexane (H), dichloromethane (DC), ethyl acetate (EA), acetone (AC) and methanol (M), twice at room temperature for three consecutive days.

The extracts were filtered and evaporated under reduced pressure using rotary evaporator at a temperature of 50 °C to obtain concentrate masses of *HV*-H, *HV* -DC, *HV* -EA, *HV* -AC and *HV*-M (shown in Fig. 1). The *HV*-H extract was analyzed through GC-MS spectra.

2.3. Phytochemical analysis of Hordeum vulgare seed extracts

2.3.1. Identification of carbohydrates

For the identification of carbohydrate i.e. reducing sugars and polysaccharides, 50 mg of each seed extract i.e. (*HV*–H, *HV*-DC, *HV*-EA, *HV*-AC and *HV*-M) of *Hordeum vulgare* plant was taken and dissolved in 20 ml of distilled water followed by filtration. The filtrate of each seed extract was then treated with the following reagents [28].

2.3.2. Benedict's test

In this test, 1 ml of each filtered extract was taken in separate test tubes respectively, which were then treated with 5 ml Benedict's reagent. Each extract was then heated for a period of 5 min. The occurrence of reddish orange color precipitates confirmed the presence of reducing sugars [28].

2.3.3. Fehling's test

In Fehling's test, each filtered extract was taken in separate test tubes respectively. Hydrochloric acid (HCl) was added in all test tubes followed by neutralization with alkali. After that, Fehling's A and Fehling's B solution were added in all respective test tubes. All the test tubes were gently heated for few minutes. The occurrence of red color precipitates indicated the presence of glycosides/carbohydrates [28].

2.3.4. Iodine test

Starch and its components can be detected in sample extracts with the help of Iodine test. In this test, 50 mg of each seed extract of *HV* plant was mixed with 2 ml of iodine solution. The occurrence of intense or dark blue coloration indicates the presence of starch in seed extracts [28].

2.3.5. Identification of protein

For the identification of proteins, 50 mg of each extract of *HV* plant was dissolved in methanol and water separately. Each extract was then sonicated and filtered to perform the following test [**28**].

2.3.6. Millon's test

For the identification of phenolic amino acid i.e. tyrosine, Millon's test was performed. This test is usually performed to detect proteins. In this test, 50 mg of each seed extract was mixed with 2 ml of Millon's reagent, as a result of which white precipitates appeared, which on heating turned to reddish brown in color [28].

2.3.7. Identification of phenols & tannins

For the identification of phenols and tannins, 50 mg of each seed extract was dissolved in methanol and water separately[**28**]. Each extract was then sonicated and filtered to perform the following test[**28**].

2.3.8. Ferric chloride test

Each filtered extract was mixed with 2 ml ferric chloride (2%) solution. The appearance of blackish-green color indicates the presence of phenol which further turns to olive green in color. This change in color confirmed the presence of phenols and tannins in the seed extracts [28].

2.4. Total phenolic content

Total phenolic content has been determined using Rico, Daniel et al. method with some variations. In this method, the 1 ml of the sample extract solution corresponding to the concentration of 0.1 g/2.5 ml was mixed with Folin-reagent (2.5 ml) with the concentration of 10%. The mixture was incubated for 5 min, followed by the addition of 2.5 ml of sodium carbonate (Na_2CO_3) with the concentration of 7.5% to the mixture with continuous shaking. The mixture was left in the dark for a period of 2 h. Three consecutive readings were noted at 765 nm. Gallic acid was used as a standard (1 ml; 10 μg/ml - 100 μg/ml), and its calibration curve has also been plotted. Results are presented in terms of (GAE) Gallic acid equivalent (g/100 g) of dry extract [29].

2.5. Total flavonoid content

Total Flavonoid content has been evaluated by implying Saliu JA, Olabiyi AA et al. method with some modifications [30]. 1 ml sample extract solution with the concentration of 0.1 g/2.5 ml was mixed with 0.5 ml Aluminium chloride (10%), 0.5 ml Potassium acetate (1 M) and 4.15 ml distilled water. Immediately, absorbance was record at 415 nm using UV-Spectrophotometer, where methanol and distilled water were used as a blank. Rutin was used as a standard (10 μg/ml to 100 μg/ml) and its calibration curve has also been plotted. The results obtained have been expressed as Rutin Equivalent (RE) (gm/100 gm) of dry extract [30].

2.6. DPPH radical scavenging activity

DPPH radical scavenging activity was determined by Meda A et al., and Liu, X. et al. (2007) methods followed with some modification [31,32]. In this method, 0.5 ml sample extract (concentration ranges from 60 to 200 mg/ml) had been incorporated in separate test tubes, followed by 3.5 ml of DPPH solution respectively. The reaction mixture was allowed to stand in the dark for 30 min and immediately each sample absorbance was recorded at 517 nm. Methanol has been used as a blank in this method and calibration curve of ascorbic acid has also been determined (0.03 mM–0.27 mM). Three consecutive readings have been recorded for all sample extracts and results have been mentioned in terms of Ascorbic Acid Equivalent capacity (AAE) gm/100 gm of dry extract. The radical scavenging activity of *HV* extracts has been evaluated by calculating its Percentage inhibition, and its results have been expressed as IC_{50}.

For calculating Percentage inhibition, following formula has been used (equation (1)).

$$Percentage\ Inhibition = \left[\frac{Ac - AT}{Ac}\right] * 100 \quad (1)$$

Equation (1) Percentage inhibition.
Where, A_C = Absorbance of Control, AT = Absorbance of Test Sample.

2.7. ABTS cation de-colorization assay

Another technique used to determine the antioxidant activity of *HV* seed extracts was the assay of ABTS (2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid). For this assay, Alzoreky et al., 2001 method was used with some modifications [33]. The 7 mM of ABTS solution was mixed with 2.45 mM potassium per sulfate to make a stock solution. The mixture was kept in dark approximately for 16 h at room temperature. After the incubation period of 16 h, a dark green colored stock solution was obtained, which is further diluted by incorporating distilled water to achieve the desired absorbance i.e. 0.700 ± 0.02. On the other side, sample extract solutions were prepared in distinct concentrations ranging from 100 mg/ml to 300 mg/ml. After that, 3.5 ml of sample extract solution was taken in a vial followed by 3.5 ml of ABTS solution and the mixture was then kept in the dark for 6 min at room temperature. The absorbance was immediately recorded at 743 nm. Three consecutive readings were recorded by using methanol as a blank. The standard used was Trolox and the results were presented as Trolox Equivalent Antioxidant Capacity (TEAC) gm/100 gm of dry extract [33]. For calculating Percentage inhibition, equation (1) has been used.

2.8. GC-MS analysis

For GC-MS analysis, Agilent Technologies equipment was used bearing Model number 7000 GC-MS Triple Quad (TQQQ). The software used was Hunter workstation, B.04.00 version. The electron ionization potential used during the analysis was 70 eV. For the separation of compound, OPTIMA-5 column was used with temperature equals to 360 °C: 30 m × 250 μm x 0.25 μm. Helium was used as a carrier gas during the analysis. The run time, flow rate and split ratio of Helium gas used in the analysis was 70.714 min, 1.129 ml/min and 5:1 respectively. 2.5 μl sample volume was injected by Automatic liquid sampler. Different compounds were identified by comparing their masses with the literature data available on NIST (National Institute of Standards and Technology) database.

Identification of the compounds in the hexane extract of *HV* seeds was achieved by making the comparison of their retention indices (RI). The retention indices were calculated by applying the Kovat's formula, which uses n-alkanes (C9 – C33) (Sigma-Aldrich, Germany) as standards under similar chromatographic settings. The calculated retention indices and their masses were also matched with the literature data provided in the Standard Reference Database, (NIST) i.e. National Institute of Standards and Technology. The relative percentage of individual compound was also evaluated by the ratio of peak area of individual compound relative to the total peak area.

3. Result & discussion

3.1. Preliminary qualitative phytochemical analysis of Hordeum vulgare seed extract

Different seed extracts of *HV* plant including hexane (*HV*–H), dichloromethane (*HV*-DC), ethyl acetate (*HV*-EA), acetone (*HV*-AC) and methanol (*HV*-M) were subjected to phytochemical analysis using following tests namely; Benedict's test, Fehling's test, Iodine test, Millon's test and Ferric chloride test respectively. The hexane extracts of *Hordeum vulgare* plant (*HV*–H) showed positive (+) result in response to all the tests mentioned above, which confirms the presence of carbohydrates, glycosides, tyrosine, phenol and tannins in it. The dichloromethane extract (*HV*-DC) showed positive result (+) for Fehling's and Millon's test, which confirmed the presence of glycosides and tyrosine/protein in it and showed negative result (−) for Benedict's, Iodine and Ferric chloride test. The ethyl acetate extract (*HV*-EA) showed negative result (−) in all the tests performed. The acetone extract (*HV*-AC) showed only one positive result (+) i.e. against Millon's test which indicates the presence of tyrosine/protein in it and failed to response positive against other tests. Lastly, the methanol extract (*HV*-M) showed positive result (+) against Benedict's, Fehling's and Millon's test which confirmed the presence of reducing sugars/carbohydrates, glycosides and tyrosine/protein in it while it failed to succeed the iodine and ferric chloride test. Table .1 showed the phytochemical analysis of each seed extract against different tests performed.

Table 1
Preliminary Qualitative Phytochemical Analysis of *Hordeum vulgare* seed extracts.

S. No	Chemical Test	Identified Compound	*HV*-H Extract	*HV*-DC Extract	*HV*-EA Extract	*HV*-AC Extract	*HV*-M Extract
1.	Benedict's Test	Reducing sugars/Carbohydrates	+	–	–	–	+
2.	Fehling's Test	Glycosides	+	+	–	–	+
3.	Iodine Test	Starch/Carbohydrates	+	–	–	–	–
4.	Millon's Test	Tyrosine/Protein	+	+	–	+	+
5.	Ferric Chloride Test	Phenol & Tannins	+	–	–	–	–

Table 2
Total phenolic content of *Hordeum vulgare* seed extracts.

S. No.	Extract	GAE Conc µg\mL	GAE Conc mg\mL	TPC=CXVxDF/M
1.	**Hexane**	25.312	0.025	5.7 ± 0.008118
2.	**DC**	52.312	0.052	11.77 ± 0.084764
3.	**EA**	45.437	0.045	10.23 ± 0.021480
4.	**Acetone**	59.750	0.059	13.44 ± 0.021480
5	**Methanol**	33.812	0.033	4.22 ± 0.007812

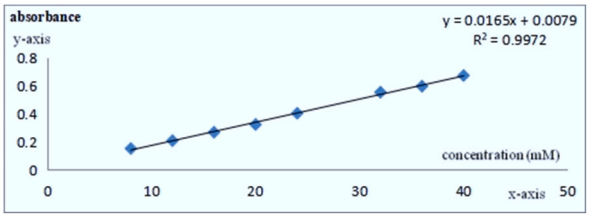


Fig. 2. Calibration curve of standard Gallic acid for TPC.

3.2. Total phenolic content

The current study investigated the total phenolic content of *HV* seed extracts. The results mentioned in Table 2, showed the highest phenolic content exhibited by the acetone extract of *Hordeum vulgare* (HV-AC) i.e. 0.059 mg/ml while; the hexane extract of *Hordeum vulgare* (HV–H) contained the minimum amongst all extracts i.e. 0.025 mg/ml. The calibration curve of Gallic acid have also been plotted (see Fig. 2), which showed a linear curve and was used in order to estimate the total phenolic content (TPC) as Gallic acid equivalent (GAE) mg/g of dry extract. The coefficient of determination (r_2) was found to be 0.999.

3.3. Total flavonoid content

The result showed that dichloromethane extract exhibited the highest flavonoid content amongst all the other extracts, i.e. 0.09 mg/ml, while ethyl acetate displayed the lowest flavonoid content i.e. 0.004 mg/ml. The order sequence of total flavonoid content displayed by all the extracts from highest to lowest is as follows; DC > H > AC > M > EA. Rutin has been used as a standard to determine the flavonoid content as RE mg/g of dried extract and its calibration curve has also been plotted which is shown in Fig. 3. The correlation coefficient value of Rutin is found to be 0.999 which represents the linearity of the curve. The total flavonoid content exhibited by different extracts of *HV* plant against Rutin standard is displayed in Table 3.

3.4. DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by evaluating different extracts of *HV* seeds. The *Hordeum vulgare* (*HV*) seed extracts were prepared in hexane (*HV*–H), dichloromethane (*HV*-DC), ethyl acetate (*HV*-EA), acetone (*HV*-AC) and methanol (*HV*-M) solvents respectively. It has been observed that at the concentration of 100 mg/ml, all the extracts showed significant amount of activity which ranges from 21.74 to 52.41%. The highest radical scavenging activity was observed with the methanol extract at the concentration of 100 mg/ml i.e. 52.41%, dichloromethane extract also showed significant radical scavenging activity at 100 mg/ml concentration i.e. 42.07%, while, the lowest radical scavenging activity was observed with the hexane extract at 100 mg/ml concentration i.e. 21.74%. The results were calculated in terms of ascorbic acid equivalent (AAE) mM/100 g as displayed in Table 4; it also

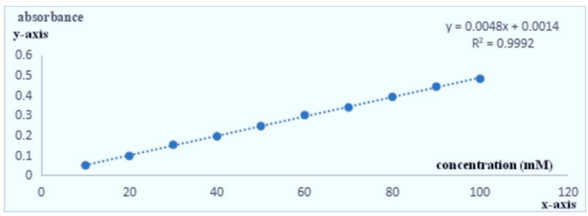


Fig. 3. Calibration curve of standard Rutin for TFC.

Table 3
Flavonoid content exhibited by different extracts of *Hordeum vulgare* plant against Rutin standard.

S. No.	Extract	RE Conc µg\mL	RE Conc mg\mL	TFC=CXVxDF/M
1.	Hexane	82.1	0.082	4.10 ± 0.005
2.	DC	93.1	0.093	4.65 ± 0.137568
3.	EA	4.2	0.004	0.10 ± 0.120657
4.	Acetone	60.3	0.060	3.01 ± 0.028431
5.	Methanol	59.4	0.059	1.48 ±0 .00144

Table 4
Antioxidant DPPH assay obtained from *Hordeum vulgare* seed extracts.

S. No.	Extract	Conc mg/mL	Percent inhibition %	IC_{50}	AAE (mM/100g)
1.	HV-H	20	21.32	ND	0.142
		18	18.97		0.146
		16	17.05		0.150
		14	16.51		0.151
		12	18.7		0.147
		10	21.74		0.141
		8	18.74		0.147
		6	17.47		0.149
2.	HV-DC	20	43.1	ND	0.106
		18	39.07		0.114
		16	37.83		0.117
		14	37.43		0.117
		12	40.02		0.112
		10	42.07		0.108
		8	38.16		0.116
		6	38.38		0.116
3.	HV-EA	20	35.84	ND	0.123
		18	31.75		0.131
		16	31.82		0.131
		14	31.46		0.132
		12	32.36		0.130
		10	32.18		0.130
		8	30.02		0.135
		6	31.28		0.132
4.	HV-AC	20	38.43	50.21	0.129
		18	22.24		0.165
		16	26.02		0.157
		14	25.66		0.158
		12	24.94		0.159
		10	26.29		0.156
		8	22.83		0.164
		6	22.57		0.165
5.	HV-M	20	38.78	53.8	0.124
		18	40.04		0.121
		16	42.05		0.117
		14	36.19		0.129
		12	42.29		0.116
		10	52.41		0.094
		8	33.06		0.136
		6	32.34		0.138

ND=Not determined.

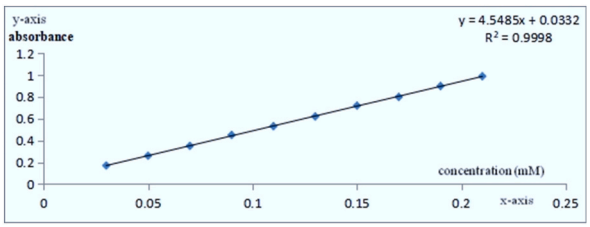


Fig. 4. Calibration curve of standard Ascorbic acid for DPPH assay.

described the antioxidant profile of all the seed extracts of *HV* plant. The calibration curve of ascorbic acid has also been plotted, shown in Fig. 4.

The above findings suggested that the methanol extract possess significant radical scavenging activity. Depending upon the concentration that ranges from 60 to 200 mg/ml, the DPPH radical scavenging activity of *HV*-H, *HV*-DC, *HV*-EA, *HV*-AC and *HV*-M was determined in the range of 16.51–21.74%, 37.43–43.1%, 31.28–35.84%, 22.24–38.43%, 32.34–52.41% respectively. While the antioxidant activity profile of the above extracts exhibited in the range of 0.141–0.151 mM AAE/100 g, 0.106–0.117 mM AAE/100 g, 0.123–0.135 mM AAE/100 g, 0.129–0.165 mM AAE/100 g and 0.116–0.138 Mm AAE/100 g respectively. The collective antioxidant content in all *HV* seed extracts was found to be in the range of 0.106–0.165 mM AAE/100 g. A significant increase in the percentage scavenging activity and antioxidant content in DPPH assay was observed in all the extracts at the concentration of 200 mg/ml except methanol extract.

The IC_{50} was determined by the acetone and methanol extract of *HV* plant which was found to be 50.21 and 53.8 mg/ml respectively. In the current study, it has been found that the highest IC_{50} was found in the methanol extract amongst all other seed extracts of *HV* plant. The percentage scavenging activity and antioxidant profile exhibited by different extracts of *HV* plant has been displayed in Table 4.

3.5. ABTS cation decolorization assay

The *HV* seed extracts were prepared in hexane (H), dichloromethane (DC), ethyl acetate (EA), acetone (AC) and methanol (M) respectively. All the extracts were tested for ABTS assay at four different concentrations, which ranges from 100 to 300 mg/ml. The acetone extract showed significantly high percent inhibition as compared to other extracts i.e. 70% at the concentration of 200 mg/ml, while methanol extract also showed considerable percent inhibition i.e. 56.48% at the concentration of 100 mg/ml. The range of percent inhibition of the following hexane, dichloromethane, ethyl acetate, acetone and methanol extract were 19.09–40.63%, 15.42–31.49%, 22.9–31.2%, 37.88–70.42% and 35.81–56.48% respectively. The IC_{50} was calculated by the help of graph plotted between concentration and percent inhibition. The IC_{50} was determined by the acetone and methanol extract of *HV* plant i.e. 1.7 and 1.62 mg/ml. Trolox, considered as a standard in this assay, its calibration curve has been plotted with coefficient correlation value of $r_2 = 0.9993$ (see Fig. 5). The antioxidant content was determined in terms of trolox equivalent antioxidant content i.e. TEAC mM/100 g. The current study showed significant antioxidant content was displayed by all the extracts of *HV* plant, enlisted in Table 5. The antioxidant content for hexane, dichloromethane, ethyl acetate, acetone and methanol ranges from 0.080 to 0.100 mM/100 g, 0.081–0.103 mM/100 g, 0.082–0.103 mM/100 g, 0.035–0.083 mM/100 g and 0.052–0.076 mM/100 g respectively.

3.6. Correlation between total phenolic content, total flavonoid content and antioxidant activities

Correlation between TPC and various antioxidant activities of *Hordeum vulgare* seeds extract have been determined (Table 6). TPC had a negative correlation ($r = -0.01637$) with DPPH activity values with a level of significance 95% showing that phenolic compounds present in *Hordeum vulgare* seeds extract are not the major contributing factor for antioxidant effect. Moreover, there was a weak correlation between TFC and DPPH, $r = 0.040$. The results indicate that DPPH radical scavenging activity of the plant is dependent on flavonoids present in the plant composition to some extent. The TPC *Hordeum vulgare* seeds extract also showed a negative correlation with ABTS radical scavenging activity. Whereas TFC is positively corelated with ABTS activity.

The amount of phenolic compounds, which are known for their antioxidant properties, in a sample is measured by its total phenolic content [34]. The antioxidant activity of a material determines its ability to scavenge free radicals, and many phenolic compounds have high antioxidant activity. As a sample's total phenolic content increases, it is therefore expected that it will have a higher DPPH or ABTS activity, indicating increased antioxidant potential [34]. It is significant to note that there is not always a clear correlation between total phenolic content and antioxidant activity [35,36]. The overall antioxidant activity can be influenced by a variety of other variables, including the precise types of phenolic compounds present, their structural variations, and their interactions. Antioxidant activity can also be impacted by the presence of additional non-phenolic antioxidants in a sample [34,37]. Flavonoids are the subclass of phenolic compounds, also well known for its antioxidant properties. But the activity can also be obstructed by the presence of other antioxidants that are not flavonoids. Hence, this lead to explore the non-phenolic and non-flavonoids that can be responsible for antioxidant activity through GC-MS technique.

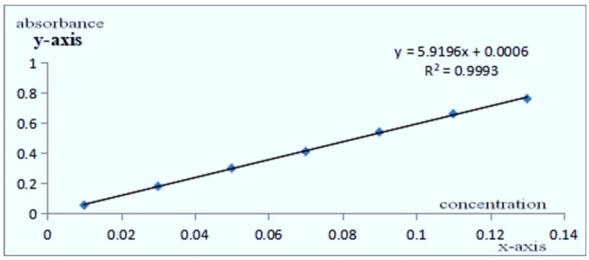


Fig. 5. Calibration curve of standard Trolex for ABTS assay.

Table 5
Antioxidant ABTS assay obtained from *Hordeum vulgare* seed extracts.

S. No.	Extract	Conc mg/mL	Percent inhibition %	IC_{50}	TROLEX (mM/100g)
1.	HV-H	3	22.4	ND	0.096
		2.5	25.12		0.092
		2	19.09		0.100
		1	40.63		0.080
2.	HV-DC	3	31.49	ND	0.081
		2.5	30.23		0.083
		2	15.42		0.101
		1	22.98		0.103
3.	HV-EA	3	29.71	ND	0.084
		2.5	31.12		0.082
		2	25.9		0.088
		1	22.9		0.103
4.	HV-AC	3	40	1.7	0.071
		2.5	38.91		0.072
		2	70.42		0.035
		1	37.88		0.083
5.	HV-M	3	44.06	1.62	0.067
		2.5	55.78		0.052
		2	35.81		0.076
		1	56.48		0.058

ND= Not determined.

Table 6
Correlation between TPC, TFC and Antioxidant activities.

	TPC	TFC	DPPH	ABTS
TPC	1			
TFC	−0.10865	1		
DPPH	−0.01637	0.040442	1	
ABTS	−0.506	0.203452	0.318251	1

The study therefore advances our knowledge of the antioxidant potential of diverse natural products and can guide when it comes to selecting substances with higher antioxidant capacities for usage, in the food industry, dietary supplements and the production of natural health care products.

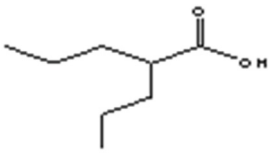
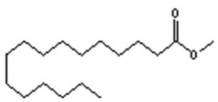
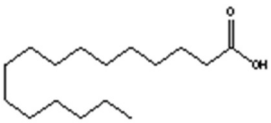
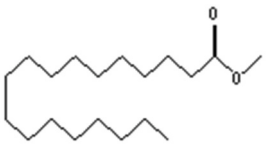
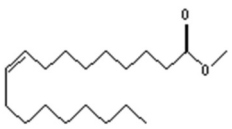
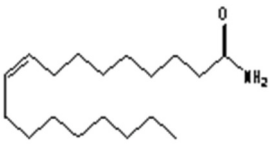
3.7. Composition and characterization

Fresh *HV* seeds were extracted with n-hexane and were analyzed through GC-MS spectra. The analysis of *Hordeum vulgare* seeds of hexane extract (*HV*–H) through GC-MS technique was conducted, which showed various significant compounds. These compounds were also compared with the NIST database. The identified compounds were associated with diverse chemical classes as shown in Table 7. The relative percentages of most prevailing compounds have been mentioned in Table 8 according to their peak heights. The compounds determined in the *HV*-H extract includes carboxylic acid (6.25%), fatty acids (37.5%), carboxylic acid amide derivatives (6.25%), phytosterols (6.25%), triterpinoids (18.75%), stigmastanes (6.25%), cycloartanol (6.25%), beta-diketones (6.25%), hydrocarbons and fat soluble vitamins (6.25%).

The hexane extract of *Hordeum vulgare* plant composed of 16 compounds. Compound (1) contained carboxylic acid, compounds (2–7) were fatty acids, compound (8) was carboxylic acid amide derivative of fatty acid, compound (9, 12 and 14) belonged to a class of organic compound known as triterpinoids, compound (10) was a fat soluble vitamin, compound (11) was a phytosterol, and compounds (13, 15 and 16) belonged to different classes of organic compounds namely; stigmastanes, beta diketones, and cycloartenol respectively. The most abundant compound includes Hexadecanoic acid, methyl ester (6.84%), n-Hexadecanoic acid (8.58%), 9,12-Octadecanoic acid (Z,Z)-, Methyl Ester (8.04%), 9,12-Octadecadienoic acid (Z,Z) (57.01%), Lup-20(29)-en-3-one (3.57%), γ-Sitosterol (3.31%) respectively-MS Chromatograms peaks and retention time of n-hexane extract are shown in Fig. 6.

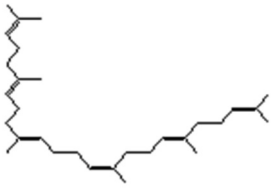
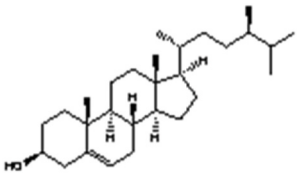
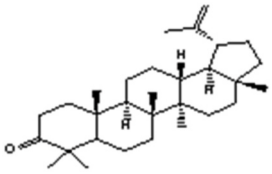
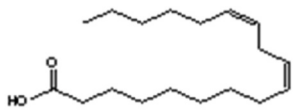
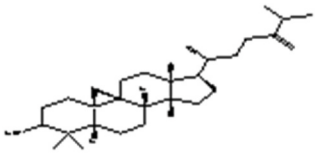
Hexane extraction is often used in the isolation of phenolic and non-phenolic compounds from various natural sources. Moreover it is a non-polar solvent, making it well-suited for the extraction of lipophilic compounds and these lipophilic (fat-soluble) compounds, can also exhibit antioxidant activity by inhibiting lipid peroxidation, a chain reaction that can cause damage to cell membranes [77]. A study suggested synergistic antioxidant effect of both lipophilic and hydrophilic components [78]. Many of the researches focuses polar solvent extraction of HV, a very few data is available that investigates the direct extraction of HV using hexane [79–81]. These studies classified the phytochemicals into various classes such as phenolic, flavonoids, sterols and phytosterols, lignans and folates [82]. Surprisingly the current study reports fatty acids and their esters as a major constituent that may be responsible for the antioxidant activity. Moreover, It is the first report that identified constituents such as Lup-20(29)-en-3-one, campesterol and squalene which were not previously reported [83,84].

Table 7
Identified non-polar compounds from hexane extract of *Hordeum vulgare*.

S.No	Compound	RT	RI	RI (reference)	Area %	Match %	Antioxidant Activity
1	 Valproic acid **(1)**	12.803	–	–	0.23	86	[38,39]
2	 2,4-Decadienal,(E,E) **(2)**	16.845	984.095	–	1.85	91	[40,41]
3	 Hexadecanoic acid, methyl ester **(3)**	27.016	1460.25	–	6.84	94	[41,42]
4	 n-Hexadecanoic acid **(4)**	27.953	1518.66	–	8.58	95	[42–46]
5	 9,12-Octadecanoic acid (Z,Z)-, Methyl Ester **(5)**	30.416	1666.26	–	8.04	93	[41]
6	 9-Octadecenoic acid (Z,Z)-, Methyl Ester **(6)**	30.549	1673.69	–	1.80	94	[41]
7	 9-Octadecenamide,(Z)- **(7)**	51.097	2642.71	–	1.33	89	[45,47]

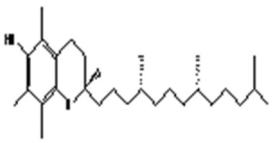
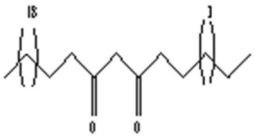
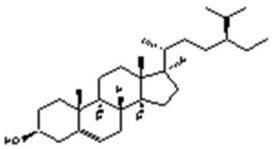
(*continued on next page*)

Table 7 (*continued*)

S.No	Compound	RT	RI	RI (reference)	Area %	Match %	Antioxidant Activity
8	 Squalene **(8)**	51.74	2702.61	–	2.34	93	[48–51]
9	 Campesterol**(9)**	56.113	3010.56	–	0.79	91	[55,56]
10	 Lup-20(29)-en-3-one **(10)**	56.48	3019.949	–	3.57	75	[57]
11	 9,12-Octadecadienoic acid (Z,Z)- **(7)**	32.449	1759.29	–	57.01	89	[41,45,46]
12	 1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)- (.+/-.)- **(14)**	57.331	3041.71	3030	0.49	86 (RI)	[51]
13	 9, 19-Cyclolanostan-3-ol, 24-methylene-, (3β)- **(16)**	58.835	3080.18	3108	1.25	92 (RI)	[58]

(*continued on next page*)

Table 7 (*continued*)

S.No	Compound	RT	RI	RI (reference)	Area %	Match %	Antioxidant Activity
14	 Vitamin E **(10)**	55.201	2964.86	–	1.84	81	[52–54]
15	 14, 16-Hentriacontanedione **(15)**	57.819	3054.19	–	0.63	85	[58]
16	 γ-Sitosterol**(13)**	57.173	3037.67	–	3.31	92	[43]

The nature of compound and reported biological activities (other than antioxidant activity) of the identified compounds through GC-MS technique are mentioned in Table 8 for future consideration.

4. Conclusion

The above findings revealed that *HV* seed extracts possess high antioxidant activity which may be due to the presence of chemical entities like Vitamin E, terpenes and polyunsaturated fatty acids in it. The high antioxidant activity of its seed extracts has made this plant pharmacologically important. As it contains several nutritious and medicinal values, it can help treat various health disorders. This study contributes to our understanding of the antioxidant capabilities of various natural products. It helps in identifying substances with greater antioxidant potential, which is valuable for applications in the food and nutrition industry and the development of natural health products. The current study reports fatty acids and their esters as a major constituent that may be responsible for the antioxidant activity. Moreover, it is the first report that identified constituents such as Lup-20(29)-en-3-one, campesterol and squalene which were not previously reported. Conclusively, there is a vast scope to further explore the active principals of barley so that more of its pharmacological properties can be identified.

Data availability statement

All the data underlying the results are available as a part of this article and no additrion data source are required.

CRediT authorship contribution statement

Saman Shahab Farooqi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Safila Naveed:** Supervision, Conceptualization. **Fatima Qamar:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Aisha Sana:** Visualization, Validation, Methodology, Investigation, Data curation. **Shahab H. Farooqi:** Validation, Software, Resources, Formal analysis, Data curation. **Neelam Sabir:** Writing – review & editing, Funding acquisition. **Asra Mansoor:** Writing – review & editing, Funding acquisition. **Halima Sadia:** Writing – review & editing.

Table 8
Nature & biological activities of compounds identified through GC-MS analysis.

S. No	Compound	Nature of Compound	Biological activity
1	Valproic acid	Fatty Acid [59]	Anti-cancer activity [60], Anti-convulsant [61]
2	2,4-Decadienal,(E,E)	Polyunsaturated Fatty Aldehydes [62]	Nematicidal activity [63], High odor activity [40]
3	Hexadecanoic acid, methyl ester	Fatty Acid Methyl Ester [64]	Anti-inflammatory activity [65], Hypocholesterolemic [66], Nematicide [67], Anti-bacterial [64], Anti-fungal [64]
4	n-Hexadecanoic acid(palmitic acid)	Fatty Acid [68]	Anti-bacterial activity [44] Anti-inflammatory activity [42] Hypocholesterolemic [42], 5-α reductase inhibitor activities agents [42] Anti-cancer activity, Larvicidal property [57] Nematicide [68], Anti-microbial [68], Anti-androgenic [68].
5	9,12-Octadecanoic acid (Z,Z)-, Methyl Ester (Linoleic acid methyl ester)	Fatty acid [69]	Anti-inflammatory [70], Hepatoprotective [70], Hypocholesterolemic [70], Anti-arthritic [70], Anti-histamine [70]
6	9-Octadecenoic acid (Z,Z)-, Methyl Ester	Fatty acid [71]	Anti-microbial [71] Anti-cancer [71]
7	9-Octadecenamide	Amide compound [47]	Anti-microbial [47]
8	Squalene	Triterpene	Anti-microbial [48], Anti-cancer [48,50] Anti-tumor [50]
9	Campesterol	Phytosterol	Anti-inflammatory activity [72]
10	Lup-20(29)-en-3-one	Triterpene [57]	Anti-bacterial [73], Anti-fungal [73]
11	9,12-Octadecadienoic acid	Fatty Acid	Anti-inflammatory [57], Anti-arthritic property [46,57] Anti-bacterial [46], Anti-fungal [46], Anti-coronary [46], Hepatoprotective [46], Anti-eczemic [46], Anti-depressant [46], Cancer preventive [46], Anti-histaminic [46]
12	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/−.)-	Triterpenoid	Anti-microbial [46], Anti-arthritic [46], Anti-inflammatory [46], Cytotoxic [46], Insecticidal [46] Chemopreventive [46]
13	9, 19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	Triterpene [58]	Anti-microbial [58]
14	Vitamin E	Fat soluble vitamin [52]	Anti-cancer [52], Anti-proliferative [52], Anti-inflammatory [52,74], lowers cholesterol level [52], Anti-microbial [58], Immune stimulatory [58], Anti-spasmodic [58]
15	14, 16-Hentriacontanedione	Beta diketone [75]	Anti-microbial [75], Anti-inflammatory [75]
16	γ-Sitosterol	Stigmastanes and Derivatives [76]	Anti-hyperlipidemic [76]

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

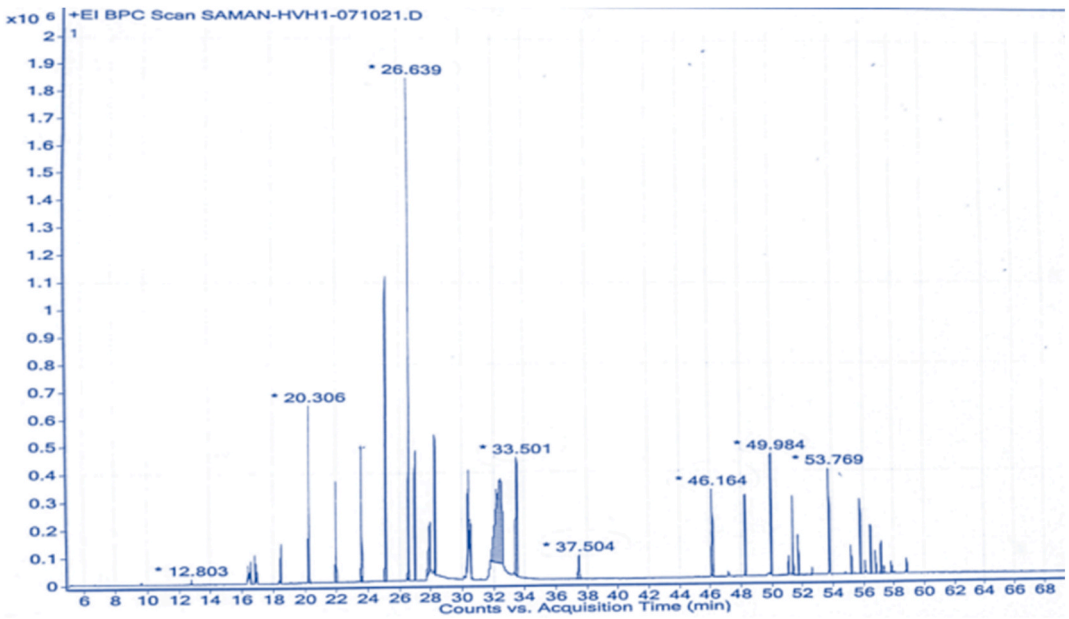


Fig. 6. GC-MS Chromatogram showing peaks and retention time of n-hexane extract of *HV* seeds.

List of Abbreviations

ABTS	2-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid
AAE	Ascorbic Acid Equivalent
AC	Acetone
A_C	Absorbance of Control
A_T	Absorbance of Test Sample
DPPH	1, 1-diphenyl-2-picryl-hydrazyl free radical
DC	Dichloromethane
EA	Ethyl Accetate
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography-Mass Spectrometry
GPR	General Purpose Reagent
HCl	Hydrochloric acid
HV	Hordeum vulgare
IC_{50}	Half Maximal Inhibitory Concentration
NIST	National Institute of Standards and Technology
QE	Quercetin Equivalent
RE	Rutin Equivalent
RI	Retention Indices
ROS	Reactive Oxygen Species
RT	Retention Time
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TEAC	Trolox Equivalent Anti-Oxidant Capacity
TQQQ	Triple quad
WHO	World Health Organization

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